

Legume Production in Semi-Arid Areas: Comparative
Study of the Physiology of Drought Tolerance in
Common Bean (*Phaseolus vulgaris* L.) and Mungbean
(*Vigna radiata* (L.) Wilczek)

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ABSTRACT

Context: Approximately one billion people live in semi-arid and arid regions, and of these about 40% live on less than a dollar a day.

Legumes crops are an important component of sustainable agriculture, but they are often grown under intermittent or terminal droughts. Thus, improving drought tolerance in legumes has the potential to improve food security and sustainability of agricultural systems.

Objective: This study compares the response of two legume crops, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek), to water stress conditions under field and controlled-environment conditions.

Methods: Field experiments were conducted in the Fergana valley, Uzbekistan, and controlled environment experiments were conducted at the Macdonald campus of McGill University, Ste-Anne-de-Bellevue, Canada, and at the Hermitage Research Station in Warwick, Australia.

Results: Our results demonstrate that alternate furrow irrigation maintains yields, yet decreases water applied by 25%. In addition, mungbean showed the highest yields in the moderate deficit irrigation treatment in 2003 and severe deficit irrigation treatment in 2004 under field conditions in Uzbekistan. Common bean also showed a capacity to maintain yields under moderate deficit irrigation in both years. Further characterization of the legume responses to water deficit stress in controlled-environment experiments indicated that mungbean's higher tolerance is attributable to higher transpiration efficiency, a more conservative water use in the vegetative stage, and a higher root-to-shoot ratio when compared to common bean. Root characteristics might also play an important role, although we have observed a large variability between genotypes. An

additional field experiment in Uzbekistan demonstrated that an early maturing Canadian soybean cultivar could be grown after the harvest of winter wheat, and thus contribute to food security. It has also been demonstrated that inoculation with rhizobia increases yields of soybean.

Conclusion: Legumes can be grown after the harvest of winter wheat in Uzbekistan, and mungbean is better adapted to drought than common bean. Short season soybean could also be produced in this cropping sequence.

RÉSUMÉ

Contexte : Environ un milliard de personnes vivent dans les régions arides et semi-arides, et 40% d'entre eux vivent avec moins d'un dollar par jour. Les cultures de légumineuses sont une part importante de l'agriculture durable, mais ces cultures sont souvent produites dans des conditions de sécheresse intermittente ou terminale. Améliorer la tolérance des légumineuses à la sécheresse peut donc augmenter la sécurité alimentaire et la durabilité des systèmes agricoles.

Objectif : Cette étude compare la réponse de deux légumineuses, le haricot commun (*Phaseolus vulgaris* L.) et le haricot doré (*Vigna radiata* (L.) Wilczek), lors de conditions de stress hydrique imposées au champ et en environnement contrôlé.

Méthodes : Les expériences au champ furent conduites dans la vallée de Fergana, en Ouzbékistan, et les expériences en environnement contrôlé furent conduites au campus Macdonald de l'Université McGill, à Ste-Anne-de-Bellevue, Canada, et à la station de recherche Hermitage, à Warwick, Australie.

Résultats : Nos résultats démontrent que l'irrigation alternante maintient les rendements tout en diminuant de 25% l'apport en eau. De plus, le haricot doré a démontré les meilleurs rendements sous les traitements d'irrigation déficitaire moyen en 2003 et sévère en 2004 dans les expériences au champ. Le haricot commun a également démontré la capacité de maintenir ses rendements sous traitement d'irrigation déficitaire moyen les deux années. Une caractérisation plus poussée de la réponse des légumineuses au stress hydrique en milieu contrôlé a indiqué que la tolérance accrue du haricot doré est attribuable à une meilleure efficacité transpirationnelle, une utilisation plus limitée de l'eau

lors de la période végétative, et une proportion plus élevée de biomasse en racines lorsque comparé au haricot commun. Les caractéristiques racinaires semblent également jouer un rôle important, bien que nous ayons observé une grande variation entre génotypes. Une expérience additionnelle en Ouzbékistan a démontré qu'un cultivar canadien de soya de courte maturité peut être produit après la récolte du blé d'hiver, et ainsi contribuer à la sécurité alimentaire. Il a également été démontré que l'inoculation rhizobiale augmente les rendements du soya.

Conclusion : Les légumineuses peuvent être cultivées après la récolte du blé d'hiver en Ouzbékistan, et le haricot doré est mieux adapté à la sécheresse que le haricot commun. Le soya de courte maturité peut également être produit dans cette rotation.

DEDICATION

I would like to dedicate this thesis to Nigora and Feruza, and other Uzbek girls of their generation who will face challenges of poverty and inequality that I have never faced.

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TABLE OF CONTENTS

DEDICATION	6
TABLE OF CONTENTS	10
LIST OF TABLES	16
LIST OF FIGURES.....	17
LIST OF ACRONYMS	19
CONTRIBUTIONS OF CO-AUTHORS	22
1. GENERAL INTRODUCTION	24
1.1 Introduction	24
1.2 Hypotheses	35
1.3 Objectives	36
2. LITERATURE REVIEW.....	37
Preface to chapter 2.1.....	40
2.1 HOW CAN AGRICULTURAL R&D HELP FULFILL UN MILLENNIUM DEVELOPMENT GOALS ? A CROP PHYSIOLOGIST PERSPECTIVE	41
2.1.1 Abstract	41
2.1.2 Today's great challenges: Poverty, water scarcity, and climate change	42
2.1.3 The UN millennium development goals.....	45
2.1.4 The role of agricultural research and development	47
2.1.5 Subsistence farming and improved stress tolerance.....	48
2.1.6 Micro-irrigation technologies and small-scale competitive advantages	50
2.1.7 Conclusions	52
Preface to chapter 2.2.....	54
2.2 TECHNICAL SOLUTIONS FOR BETTER WATER MANAGEMENT: THE ARAL SEA BASIN AND UZBEKISTAN AS A CASE STUDY.....	55
2.2.1 Abstract	56
2.2.2 The ecological disaster of the Aral Sea.....	56
2.2.2.1 The environment	56
2.2.2.2 Impacts on the health of human populations.....	58
2.2.2.3 Can the Sea be saved?.....	60
2.2.3 Historical, political and economic context of agriculture in the Aral Sea basin.....	61
2.2.3.1 The Soviet era	61

2.2.3.2 Today: Newly independent republics.....	62
2.2.3.3 Water management in newly independent republics.....	63
2.2.4 Policy options for better water management.....	64
2.2.4.1 Land reforms.....	64
2.2.4.2 Demand-based management and water user associations.....	65
2.2.4.3 Water pricing.....	66
2.2.5 Technical options for better water management.....	67
2.2.5.1 Modernization of irrigation infrastructures.....	67
2.2.5.2 On-farm techniques: alternate furrow irrigation and regulated deficit irrigation.....	68
2.2.6 Crop choices for better water management.....	72
2.2.7 The way forward.....	73
Preface to chapter 2.3.....	74
2.3 LEGUMES IN SEMI-ARID AND ARID REGIONS: PHYSIOLOGICAL UNDERSTANDING OF DROUGHT TOLERANCE MECHANISMS, BREEDING TECHNOLOGIES AND LIMITS TO KNOWLEDGE.....	75
2.3.1 Abstract.....	76
2.3.2 Legumes in semi-arid and arid regions.....	77
2.3.2.1 Common bean (<i>Phaseolus vulgaris</i> L.).....	78
2.3.2.2 Chickpea (<i>Cicer arietinum</i>).....	80
2.3.2.3 Cowpea (<i>Vigna unguiculata</i>).....	80
2.3.2.4 Pigeon pea (<i>Cajanus cajan</i>).....	81
2.3.2.5 Mungbean (<i>Vigna radiata</i> (L.) Wilczek).....	81
2.3.2.6 Lentil (<i>Lens culinaris</i>).....	82
2.3.2.7 Faba bean (<i>Vicia faba</i>).....	83
2.3.2.8 Soybean (<i>Glycine max</i> L. Merr).....	83
2.3.2.9 Groundnut (<i>Arachis hypogaea</i>).....	84
2.3.3 Drought: a complex environmental stress.....	84
2.3.4 Drought resistance framework: Drought escape.....	86
2.3.5 Drought resistance framework: Dehydration avoidance.....	86
2.3.5.1 Leaf area development response.....	87
2.3.5.2 Root characteristics and growth response.....	88
2.3.5.3 Stomatal response.....	89
2.3.5.4 Leaf characteristics and leaf movement.....	91

2.3.5.5 Nitrogen fixation response.....	92
2.3.5.6 Osmotic adjustment.....	94
2.3.6 Drought resistance framework: Dehydration tolerance	95
2.3.7 Yield component frameworks	96
2.3.8 Clues from molecular biology	98
2.3.8.1 Clues from microarray data	98
2.3.8.2 Signalling pathways in response to dehydration stress.....	100
2.3.8.3 Genetic engineering of drought tolerance traits.....	104
2.3.8.4 Quantitative trait loci (QTLs) and marker assisted selection (MAS)	105
2.3.8.5 Crop physiology and genomics: linking gene networks to whole plant response.....	106
2.3.9 Conclusions.....	108
Preface to chapter 3.....	110
3. LEGUME PRODUCTION AND IRRIGATION STRATEGIES IN A SEMI-ARID ENVIRONMENT: YIELD, YIELD COMPONENTS, WATER RELATIONS, AND CROP DEVELOPMENT OF COMMON BEAN (<i>Phaseolus vulgaris</i> L.) AND MUNGBEAN (<i>Vigna radiata</i> (L.) Wilczek)	112
3.1 Abstract.....	112
3.2 Introduction	113
3.3 Materials and methods.....	117
3.3.1 Environment	117
3.3.2 Experimental design	118
3.3.3 Irrigation scheduling	118
3.3.4 Cultural practices.....	120
3.3.5 Common bean and mungbean seeds	121
3.3.6 Measurements.....	121
3.3.7 Statistical analyses.....	123
3.4 Results	123
3.4.1 Yield	123
3.4.2 Yield components	124
3.4.3 Harvest index	125
3.4.4 Stem water potential.....	125
3.4.5 Stomatal conductance.....	126

3.4.6 Crop development	127
3.4.7 Nodule development	129
3.4.8 Water use efficiency	129
3.5 Discussion	129
3.6 Conclusions.....	135
Preface to chapter 4.....	146
4. EFFECT OF INOCULATION ON A SHORT-SEASON CANADIAN CULTIVAR OF SOYBEAN (<i>Glycine max</i> [L.] Merr.) GROWN IN UZBEKISTAN	148
4.1 Abstract.....	148
4.2 Introduction	148
4.3 Materials and methods.....	150
4.4 Results and discussion	153
4.5 Conclusion	155
Preface to chapter 5.....	158
5. EFFECTS OF DEFICIT IRRIGATION AND SALINITY STRESS ON COMMON BEAN (<i>Phaseolus vulgaris</i> L.) AND MUNGBEAN (<i>Vigna radiata</i> (L.) Wilczek) GROWN IN A CONTROLLED ENVIRONMENT	161
5.1 Abstract.....	161
5.2 Introduction	162
5.3 Materials and methods.....	165
5.3.1 Drought experiment.....	165
5.3.1.1 Experimental design	165
5.3.1.2 Plant growth	165
5.3.1.3 Watering schedule.....	166
5.3.1.4 Measurements.....	166
5.3.2 Drought and Salinity experiment	167
5.3.2.1 Experimental design	167
5.3.2.2 Plant growth	167
5.3.2.3 Salinity stress	168
5.3.2.4 Water scheduling.....	168
5.3.2.5 Measurements.....	169
5.3.3 Statistical analysis	169
5.4 Results and discussion	170
5.4.1 Yield and yield components under regulated deficit irrigation	170

5.4.2 Leaf area, biomass, and harvest index under regulated deficit irrigation	171
5.4.3 Water potential, photosynthesis, transpiration and specific leaf weight under regulated deficit irrigation	172
5.4.4 Salinity stress	173
5.5 Conclusions.....	175
Preface to chapter 6.....	184
6. COMPARATIVE STUDY OF COMMON BEAN (<i>Phaseolus vulgaris</i> L.) AND MUNGBEAN (<i>Vigna radiata</i> (L.) Wilczek) RESPONSE TO SEVEN WATERING REGIMES IN A CONTROLLED ENVIRONMENT.....	186
6.1 Abstract	186
6.2 Introduction	187
6.3 Materials and methods.....	190
6.3.1 Experimental design	190
6.3.2 Plant growth	190
6.3.3 Watering schedule.....	191
6.3.4 Measurements.....	192
6.3.5 Statistical analyses	193
6.4 Results	193
6.4.1 Water consumption	193
6.4.2 Leaf area and biomass	194
6.4.3 Water potential and relative water content	194
6.4.4 Gas exchange measurements	195
6.5 Discussion.....	196
6.6 Conclusion	201
Preface to Chapter 7	208
7. COMPARATIVE STUDY OF THE EARLY ROOT MORPHOLOGY IN COMMON BEAN (<i>Phaseolus vulgaris</i>) AND MUNGBEAN (<i>Vigna radiata</i> (L.) Wilczek).....	210
7.1 Abstract.....	210
7.2 Introduction	211
7.3 Materials and methods.....	213
7.3.1 Experimental design	213
7.3.2 Plant growth and measurements.....	213
7.3.3 Statistical analyses	214

7.4 Results	214
7.5 Discussion.....	216
7.6 Conclusion	218
8. GENERAL DISCUSSION.....	222
8.1 Role of R&D in poverty alleviation.....	222
8.2 On-farm water management: importance of monitoring inflows.....	223
8.3 Challenges in applying water stress in field and controlled-environment experiments.....	224
8.4 Mechanisms of drought tolerance in legumes: osmotic adjustment.....	225
8.5 Mechanisms of drought tolerance in legumes: transpiration efficiency ...	227
8.6 Improvement of mungbean germplasm	228
9. CONCLUSIONS.....	231
10. CONTRIBUTIONS TO KNOWLEDGE	233
11. DIRECTIONS FOR FUTURE RESEARCH.....	238
12. REFERENCES CITED.....	240
APPENDIX A: SOIL ANALYSIS RESULTS	262

LIST OF TABLES

Table 2.3.1: World production, average yields, and major producers of selected legume crops in 2007 according to FAOSTAT	109
Table 3.1: Alternate furrow irrigation as compared to every furrow irrigation on yield, yield components, and water relations of common bean and mungbean.....	143
Table 3.2: Yield components of common bean and mungbean under three levels of regulated deficit irrigation in the Fergana Valley, Uzbekistan.....	144
Table 3.3: Water relations of common bean and mungbean under three levels of regulated deficit irrigation	145
Table 4.1: Yield and yield components of soybean (cv. Costaud) grown in the Fergana Valley, Uzbekistan.....	156
Table 4.2: Average number (per 0.5 m row) and dry weight of nodules found in soybean (cv Costaud) grown in Uzbekistan in 2004	157
Table 5.1: Yield components of common bean and mungbean subjected to regulated deficit irrigation.	182
Table 5.2: Proportion of biomass allocated to reproductive structures and harvest index in common bean and mungbean subjected to water stress.....	183
Table 6.1: Evapotranspiration, leaf area, dry biomass, relative water content, and osmotic potential of common bean and mungbean subjected to seven watering regimes.	206
Table 7.1: Root characteristics of common bean and mungbean 13 days after seeding.	220

LIST OF FIGURES

Figure 3.1: Map of the Aral Sea Basin and location of experimental area (Fergana Valley).....	137
Figure 3.2: Climatic data for the growing seasons of 2003 and 2004 in the Fergana Valley, Uzbekistan (40°23'N, 71°45'E) from the beginning of July until the end of October.....	138
Figure 3.3: Seed yields of common bean and mungbean in 2003 and 2004 subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.....	139
Figure 3.4: Harvest index of common bean and mungbean in 2003 and 2004 subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.....	140
Figure 3.5: Crop development (biomass A, leaf area index B, crop height C, and number of pods D) of common bean and mungbean subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.....	141
Figure 5.1: Yield of common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.....	176
Figure 5.2: Leaf area of common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.....	178
Figure 5.3: Photosynthesis as a function of average soil available water depletion level in common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.....	180
Figure 5.4: Relationship between photosynthesis and transpiration in common bean and mungbean subjected to regulated deficit irrigation.....	181
Figure 6.1: Photosynthetic rates prior to a watering event in common bean and mungbean subjected to seven watering regimes at Hermitage Research Station, Warwick, Australia.....	202
Figure 6.2: Transpiration rates after a watering event in common bean and mungbean subjected to seven watering regimes at Hermitage Research Station, Warwick, Australia.....	204

Figure 7.1: Typical root systems of mungbean (top row) and common bean (bottom row) scanned with the WinRHIZO system 13 days after seeding..... 219

LIST OF ACRONYMS

ABA	Abscisic acid
ABRE	ABA-responsive element
AREB	ABA-responsive-element binding protein
AFI	Alternate furrow irrigation
ANOVA	Analysis of variance
bZIP	Basic region / leucine zipper protein
C	Crop growth rate
CDPK	Calcium-dependent protein kinase
CGIAR	Consultative group on international agricultural research
CIAT	Centro internacional de agricultura tropical
CIDA	Canadian international development agency
DAS	Days after seeding
Dr	Duration of reproductive growth
DRE	Dehydration-responsive element
DREB	Dehydration-responsive-element binding protein
EC _e	Electrical conductivity
EC-IFAS	Executive committee of the International Fund for the Aral Sea
ERD	Early responsive to dehydration
ET	Evapotranspiration
FAO	Food and Agriculture Organization of the United Nations
FQRNT	Fonds québécois de recherches en nature et technologies

HI	Harvest index
ICARDA	International center for agricultural research in the dry areas
IFAD	International fund for agricultural development
LEA	Late embryogenesis abundant
MANOVA	Multivariate analysis of variance
MAP	Mitogen-activated protein
MAS	Marker-assisted selection
MDG	Millennium development goal
OA	Osmotic adjustment
p	Proportion biomass allocated to yield
QTL	Quantitative trait loci
R&D	Research and development
RDI	Regulated deficit irrigation
RI	Radiation intercepted
RPK	Receptor-like kinase
RUE	Radiation use efficiency
RWC	Relative water content
SANIIRI	Central Asian Scientific Research Institute of Irrigation (acronym letters from original name in Russian)
SIC-ICWC	Scientific information center of the InterState Coordination Water Commission
SLW	Specific leaf weight
SWP	Stem water potential
TE	Transpiration efficiency

UN	United Nations
W	Water used
WUAs	Water user associations
Y	Yield

CONTRIBUTIONS OF CO-AUTHORS

I have prepared all the manuscripts presented in this thesis, and have been involved in the design and planning of all experiments, data collection and analysis as well as in the discussion of the results. However, this work could not have been achieved without the contribution of several colleagues who share authorship on the manuscripts. Their contributions are described below.

Heidi Webber is a graduate student from the Bioresources Engineering Department. Heidi Webber's contribution has been invaluable in all stages of these experiments, and this collaboration has allowed us to go beyond individual disciplines. It has certainly provided me with a greater perspective on water and irrigation issues. Specifically, Heidi Webber was in charge of the collection of soil moisture data and the irrigation scheduling which were at the center of the treatment imposition for the experiments described in chapters 3 and 5 of this thesis. She was also responsible for the irrigation of the soybean experiment described in chapter 4. Furthermore, close interaction with Heidi Webber has allowed thorough discussion of several key issues from these papers.

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Mikhail Horst is an irrigation scientist at the Scientific Information Center of the InterState Coordination Water Commission (SIC ICWC) in Tashkent, Uzbekistan, and contributed irrigation expertise to the field experiments, a contribution that proved essential.

Galina Stulina is soil scientist also at the SIC ICWC, and was instrumental in the establishment of the field experiments in both years in the Fergana valley. Galina Stulina's very practical advice, local experience and logistics skills were very helpful in the proper execution of the experiments.

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1. GENERAL INTRODUCTION

1.1 Introduction

Extreme poverty is afflicting 1.1 billion people, a disproportionate number of which live in rural areas and depend on agriculture for a living (IFAD, 2007). At the same time, freshwater resources are under increasing pressures not only from population growth but also industrial and urban development (Postel, 1997; Wallace, 2000; Shady, 2001), a situation that is expected to be exacerbated by climate change in most dry areas (Ragab and Prud'homme, 2002). Agricultural research and development programs and growth in agricultural productivity are unanimously viewed as necessary (although not sufficient) for poverty alleviation (Johnston and Mellor, 1961; Adelman, 1984; Alexandratos, 1999; Rola-Rubzen et al., 2001; Pingali, 2007; Thirtle and Piesse, 2007). Specifically, access to irrigation water and improved crop varieties are seen as two factors that contribute to poverty alleviation and that have positive effects on several of the UN millennium development goals (Courtois et al., 2001; Amarasinghe et al., 2005; Polak and Yoder, 2006; Ceccarelli et al., 2007; Hussain, 2007a, Joshi et al., 2007; Narayanamoorthy, 2007). To sustain the current use and the increased demand for the growing population, irrigation water needs to be managed carefully at the institutional, basin-wide, and farm levels (Postel, 1997; Wallace, 2000).

This is especially true in Central Asia where the expansion of irrigation networks and mismanagement of irrigation water have led to the desiccation of the Aral Sea which, in turn, has caused serious economic and health problems in the lower reaches of the Amu-Darya and Syr-Darya river systems (Elpiner, 1999; Glantz, 1999; Micklin, 2000; Small et al., 2001; Dukhovny, 2003). Because of the importance of irrigation for agriculture in the region, and because the agricultural sector is so

important for the economy of Central Asian countries, agricultural growth and, thus, economic development will require improvements in irrigation water management (Spoor, 1993; Micklin, 2000). With surface irrigation being used on the vast majority of farms (EC-IFAS, 1999), two accessible water saving irrigation techniques are alternate furrow irrigation (AFI) and regulated deficit irrigation (RDI).

AFI, which consists of surface irrigation systems supplying water to every second furrow, has been found to have great potential to improve water productivity in dry areas (Grimes et al., 1968; Crabtree et al., 1985; Kang et al., 2000; Horst et al., 2005; Webber et al., 2006). Further, by alternating the irrigated furrows, root growth is stimulated and this probably helps offset the negative effects of reduced water supply (Kang et al., 2000). In split-root experiments, both Kang et al. (1998) and Kirda et al. (2004) have suggested that by having half of its root system in dry soil, the plant continues to synthesize abscisic acid in the roots, which reduces its transpiration rate.

Through RDI, growers allow some degree of water stress to be experienced by the crop, but the water saved should allow an increase in the area irrigated (English and Raja, 1996), or it could be put to more productive use, in industrial activities for example (Molden et al., 2003). Indeed, with climate change and the increasing demand for water from other sectors, irrigation with limited amounts of water and below full crop water requirements might become the norm rather than the exception (Shady, 2001; Fereres and Soriano, 2007). The method is more complicated than AFI however, and it is important that farmers have control over the timing of irrigations and amount of water applied, as well as the tools for proper irrigation scheduling. Precise information is needed regarding the sensitivity of crops to both the intensity and the timing of water stress (Kijne et al., 2003). Studies performed on a number of

species suggest RDI, when properly performed, is economical, and can lead to substantial gains in water use efficiency, often without significant decreases in yields (Webber et al., 2006; Costa et al., 2007; Fereres and Soriano, 2007; Karam et al., 2007; Vazifedoust et al., 2008).

Further, for the extremely poor farmers, living with less than a dollar a day, diversification into minor but profitable crops, such as legumes, can greatly contribute to increased family income when they have access to markets (Belshaw, 2002; Polak and Yoder, 2006; Baudoin et al., 2007). However, legumes tend to be grown in more marginal environments, under various biotic and abiotic stresses, and as such their yield is often much below their potential (Subbarao et al., 1995). Yet, legumes play a vital role in sustainable agricultural systems because of their nitrogen-fixing symbiosis with rhizobia (Subbarao et al., 1995). As importantly, they often fit in as second crops.

Gains in crop breeding in semi-arid areas could be greatly enhanced by a better physiological understanding of the mechanisms of tolerance to water stress (Rao, 2002; Richards, 2006). In addition, the mechanisms of drought tolerance in legumes have not been as well characterized as in cereals, and the use of physiological and genetic markers for improved legume germplasm has been limited (Turner et al., 2003). A greater understanding of legume responses to drought would likely provide high returns.

Broadly speaking, an agricultural drought which decreases crop yields substantially is a combination of atmospheric conditions, soil moisture conditions, and crop conditions: high temperature and high vapour pressure deficit are combined with low soil moisture and increased soil hardness, and the stress experienced is further affected by the crop development stage and crop planting density (Begg and Turner, 1976;

Thomas, 1997; Turner, 2003). Both the timing and the intensity of the water stress can be important and highly variable. It is not surprising, thus, that the physiological responses of crops to drought are at least as complex. To deal with this complexity, frameworks have been developed to dissect either the response of various processes to water stress (drought tolerance framework), or the contribution of specific traits to yield (yield component frameworks) (Begg and Turner, 1976, Jones et al., 1981; Turner, 1986; Kramer, 1990; Turner, 2003).

The drought tolerance framework divides the responses into processes associated with stress escape, avoidance or tolerance. Drought escape refers to characteristics that allow crop development to be well matched with the water availability (Begg and Turner, 1976; Jones et al., 1981; Turner, 2003). In general, this is achieved by reducing days to maturity. Indeed, early maturity is probably the single most important factor contributing to improved yields of legume crops in semi-arid areas (Silim and Saxena, 1993 and Berger et al., 2007 in chickpea; Silim et al., 1993 in lentil; Ehlers and Hall, 1997 in cowpea). However, an indeterminate growth habit and the associated ability to recover from stress have been argued for as characteristics that might contribute to higher yields in semi-arid areas (Poehlman, 1991 in mungbean; Turner et al., 2003 in legumes in general).

Dehydration avoidance refers to mechanisms that enable a crop to maintain a high relative water content, or high water potential. This can be achieved by maintaining water uptake or by reducing water loss (Jones et al., 1981). Related characteristics include leaf area development response to declining soil moisture, root characteristics and growth response, leaf characteristics and leaf movement, stomatal response, nitrogen fixation response, and osmotic adjustment.

Leaf area development tends to be more responsive to water deficit stress than photosynthesis or stomatal conductance (Begg and Turner, 1976; Blum, 1996). A lower leaf area might not be solely a consequence of a lower growth rate due to water deficit stress, but might be an adaptive mechanism related to a higher relocation of resources to root growth, or a conservation mechanism to reduce water loss from transpiration (Begg and Turner, 1976). Further, the active senescence of leaves under water stress can allow an organized translocation of resources to developing seeds (Turner, 1986; Tardieu, 1996). Indeed, the higher leaf area developed in mungbean and soybean irrigated during the vegetative stage led to a higher water loss from a larger canopy, and led to an overall reduction in radiation use efficiency when compared to treatments watered during other stages of development (De Costa et al., 1999 in mungbean; De Costa and Shanmugathan, 2002 in soybean). However, it has also been well demonstrated that the delay in the onset of leaf senescence in sorghum is associated with higher yields under water stress, but not under well-watered conditions (Borrell et al., 2000; Bennett, 2003; Richards, 2006). This suggests, on the contrary, that the maintenance of leaf area, and of photosynthetic capacity, is an adaptive mechanism to water stress.

Some of the most important and yet least researched components of plant response to drought relate to root development and architecture (Passouira, 1983; Sponchiado et al., 1989; White and Castillo, 1989). A grafting experiment with common bean performed suggested that most of the variability in drought tolerance is attributable to root stocks (White and Castillo, 1989). Several studies observing root systems of both cereals and legumes have shown that drought tolerant cultivars tend to have a greater root biomass, a greater root-to-shoot ratio and deeper root profiles (Grzesiak et al., 1997 in common bean and pea; Matsui and Singh, 2003 in cowpea; Benjamin and Nielsen, 2006 in soybean, pea and chickpea; Kashiwagi et al., 2006 in chickpea). It is also well established that root

growth is less inhibited than shoot growth under moisture stress (Bartels et al., 1996; Blum 1996; Thomas, 1997). For example, De Costa and Shanmugathan (1999) showed that root weight and root-to-shoot ratio in mungbean were highest in the rainfed treatment and lowest in the fully irrigated treatment. However, Passouira (1983) suggested the optimal resource allocation might not be towards the maximum root biomass, but rather to maximize the return on investment on carbon allocated to roots rather than leaves. Crops grown on residual moisture might also deplete the soil water earlier and thus suffer from severe water stress at the reproductive stage (Huang, 2000). Indeed, a breeding retrospective in tropical maize showed that selection for high grain yield in eight cycles of selection resulted in a reduction of the root biomass in the top 50 cm of the soil profile (Bolanos et al., 1993).

One of the most universal responses of plants to water stress is stomatal closure. This is believed to be an integrative response of hydraulic and chemical signals, responding both to a 'long-term' abscisic acid (ABA) signal originating from the roots, and 'short-term' changes in evaporative demands (Tardieu and Davies, 1993). The analysis of the optimal stomatal behaviour is complex because stomata are the site of carbon dioxide uptake as well as water loss, and as such, restriction of water loss might lead to a proportional inhibition of carbon assimilation. Comparative studies among *Phaseolus* species or among *P. vulgaris* cultivars have shown that tolerant plants tend to exhibit a faster stomatal closure in response to decreasing soil water potentials (Markhart, 1985; Lizana et al., 2006). On the contrary, Cruz de Carvalho et al. (1998) showed that the susceptible *P. vulgaris* genotypes exhibited a greater decrease in stomatal conductance than the more tolerant cowpea cultivars. A study on soybean suggested that drought tolerance was associated with a limitation of the maximum transpiration rate (Fletcher et al., 2007). Yet other studies showed that tolerant genotypes exhibited higher rates of

stomatal conductance early in the morning, but lower rates at midday and during the afternoon (Bates and Hall, 1992 in cowpea; Pimentel et al., 1999 in common bean), suggesting an important role for circadian rhythms (Mencuccini et al., 2000), in addition to the response to declining soil moisture. Furthermore, the lack of stomatal response to dry soils after flowering, in several crops, suggests that limitation of water use through decreased stomatal conductance might not lead to higher yields under drought conditions (Ludlow and Muchow, 1990; Blum, 1996).

Some characteristics of the cuticle are thought to have an effect on water loss when stomata are closed (Begg and Turner, 1976). Leaf characteristics that increase leaf reflectance, such as large white hairs for sunflower (Johnson, 1975), or the development of a wax bloom on sorghum (Chatterton et al., 1975) and wheat (Johnson et al., 1983; Richards et al., 1986), can decrease leaf temperature and transpiration. To our knowledge, the only example of a legume crop producing considerable epicuticular wax in response to water stress is in black gram (Ashraf and Karim, 1991), although differences in leaf reflectance are sometimes reported (eg. Phogat et al., 1984 in cowpea and mungbean). In spite of the fact that these constitutive and adaptive traits of the leaf epidermis were identified decades ago, the mechanisms related to cuticular characteristics have not been thoroughly researched. Similarly, the movement of leaves to align them parallel to incident light decreases the irradiation load and might help in reducing leaf temperature and water loss. All legumes manifest some degree of paraheliotropy (Subbarao et al., 1995), but some comparative studies suggest that this is an adaptive trait to water stress deficits (Smith et al., 1988), while others have associated it with higher susceptibility to stress (Lizana et al., 2006).

Biological nitrogen fixation might be more suitable than mineral nitrogen fertilization in drought-prone areas (Lodeiro et al., 2000; Kirova et al.,

2008). For example, a fertilized bean crop showed higher leaf development, and consequently more severe water stress during pod filling, compared to the inoculated crop (Lodeiro et al., 2000). Other mechanisms might also be involved. However, nitrogen fixation is also highly susceptible to drought stress, and this can substantially decrease the benefits of growing legume crops in semi-arid areas (Giller, 2001). Because nitrogen fixation decreases before any changes in photosynthesis are observed, the inhibition of nitrogen fixation does not seem to be related to decreased photosynthate availability, but rather seems to involve three mechanisms: 1. changes in oxygen permeability, which in turn might regulate nitrogenase activity (Pankhurst and Sprent, 1975); 2. a feedback mechanism related to ureide accumulation (Serraj and Sinclair, 1999; Sinclair et al., 2003; King and Purcell, 2005), and 3. a carbon shortage in nodules (Galvez et al., 2005). In addition, all three mechanisms seem to be related in complex interactions, involving ureides, asparagine, manganese, and potentially some yet unknown intermediate compound(s) in nodules and leaves (Vadez et al., 2000; King and Purcell, 2005; Todd et al., 2006).

Osmotic adjustment (OA) is defined as the active accumulation of solutes and consequent decrease in osmotic potential. According to theory, OA helps maintain water extraction, turgor and growth under dehydration and salt stress (Hsiao et al., 1976, Turner and Jones, 1980; Morgan, 1984; Munns, 1988; Blum et al., 1996), and could enable drought-stressed plants to keep stomata open, and thus continue to take up carbon dioxide (Hopkins, 1995). Accumulated solutes include amino acids (particularly proline), sugars, organic acids (malate, citrate), the quaternary ammonium compound glycine betaine, as well as nitrate, potassium and chlorine ions (Morgan, 1984). Osmotic adjustment has been correlated with higher yields in several cereal crops (Ludlow and Muchow, 1990; Fukai and Copper, 1995 in rice) and some legume crops (Flower and Ludlow, 1987

in pigeon pea; Lecoœur et al., 1992 in chickpea; Baigorri et al., 1999 in semi-leafless pea). However, most have reported OA in legumes, but have failed to observe the associated benefits (Wullschleger and Oosterhuis, 1991; Turner et al., 2007a; Likowse and Lawn, 2008). Indeed, the concept of OA has been the object of significant debate over the last 20 years because the physiological basis of its contribution to higher yields is still unclear. Very few solutes seem to accumulate as a result of an increase in uptake following water stress, but rather seem to accumulate as the result of decreasing leaf expansion rates (Munns, 1988; Serraj and Sinclair, 2002). Still, selection for high OA in wheat lines has led to increases in yields under drought-prone environments (Richards, 2006), and simulation models for sorghum suggest OA benefits yield under severe stress (Chapman et al., 2002). It is possible that the main role of the accumulated solutes might not be to decrease the osmotic potential and maintain turgor, but might rather be to protect cell membranes and proteins (Shabala and Lew, 2002), potentially through radical scavenging activities (Smirnov and Cumbes, 1989), or might facilitate recovery of photosynthesis and growth once the stress is relieved (Munns, 1988).

We have discussed the mechanisms that allow plants to avoid dehydration by increasing water extraction, or by reducing water loss. However, if the stress becomes severe, cells might experience dehydration. Protection of the protoplasm by compatible solutes from OA, or from proteins produced specifically for this purpose, might contribute to longer leaf survival or better growth recovery once the stress is relieved (Ludlow and Muchow, 1990). This has been evaluated in cereals and in some legumes (Shackel and Hall in cowpea; Flower and Ludlow, 1986 in pigeon pea; Sinclair and Ludlow, 1986 in cowpea, mungbean, soybean and peanut) from the measurement of lethal water status, consisting either of the relative water content or the leaf water potential at which leaves died.

Ultimately, a physiological understanding of drought tolerance should allow for the incorporation of useful traits into current elite crop germplasm. Besides the analysis of individual traits that could be beneficial under water deficit, another useful approach to consider the effects of a particular trait on economic yield. Since yield is a very complex trait (often with relatively low heritability), it might be particularly relevant to separate physiological processes (ideally into processes with relatively high heritability) contributing to yield and consider these in simple mathematical terms (Turner et al., 2003). Particularly relevant for drought-prone areas is the Passouira identity in which yield is considered the product of the water transpired (W), the transpiration efficiency (TE), and the HI (Passouira, 1977). $Y = W \times TE \times HI$

Thus, traits of possible interest for improving crop tolerance to drought would include: 1. high seedling vigour in order to allow high early radiation interception and decreased soil evaporation, 2. a large and efficient rooting system to allow high water extraction capacity, 3. a high transpiration efficiency, and 4. high harvest index (Turner et al., 2003; Richards, 2006). Recent studies in cowpea, chickpea and lentil suggest that there is genetic variability in root biomass and root length density, especially at depth, and that these traits could lead to higher yields (Matsui and Singh, 2003; Serraj et al., 2004; Ali et al., 2005; Sarker et al., 2005; Kashiwagi et al., 2006). This suggests that present cultivars are not consuming all of the available water.

The correlation between the carbon isotope discrimination (Δ) in leaves and transpiration efficiency (TE) has led to advances in breeding higher yielding wheat (Richards, 2006), and sunflower plants (C. Lambrides, personal communication). Unfortunately, there are conflicting reports regarding the relationship between Δ and TE in legumes (Hall et al., 1992;

Ismail et al., 1994; Khan et al., 2007; Krishnamurthy et al., 2007; Turner et al., 2007b), and the relationship, if it exists, might depend on growing conditions. The HI however holds particular promise. Methods have been developed to evaluate the translocation ability, allowing for the screening of a large number of genotypes (Turner et al., 2003). There seems to be considerable genetic variability in various legume crops subjected to drought stress (Constable and Hearn, 1978 in soybean; Bushby and Lawn, 1992 in mungbean). Indeed, some less-researched legume crops presently exhibit low HI, and there might be opportunities to improve their overall yield potential through improvement in the HI under all conditions.

In summary, this thesis explores farm-level technologies to reduce water use in agriculture while improving food security by growing legumes as second crops, and explores mechanisms of tolerance to water stress by comparing two legume crops, namely common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek). An effort has been made to provide easily identifiable physiological traits that could be useful in breeding programs for improved drought tolerance. The specific hypotheses and objectives of this project are listed in the following pages.

1.2 Hypotheses

This research project tested the following hypotheses:

1. Short-season legume species can be grown after the harvest of winter wheat in the Aral Sea basin;
2. Mungbean, as a locally adapted crop reputed for its drought tolerance, yields better under limited irrigation than common bean;
3. Deficit and alternate furrow irrigation techniques can maintain yields of common bean and mungbean, or at least increase water use efficiency, defined as seed yield per unit of water applied;
4. Inoculation with rhizobia increases yields of a short-season Canadian soybean cultivar grown after the harvest of winter wheat in the Aral Sea basin;
5. Mungbean, being more drought tolerant, is also more tolerant to salinity stress than common bean;
6. Mungbean's better tolerance to drought is due to a greater capacity for osmotic adjustment, a stomatal behaviour more responsive to water stress, and a larger root system compared with common bean;

1.3 Objectives

Based on the hypotheses just stated, the objectives of this project were:

1. To evaluate the potential yield of three legumes species, namely common bean, mungbean and soybean grown after the harvest of winter wheat in the Aral Sea basin;
2. To evaluate the effects of deficit and alternate furrow irrigation techniques on the development and yield of common bean and mungbean;
3. To evaluate the potential yield gains from inoculation of a short-season Canadian soybean cultivar grown in the Aral Sea basin;
4. To evaluate the response and potential interaction in responses to drought stress and salinity stress by common bean and mungbean;
5. To evaluate the osmotic adjustment, photosynthetic and transpirational responses of common bean and mungbean subjected to varying degrees of water stress;
6. To characterize early root development in common bean and mungbean, and determine root characteristics that might be associated with greater water stress tolerance;

2. LITERATURE REVIEW

The following chapter contains three review papers that are being submitted to scientific journals. The first one presents an overview of the problems of poverty, water scarcity, and climate change, and suggests ways agricultural research and development programs can improve productivity (through sustainable irrigation management and improved crop varieties) with the specific aim to reduce poverty and contribute to the UN millennium development goals. Specifically, we suggest that more efforts are needed to understand stress physiological responses, and responses to limited supplemental irrigation, especially in non-cereal crops that have potential to increase poor farming families' incomes.

The second paper reviews the literature related to the ecological disaster of the Aral Sea and the socio-politico-economic context of water management in Uzbekistan, and suggests technical solutions for better management, and among others, a simple yet effective way to improve food self-sufficiency in the region: growing a legume crop after the harvest of winter wheat in the current cropping system. We also argue for more investments in the irrigation infrastructure, as it presently is in a deplorable state.

Finally, the third paper describes the extent of the scientific knowledge on physiological responses to water stress, as well as strategies used to breed crops that are more tolerant to drought conditions. This paper examines drought resistance from a classical crop physiology perspective by considering drought resistance and yield component frameworks, and looks at recent progress using molecular biology tools. Characterization of the molecular response to water deficit stress has so far failed to improve our knowledge of mechanisms of drought tolerance at the whole plant level, but by working together, crop physiologists and molecular

biologists might develop a better understanding of drought tolerance, and might identify traits that could serve as physiological markers for the genetic improvement of legumes in dry areas.

The first and second manuscripts in this literature review relate to the context of this research. We face today great challenges, and agricultural scientists are in a position to help reduce hunger and poverty. However, this will require a fundamental shift in thinking from large-scale, high-input, and mechanized agriculture, to research programs aimed specifically at the rural poor and addressing research priorities such as stress physiology and minor crops (chapter 2.1). These ideas have been central to this research project. Further, it is essential that research projects, and particularly international development projects, consider how the socio-politico-economic context will affect the adoption of the introduced technologies. It is from this analysis, for example, that we have determined the importance of government control in the cropping systems in Uzbekistan (chapter 2.2). The only window of opportunity for the introduction of legumes is after the harvest of winter wheat, but this had never been tested (discussed in section 2.2.6). This, thus, has led to the first and third objectives of this thesis (section 1.3), i.e. to evaluate the potential yield of common bean, mungbean and soybean grown after the harvest of winter wheat in the Aral Sea basin. Because of the particular problems of water management in Central Asia, it was important to test accessible water saving techniques, alternate furrow irrigation and regulated deficit irrigation (chapter 2.2), and to determine their effects on the development and growth of the two legume crops we proposed to introduce. This is indeed the second objective (section 1.3). Thus, the first three objectives are addressed in the field experiments in Uzbekistan and are presented in chapters 3 and 4 of this thesis.

The third manuscript in this literature review addresses the mechanisms of

drought tolerance in legume crops (particularly sections 2.3.4 to 2.3.8), and has served, with data from the field experiment presented in chapter 3, as the basis for the identification of traits that could contribute to mungbean's better stress tolerance observed in the field. Specifically, the stomatal response to water stress, the leaf area development response, the osmotic adjustment capacity, and the root development (discussed in chapter 2.3) of common bean and mungbean under water and salinity stress are at the center of the fourth, fifth and sixth objectives of this thesis (section 1.3), and are addressed in chapters 5, 6 and 7.

Preface to chapter 2.1

The following manuscript is under preparation for submission to the *Agronomy Journal*. Prof. Donald L. Smith, my supervisor, is co-author on the manuscript and provided constructive feedback on the ideas presented and editorial assistance.

2.1 HOW CAN AGRICULTURAL R&D HELP FULFILL UN MILLENNIUM DEVELOPMENT GOALS? A CROP PHYSIOLOGIST PERSPECTIVE

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Outline

2.1.1 Abstract	41
2.1.2 Today's great challenges: Poverty, water scarcity and climate change	42
2.1.3 The UN millennium development goals	45
2.1.4 The role of agricultural research and development	47
2.1.5 Subsistence farming and improved stress tolerance	48
2.1.6 Micro-irrigation technologies and small-scale competitive advantages	50
2.1.7 Conclusions	52

2.1.1 Abstract

Poverty is afflicting a disproportionate number of people in the rural areas and improved agricultural productivity is unanimously viewed as necessary (although not sufficient) for poverty alleviation. At the same time, freshwater resources are under increasing pressures, not only from population growth, but also industrial and urban development, a situation that is expected to be exacerbated in most dry areas due to climate change. Access to irrigation water and improved crop varieties are seen

as two factors that contribute to poverty alleviation and that have effects on several of the UN millennium development goals. This paper reviews agricultural research strategies that could contribute to poverty alleviation, with a special emphasis on crop physiological traits that could be useful to poor rural farmers. More specifically, we argue that because poor farmers share low fertility soils prone to erosion and challenging climatic conditions, more research is needed in the area of drought, heat, and low nutrient tolerance. Research has mainly focused on staple crops and these remain important for subsistence agriculture, but more research is needed on minor crops that can allow poor farmers to be competitive in agricultural markets.

2.1.2 Today's great challenges: Poverty, water scarcity, and climate change

An estimated 1.1 billion people live in extreme poverty, defined by the World Bank as living with less than a dollar a day. The extreme poor are unable to meet their basic needs for food, water, clothing, shelter, sanitation and education. In 1990, this represented 28% of the world population, today it represents about 19%, although the absolute number of people living in extreme poverty has not decreased significantly because of population growth (UN, 2006). Of these, between 824 and 852 million people are still affected by chronic hunger (UN, 2006; Bongaarts, 2007), and approximately five million children die from malnutrition and related causes every year (UN, 2008). The extreme poor are caught in a vicious circle, a "poverty trap", where people are poor because of the high incidence of disease, the lack of roads and infrastructures, as well as unfavourable climatic conditions and environmental degradation. Poverty, in turn, makes them more vulnerable to disease and extreme weather events and, in the long term, pushes them into unfavourable environments (Sachs, 2005).

The vast majority (93% by 2003 numbers) of the extreme poor live in East Asia, South Asia and sub-Saharan Africa (Sachs, 2005). Of these, about 75% live in rural areas and are dependent on agriculture for their livelihood (IFAD, 2007). Indeed, although world food production is sufficient to feed us all, and food products are traded on the world market, failures of local agricultural production are the underlying causes of food insecurity (Alexandratos, 1999). It seems clear thus that agricultural research and development (R&D) has an important role to play in the eradication of extreme poverty.

Water resources are also increasingly becoming scarce in many parts of the world. It is presently estimated that a third of the world's population lives in conditions of water shortage (Gonzalez, 2001). Simply due to population growth, this proportion is expected to rise to two thirds in 2050, representing between 5 and 7.5 billion people, almost certainly exacerbating problems of extreme poverty (Wallace, 2000). The appropriation of accessible runoff on earth by humans has been estimated at 54%, and is expected to be over 70% in 2050 (Postel et al., 1996). In addition, good agricultural land is likely to be lost to urban sprawl (Shady, 2001) and an estimated 2 million hectares are lost to waterlogging and salinization every year (Postel, 1997). It seems very clear thus that there are limits to the expansion of land and water resources, and increasing water availability will have to come from water conservation (Postel et al., 1996).

To make matters worse for the extreme poor, global climate change now adds a new uncertainty to the problem of world water scarcity and agriculture. Important changes will come from changes in patterns of distribution of precipitation, evapotranspiration, river runoff, and soil moisture, and these will undeniably affect patterns of world crop

productivity and affect human populations (Ragab and Prud'homme, 2002). Indeed, as Ragab and Prud'homme (2002) point out, even if precipitation increases slightly, the increase in evapotranspiration due to higher temperatures might more than offset higher rainfall and result in lower soil moisture. They also note that a temperature increase of 1 to 2°C, combined with a decrease of 10% in precipitation could decrease river runoff by 40 to 70%. In addition, glaciers are an important source of irrigation water in the summer in several hydrological basins. There are growing concerns that when the glaciers have finished melting because of the rise in temperature, there will be an important reduction in irrigation water availability for summer crops (Kharlamova and Revyakin, 2004). It seems generally accepted that tropical and sub-tropical regions will be more negatively affected by global climate change than temperate regions (Ragab and Prud'homme, 2002). It is unfortunate that these regions are also, in general, less developed regions, less able to adapt to new conditions (Reddy and Hodges, 2000).

It has been suggested that higher carbon dioxide concentrations, an important driver of climate change, and higher temperatures could enhance crop productivity (Kimball and Idso, 1983; Reddy and Hodges, 2000). However, the growing consensus is that climate change effects on agriculture might not be beneficial, and that the carbon dioxide fertilization might not be enough to compensate for the other effects of climate change (Iglesias et al., 1996; Brown and Rosenberg, 1997; Matthews et al., 1997; Tubiello et al., 2002; Ziska and Bunce, 2007). While higher temperatures might be beneficial in temperate regions, higher temperatures in semi-arid and arid regions will be detrimental to crop production in particular if the rise in temperature is accompanied by decreasing precipitation (Reddy and Hodges, 2000; Polley, 2002). Thus the agricultural community should be preparing itself for a hotter and drier world.

2.1.3 The UN millennium development goals

In September 2000, political leaders of the United Nations made a commitment to create “a world with less poverty, hunger and disease, greater survival prospects for mothers and their infants, better educated children, equal opportunities for women, and a healthier environment; a world in which developed and developing countries worked in partnership for the betterment of all” (UN, 2006). This vision was translated into eight goals, called the UN Millennium Development Goals (MDGs), with time-bound and measurable targets:

1. Eradicate extreme poverty and hunger: Halve, between 1990 and 2015, the proportion of people whose income is less than \$1 per day; halve, between 1990 and 2015, the proportion of people who suffer from chronic hunger;
2. Achieve universal primary education: Ensure that, by 2015, children everywhere, boys and girls alike, will be able to complete a full course of primary schooling;
3. Promote gender equality and empower women: Eliminate gender disparity in primary and secondary education, preferably by 2005, and in all levels of education no later than 2015;
4. Reduce child mortality: Reduce by two-thirds, between 1990 and 2015, the under-five mortality rate;
5. Improve maternal health: Reduce by three-quarters, between 1990 and 2015, the maternal mortality ratio;
6. Combat HIV/AIDS, malaria and other diseases: Have halted and by 2015 begun to reverse the spread of HIV/AIDS; have halted and by 2015 begun to reverse the incidence of malaria and other major diseases;
7. Ensure environmental sustainability: Integrate the principles of sustainable development into country policies and programmes and reverse the loss of environmental resources; halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation; by 2020, to have achieved a significant improvement

in the lives of at least 100 million slum-dwellers;

8. Develop a global partnership for development: Address the special needs of the least developed countries, landlocked countries and small island developing states; develop further an open, rule-based, predictable, non-discriminatory trading and financial system; deal comprehensively with developing countries' debt; in cooperation with developing countries, develop and implement strategies for decent and productive work for youth; in cooperation with pharmaceutical companies, provide access to affordable essential drugs in developing countries; in cooperation with the private sector, make available the benefits of new technologies, especially information and communications.

(UN, 2006)

Half way to the deadline of 2015, there has been progress towards reaching those goals, but progress towards the eradication of poverty and hunger, which holds particular interest in this discussion, has been very limited in sub-Saharan Africa and South Asia, where most of the extreme poor live (UN, 2006). In fact, because of population growth, the absolute number of people living in extreme poverty has increased by 140 million in sub-Saharan Africa (UN, 2006).

Although agricultural R&D impacts directly on the first MDG, indirect effects of improved agricultural production and nutrition extends to most other goals. Indeed, for a family, improved nutrition should lead to better health, and as such lower maternal and child mortality rates, but also lower incidence of diseases such as malaria (Caufield et al., 2004). Rural children account for 82% of children out of school, possibly because of the distance to school, but probably also because child labour in small-scale agriculture is still very much a reality (UN, 2006). Increased agricultural production, if combined with appropriate social programs might also increase gender equity (Hussain, 2007b; van Koppen and Hussain, 2007). It also has been proposed that because of the prevalence of HIV/AIDS in

rural populations of Africa, investments in agricultural R&D should help raise living standards and decrease the effects of the disease (Jayne et al., 2005).

2.1.4 The role of agricultural research and development

It seems undisputed among agricultural economists and in the development literature that agricultural growth *per se* is an important force for economic development of developing nations (Johnston and Mellor, 1961; Adelman, 1984; Rola-Rubzen et al., 2001; IFAD, 2007; Pingali, 2007) and seems especially important in the early stages of industrialization (Adelman, 1984; Alexandratos, 1999; Bhutto and Bazmi, 2007). Research aiming to increase crop yields has shown high rates of returns in Africa and Asia, and has shown positive impacts on poverty, more so than economic growth in the industrial or service sectors (Thirtle and Piesse, 2007). Studies have documented how agricultural research had led to direct and indirect benefits to the extreme poor. These include improved crop yields, improved food security, lower food prices, as well as increased employment opportunities and higher wages. When programs are designed to lower poverty in women and disadvantaged ethnic groups, access to irrigation water and land often results in increased equity (Hussain, 2007c; van Koppen and Hussain, 2007; Namara et al., 2007).

Yet, we hold that economic growth – and extreme poverty alleviation – is driven by technological progress (Sachs, 2005), and that much of the current advancement in agricultural technologies are directed towards the Western agricultural systems aimed at relatively large holding, mechanized and relatively conventional, high input agricultural systems, and thus not directly applicable to resource poor farmers (Stoop and Hart, 2005). It also seems clear that some of the needs of the extreme poor might be overlooked by agricultural R&D programs that do not specifically

try to answer these needs (Bellon et al., 2005; Sachs, 2005).

Although other factors such as disease, roads and other infrastructure, basic sanitation and education are also important, and arguably just as necessary as agricultural technologies to break the “poverty trap” (Sachs, 2005; Sanchez et al., 2007), we would like to take a very technical approach as crop scientists and ask specifically: *what are the needs of smallholder subsistence farmers that are not being answered by current agricultural R&D programs?*

Studies have suggested that even the international R&D programs aiming at reducing poverty have been using management practices and locating trials in environments more favourable than those available to the extreme poor – which is clearly and fully understandable for practical reasons – but may not result in technologies and crop varieties directly suitable to the rural poor (Bellon et al., 2005; Stoop and Hart, 2005). Polak and Yoder (2006) have also stressed that poverty eradication will only be achieved if we seriously consider the particular challenges faced by farms smaller than one hectare.

While there has been an undeniable contribution by the Consultative Group on International Agricultural Research (CGIAR) centers to research aimed at resource poor farmers (and indeed many of the success stories presented below are from the experiences of CGIAR centers), the extent of today’s extreme poverty and the international commitment to end hunger by 2025 requires greater efforts, greater participation, and greater collaboration within the plant science community. The MDGs should not be seen as a CGIAR mandate exclusively, especially considering the decreasing budgets allocated to international agricultural R&D.

2.1.5 Subsistence farming and improved stress tolerance

Although smallholding farmers are located in a range of environments, they tend to have in common unfavourable conditions such as sloping hills, poor soil fertility and a greater susceptibility to extremes of temperatures and climate, either too cold or too warm, too dry or too wet, as well as a greater susceptibility to insects and diseases due to their lack of resources for the purchase of agro-chemicals and the poorer growth of the crops due to low fertility conditions (Ceccarelli et al., 2000; Bellon, 2006; Ceccarelli et al., 2007). They also often share unreliable, low quality and/or low supply of irrigation water (Hussain, 2007a). Farmers in remote areas also depend on their own production to fulfill their nutritional needs, and thus will need to produce adequate levels of staple crops, and ideally a minimum amount of legumes, vegetables, cash crops and sometimes livestock for proteins, vitamins and other needs. As these people tend to be vulnerable to climate variability, reliable yields are often more important than high yields (Wale and Yalew, 2007), and this partly explains why rates of adoption of improved germplasm (which is often selected under more favourable environments) and other agricultural technologies have sometimes been very low (Kydd, 1989; Lipton and Longhurst, 1989; Ortiz-Perez et al., 2006).

Given the situation of subsistence farming families, breeding programs should focus on improving the robustness of crops to relatively extraordinary fluctuations in climate. Thus, more research on stress tolerance is needed, and particularly on stresses such as high temperature, water deficit, waterlogging, and low fertility stress, but also on low temperature stress as well as disease, insect, and nematode resistance. Therefore, laboratories across the planet have the potential to provide knowledge (and germplasm) to pro-poor breeding programs, although progress in water deficit and low fertility stress has been slow due in part to the higher complexity of these stresses.

To address the diversity of the rural poor environments and needs, as well as the discrepancies between results obtained from controlled experiments on research stations and on farmers' fields, breeding programs have increasingly been working in collaboration with farmers for part of the testing and selection processes, a method referred to as participatory varietal selection with a level of success higher than that of conventional breeding programs (Courtois et al., 2001; Morris and Bellon, 2004; Murphy et al., 2005; Ortiz-Perez et al., 2006; Singh et al., 2006; Ceccarelli and Grando, 2007; Ceccarelli et al., 2007; Joshi et al., 2007). This provides highly adapted local varieties and the delivery to farmers is much faster (Ceccarelli and Grando, 2007). The approach has the added potential to empower farmers (Ceccarelli and Grando, 2007) through an opportunity for further training (Humphries et al., 2005), and does not seem to decrease the genetic biodiversity of the crops (Witcombe et al., 2001; Fufa et al., 2007). It also seems clear that plant scientists, breeders, physiologists, pathologists and molecular biologists should all have a supporting role in such breeding programs; indeed there is potential for great gains from increased collaboration and open communication between scientists, and also between programs. We further argue that participatory breeding programs need to expand their focus to include several more minor crops of important food value since these substantially contribute to farming families' nutritional needs, and often to income as well (Polak and Yoder, 2006; Graham et al., 2007).

2.1.6 Micro-irrigation technologies and small-scale competitive advantages

Irrigation technologies have contributed to the success of the Green Revolution, and indeed regions where irrigation networks, fertilizers and improved germplasm have been adopted are mostly extreme-poverty-free. Unfortunately, many of the modern irrigation technologies are suitable for

large areas of flat land, where farmers and/or governments have had the funds to install the systems, but unsuitable on smallholder plots on sloping hills, and largely too expensive to farmers trapped in poverty (Namara et al., 2007). Not surprisingly, thus, the development and distribution of affordable micro-irrigation technologies, such as the treadle pump developed by International Development Enterprises but locally made and distributed with local and affordable materials, has had a noticeable impact in poverty alleviation (Kgole and Walker, 2000; Postel et al., 2001; Mofoke et al., 2004; Amarasinghe et al., 2005; Liu et al., 2005; Polak, 2005; Polak and Yoder, 2006; Narayanamoorthy, 2007, Biggs, 2008). These have provided access to “new” irrigation water, either from waste water, harvested rain water or shallow groundwater, and have not only increased the agricultural production of smallholding families, and thus increased their income, but it has also created an agricultural market sector to supply with micro-irrigation technologies at various levels of affordability (Postel et al., 2001; Polak and Yoder, 2006). While not all of these technologies are equally efficient, they are nonetheless affordable enough for even the extreme poor, and some level of unevenness in the distribution of water can be quite acceptable to farmers, as it may contribute to a sequential harvesting of the crops (Kgole and Walker, 2000). It is also interesting to note that this new irrigation water is not generally applied to staple crops, but rather to high-value, labour-intensive, marketable crops such as vegetables, and indeed the access to markets is one of the main factors contributing to increased family incomes (Polak and Yoder, 2006).

Because of their small holdings, the rural poor do not have the same access to the “supermarket chain”, and their competitive advantage mostly lies in their proximity to local markets and the quality of their produce (Magistro et al., 2007). Indeed, the competitive advantage of small farmers has been well demonstrated in coffee and tea productions (Belshaw,

2002), and to some extent, some commercial pulse productions in the developed world have difficulties matching the grain quality of hand-harvested legumes. Mungbean in Australia is an example (C. Douglas, personal communication). It is also important to stress that labour is not limiting and, quite the contrary, technologies that enhance rural employment might be especially beneficial to the extremely poor landless rural labourers (von Braun, 1992; Lipton, 2007; Thirtle and Piesse, 2007). Thus, there is a potential to help poor farmers get out of poverty with more research on so-called less important crops, such as legumes, vegetables, fruits, nuts, spices, medicinal plants, cut flowers, and aromatics (Belshaw, 2002; Baudoin et al., 2007). As with staple crops, we need to determine the response of these crops to water deficits as well as their response to other biotic and abiotic stresses, and breed varieties with improved nutritional value (Graham et al., 2007), better quality (Polak and Yoder, 2006), and greater stress tolerance. We might also need to integrate concepts from organic agriculture (Stoop and Hart, 2005) and permaculture to develop appropriate production systems for the small size of a one-hectare farm and the low mechanization that can be deployed on it. Our contribution as plant scientists clearly could be quite substantial, but this might require a fundamental shift in thinking from large-scale, high-input, mechanized, broadly applicable technological concepts to more tailored programs aimed specifically at the poor, and applicable to small-scale, low-input, and labour-intensive farming.

2.1.7 Conclusions

Although today's world presents great challenges, agricultural scientists are also in a position to help address those challenges and make a difference. Let us encourage more plant scientists to embark in such an endeavour. The responsibility towards the extreme poor should not lie only with the CGIAR centers.

The particular situation of the extreme poor is as follows: most live in rural areas on holdings of less than one hectare, and farm under unfavourable climatic conditions that make them particularly vulnerable to crop failures from extreme of temperatures, drought, crop disease or insect pests. In some cases, they have access to small amounts of irrigation water and might have access to local markets. Research needs include greater understanding of cultivars adapted to abiotic and biotic stresses, particularly with regards to crops that are deemed more minor, but that might represent a way out of poverty for the extreme poor because of their competitive marketplace advantages. Agricultural science for poverty alleviation requires a major shift in thinking towards small-scale, affordable, low-input, and labour-intensive technologies.

Preface to chapter 2.2

The following manuscript is under preparation for submission to the European Journal of Agronomy. Dean Chandra A. Madramootoo, Dr. Heidi A. Webber, and Prof. Donald L. Smith contributed meaningful comments on the ideas presented. Prof. Donald L. Smith also provided editorial assistance. Galina Stulina and Mikhail G. Horst both provided insights into water management in Central Asia through numerous informal conversations, and provided feedback on the manuscript.

2.2 TECHNICAL SOLUTIONS FOR BETTER WATER MANAGEMENT: THE ARAL SEA BASIN AND UZBEKISTAN AS A CASE STUDY

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Outline

2.2.1 Abstract	56
2.2.2 The ecological disaster of the Aral Sea	56
2.2.2.1 The environment	56
2.2.2.2 Impacts on the health of human populations	58
2.2.2.3 Can the Sea be saved?	60
2.2.3 Historical, political and economic context of agriculture in the Aral Sea basin	61
2.2.3.1 The Soviet era	61
2.2.3.2 Today: Newly independent republics	62
2.2.3.3 Water management in new independent republics	63
2.2.4 Policy options for better water management	64
2.2.4.1 Land reforms	64
2.2.4.2 Demand-based management and water user associations	64
2.2.4.3 Water pricing	66

2.2.5 Technical options for better water management	67
2.2.5.1 Modernization of irrigation infrastructures	67
2.2.5.2 On-farm techniques: alternate furrow irrigation and regulated deficit irrigation	68
2.2.6 Crop choices for better water management	72
2.2.7 The way forward	73

2.2.1 Abstract

Perhaps the most extreme contemporary example of the potential negative impacts of agriculture is the drying of the Aral Sea due to the expansion of irrigation networks in the Soviet era. This paper looks at the ecological disaster of the Aral Sea disappearance, the socio-political-economic context of water management in Central Asia, and suggests technical solutions to the problems of water management and of food insecurity. In particular, irrigation techniques such as alternate furrow irrigation and deficit irrigation, as well as the introduction of legumes as double crops with winter wheat are presented as viable, low-cost alternatives to incorporate into the present cropping system. It also stresses that appropriate investments in the repair of the irrigation and drainage infrastructures are urgently needed.

2.2.2 The ecological disaster of the Aral Sea

2.2.2.1 The environment

Once the fourth largest inland water body, the Aral Sea formerly supported an important fishery, served as a key transportation route, and its extensive deltas housed a great diversity of flora and fauna, which co-habited with irrigated agriculture, animal husbandry, hunting, trapping and

the harvesting of reeds (Micklin, 1988; Kotlyakov, 1991; Micklin, 2006). *The Shores of Lake Aral* by Major Wood, written in 1876, is an account of the exploration of an exotic land and a lake surrounded by a lush vegetation and diverse wildlife, a picture which contrasts sharply with present environmental conditions.

Since the start of the ambitious plan by the Soviet Union to expand the irrigation networks in the dry steppes of Central Asia in the early 1960s, the Aral Sea has shrunk steadily. The latest numbers claim its level has dropped by more than 17 m, has lost 74% of its area, and 90% of its original volume and it is now divided into three parts (Micklin, 2006; Micklin and Aladin, 2008). The salinity of the water, originally around 10 g L⁻¹, has increased because of evaporation to over 100 g L⁻¹ in the southern parts, which has led to the extinction of the freshwater fish species once present (Micklin, 1988; Kotlyakov, 1991; Kotlyakov et al., 1992; Vinogradov and Langford, 2001).

The drying of the sea has also led to significant changes in the climate of former shoreline human settlements. The climate is now more continental with longer colder winters, and hotter and drier summers (Zolotokrylin, 1999). The frost-free period has decreased to 170 days, below the 200 frost-free days required for the cultivation of cotton (Kotlyakov, 1991; Vinogradov and Langford, 2001). Most importantly, the more arid climate is leading to an increased frequency in dust storms, which carry sediments from the dried sea bottom for several hundred kilometres, sediments which include toxic residues of pesticides, herbicides and other chemicals (Zolotokrylin, 1999; Vinogradov and Langford, 2001; Wiggs et al., 2003). These storms are affecting crop production, pastures, and both animal and human health (see section 2.2.2.2).

What is perhaps most striking about the disaster of the Aral Sea is the

speed at which environmental change has occurred. The commercial fishery, which in 1960 exploited 22 species of fish, closed in 1982. In 1950, catches amounted to nearly 50,000 metric tons of various species of fish; by 1994, catches were limited to 5,000 metric tons of carp from polluted lakes (Vinogradov and Langford, 2001). Few species – and few jobs - remain. Fish were previously transported from the Baltic Sea, and the Pacific and Arctic oceans into Muynak for processing (Micklin, 1988), but this expensive measure was stopped in 1994 (Glantz, 1994). The collapse of the fisheries has affected directly and indirectly 60,000 people in the area (Micklin, 1988; Vinogradov and Langford, 2001). Navigation was abandoned as the shore receded more than 100 km from Muynak making past harbours and ports unreachable (Small et al., 2001). The natural vegetation is now being replaced by halophytic and xerophytic plants, which are better adapted to these saline and arid conditions (Micklin, 2000). It has been estimated that over 200 species of flora and fauna have become extinct in this area (Kotlyakov et al., 1992; Micklin and Aladin, 2008).

2.2.2.2 Impacts on the health of human populations

Over 400,000 km² and 5 million people are affected directly by this ecological disaster, most of them belonging to the Karakalpakstan autonomous zone in Uzbekistan, an ethnic group that is distinct from the Uzbeks in both culture and language (Micklin, 2000). The proliferation of various diseases and infections is so prevalent that between 70 and 80% of the population is estimated to be sick in one way or another (Kotlyakov et al. 1992; Vinogradov and Langford, 2001). These people are suffering from acute respiratory, arthritic, cardio-vascular, intestinal, kidney and liver diseases, eye problems, and cancers (Elpiner, 1999; Micklin 2000; Ataniyazova et al., 2001a; O'Hara et al., 2002; Whish-Wilson, 2002). The increases in the incidence of these diseases vary, but are typically

mentioned in terms of 'folds' rather than in percentage increase. For instance, viral hepatitis has increased 7 fold during the 1973-1988 period, and typhoid fever increased as much as 29 fold in some areas (Elpiner, 1999), various cancers have increased 30 to 40 fold, arthritic diseases by 60 fold, and chronic bronchitis by 30 fold (Vinogradov and Langford, 2001). Infant mortality rates range from 25 to more than 100 per 1000 live births in some regions (Elpiner, 1999; Whish-Wilson, 2002). There has also been an increase in innate malformations and other genetic problems (Elpiner, 1999). Maternal mortality rates are also high, at around 120 per 100,000 live births. Between 70 and 80% of the women suffer from anaemia (Elpiner, 1999; O'Hara et al., 2002). One third of women have suffered a miscarriage (Elpiner, 1999). Understandably, people also suffer from depression and other psychological illnesses (Small et al., 2001; Crighton et al., 2003).

This high morbidity and mortality seems to be explained by the high frequency of wind storms, which carry 15 to 75 million metric tons of agro-chemical bearing dust annually (Kotlyakov et al., 1991). It has been reported that chemicals such as organochlorine pesticides (HCH, β -HCH, *pp*-DDE) and the dioxin 2,3,7,8-TCDD have been found at high levels in maternal and foetal cord blood (Ataniyazova et al., 2001b). In addition, Karakalpakstan has problems typical of third world nations: medical facilities lack funding, nutrition is poor and lacks variety, and sewage systems are generally inadequate (Kotlyakov et al., 1992; Micklin, 2000; Small et al., 2001). To complicate matters, the Soviet government has used one of the Aral Sea islands, Vozrojdeniya island, as its base for its biological warfare program (Pala, 2005), and since June 2001, this island is a peninsula. There are fears that bacteria have survived and might propagate through local rodents for example, and create an epidemic (Whish-Wilson, 2002; Pala, 2005). Unfortunately, although large amounts of international aid money have been transferred to the countries affected

by this crisis, the health of people living in the most affected zone has not improved (Micklin, 2000; Ataniyazova et al., 2001a). Several authors have repeatedly argued that this is a humanitarian crisis that requires immediate international action. Efficient sources of clean water and local production of contaminant-free food are urgently needed (Ataniyazova et al., 2001a), in addition to appropriate medical aid.

2.2.2.3 Can the Sea be saved?

It has been estimated that the amount of annual water inflow that would be required to restore the Aral Sea to its original size is 50 km^3 , a little less than half of the total annual renewable available water (Bortnik, 1999; Micklin, 2000). Since such an increase in inflow into the sea is unattainable, considering the importance of agriculture (and therefore irrigation water) in Central Asian economies, many have accepted the death of the Aral Sea as it once was (Aladin et al., 2005). Kazakh authorities, with the help of the World Bank, have been building a dam to retain the water in the northern, or small Aral sea, and this has already led to substantial increases in the level of the small sea (Micklin and Aladin, 2008). Salinity levels are around 20 g L^{-1} and are expected to stabilize around 14 g L^{-1} (Aladin et al., 2005; Micklin and Aladin, 2008). This has allowed a resurrection of commercial fisheries, and a processing factory in Aralsk (Kazakhstan) has reopened, contributing to some economic relief for the region (Micklin and Aladin, 2008). As the salinity of the lake decreases, native freshwater species should come back, and the introduced saltwater fish should slowly disappear (Micklin and Aladin, 2008). In fact, the idea of focusing efforts on saving smaller but productive areas was put forward several years ago (Bortnik 1999). In the south, Micklin and Aladin (2008) have suggested the construction of a dam near Muynak with a canal that would link it with the western part of the sea, which is smaller, but deeper, and where there is still some hope

of restoration. The eastern part of the large Aral Sea, unfortunately, is broad and shallow and the inflow required to balance the evaporation is not realistically achievable (Micklin and Aladin, 2008).

2.2.3 Historical, political and economic context of agriculture in the Aral Sea basin

2.2.3.1 The Soviet era

Irrigation has probably been practiced in the area since the Bronze Age, and there is evidence that at some point, the irrigated area had reached 3.5 to 3.8 million ha on the Amu Darya (Micklin, 2000). The irrigation systems were destroyed and rebuilt numerous times as periods of war and peace alternated, and by the early 20th century, with the Russian conquest, irrigation systems were being rebuilt. By the mid-20s, the traditional system had been preserved and the 2-3 ha farms were independently managed and organized in fields of 0.3 to 0.8 ha lined with trees to reduce wind erosion and waterlogging (Micklin, 2000). Water distribution was overseen by a hierarchy of water bureaucrats. This system however was seen as primitive, backward, ineffective, feudal and oppressive of the peasantry, and the Russian authorities replaced it with one based on an industrial model with tight government control. Land was nationalized, agricultural workers organized into teams, and trees were uprooted to increase the size of fields to 3.5 ha on average (Spoor, 1993; Micklin, 2000). With increased mechanization, fields would be further increased in the following years to 20, 40, 60 and sometimes up to 100 ha, making uniform distribution of water impossible (Zonn, 1999).

During the same period, cotton self-sufficiency quickly became high priority in irrigation and development policies (Zonn, 1999), and the proportion of land allocated to cotton increased from 17 % in 1913 to approximately 50% in 1933 (Micklin, 2000). Until then, the increase in

irrigated area came mostly from the conversion of field borders and fallows into productive fields (the irrigated area actually decreased during WWII), but the expansion of irrigation networks was particularly accelerated between 1950 and 1965, yet still tended to focus on older irrigated areas. It was in the twenty years that followed that rapid development into new desert lands (from the construction of the Kara-Kum canal into Turkmenistan) that total irrigated area increased from nearly 5 million to nearly 8.0 million hectares (Micklin, 2000). Calculations by Soviet experts had estimated that there were as much as 20.0 million hectares of potentially irrigable land in Central Asia (Zonn, 1999), and the expansion of irrigation networks only slowed down with Gorbachev's refusal to implement a project to divert Siberian waters into the Aral Sea (Micklin, 2000; Pala, 2005). At the same time, salinization of agricultural soils was increasing, and the disappearance of the sea was beginning to draw public attention. Optimist experts that had predicted insignificant impacts from the drying of the Sea, and had hoped to farm the sea bottom, were proved wrong as changes in climate led to wind storms that in turn led to dramatic consequences for the health of the local population (Micklin, 1988).

2.2.3.2 Today: Newly independent republics

Following the collapse of the Soviet Union in December 1991, the newly independent republics, and especially Uzbekistan, inherited a dysfunctional economy, primarily based on the export of one raw agricultural commodity, cotton. Agriculture employs more than 40% of the population and accounts for 25 to 30% of the gross domestic product, yet the sector is receiving less than 7% of total investment (O'Hara and Hudson, 1999). Thus, today, the irrigation infrastructure built in the Soviet era is in great need of repair.

Furthermore, land degradation is now obvious throughout the basin. It appears that 74% of the area has a water table higher than 3 m (EC-IFAS, 1999), and 31% higher than 2 m (Savoskul et al., 2004). It is estimated that, in the last decade, the proportion of the irrigated area affected by salinization has increased from 25 to 50% (EC-IFAS, 1999; Savoskul et al., 2004). Water is used to leach the salts out of the soil, but this in turn increases the salinity of the drainage water and its quality decreases for downstream uses. In addition, this leads to a need for increased water use for leaching, and applications of water to fields downstream have been estimated as high as 50,000 m³ per hectare (Zonn, 1999). In order to leach salts away successfully however, drainage systems are necessary. Unfortunately, although 93% of the irrigated area has installed drainage, 32% of the open ditches are out of order, 46% of subsurface drainage systems are no longer functional, and all of the vertical drainage systems, which were not very common, are broken (EC-IFAS, 1999; Dukhovny et al., 2000; Dukhovny et al., 2007). Yield losses due to waterlogging and salinization are estimated to be 20 to 30% (Savoskul et al., 2004).

2.2.3.3 Water management in newly independent republics

The break-up of the Soviet Union has resulted in a new complexity in the management of the irrigation systems. In February 1992, shortly after independence, the Interstate Coordination Water Commission (ICWC) was created to allocate water between the five newly-independent republics according to water availability (Dukhovny, 2003). With independence, however, republics are at liberty to seek their own interests in water resources, interests which can sometimes be in conflict. The best example is probably between Kyrgyzstan and the two downstream states, Uzbekistan and Kazakhstan. While Uzbekistan and Kazakhstan need the water for irrigation during the summer, Kyrgyzstan, poor in fossil fuels,

needs the water to produce the hydroelectricity from the Toktogul reservoir. The release of water in winter would produce hydroelectricity to warm Kyrgyz homes, but this would diminish Uzbekistan and Kazakhstan supply of irrigation water in summer. The three countries have signed an agreement about this, but each side has been accusing the other of non-respect of the agreement (Micklin, 2000; Savoskul et al., 2004). This has led numerous (and perhaps too many) World Bank and internationally-funded projects to focus on promoting cooperation between the five states (Small and Bunce, 2003), yet cooperation has been developing, and claims of an imminent war for water are grossly exaggerated (Dukhovny, 2003).

2.2.4 Policy options for better water management

2.2.4.1 Land reforms

It has been proposed that privatization of agriculture could significantly enhance land and water productivity in Central Asia (van Atta, 1993; Lerman et al., 1996). Uzbek household plots are very productive, representing 8.6% of the agricultural area, but accounting for 41% of total agricultural production (Lerman et al., 1996). While the government has, at least in rhetoric, embarked on a transition toward a market-based economy, land and water reforms have been limited (Lerman et al., 1996; O'Hara and Hudson, 1999). While the Uzbek government passed a law on Peasant Farms in July 1992 to officially transfer ownership of land from the state to peasant families, the Uzbek constitution still stresses that land is useless without water, and that water is distributed by the state (Lerman et al., 1996). Indeed because of the dependence of agriculture on irrigation water, some have also argued that privatization of land, in the current context, might not be appropriate at all (Spoor, 1993). In 1998, no more than 6% of the agricultural area had been converted to private farms (Koopman, 1998). Farmers, private or in collective farms, are still subject

to production targets set by the government for cotton and wheat, and need to buy inputs, borrow assets such as tractors, and sell products to state monopolies (Koopman, 1998). Furthermore, land privatization, to be effective, would need to be combined with a redistribution of farm assets (Lerman et al., 1996). In addition, there seems to be no desire from farmers themselves to demand a complete redistribution of land. It is thus doubtful that a drastic change towards full privatization of agriculture, even if combined with full liberalization of agricultural markets, would lead to improved water use efficiency, let alone improved economic conditions. Instead, it has been proposed (repeatedly) that Central Asian republics need to revisit their overall development strategy, and diversify to decrease their dependency on agriculture and irrigation water (Kotlyakov et al., 1992; Levintanus, 1992; Spoor, 1993). However, it has also been demonstrated that growth in agricultural productivity is necessary for poverty alleviation (Johnston and Mellor, 1961; Adelman, 1984; Alexandratos, 1999; Rola-Rubzen et al., 2001; Pingali, 2007; Thirtle and Piesse, 2007), and this growth is dependent on proper irrigation management (Spoor, 1993). Better water management through policy options, thus, might well be achieved by adopting development strategies that deal minimally with water, but only once the agricultural sector has been strengthened.

2.2.4.2 Demand-based management and water user associations

Inconsistencies in the availability of irrigation water leads farmers to irrigate when water is present, and tends to result in over-irrigation and in scheduling that has little to do with crop requirements (Pereira et al., 2002). Although challenging, it seems necessary that irrigation systems provide greater flexibility so that water is distributed at the right time, at the right place, and in the right amounts. Recently, much effort has been devoted to the creation of water user associations (WUAs). The

underlying assumption of these projects is that WUAs, if successful, could coordinate the operation and maintenance of irrigation systems as well as the equitable distribution of water more effectively and at a lower cost than the central government. They could also empower rural communities by providing their members with extension services, supply agricultural products and equipment, coordinate marketing, and provide financial services such as microcredit to small farmers. Eventually, WUAs could also represent the rural community and act to lobby for the interests of this group (Carney and Farrington, 1998; Micklin, 2000). Such WUAs have been implemented with limited success in Central Asia (Horinkova and Abdullaev, 2003; Hassan et al., 2005; Nizamedinkhodjayeva, 2007; Sehring, 2007; Yakubov and Hassan, 2007). Often, the creation of WUAs has been associated with the implementation of water fees to cover the costs of operation and maintenance of the irrigation systems, and as most farmers are too impoverished to pay their share, WUAs and associated activities have not been overly popular (Nizamedinkhodjayeva, 2007; Sehring, 2007). In addition, in certain cases, WUAs have perpetuated inequalities already present in the social hierarchy by asymmetries in the information received (Hassan et al., 2005). This too has led to WUAs being unpopular and unsustainable.

However, it has also been found that when projects made a conscious effort towards social mobilization, real benefits were achieved in terms of improved knowledge and awareness about water and irrigation management by water users. In addition, although improved irrigation management and reduced water use are not apparent yet, water users are at least better equipped to face the challenge to improve water management and deliveries (Yakubov and Hassan, 2007).

2.2.4.3 Water pricing

It has been proposed that one of the main causes of the degradation of land and water resources is the lack of responsibility in water management at the farm level. Water is managed as if plentiful because farmers do not pay the actual cost of irrigation water. This has led several scientists in the region to argue for a system of water pricing that would give incentives to farmers to save water. While water pricing is present in some Central Asian countries, and water is sometimes managed by WUAs as discussed above, the fees imposed are generally much too low to cover operation and maintenance costs. In addition, no monitoring equipment is installed at farm gates, such that water taxes are based on estimated use, calculated from norms of consumption of different crops multiplied by the area cultivated, rather than actual use (Micklin, 2000). However, as mentioned previously, farmers have limited means of paying for water fees, and traditionally peoples of Central Asia have valued water and still realize that it is a scarce resource (Dukhovny, 2003). In addition, we believe it is also important to protect people's right to water for basic needs, and we feel that to rely exclusively on the most impoverished fraction of society to deal with water management problems is ethically questionable. Therefore, water pricing might be part of a solution in the long-term, but in the short-term, other interventions must be attended to.

2.2.5 Technical options for better water management

2.2.5.1 Modernization of irrigation infrastructures

One of these interventions is the restoration of the irrigation systems, which are in great need of maintenance and repairs. It is estimated that 50% of the water withdrawn from the rivers never reaches the fields because of leakage (EC-IFAS, 1999); we have already mentioned the poor state of drainage systems (Dukhovny et al., 2007). The cost of reconstruction has been estimated at \$US 16 billion (Micklin, 2000), and the five republics that reside in the Aral Sea basin do not have the

resources to undertake such an effort. Unfortunately, without massive investments to significantly reduce the leakage from the irrigation channels, improvements in institutional management or in management at the farm level (see following sections) will be limited. It is unfortunate that investments in agricultural research are declining globally, and that irrigation and drainage projects, in particular, are becoming unpopular. Given the importance of irrigation water for agriculture and of agriculture for the economy of Uzbekistan and Central Asia, it seems that economic (and social) development will be much more difficult to achieve with leaking irrigation systems and a weak agricultural sector as a consequence.

2.2.5.2 On-farm techniques: alternate furrow irrigation and regulated deficit irrigation

Proper irrigation scheduling is defined as applying the right amount of irrigation water at the right time. Unfortunately, it seems that some farmers have little or incorrect knowledge of proper irrigation scheduling (EC-IFAS, 1999; O'Hara and Hudson, 1999), and many farmers in dry areas prefer to over-irrigate to avoid crop water stress (Horst et al., 2005). For example, while winter wheat is a less water-demanding crop than cotton, many farm managers believe that wheat requires more water, so that it is often over-irrigated (EC-IFAS, 1999; O'Hara and Hudson, 1999). Surface irrigation is the most widely used irrigation technique and will likely remain so in the near future (EC-IFAS, 1999). Surface irrigation requires minimal capital investment and is not affected by wind speed or the level of sediments in the water, as might be sprinkler and drip irrigation systems. However, it is typically less efficient, and more labour-intensive. Small irrigation depths (beneficial towards the beginning and the end of the season) are difficult to apply. Innovations in these millennia-old irrigation systems have been limited, and often consist of greater

monitoring, automation, and computerized feedback systems to control water supply in furrows (Pereira, 1999). Most innovations are too expensive to be used in developing countries, although laser levelling has shown great results in improving irrigation efficiency and uniformity (Abdullaev et al., 2007).

In recent years, however, alternate furrow irrigation (AFI) has been found to have great potential for improving water productivity in dry areas. It is a technique that is available to all. AFI consists of surface irrigation systems supplying water to every second furrow. Further, by alternating irrigated furrows, root growth is stimulated and this probably helps offset the negative effects of reduced water supply (Kang et al., 2000). In a controlled environment with a divided root system, Kang et al. (1998) showed that water consumption by maize plants subjected to partial root drying was decreased by 34 to 37% while yields only decreased 6 to 11%. They also showed that transpiration rate decreased compared to well-watered controls, but that the photosynthetic rate and leaf water content remained the same, thus leading to significant increases in water use efficiency, root development and distribution and shoot biomass production compared to controls. Another split-root experiment, Kirda et al. (2004) showed no significant decrease in the yield of tomato subjected to half of the irrigation water, when each side received water alternately. Both groups suggested that by having half of its root system in dry soil, the plant continues to synthesize abscisic acid in the roots, which reduces its transpiration rate. However, because water is available, growth is less affected.

Field experiments have also confirmed these findings: Grimes et al. (1968) reported a 23% decrease in water use with no decrease in yields; Kang et al. (2000) reported a 50% decrease in applied water with no significant decrease in maize yields; Crabtree et al. (1985) reported an 'acceptable

tradeoff in soybean: water use had decreased 40 to 50% while yields decreased 7 to 10%. Graterol et al. (1993), however, showed that AFI required more irrigation events than the conventional every furrow irrigation. Horst et al. (2005) also demonstrated that AFI improved application efficiency and distribution uniformity, and led to water savings of 200 to 300 mm for the whole season in cotton. Finally, Webber et al. (2006) demonstrated higher water use efficiency and no significant decrease in yield in common bean and mungbean subjected to AFI when compared to the conventional every furrow irrigation.

Another water-saving irrigation technique is regulated deficit irrigation (RDI). Through RDI, growers allow some degree of water stress to be experienced by the crop, but the water saved should allow an increase in the area irrigated, or can be used elsewhere (Pereira et al., 2002; Kijne et al., 2003). Indeed, with climate change and the increasing demand for water from other sectors, irrigation with limited amounts of water and below full crop water requirements might become the norm rather than the exception (Shady, 2001; Fereres and Soriano, 2007). The method is more complicated than AFI however, and it is important that farmers have control over the timing of irrigations and amount of water applied, as well as the tools for proper irrigation scheduling. Precise information is needed on the sensitivity of crops to both the intensity and the timing of water stress (Kijne et al., 2003). Studies performed on a number of species suggest RDI, when properly performed, is economical, and can lead to substantial gains in water use efficiency, often without significant decreases in yields (Webber et al., 2006; Costa et al., 2007; Fereres and Soriano, 2007; Karam et al., 2007; Vazifedoust et al., 2008). In trees and vines particularly, RDI has been shown to increase fruit quality and decrease vegetative growth (Cifre et al., 2005; Chaves et al., 2007; Costa et al., 2007; Spreer et al., 2007).

While the concept of RDI is relatively straightforward, its applicability in the field is not always consistent. Studies on RDI are usually performed by either reducing the amount of water that is applied to crops to a fraction of the full evapotranspiration (ET), but otherwise keeping the same frequency of irrigations (for example, Pandey et al., 1984; Shani and Dudley, 2001; de Souza et al., 2003; Oktem et al., 2003; Oweis et al., 2004; Chaves et al., 2007), or by withholding irrigation at specific growth stages (for example, Nielson and Nelson, 1998; Calvache and Reichardt, 1999; Pandey et al., 2000; De Costa et al., 1999; Boutraa and Sanders, 2001; Xue et al., 2003; Karam et al., 2005; Karam et al., 2007). While applying a fraction of the ET might be a practical way to impose RDI with sprinkler and drip irrigation, in surface irrigation systems very small irrigation depths are not technically feasible. Further, these small irrigation depths do not bring the soil profile back to field capacity, but rather wet the upper layers, and result in a soil depletion that is increasing over time. This could potentially lead to severe damage at the yield formation stage. On the other hand, withholding irrigation water at specific growth stages is too simplistic. Even if rainfall is negligible, air temperature, wind speed, irradiation, relative humidity, crop ground cover, soil water holding capacity and fertility conditions are all factors that affect ET (Allen et al., 1998) and that might vary considerably from year to year. The intensity of the water stress is thus hardly reproducible, and generalizations of irrigation requirements from such studies should be taken with caution.

RDI using increased time intervals between irrigation events, based on the water balance method for irrigation scheduling and greater depletion factors (as proposed by Allen et al., 1998, and used in Panda et al., 2003 and Webber et al., 2006), is probably a better approach because: 1. the method is available no matter what water application technology (or lack thereof) is being used, 2. the water stress is reproducible over soil types and climatic conditions, 3. filling the entire soil profile potentially

encourages deeper root growth, 4. the lower frequency of irrigation events lowers the water losses from evaporation, and 5. crop producers will not grow a control plot to calculate the ET, and then apply a fraction of it to the rest of their fields, as would be required using a fraction of the full ET.

2.2.6 Crop choices for better water management

As discussed previously, the role of cotton in the economy of Central Asia probably needs to be reduced over time, and the agricultural sector might benefit from a shift toward greater food self-sufficiency, less water-intensive, and more salt tolerant crops (Kotlyakov, 1991). In particular, the development of fruit and vegetable export markets could revive the agricultural sector, but will most likely require state intervention and political will (Spoor, 1993). In the short-term however, it seems clear that the government of Uzbekistan is not lessening its dependence on cotton, and as such, the only window of opportunity available to growers is after the harvest of winter wheat, from late June to the onset of cold temperatures in October. As such, short duration crops like legumes can be introduced into the current cropping system without compromising growers' ability to provide state prescribed cotton and winter wheat (chapter 3; chapter 4; Webber et al., 2006).

The benefit of legumes in cropping systems has long been recognized. Food legumes not only can provide a good source of protein for human consumption, but their residues can also improve the fertility of the soil, due in great part to their ability to form a nitrogen-fixing symbiosis with rhizobia (Senthong and Pandey, 1989; Haqqani and Pandey, 1994a; Subbarao et al., 1995). In addition, legumes in rotation with other crops can break disease cycles and encourage mycorrhizae (Subbarao et al., 1995). Often, legumes are short-season crops, and can be grown after main cash crops, such as rice in subtropical Asia and parts of Africa

(Senthong and Pandey, 1989), or after winter wheat in the government prescribed cotton-wheat rotation in Uzbekistan. Unfortunately, the economics of their production often restricts them to less productive lands and/or periods of growth (Subbarao et al., 1995). As such, their productivity is limited in semi-arid and arid regions (Senthong and Pandey, 1989). We have argued previously that breeding programs for legumes (and indeed for other crops as well) should put greater emphasis on improved resilience under water scarcity (chapter 2.1), and germplasm from Central Asia which, to date, remains underrepresented in many collections (Kashiwagi et al., 2005), has the potential to provide useful genetic diversity in traits related to drought tolerance.

2.2.7 The way forward

In this paper, we have described how the expansion of irrigation networks has led to the Aral Sea's disappearance, and how this has negatively affected the 5 million people living in the area. Development projects aiming at improved water management in the region have focused on enhanced cooperation between the newly independent States. However, we have argued that massive investments in the irrigation infrastructure are necessary to complement water savings from improved on-farm irrigation methods such as alternate furrow irrigation and deficit irrigation. In addition, we have discussed how the introduction of legumes as double crops with winter wheat is a viable, low-cost alternative that can be easily incorporated into the present cropping system. Thus, we suggest that agricultural research and development projects in the region should focus on developing crops and related markets other than cotton and winter wheat, and improving water stress tolerance in these same crops.

Preface to chapter 2.3

The following manuscript is under preparation for submission to Crop and Pasture Science (continuing the Australian Journal of Agricultural Research). Prof. Donald L. Smith, my supervisor, is co-author on the manuscript and provided constructive feedback on the ideas presented and editorial assistance.

**2.3 LEGUMES IN SEMI-ARID AND ARID REGIONS: PHYSIOLOGICAL
UNDERSTANDING OF DROUGHT TOLERANCE MECHANISMS,
BREEDING TECHNOLOGIES AND LIMITS TO KNOWLEDGE**

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Outline

2.3.1 Abstract	76
2.3.2 Legumes in semi-arid and arid regions	77
2.3.2.1 Common bean (<i>Phaseolus vulgaris</i> L.)	78
2.3.2.2 Chickpea (<i>Cicer arietinum</i>)	79
2.3.2.3 Cowpea (<i>Vigna unguiculata</i>)	80
2.3.2.4 Pigeon pea (<i>Cajanus cajan</i>)	80
2.3.2.5 Mungbean (<i>Vigna radiata</i> (L.) Wilczek)	81
2.3.2.6 Lentil (<i>Lens culinaris</i>)	82
2.3.2.7 Faba bean (<i>Vicia faba</i>)	82
2.3.2.8 Soybean (<i>Glycine max</i> L. Merr)	83
2.3.2.9 Groundnut (<i>Arachis hypogaea</i>)	84
2.3.3 Drought: a complex stress	84
2.3.4 Drought resistance framework: Drought escape	86
2.3.5 Drought resistance framework: Drought avoidance	86
2.3.5.1 Leaf area development response	87

	76
2.3.5.2 Root characteristics and growth response	88
2.3.5.3 Stomatal response	89
2.3.5.4 Leaf characteristics and leaf movement	91
2.3.5.5 Nitrogen fixation response	92
2.3.5.6 Osmotic adjustment	94
2.3.6 Drought resistance framework: Dehydration tolerance	95
2.3.7 Yield component frameworks	96
2.3.8 Clues from molecular biology	98
2.3.8.1 Clues from microarray data	98
2.3.8.2 Signalling pathways in response to dehydration stress	100
2.3.8.3 Genetic engineering of drought tolerance traits	104
2.3.8.4 Quantitative trait loci (QTLs) and marker assisted selection (MAS)	105
2.3.8.5 Crop physiology and genomics: linking gene networks to whole plant response	106
2.3.9 Conclusions	108

2.3.1 Abstract

Approximately one billion people live in semi-arid and arid regions and of these about 40% live below the poverty line. Pulse crops are an important source of protein in the diet of people who cannot afford to eat meat, or choose not to. In addition, legumes play a vital role in sustainable agricultural systems, but they are often grown in marginal environments, under various abiotic and biotic stresses, and as such their yields are often much below their potential. Improving drought tolerance in legumes

has the potential to improve food security and the sustainability of agricultural systems. However, the mechanisms of drought tolerance and the genetic variability of traits conferring tolerance have not been as well characterized in legumes as in cereals, and the use of physiological and genetic markers for improved legume germplasm has been limited. This paper examines drought resistance from a classical crop physiology perspective by considering drought resistance and yield component frameworks, and looks at recent progress resulting from the use of molecular biology tools. The characterization of the molecular responses to water deficit stress has so far failed to improve our knowledge of mechanisms of drought tolerance at the whole plant level, but by working together, crop physiologists and molecular biologists can develop a better understanding of drought tolerance, and might identify traits that could serve as physiological markers for the genetic improvement of legumes in dry areas.

2.3.2 Legumes in semi-arid and arid regions

Approximately one billion people live in semi-arid and arid regions, and of these about 40% live below the poverty line (Erskine, 2003). Pulse crops are an important source of protein in the diet of people who cannot afford to eat meat, or choose not to. Food legumes are usually grown in rotation with cereals. In Asia and Africa, for example, legume crops are often grown on residual soil moisture following wheat, rice, maize, sorghum or pearl millet (Angus et al., 1983; Senthong and Pandey, 1989; Jeyaraman et al., 1994; DeCosta et al., 1999). Alternatively, they can be grown in intercropping or mixed cropping systems, often in combination with cereal crops, but sometimes with other pulses (Adams et al., 1985; Lawn and Ahn, 1985). In Australia, legumes are grown as summer crops or winter crops in rotation with wheat, barley or sorghum (Bushby and Lawn, 1992). The climate in semi-arid and arid environments is often variable with high

frequencies of intermittent or terminal droughts (Erskine, 2003; Turner, 2003). Depending on the cropping season, food legumes can also be subject to cold stress, as well as sometimes high insect and disease pressures (Siddique et al., 2001; Liu et al., 2008).

The benefit of legumes in cropping systems has long been recognized. Their residues can improve the fertility and physical condition of the soil, due in great part to their ability to form a nitrogen-fixing symbiosis with rhizobia. They can also break disease cycles and encourage mycorrhizae (Subbarao et al., 1995; Hedley, 2001). As the price of nitrogen fertilizer increases, they are considered increasingly valuable and profitable crops, because of their lower input requirements. Furthermore, in developing countries, diversification into more horticultural crops, such as legumes, plus access to markets, are seen as particularly effective in helping the poor break out of the poverty trap (Polak, 2005; Sachs, 2005; Sanchez et al., 2007).

Most legume crops of economic importance belong to one of two taxonomic tribes: the *Vicieae* and the *Phaseoleae*. Exceptions include chickpea (*Cicer arietinum*), from the tribe *Cicereae*, a separate offshoot, and groundnut (*Arachis hypogaea*) from the tribe *Aeschynomeneae*, which is morphologically very distinct. In addition, in the *Phaseoleae*, the genera *Phaseolus* and *Vigna* have been redefined since the 1970s, and the genus *Phaseolus* has been narrowed to plants of American origin while the genus *Vigna* has been expanded (Polhill and van der Maesen, 1985). Based on their utilization, soybean and groundnut are generally classified as oilseed crops, separate from pulses. Here, we will consider them as included in the food legumes.

2.3.2.1 Common bean (*Phaseolus vulgaris* L.)

Common bean is a very diverse pulse crop, with approximately 500 varieties. It was domesticated independently in the Andes and in Central America (Polhill and van der Maesen, 1985), possibly from distinct wild progenitors within these same areas (Miklas and Singh, 2007). It is the most important pulse crop in the world, being eaten directly more than any other legume crop (Hedley, 2001; Broughton et al., 2003). It is particularly important in Latin America where it is a key part of national diets, but it is also widely cultivated throughout the world (Adams et al., 1985).

Most producers in developing countries cultivate unimproved varieties (i.e. landraces) in complex multi-cropping systems, often climbing on maize, sugar cane, coffee or plantains, but also intercropped with potato, amaranth and other subsistence crops. Common bean yields on average less than 1000 kg ha⁻¹ in such systems (Adams et al., 1985; Graham and Ranalli, 1997). In contrast, in North America and Europe crops are grown on level land with mechanization, fertilizer and pesticide inputs, sometimes with irrigation, and yields can be over 3000 kg ha⁻¹ (Adams et al., 1985; Graham and Ranalli, 1997; Miklas and Singh, 2007). In North America, breeding programs have emphasized earlier maturity and seed quality rather than higher yields (Adams et al., 1985). Improvement of germplasm has been further complicated by local preferences for seed types and colors such that overall yield potential has not been greatly improved (Graham and Ranalli, 1997).

Common bean is not noted for its drought tolerance, yet a recent studies suggest that only 7% of the growing area receives adequate rainfall (Broughton et al., 2003), and 60% of the production occurs under severe drought stress (Graham and Ranalli, 1997). Other *Phaseolus* species of lesser importance are tepary bean (*P. acutifolius*), runner bean (*P. coccineus*), and lima bean (*P. lunatus*). Tepary bean is considered more heat and drought tolerant than common bean, and could represent an

interesting source of genetic variability to incorporate into cultivars of common bean for improved performance under drought conditions (Castonguay and Markhart, 1992; Camacho Barron and Gonzalez de Mejia, 1998; Giller, 2001; Micheletto et al., 2007).

2.3.2.2 Chickpea (*Cicer arietinum*)

Chickpea comes from two centers of origin: the Iran – Turkey region and the Central Asia – Afghanistan region (Polhill and van der Maesen, 1985). It is produced extensively in India, where it is the most important legume crop in terms of production (Giller, 2001). It is widely grown in semi-arid areas, and increasingly in more temperate regions such as Canada and New Zealand (Anwar et al., 2003). It is increasingly being grown as a winter crop in tropical settings to avoid severe water and high temperature stress, but as a result, sometimes suffers from cold stress, especially at flowering (Erskine, 2003). There are two types of chickpea: the desi types, with angular seeds, which accounts for about 85% of the production, and the kabuli type which has large round seeds (Giller, 2001). The desi type is reported to be more drought tolerant, partly because of better remobilization of carbon and nitrogen to seeds under stress (Davies et al., 2000a).

2.3.2.3 Cowpea (*Vigna unguiculata*)

Cowpea was domesticated in West Africa, but also has a significant center of diversity in India (Polhill and van der Maesen, 1985; Giller, 2001). As a member of the genus *Vigna*, it is relatively tolerant to high temperature and drought stress, and as such it is cultivated in semi-arid and arid areas. Two thirds of the production occurs in Africa, with important production in South America (principally Brazil), Asia, Mexico and the Southern United States (Elhers and Hall, 1997). Cowpea is also tolerant of low fertility due

to high rates of nitrogen fixation, facilitated by prosmisicous nodulation and efficient mycorrhizal symbioses (Elhers and Hall, 1997; Hall et al., 2003). The crop is mainly consumed as dry grains, but leaves as well as fresh seeds and pods are also consumed (Elhers and Hall, 1997). Cowpea breeding programs have produced early-maturity photoperiod-insensitive cultivars which exhibit resistance to insects and diseases (Hall et al., 2003).

2.3.2.4 Pigeon pea (*Cajanus cajan*)

Pigeon pea probably originated from India, but spread to Africa before 2000 BC, where there is a second center of diversity (van der Maesen, 1990). From West Africa, it spread to the West Indies, where it is an important crop (Giller, 2001). Between 70 and 85% of its production occurs in India, but the crop holds promise in Africa, as it is quite drought tolerant by legume standards (Giller, 2001; Odeny, 2007) and seems to decrease the pressure from the weed *Striga* in subsequent cereal crops (Odeny, 2007). It is also increasingly grown in Australia and in the United States (Flower and Ludlow, 1987). It is a versatile crop with multiple uses: split seeds are used in dhal, green seeds and pods as vegetables, crushed seeds and leaves to feed livestock. Tall shrubby perennial landraces are also used as living fences with stems being used as fuel wood or to make huts and baskets (Nene and Sheila, 1990). Early maturing cultivars have been developed but seem to be more susceptible to drought (Subbarao et al., 2000).

2.3.2.5 Mungbean (*Vigna radiata* (L.) Wilczek)

Mungbean, also known as green gram, is a small-seeded crop less known in the Americas, but widely cultivated in Asia, particularly in India and Myanmar, where it is thought to have originated (Lawn and Ahn, 1985;

Hafeez et al., 1991; Poehlman, 1991). Central Asia appears to be a secondary center of origin, and it is also cultivated to some degree in the United States and in Australia, and is often consumed as sprouts (Lawn and Ahn, 1985; Poehlman, 1991). The crop is known as one that performs well under conditions of low soil moisture. It is closely related to black gram (*Vigna mungo* (L.) Hepper). Despite their long history in Asian agriculture, neither crops received much research attention prior to 1970 (Lawn and Ahn, 1985), and the research effort has decreased since 1985. Both are also mostly cultivated as subsistence crops with very little inputs, and average yields are low, around 400 kg ha⁻¹ (Lawn and Ahn, 1985; Lambrides and Godwin, 2007). The yield potential of mungbean seems relatively low, rarely exceeding 2500 kg ha⁻¹, even under experimental conditions. Most accessions of mungbean exhibit photoperiod sensitivity, some degree of lodging and pod shattering, plus a continuous production of pods and sometimes weather damage at harvest (Lawn, 1979; Lawn and Ahn, 1985; Ellis et al., 1994; Lambrides and Godwin, 2007). Breeding programs have emphasized seed quality characteristics (Lambrides and Godwin, 2007) and to some extent characteristics that relate to an easier harvest through mechanization such as an erect and determinate growth habit (M. Ryan, personal communication). Disease and insect resistance are also breeding objectives (Lawn and Ahn, 1985; Poehlman, 1991; Lambrides and Godwin, 2007).

2.3.2.6 Lentil (*Lens culinaris*)

Lentil is one of the most ancient domesticated food crops, and is from the Mediterranean area (Muehlbauer et al., 1985; Hedley, 2001). There is evidence of co-domestication with cereal crops such as wheat and barley (Muehlbauer et al., 1985; Muehlbauer et al., 2006). It is grown in a wide range of environments throughout Europe, Asia and North Africa (Hedley, 2001), as well as in the Americas, Australia and New Zealand (Siddique et

al., 201; Ford et al., 2007). India is the most important producer of lentil, mostly for domestic consumption. The production of lentil in Australia and Canada is fairly recent, but is increasing rapidly (Muehlbauer et al., 2006). In most semi-arid areas, lentil is sown in the winter under rainfed conditions, so that it regularly suffers from water stress at the reproductive stage (Silim et al., 1993; Erskine et al., 1994), and to a lesser degree, may also be subjected to cold stress in the vegetative phase (Erskine et al., 1994).

2.3.2.7 Faba bean (*Vicia faba*)

The wild progenitor of faba bean, also called broad bean, remains unknown, but the crop is thought to originate from West or Central Asia (Bond et al., 1985). Faba bean is an important crop in temperate regions, and is often grown during cooler periods in the semi-arid regions (Bond et al., 1985), and therefore, it is not regularly exposed to drought stress except in Australia and China (Johansen et al., 1994). Cropping systems, like most other legumes, range from subsistence agriculture to modern high-input monocultures. The crop is particularly susceptible to water stress and heat stress when compared to other cool-season legumes, such as lentil or chickpea (Johansen et al., 1994).

2.3.2.8 Soybean (*Glycine max* L. Merr)

Soybean was domesticated in China, and is now the most widely cultivated legume crop worldwide by far (Giller, 2001; Hedley, 2001; FAOSTAT, 2009; see Table 2.3.1 for world production values of legumes discussed here). It is becoming increasingly important as a cash crop in many parts of the world, as it is used as food for human consumption, as a source of protein in animal feed, and is the world's most important source of vegetable oil (Giller, 2001). Most of the production is concentrated in

the United States of America, China, Brazil and Argentina (Giller, 2001; Hedley, 2001; FAOSTAT, 2009), with China being the most important consumer, importing half of its domestic demand (Liu et al., 2008). In developing countries, soybean prices are often greater than those of other legume crops or cash crops such as coffee (Ndakidemi et al., 2006). While soybean is not particularly drought tolerant, some cultivars have shown good resistance to water deficit stress in the Southern United States, and the crop is often used in studies of legume nitrogen fixation responses to drought (Serraj and Sinclair, 1999; Purcell et al., 2000; Sinclair et al., 2003). However, recent interest in soybean for biofuel production is shifting the emphasis to higher oil content in breeding programs (Ray et al., 2008). Whether or not this is going to be achieved in combination with traits related to good performance in dry environments remains to be seen.

2.3.2.9 Groundnut (*Arachis hypogaea*)

The center of diversity for groundnut, or peanut, is Brazil (Bunting et al., 1985; Giller, 2001). From the New World, it was taken to Africa, India, and Asia by Spanish and Portuguese explorers (Bunting et al., 1985). It is the world's second most important source of vegetable oil, after soybean (Giller, 2001). Seeds are eaten raw, roasted or boiled. Peanut is also used as animal feed, or in industrial production of soap, detergent or cosmetics (Maiti and Wesche-Ebeling, 2002). Ninety percent of its production occurs in developing countries (Giller, 2001), and two-third occurs under rainfed conditions in the semi-arid tropics (Songsri et al., 2008). Drought can also increase the susceptibility of peanut plants to the fungus *Aspergillus flavus* and lead to the accumulation of aflatoxin (Bunting et al., 1985).

2.3.3 Drought: a complex environmental stress

The improvement of drought tolerance in legumes, here defined as relatively high yields under unfavourable moisture regimes (Erskine, 2003) and our physiological understanding of crop response to drought, have been made difficult by the complexity of drought as an environmental stress. An agricultural drought which decreases crop yields substantially is a combination of atmospheric conditions, soil moisture conditions, and crop conditions: high temperature and high vapour pressure deficit are combined with low soil moisture and increased soil hardness, and the stress experienced is further affected by the crop development stage and crop planting density (Begg and Turner, 1976; Thomas, 1997; Turner, 2003). In addition, rainfall in semi-arid areas is characteristically unpredictable (Erskine, 2003). Thus, drought is not only complex, but both timing and intensity of the water deficit stress experienced by the crop can be important and highly variable. Most plant processes are affected by drought, and a large number of traits and mechanisms have been suggested to contribute to better performance under drought stress. To deal with this complexity, frameworks have been developed to dissect the response of various processes to water deficit stress, and have often been discussed with an emphasis on breeding crops for greater drought tolerance (Begg and Turner, 1976; Jones et al., 1981; Turner, 1986; Kramer, 1990; Turner, 2003). In the following sections, we will review these frameworks with a particular emphasis on understanding the physiology and the contribution of traits to yield in legumes in semi-arid areas. Some references are also made to mechanisms of drought tolerance in cereals, because traits and mechanisms have been better characterized and breeding efforts have included a physiology approach (Turner et al., 2003).

We will first consider the drought resistance framework. This framework considers separately traits that enable crops to escape drought by completing development before high evaporative demand, for example

(discussed in section 2.3.4), or to either avoid (discussed in section 2.3.5) or tolerate dehydration (discussed in section 2.3.6).

2.3.4 Drought resistance framework: Drought escape

Drought escape refers to characteristics that allow crop development to be well matched to water availability. In general, this is achieved by reducing days to maturity. In some cases, early maturity allows the crop to complete its development before the onset of very high temperatures and high evaporative demand. This allows an 'escape' from the severe water deficit stress that might occur, especially if the crop is grown on residual soil moisture. Early maturity is probably the single most important factor contributing to improved yields of legume crops in semi-arid areas (Silim and Saxena, 1993 in chickpea; Silim et al., 1993 in lentil; Ehlers and Hall, 1997 in cowpea; Berger, 2007 in chickpea).

Earliness might not always be optimal, however, and in years where rainfall or irrigation is sufficient, yields might be limited (Ehlers and Hall, 1997; Turner et al., 2003). In the face of intermittent droughts, developmental plasticity and the ability to recover from stress might be particularly important if the stress occurs during reproductive development. Indeed, the maintenance of an indeterminate growth habit in legumes (Poehlman, 1991 in mungbean and black gram; Turner et al., 2003 in general) has been argued for, although uneven maturity might present important challenges for mechanized agriculture.

2.3.5 Drought resistance framework: Dehydration avoidance

Also referred to as dehydration postponement, dehydration avoidance refers to mechanisms that enable a crop to maintain a high relative water content, or high water potential. This can be achieved by maintaining

water uptake or by reducing water loss (Jones et al., 1981). Related characteristics include leaf area development response to declining soil moisture, root characteristics and growth response, leaf characteristics and leaf movement, stomatal response, nitrogen fixation response to water deficit, and osmotic adjustment. Each of these are discussed in detail below.

2.3.5.1 Leaf area development response

Leaf area development tends to be more responsive to water deficit stress than photosynthesis or stomatal conductance (Begg and Turner, 1976; Blum, 1996). A lower leaf area might not be solely a consequence of a lower growth rate due to water deficit stress, but might be an adaptive mechanism related to a higher relocation of resources to root growth for example. In addition, a lower leaf area provides less area for transpiration, and thus might help conserve water (Begg and Turner, 1976). Further, the active senescence of leaves under water stress can allow an organized translocation of resources from senescing leaves to developing seeds (Turner, 1986; Tardieu, 1996). Indeed, the higher leaf area developed in mungbean and soybean irrigated during the vegetative stage led to a greater water loss from a larger canopy, and an overall reduction in radiation use efficiency when compared to treatments watered during other stages of development (De Costa et al., 1999 in mungbean; De Costa and Shanmugathan, 2002 in soybean). Further, a comparative study between a conventional and a semi-leafless pea cultivar showed that the semi-leafless cultivar was more tolerant to water stress (Baigorri et al., 1999).

While a low leaf area can be a constitutive (as in semi-leafless peas) or an adaptive mechanism to limit water loss, it has also been well demonstrated that the delay in the onset of leaf senescence in sorghum is

associated with higher yields under water stress, but not under well-watered conditions (Borrell et al., 2000; Bennett, 2003; Richards, 2006). This suggests, on the contrary, that the maintenance of leaf area, and of photosynthetic capacity, is an adaptive mechanism to water deficit stress.

2.3.5.2 Root characteristics and growth response

Some of the most important and yet least researched components of plant response to drought relate to root development and architecture (Passouira, 1983; Sponchiado et al., 1989; White and Castillo, 1989). A grafting experiment with common bean performed by White and Castillo (1989) suggested that most of the variability in drought tolerance is attributable to root stocks. Several studies observing root systems of both cereals and legumes have shown that drought tolerant cultivars tend to demonstrate a greater root biomass, a greater root-to-shoot ratio and deeper root profiles (Grzesiak et al., 1997 in common bean and pea; Matsui and Singh, 2003 in cowpea; Benjamin and Nielsen, 2006 in soybean, pea and chickpea; Kashiwagi et al., 2006 in chickpea). According to Kashiwagi et al. (2008), various alleles would have additive effects to increase the root biomass and the root length density of chickpea.

It is also well established that root growth is less inhibited than shoot growth under moisture stress (Bartels et al., 1996; Blum 1996; Thomas, 1997). For example, De Costa and Shanmugathan (1999) showed that root weight and root-to-shoot ratio in mungbean were highest in the rainfed treatment and lowest in the fully irrigated treatment. Similarly, Grzesiak et al. (1999) showed that the accumulation of carbon in roots declined less in tolerant cultivars of faba bean and pea than susceptible ones; Mohamed and Tawfik (2007) showed that higher yielding lines of tepary bean allocated more biomass to roots in response to water deficit

stress. Thus, while a large root system is probably a constitutive trait associated with dehydration avoidance, increased root growth (even relative to shoot growth) is also an adaptive trait to cope with declining soil moisture.

However, Passouira (1983) suggested the optimal resource allocation might not be towards the maximum root biomass, but rather to maximize the return on investment on carbon allocated to roots rather than leaves. Crops grown on residual moisture might also deplete the soil water early and thus suffer from severe water stress at the reproductive stage (Huang, 2000). Indeed, a breeding retrospective on tropical maize showed that selection for high grain yield in eight cycles of selection resulted in a reduction of the root biomass in the top 50 cm of the soil profile (Bolanos et al., 1993). Other root characteristics that might play a role in drought responses by crops subjected to drought stress include root hair development, hydraulic conductivity, fine root development and viability, and various aspects of root cell metabolism (Huang, 2000). For example, in wheat, selection for small xylem diameter in seminal roots has led to cultivars that use soil water more slowly. These show yield gains of 8% in dry environments with no yield penalty in more favourable environments (Richards and Passouira, 1989; Richards, 2006). The contribution of such traits to the maintenance of legume yields under water deficit stress has not been established.

2.3.5.3 Stomatal response

One of the most universal responses of plants to water deficit stress is stomatal closure. This is believed to be an integrative response of hydraulic and chemical signals, responding both to a 'long-term' abscisic acid (ABA) signal originating from the roots, and 'short-term' changes in evaporative demands (Tardieu and Davies, 1993). Recent research

efforts have considerably enhanced our knowledge of the molecular events associated with the loss of turgor in guard cells (see section 2.3.8 for in-depth discussion).

The analysis of the optimal stomatal behaviour is complex because stomata are the site of carbon dioxide uptake as well as water loss, and as such, restriction of water loss might lead to a proportional inhibition of carbon assimilation. Comparative studies among *Phaseolus* species or among *P. vulgaris* cultivars have shown that tolerant plants tend to exhibit faster stomatal closure in response to decreasing soil water potentials (Markhart, 1985; Lizana et al., 2006). On the contrary, Cruz de Carvalho et al. (1998) showed that the susceptible *P. vulgaris* exhibited the largest decrease in stomatal conductance when compared to more tolerant cowpea cultivars. A study on soybean suggested that drought tolerance was associated with a limitation of the maximum transpiration rate (Fletcher et al., 2007). Yet other studies showed that tolerant genotypes exhibited higher rates of stomatal conductance early in the morning, but lower rates at midday and during the afternoon (Bates and Hall, 1992 in cowpea; Pimentel et al., 1999 in common bean), suggesting an important role for circadian rhythms (Mencuccini et al., 2000), in addition to the response to declining soil moisture. Finally, Grzesiak et al. (1999) found that tolerant cultivars of faba bean and pea, when compared with susceptible cultivars, showed lower photosynthetic and transpiration rates five days after stress imposition, but a higher rates ten days after stress imposition. In addition, the lack of stomatal response to dry soils after flowering, in several crops, suggests that limitation of water use due to decreased stomatal conductance might not lead to higher yields under drought conditions (Ludlow and Muchow, 1990; Blum, 1996).

Further, a closed canopy creates a boundary layer whose thickness depends on weather conditions such as relative humidity and wind speed.

This boundary layer can decrease the plant's control over transpiration rates substantially, but might also increase transpiration efficiency by increasing relative humidity around the leaf (Subbarao et al., 1995; Jones, 1998; Polley, 2002). Light, relative humidity, and plant density are often quite different in controlled-environment studies and as such, transpiration rates observed in response to water stress might be considerably different than in the field (Jones, 1998). It seems that further understanding of stomatal response to water deficit stress, and of what might constitute an 'optimal' behaviour (if it exists), will require intensive monitoring (in minutes, and over an entire growing season) both in controlled-environment studies and in field experiments. This has not been possible with gas exchange measurements reported in the literature.

2.3.5.4 Leaf characteristics and leaf movement

Some characteristics of the cuticle are thought to have an effect on water loss when stomata are closed (Begg and Turner, 1976). Leaf characteristics that increase leaf reflectance, such as large white hairs in sunflower (Johnson, 1975), or the development of a wax bloom in sorghum (Chatterton et al., 1975) and wheat (Johnson et al., 1983; Richards et al., 1986), can decrease leaf temperature and transpiration. To our knowledge, the only example of a legume crop producing considerable epicuticular wax in response to water stress is in black gram (Ashraf and Karim, 1991), although differences in leaf reflectance are sometimes reported (eg. Phogat et al., 1984 in cowpea and mungbean). In spite of the fact that these constitutive and adaptive traits of the leaf epidermis were identified decades ago, the mechanisms related to cuticular characteristics have not been thoroughly researched.

Similarly, the movement of leaves to align them parallel to incident light decreases the irradiation load and might help in reducing leaf temperature

and water loss. All legumes manifest some degree of paraheliotropy (Subbarao et al., 1995), but some comparative studies suggest that this is an adaptive trait to water deficit stress (Smith et al., 1988), while others have associated it with higher susceptibility to this stress (Lizana et al., 2006).

2.3.5.5 Nitrogen fixation response

Biological nitrogen fixation might be more suitable than mineral nitrogen fertilization in drought-prone areas (Lodeiro et al., 2000; Kirova et al., 2008). For example, a fertilized bean crop had greater leaf development, and consequently experienced more severe water stress during pod filling, compared to the inoculated crop (Lodeiro et al., 2000). Other mechanisms might also be involved: Figueiredo et al. (1999) showed that some bradyrhizobial strains alleviated the effects of water stress in cowpea; Curtis et al. (2004) showed that proline which accumulates in some drought stressed crops (see section 2.3.5.6) is catabolized by bacteroids, and this has positive effects on nitrogen fixation under mild water deficit stress; Matiru and Dakora (2005) suggested lumichrome acts as a bacterial signal, and is involved in regulating root respiration, stomatal conductance and photosynthetic rates.

However, nitrogen fixation is also highly susceptible to drought stress, and this can substantially decrease the benefits of growing legume crops in semi-arid areas (Giller, 2001). It is clear that the ability to yield well under drought is related to the maintenance of biological nitrogen fixation by rhizobia in legume nodules, and genetic variability in this trait has been demonstrated in both legumes (Sinclair et al., 2007 in soybean; Pimratch et al., 2008 in groundnut) and bradyrhizobial strains (Hungria and Vargas, 2000; Curtis et al., 2004).

Because nitrogen fixation decreases before any changes in photosynthesis are observed, the inhibition of nitrogen fixation does not seem to be related to decreased photosynthate availability, but rather seems to involve three mechanisms: 1. changes in oxygen permeability, which in turn might regulate nitrogenase activity (Pankhurst and Sprent, 1975); 2. a feedback mechanism related to ureide accumulation (Serraj and Sinclair, 1999; Sinclair et al., 2003; King and Purcell, 2005), and 3. a carbon shortage in nodules (Galvez et al., 2005). In addition, all three mechanisms seem to interact in complex a fashion. For example, in both soybean (Sinclair et al., 2003; King and Purcell, 2005) and chickpea (Thavarajah and Ball, 2006), the decrease in nitrogen fixation is associated with the accumulation of ureides, allantoin and allantoate, in leaves. This is thought to lead to a feedback mechanism from the conversion of ureides to some yet unknown intermediate compound(s). A possible candidate is asparagine (Asp) which could chelate manganese (Mn) in nodules, which in turn would lead to lower malate availability to bacteroids (Todd et al., 2006), and thus a carbon limitation in nodules.

It has also been shown that Mn fertilisation can alleviate the effects of water deficit stress on the decline in nitrogen fixation (Vadez et al., 2000). The same group has further shown that in 6 of 8 soybean lines exhibiting nitrogen fixation tolerance to water stress, the ureide accumulation in leaves is independent of Mn nutrition, suggesting tolerant cultivars use a Mn-independent pathway for ureide catabolism (Sinclair et al., 2003). However, no enzyme has been described to date that does not require Mn for ureide catabolism (Todd et al., 2006). Better understanding of the mechanisms of inhibition of nitrogen fixation could lead to improved yields of legumes, and greater benefits for sustainable agriculture in semi-arid areas. Further research to identify high nitrogen fixing legume cultivar-rhizobial strain combinations in dry environments is clearly needed.

2.3.5.6 Osmotic adjustment

Osmotic adjustment (OA) is defined as the active accumulation of solutes and consequent decrease in osmotic potential. According to theory, OA helps maintain water extraction, turgor and growth under dehydration and salt stress (Hsiao et al., 1976, Turner and Jones, 1980; Morgan, 1984; Munns, 1988; Blum et al., 1996), and could enable plants to keep stomata open, and thus continue to take up carbon dioxide (Hopkins, 1995). Accumulated solutes include amino acids (particularly proline), the quaternary ammonium compound glycine betaine, sugars, organic acids (malate, citrate), as well as nitrate, potassium and chlorine ions (Morgan, 1984). Osmotic adjustment has been correlated with higher yields in several cereal crops (Ludlow and Muchow, 1990; Fukai and Copper, 1995 in rice), and some legume crops (Flower and Ludlow, 1987 in pigeon pea; Lecoœur et al., 1992 in chickpea; Baigorri et al., 1999 in semi-leafless pea). However, several publications have reported OA in legumes, but have failed to observe the associated benefits: Wullschleger and Oosterhuis (1991) observed OA in leaves and roots of common bean and in roots of pea, but found that this was not associated with the maintenance of leaf growth in either crop; Likowse and Lawn (2008) found that both pigeon pea and soybean adjusted osmotically, but this was of no benefit for leaf survival in soybean; Turner et al (2007a) found that OA did not result in yield benefits under terminal drought, and expressed doubt that OA is an active accumulation of solutes.

Indeed, the concept of OA has been the object of significant debate over the last 20 years. The physiological basis of a contribution to higher yields from OA is still unclear. Very few solutes seem to accumulate as a result of an increase in uptake following water stress, but rather seem to accumulate as the result of decreasing leaf expansion rates (Munns, 1988; Serraj and Sinclair, 2002). If solutes accumulate specifically to

decrease the osmotic potential, they should thus divert resources from productive processes, and would not contribute to maintaining growth (Munns, 1988). Further, given the range of changes in osmotic potential observed in most crops, it is doubtful that OA contributes significantly to increased water extraction, except if OA occurs in root tips and increases root growth (Serraj and Sinclair, 2002). Finally, it seems that OA does not allow for the maintenance of high stomatal conductance under stress. Rather plants either reduce stomatal conductance to maintain leaf water potential, or reduce stomatal conductance and osmotic potential simultaneously (Tardieu, 1996).

Still, selection for high OA in wheat lines has led to increases in yields under drought-prone environments (Richards, 2006), and simulation models in sorghum suggest OA benefits yield under severe stress (Chapman et al., 2002). It is possible that the main role of the accumulated solutes might not be to decrease the osmotic potential and maintain turgor, but might rather be to protect cell membranes and proteins (Shabala and Lew, 2002), potentially through radical scavenging activities (Smirnoff and Cumbes, 1989), or might facilitate recovery of photosynthesis and growth once the stress is relieved (Munns, 1988).

2.3.6 Drought resistance framework: Dehydration tolerance

We have discussed the mechanisms that allow plants to avoid dehydration by increasing water extraction, or by reducing water loss. However, if the stress becomes severe, cells might experience dehydration. Protection of the protoplasm by compatible solutes from OA, or from proteins produced specifically for this purpose (discussed in section 2.3.8), might contribute to longer leaf survival or better growth recovery once the stress is relieved (Ludlow and Muchow, 1990). This has been evaluated in cereals and in some legumes (Shackel and Hall, 1983 in cowpea; Flower and Ludlow,

1986 in pigeon pea; Sinclair and Ludlow, 1986 in cowpea, mungbean, soybean and peanut) from the measurement of lethal water status, consisting either of the relative water content or the leaf water potential at which leaves died.

Another mechanism relates to cell wall elasticity and the ability of cell membranes to withstand dehydration. Electrolyte leakage has been examined by Srinivasan et al (1996), who found considerable genetic variability in chickpea, groundnut, pigeon pea and soybean. However, lower electrolyte leakage has yet to be correlated with drought tolerance observed in the field (Turner et al., 2001).

2.3.7 Yield component frameworks

Ultimately, a physiological understanding of drought tolerance should allow for the incorporation of useful traits into current elite crop germplasm. Besides the analysis of individual traits that could be beneficial under water deficit, another useful approach is to consider the effects of a particular trait on economic yield. Since yield is a very complex trait (often with relatively low heritability), it might be particularly relevant to separate physiological processes (ideally into processes with relatively high heritability) contributing to yield and consider these in simple mathematical terms (Turner et al., 2003). Duncan et al. (1978), for example, considered yield (Y) as the product of the crop growth rate (C), the duration of the reproductive growth (Dr) and the proportion of the biomass partitioned to yield (p).

$$Y = C \times Dr \times p$$

Monteith (1977) considered yield as the result of the radiation intercepted by the crop (RI), the efficiency of the conversion of this radiation into biomass (RUE), and the partitioning of the biomass into harvestable yield (HI).

$$Y = RI \times RUE \times HI$$

Particularly relevant for drought-prone areas is the Passouira identity in which yield is the product of the water transpired (W), the transpiration efficiency (TE), and the HI (Passouira, 1977).

$$Y = W \times TE \times HI$$

Thus, traits of possible interest for improving crop tolerance to drought would include: 1. high seedling vigour in order to allow high early radiation interception and decreased soil evaporation, 2. a large and efficient rooting system to allow high water extraction capacity, 3. a high transpiration efficiency, and 4. high harvest index (Turner et al., 2003; Richards, 2006). As mentioned previously, however, depending on the soil moisture regime, early growth that is too vigorous could deplete soil water resources and decrease the HI. Recent studies in cowpea, chickpea and lentil suggest that there is genetic variability in root biomass and root length density, especially at depth, and that these traits could lead to higher yields (Matsui and Singh, 2003; Serraj et al., 2004; Ali et al., 2005; Sarker et al., 2005; Kashiwagi et al., 2006). This suggests that present cultivars are not consuming all of the available water.

The correlation between the carbon isotope discrimination (Δ) in leaves and transpiration efficiency (TE) has led to advances in breeding higher yielding wheat (Richards, 2006), and sunflower plants (C. Lambrides, personal communication). Unfortunately, there are conflicting reports regarding the relationship between Δ and TE in legumes (Hall et al., 1992; Ismail et al., 1994; Khan et al., 2007; Krishnamurthy et al., 2007; Turner et al., 2007b), and the relationship, if it exists, might depend on growing conditions. The HI however holds particular promise. Methods have been developed to evaluate the translocation ability, allowing for the screening of a large number of genotypes (Turner et al., 2003). There seems to be considerable genetic variability in various legume crops subjected to

drought stress (Constable and Hearn, 1978 in soybean; Bushby and Lawn, 1992 in mungbean). Indeed, some less-researched legume crops presently exhibit low HI, and there might be opportunities to improve their overall yield potential through improvement in the HI under all conditions.

2.3.8 Clues from molecular biology

2.3.8.1 Clues from microarray data

Microarray data have identified a large number of genes differently expressed during dehydration stress. Most of the work has been performed on *Arabidopsis thaliana* (reviewed by Bray, 2002) or on the resurrection plant *Craterostigma plantagineum* (reviewed by Bartels and Salamini, 2001). Genes up-regulated by dehydration stress can be categorized in several groups: genes related to metabolism, genes related to photosynthesis, transcription factors, protein degradation and protection, transport, cell communication, genes related to the response to oxidative stress and/or defense against pathogens, genes coding for proteins related to cell wall functions and characteristics, and encoding late-embryogenesis-abundant proteins, which are otherwise still unclassified (Bray, 2002). These are discussed in more detail below. Other genes related to cellular communication and a number of transcription factors are also up-regulated in response to dehydration stress, but these will be discussed in the following section.

In the first category, genes related to the general metabolism, most up-regulated genes in response to dehydration stress are involved in amino acid, phenylpropanoid and fatty acid metabolism (Bray, 2002). Nitrate reductase activity has also been shown to be down-regulated in a number of species (Foyer et al., 1998).

The vast majority of genes that change expression patterns in response to dehydration stress are down-regulated. In particular, there is an important decrease in the transcription of the mRNA that encodes the small subunit of Rubisco (Bartholomew et al., 1991). Two exceptions include the up-regulation of genes coding for the oxygen evolving complex (Reymond et al., 2000), and the reaction center subunit II of photosynthesis system I (Seki et al., 2001).

Cysteine proteases (Seki et al., 2001) and metallopeptidases (Reymond et al., 2000) have been shown to be up-regulated during dehydration stress. These are thought to protect cell metabolism by degrading proteins that have been damaged by dehydration stress (Bray, 2002). In addition, they may play a role as chaperones to protect existing proteins. This role has also been suggested for heat shock proteins (Bartels et al., 1996). In *Craterostigma*, Bartels and Salamini (2001) suggest the observed conversion of 2-octulose to sucrose upon dehydration plays an important role in the protection of cellular organization.

The only up-regulated genes related to transport identified in microarray studies relate to water and sugar transport (Seki et al., 2001; Bray et al. 2002). Aquaporins are membrane proteins that facilitate the passive diffusion of water from cell to cell. Aroca et al. (2006) cloned three plasma membrane intrinsic proteins (aquaporins) from *Phaseolus vulgaris*, and found that their expression were up-regulated in leaves in response to ABA, methotrexate (a stomatal inhibitor), and water deficit stress from lack of re-watering. In roots, however, only the lack of re-watering increased the gene expression and protein abundance of the three aquaporins cloned.

In microarray studies by Reymond et al. (2000) and Seki et al (2001), the two largest groups of up-regulated genes were related to the response to

oxidative stress and the common defense pathways. Phenolics and phenylpropanoids also have a potential role as antioxidants (Bray 2002).

Microarray data has also shown the up-regulation of a polygalacturonase-like gene product, a polygalacturonase inhibitor, beta-glucosidase, and a gene product with pectinesterase-like activity (Seki et al., 2001), and these are thought to modify cell walls to maintain some level of growth under reduced turgor (Bray 2002). Proline-rich proteins have also been identified in bean cell walls subjected to water deficit stress, and were shown to interact with a membrane protein (Garcia-Gomez et al., 2000).

Finally, late-embryogenesis-abundant (LEA) proteins are a heterogeneous group of proteins that were first discovered to accumulate in high quantities in the late stages of embryo development. Several classes of LEA proteins have been found to be up-regulated in response to dehydration stress in *Arabidopsis* (Bray, 2002), and in *Craterostigma* (Bartels and Salamini, 2001). Their molecular and biochemical features suggest a protective role (Bartels and Salamini, 2001), although they also exhibit different characteristics that suggest they may play distinct roles (Bray, 2002). More research is warranted to elucidate their role in crop tolerance to drought under field conditions (Colmenero-Flores et al., 1997; Bray, 2002).

2.3.8.2 Signalling pathways in response to dehydration stress

The signalling networks induced by dehydration stress have been the object of considerable research interest. Several classes of transcription factors induced by water stress have been identified from molecular studies in *Arabidopsis*. These include the ABA-responsive-element binding protein 1 (AREB1), the ABA-responsive-element binding factor 2 (ABF2), the dehydration-responsive-element binding protein 2A

(DREB2A), homeobox leucine zipper (bZIP) proteins 6, 7 and 12 (ATHB-6, -7, and -12), AP2 domain (DREB2A), NAC, MYB and MYC-related factors, and protein kinases such as the receptor-like kinase 1 (RPK1), SNF1-related protein kinase 2C (SRK2C), the guard cell-expressed calcium dependent protein kinases 3 and 6 (CDPK3, 6), (Bray, 2002; Bray, 2004; Seki et al. 2007; Shinozaki and Yamaguchi-Shinozaki, 2007). These are involved in ABA-dependent and ABA-independent signalling pathways, which are not fully independent from each other (Bajaj et al., 1999; Pastori and Foyer, 2002; Shinozaki and Yamaguchi-Shinozaki, 2007). The promoters of dehydration-inducible genes contain two major cis-acting elements: an ABA-responsive element (ABRE) and a dehydration-responsive element (DRE) (Yamaguchi-Shinozaki and Shinozaki, 1994; 2005). In at least one ABA-dependent signalling pathway, some as yet unknown ABA-receptor and/or intermediate is required to activate basic region/leucine zipper (bZIP) transcription factors that then turn on gene expression by binding to ABRE in the promoter region of dehydration-inducible genes (Bajaj et al., 1999). It is thought that a second ABA-dependent pathway results from the activation of MYC and MYB, and/or bZIP transcription factors that bind to DNA regions other than ABRE (Abe et al., 1997; Shinozaki and Yamaguchi-Shinozaki, 2007). The ABA-independent pathways are less well understood, but are believed to involve DREB2A which would bind to DRE in promoter regions of early-responsive-to-dehydration (ERD) genes (Jensen et al., 1996; Shinozaki and Yamaguchi-Shinozaki, 1996). In all pathways, many genes up-regulated are involved in mitogen-activated protein (MAP) cascades, and might involve calcium as a secondary signal (Bray, 2002).

There have been considerable efforts to identify the initial steps of the perception of plants to drought stress. We have yet to identify with confidence a receptor protein that would bind to ABA (Christmann et al., 2003). However, there is increasing evidence that active oxygen species

such as H₂O₂ (Pastori and Foyer, 2002), or NO (Desikan et al., 2004), ethylene (Sharp, 2002), as well as pH (Wilkinson, 1999) and sugars (Verslues and Zhu, 2005) may play an important role as key signals in the response to ABA and drought.

Pastori and Foyer (2002), from analyses of the transcription factors discussed above, propose that the role of ABA is not restricted to drought response, but is rather involved in common defense pathways that are part of tolerance responses to various stresses. A common feature of the response to biotic and abiotic stresses is the production of active oxygen species, and the signalling networks in response to oxidative stress are worthy of further investigations (Pastori and Foyer, 2002).

Sharp (2002) also argues that the role of endogenous ABA produced by plants under water deficit stress might not be to reduce growth as first thought, but rather to limit ethylene production. He suggests ethylene rather than ABA reduces growth. Sharp (2002) further supports his argument with the old observations that ABA deficient mutants, which are often shorter and smaller, can have similar leaf and stem growth to their corresponding wild types with the application of exogenous ABA (Imber and Tal, 1970; Bradford, 1983, Quarrie, 1987). Thus, although ABA is associated with decreased growth under water deficit stress, the hormone probably acts to maintain growth under suboptimal conditions.

In parallel, Wilkinson (1999) demonstrated that increases in pH in the xylem sap of plants subjected to various abiotic stresses results in the liberation of ABA already present in conjugated forms in the cells adjacent to guard cells. This, then, activates ABA signalling in guard cells, leading to stomatal closure. She argues that without ABA, the increase in pH would lead to an increase in stomatal conductance and consequent water loss.

Further, there has been considerable progress in determining the signalling pathways specifically in guard cells. There appear to be two pathways controlling the loss of turgor, although, again, they share a number of common components (Christmann et al., 2003): one involves calcium oscillations induced by ABA, which then result in an outward flow of K⁺ (Blatt and Armstrong, 1993; Luan, 2002) and changes in anion channel activities; the other is characterized by changes in the phosphorylation status of transcription factors in the nucleus (Christmann et al., 2003). Recently, the tandem production of H₂O₂ and NO has been proposed as a signal upstream of calcium and protein kinases (Desikan et al., 2004).

It is clear that there has been considerable progress to describe the molecular responses and signalling pathways related to the response to water deficit stress and dehydration stress in plants. There are still discoveries being made (Desikan et al., 2004), and this field of research appears to be a particularly exciting one for molecular biologists. However, many molecular studies have been criticized for imposing an unrealistically severe and rapid dehydration stress on plants (Bray, 2002). In several cases, this has been imposed by leaving a detached leaf drying on a laboratory bench (Seki et al., 2001 among others). Hanson and Hitz (1982) have suggested that most observed responses in such treatment imposition would be injury-related rather than adaptive responses to slow-developing water deficit stress. Indeed, Talame et al. (2007) compared transcript profiles of barley plants subjected to a rapid shock-like treatment (imposed by pulling out plants and leaving them to dry for 6 hours), and a gradual decline in soil moisture availability. They found that only 10% of the transcripts were commonly up-regulated or down-regulated in the two treatments. Similarly, a microarray experiment considering three water deficit stress treatments (desiccation of detached leaf, mannitol treatment,

and slow water deficit) found no more than 1.4% commonly induced or repressed genes under all three treatments (Bray, 2004). As mentioned previously, physiologists have also argued that in most agricultural cases, water deficit stress does not induce molecular dehydration until the stress is quite severe, or unless there is a rapid rise in evaporative demand (Tardieu, 1996).

2.3.8.3 Genetic engineering of drought tolerance traits

Most genetic transformation of plants to improve drought tolerance has involved the overproduction of enzymes responsible for the biosynthesis of compatible osmolytes, LEA proteins, or detoxification enzymes (Bajaj et al., 1999). These were thought to be relatively simple traits that could be relatively easily inserted in plant genomes. Examples include enzymes involved in the production of mannitol (Tarczynski et al., 1993; Thomas et al., 1995; Karakas et al., 1997), proline (Kavi Kishor et al 1995; Zhu et al 1997), fructans (Pilon-Smits et al., 1995), trehalose (Romero et al., 1997; Pilon-Smits et al., 1998), glycine betaine (Lilius et al., 1996; Hayashi et al., 1997; Alia et al 1998; Sakamoto et al 1998), and LEA proteins (Zhu et al., 1998). Some studies have also transformed plants to overproduce transcription factors involved in the signalling pathway (Shinozaki and Yamaguchi-Shinozaki, 2007). However, in at least one case, the overexpression of such transcription factor, DREB1A resulted in severe growth retardation under normal conditions under constitutive expression (Kasuga et al., 1999). This has led Bajaj et al. (1999) to argue for the use of stress-inducible promoters.

However, even with better genetic transformation, there is considerable skepticism about the possibilities of increasing crop yields using this approach. To date, there still has not been one instance where this has led to consistent improvement of yields in the field (Sinclair et al., 2004).

This might be because the osmolytes overexpressed play a role in plant survival rather than plant growth, and/or because there are several levels of regulation and complexity that interact and ultimately determine crop yields (Sinclair et al. 2004; Chapman, 2008).

2.3.8.4 Quantitative trait loci (QTLs) and marker assisted selection (MAS)

Two complementary approaches that have been more popular with plant breeders than genetic transformation are the identification of quantitative trait loci, and marker assisted selection (Tuberosa et al., 2002; Eathington et al., 2007). Through various molecular techniques, segments of DNA (i.e QTLs) can be statistically associated with a quantitative trait such as high yield, for example. When identified, these segments can be introgressed into elite germplasm by recurrent selection for this QTL (i.e marker assisted selection, or MAS). This approach has been used successfully in commercial breeding programs for simple traits such as disease resistance (Eathington et al., 2007), and there are high hopes that the identification of QTL and MAS will improve breeding efficiency for drought tolerance (Tuberosa et al., 2002; Ribault and Ragot, 2007; Tuberosa et al., 2007), especially for traits that are difficult and expensive to phenotype, and for adaptive traits that might not always be expressed in breeding programs.

There are more than 150 studies to date (April 2009) that have examined QTLs for a number of quantitative traits related to drought tolerance in several species. In legumes specifically, Schneider et al. (1997) has identified four chromosomal regions in a population of common bean, and five regions in another population. They demonstrated that using MAS led to higher yield gains than conventional breeding in Michigan, but not in Mexico. Although both environments were characterized by severe

drought, they conclude that significant genotype by environment interaction had modified the expression of the QTL. Not surprisingly, several more studies have identified QTLs related to drought tolerance in cereals (Tuberosa et al., 2002, 2003, 2007). For example, specific chromosome regions have been identified that relate to ABA concentrations in the xylem sap (Lebreton et al., 1995) and in leaves (Lebreton et al., 1995; Tuberosa et al., 1998), or to root traits such as seminal root number, nodal root number at the base of the stem, and root pulling force (Lebreton et al., 1995; Tuberosa et al., 2002). These studies have shown that high yielding lines accumulated low levels of ABA due to better water extraction capacity (Tuberosa et al., 2002). Similarly, a gene controlling OA in wheat has been identified and a high OA wheat line has been developed from recurrent crosses into elite germplasm. Unfortunately, the gene is also linked to an endosperm peroxidase that reduces quality (Richards, 2006).

These are some of the very few examples where QTLs have been used in plant breeding to improve drought tolerance in crops (Edmeades et al., 2004). It is clear that QTL analysis and marker assisted selection are not alternatives to conventional plant breeding field testing, or to more 'classical' crop physiology, instead they are complementary approaches. In addition, QTL analysis combined with genomics approaches (transcriptome, proteome and metabolome analyses), as well as the evaluation of individual traits' contributions to yields, can enhance our ability to understand the molecular and physiological responses of crops to drought (Tuberosa et al., 2002).

2.3.8.5 Crop physiology and genomics: linking gene networks to whole plant response

Crop physiologists' strengths typically lie in the scale of whole plant processes. The agricultural community has reached a point where crop physiologists, molecular biologists, and plant breeders need to work together to contribute to current knowledge in ways that are relevant. In studies that aim to determine genes related to drought tolerance, for example, crop physiologists can help in the identification of candidate genes and crop ideotypes, in the proper design and execution of field experiments, in the imposition of meaningful drought stress treatments, and in the proper characterization of the phenotypes to be linked with genotypic information (Edmeades et al., 2004). Given the advent of molecular techniques, it would also make sense for crop physiologists to start studying marker characterized segregating populations and marker specific near isogenic lines instead of varieties.

Edmeades et al. (2004) suggest crop models might represent the 'great unifier' between crop physiologists, molecular biologists, and plant breeders. We have discussed above that the genetic transformation of single genes has not led to improvement in yields in the field. While these have not been reported, interactions with the rest of the genome and with the environment have probably precluded their use in breeding programs. Crop models that integrate the effects of genes, of the environment and of crop management practices are being developed. These are used to simulate plant breeding programs, and help breeders make decisions about lines to carry forward in the breeding process, for example (Cooper et al., 2002; Chapman et al., 2002; Edmeades et al., 2004; Chapman 2008).

Chenu et al (2008) demonstrate the application of this integration by the development of an integrated model encompassing the existing models of leaf development (in general) and leaf 6 expansion in maize in response to short term environmental variations. They have further incorporated a

model of three QTLs associated with leaf expansion (Chenu et al., submitted). Interestingly, but not surprisingly, they found that the ideal expression of those QTLs varied with the environment tested. Of particular interest for plant breeders, they have also found that the optimal level of expression of the three QTLs in one environment was not possible with the cross tested.

2.3.9 Conclusions

Pulse crops are an important source of protein in the diet of people who cannot afford to eat meat, or choose not to. Yet, the mechanisms of drought tolerance and genetic variability in traits conferring better tolerance have not been as well characterized in legumes as in cereals, and the use of physiological and genetic markers for improved legume germplasm has been limited.

Drought is a complex environmental stress, and the physiological response of crops to drought is at least as complex. One of the fundamental challenges is to distinguish between the consequences of water stress and constitutive and adaptive mechanisms that confer tolerance to drought.

Molecular biology has also improved our knowledge of some of the transcription pathways involved in the plant responses to water deficit stress, but not our understanding of responses at the whole-plant level, and their potential contribution to yield. The integration of genomic information with physiological models might however lead to breakthroughs in this area.

Legume Crop	World Production (million tons)	Average yield (kg ha ⁻¹)	Three most important producers
Dry bean	19.3	0.72	Brazil, India, China
Chickpea	9.3	0.80	India, Pakistan, Turkey
Cowpea	5.4	0.48	Nigeria, Niger, Burkina Faso
Pigeon pea	3.4	0.74	India, Myanmar, Kenya
Lentil	3.9	1.02	India, Canada, Turkey
Faba bean	4.9	1.85	China, Ethiopia, Egypt
Soybean	216.1	2.28	USA, Brazil, Argentina
Groundnut	34.9	1.49	China, India, Nigeria

Data from the estimation of the FAO for 2007, and reported in FAOSTAT (2009). Values for the production and yield of dry bean might not only contain *Phaseolus* species, but some *Vigna* species whose production is small. They would not however include peas, or bambara beans, as these are computed separately in FAOSTAT.

Preface to chapter 3

The experiment presented here was performed during the summers of 2003 and 2004 in the Fergana valley in Uzbekistan, Central Asia. As discussed previously (chapters 2.1 and 2.2), there is a need globally, and particularly in Uzbekistan, for greater food self-sufficiency and better irrigation management. Legumes can contribute to food security and better sustainability of agricultural systems. Common bean and mungbean were chosen as experimental crops as they are widely consumed in Central Asia

The objective of this study was to evaluate the potential yield of two legumes species grown after the harvest of winter wheat, and to evaluate the effect of two water saving techniques: regulated deficit irrigation and alternate furrow irrigation on these yields. The results were presented to a group of local farmers and agronomists at the end of the second season.

The study demonstrates that both techniques can be used to improve water use efficiency with legume crops. Alternate furrow irrigation did not decrease yields. In addition, mungbean was able to sustain yields under moderate stress in 2003 and severe water stress in 2004. As importantly, however, we have also demonstrated in both years that the number of irrigation events could be reduced from the recommended schedule to a moderate stress schedule without significantly affecting the yields of common bean. Clearly, a legume crop can be grown after the harvest of winter wheat in Uzbekistan, and as little as one or two irrigation events are sufficient to maintain yields. This is particularly interesting as this land allocation does not compete with government-prescribed growth of cotton and winter wheat.

The following manuscript is under preparation for submission to the

European Journal of Agronomy. A preliminary form has been presented at the WASAMED Conference in Italy in February 2007, and can be found in the conference proceedings published in *Options Méditerranéennes B* 56 (1): 223-234.

Dr. Heidi Webber, a former graduate student from the Bioresources Engineering department, was involved in the planning and execution of this experiment. Specifically, Dr. Webber was in charge of tasks related to irrigation scheduling. Furthermore, close interaction with Dr. Webber has allowed thorough discussion of several key issues from this experiment. Dean Chandra Madramootoo, who was also Heidi Webber's supervisor, contributed meaningful advice in the planning and the execution of the experiment, and provided the research funds necessary. Mikhail Horst is an irrigation scientist at the Scientific Information Center of the InterState Coordination Water Commission (SIC ICWC) in Tashkent, Uzbekistan, and contributed irrigation expertise to the field experiment, a contribution that proved essential. Galina Stulina is soil scientist also at the SIC ICWC, and was instrumental in the establishment of the field experiment in both years in the Fergana valley. Galina Stulina's very practical advice, local experience and logistics skills have been very helpful in the proper execution of the experiment. Finally, Prof. Donald L. Smith, my supervisor, contributed to the paper by meaningful advice in the design stage of the experiment and provided constructive feedback and editorial assistance on the manuscript.

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3. LEGUME PRODUCTION AND IRRIGATION STRATEGIES
IN A SEMI-ARID ENVIRONMENT: YIELD, YIELD
COMPONENTS, WATER RELATIONS, AND CROP
DEVELOPMENT OF COMMON BEAN (*Phaseolus vulgaris* L.)
AND MUNGBEAN (*Vigna radiata* (L.) Wilczek)

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3.1 Abstract

With world population expected to reach 9.2 billion people by 2050, improved irrigation methods will be needed to increase the productivity of agricultural land and improve food supply worldwide. The objective of this work was to examine the effect of regulated deficit irrigation (RDI) and alternate furrow irrigation (AFI) on the yield and yield components of two legume species (common bean and mungbean), produced as a second crop, following winter wheat. Water relations and crop development were also examined. The research was conducted during two successive growing seasons in the Fergana valley of Uzbekistan, Central Asia. Production of mungbean using the severe stress RDI treatment in combination with AFI resulted in the highest yields with the smallest use of applied water. Meanwhile, yields of common bean in the moderate stress treatment were not different from the recommended schedule, even though irrigation events were decreased from 4 to 2. AFI did not reduce

yields and, in general, mungbean yields were higher than those of common bean. The combination of AFI and RDI can allow legume production with reduced water inputs.

3.2 Introduction

World population is expected to reach 9.2 billion people by 2050. This is an increase of 2.5 billion people from the current population, an increase equivalent to the total world population in 1950, or to the populations of China and India today (UN, 2007). Because irrigated agricultural systems represent approximately 17% of the cultivated area yet contribute 40% of total crop production worldwide (Kijne et al., 2003), increased use of supplemental irrigation water in rainfed agriculture and improved irrigation methods will be needed to increase the productivity of agricultural land and improve food supply worldwide (Wallace, 2000). Unfortunately, the low efficiency of irrigation in agriculture has had, and will continue to have, environmental and social costs. Perhaps the most extreme contemporary example of these is the drying of the Aral Sea. Land and water resources in the Aral Sea basin have been degraded due to the expansion of irrigation networks, on-farm mismanagement of irrigation water, and degraded infrastructure. This has caused serious economic and health problems along the lower reaches of the two large rivers flowing into the Aral Sea (Elpiner, 1999; Glantz, 1999; Micklin, 2000; Small et al., 2001; Dukhovny, 2003). Thus, sustainable irrigated agriculture is necessary to maintain food production per capita, but will also require technological improvements in water use efficiency.

Regulated deficit irrigation (RDI) consists in finding the optimum balance between water use and crop yield. Under RDI, crop producers allow the crop to experience some water stress, but the water saved should allow an increase in the area irrigated (English and Raja, 1996), or it could be

put to more productive use, in industrial activities for example (Molden et al., 2003). ICARDA has shown that a 50% reduction in irrigation water applied decreased yields by 10 to 15%, but overall farm productivity increased by 38% when the water saved was used on other land (Pereira et al., 2002). While incomes could be greatly increased by deficit irrigation, it is important that farmers have control over the timing of irrigation events and amount of water applied, as well as the tools for proper irrigation scheduling (Kijne et al., 2003). One of these tools is precise information on factors such as the response and sensitivity of crops to water stress, critical stages of development when deficit irrigation should or should not be performed, and how much water can be conserved (Kijne et al., 2003).

Previous studies on RDI were usually performed by either reducing the amount of water that is applied to crops to a fraction of the full evapotranspiration (ET), but otherwise keeping the same frequency of irrigations (for example, Pandey et al., 1984; Shani and Dudley, 2001; de Souza et al., 2003; Oktem et al., 2003; Oweis et al., 2004; Chaves et al., 2007), or by withholding irrigation at specific growth stages (for example, Nielson and Nelson, 1998; Calvache and Reichardt, 1999; Pandey et al., 2000; De Costa et al., 1999; Boutraa and Sanders, 2001; Xue et al., 2003; Karam et al., 2005; Karam et al., 2007). While applying a fraction of the ET might be a practical way to impose RDI with sprinkler and drip irrigation, in surface irrigation systems very small irrigation depths are not technically feasible. Further, these small irrigation depths do not bring the soil profile back to field capacity, but rather wet the upper layers, and result in a soil depletion that is increasing over time. This could potentially lead to severe damage at the yield formation stage. On the other hand, withholding irrigation water at specific growth stages is too simplistic. Even if rainfall is negligible, air temperature, wind speed, irradiation, relative humidity, crop ground cover, soil water holding capacity and

fertility conditions are all factors that affect ET (Allen et al., 1998) and that might vary considerably from year to year. The water stress is thus hardly reproducible.

RDI using increased time intervals between irrigation events, based on the water balance method for irrigation scheduling and greater depletion factors (as proposed by Allen et al., 1998, and used in Panda et al., 2003 and in this study), is probably a better approach because: 1. the method is available no matter what water application technology (or lack thereof) is being used, 2. the water stress is reproducible over soil types and climatic conditions, 3. filling the entire soil profile potentially encourages deeper root growth, 4. the lower frequency of irrigation events lowers the water losses from evaporation, and 5. crop producers will not grow a control plot to calculate the ET, and then apply a fraction of it to the rest of their fields, as would be required using a fraction of the full ET.

Alternate furrow irrigation (AFI) consists of surface irrigation systems supplying water to every second furrow. By alternating irrigated furrows, root growth is stimulated and this probably helps offset the negative effects of reduced water supply (Kang et al., 2000). In a controlled environment with a divided root system, Kang et al. (1998) showed that water consumption by maize plants subjected to partial root drying was decreased by 34.4 to 36.8% while yields only decreased 6 to 11%. They also showed that transpiration rate decreased compared to well-watered controls, but that the photosynthetic rate and leaf water content remained the same, thus leading to significant increases in water use efficiency, shoot biomass production, and root development and distribution compared to controls. Another split-root experiment, Kirda et al. (2004) showed no significant decrease in the yield of tomato subjected to half of the irrigation water, when each side received water alternately. Both groups suggested that by having half of its root system in dry soil, the

plant continues to synthesize abscisic acid in the roots, which reduces its transpiration rate. However, because water is available, growth is less affected. Other field experiments have confirmed these findings (Grimes et al., 1968; Crabtree et al., 1985; Graterol et al., 1993).

The benefit of legumes in cropping systems has long been recognized. Food legumes provide a good source of protein for human consumption, and their residues can improve the fertility and physical condition of the soil, due in great part to their ability to fix nitrogen in symbiosis with rhizobia (Senthong and Pandey, 1989; Haqqani and Pandey, 1994a; Subbarao et al., 1995). Rotating legumes with other crops can break disease cycles and encourage the development of mycorrhizal associations (Subbarao et al., 1995). Often, legumes are short-season crops that can be grown after main cash crops, such as rice in subtropical Asia and parts of Africa (Senthong and Pandey, 1989). Unfortunately, the economics of their production restricts them to less productive lands and/or periods of growth (Subbarao et al., 1995). As such, their productivity is often limited in semi-arid and arid regions (Senthong and Pandey, 1989).

Both common bean and mungbean are widely consumed in Central Asia. Common bean (*Phaseolus vulgaris* L.) is a very diverse pulse crop. It is the most important pulse crop in the world, being eaten directly more than any other legume crop (Hedley, 2001; Broughton et al., 2003). The estimated value of its 18-million-tonne annual harvest is approximately US\$11 billion (CIAT, 2001). Recent studies suggest that only 7% of the growing area receives adequate rainfall (Broughton et al., 2003), and 60% of the production occurs under severe drought stress (Graham and Ranalli, 1997).

Mungbean, also known as green gram, is a small-seeded crop less known

in the Americas, but widely cultivated in Asia (Lawn and Ahn, 1985; Hafeez et al., 1991; Poehlman, 1991). It is also cultivated to some degree in the United States and in Australia and is often consumed as sprouts (Lawn and Ahn, 1985; Poehlman, 1991). The crop is known to perform well under conditions of low soil moisture availability. It remains, however, one of the least researched and most under-exploited legume crops (De Costa et al., 1999).

In Uzbekistan, agricultural policies emphasize the culture of cotton, an important component of the Uzbek economy, and to a lesser degree, winter wheat. Both are subject to state regulation through a system of quotas, and little agricultural land is left for other food crops. The main objective of this study was to evaluate the production of food legumes under RDI and AFI irrigation methods. Another objective was to confirm that being short-season, legumes could be grown after the harvest of winter wheat (early July). This study is the first to evaluate the effect of regulated deficit irrigation using the water balance method and increased time intervals between irrigation events in conjunction with alternate furrow irrigation on the yield of common bean and mungbean.

3.3 Materials and methods

3.3.1 Environment

The experiment was conducted in the Fergana valley (Figure 3.1), in Uzbekistan, Central Asia (40°23'N, 71°45'E) from the beginning of July until the onset of cold temperatures in mid-October, in the growing seasons of 2003 and 2004. During this period, the climate is hot and dry, with typical daily high temperatures being 40 °C, and typical daily lows being 20 °C. Rain is very infrequent, except in early October. From July 15th to September 30th 2003 and 2004, we recorded a total of 8.8 and 7.6 mm of rainfall, respectively, at our field sites. Climatic data (Figure 3.2)

was collected using an on-site Vantage Pro Meteorological station (Davis Instruments Corp., Hayward, USA), located approximately 200 m from the field site. Based on textural analyses, the soil at the experimental sites was a silt loam in 2003 and ranged from a sandy loam to a silt loam in 2004. The water content at field capacity and at wilting point was analysed using a pressure membrane at $-1/3$ and -15 atm respectively in the SANIIRI laboratory (Tashkent, Uzbekistan). The soils had an available water content of 96 mm in 2003 and 75 mm in 2004, in the top 60 cm. The organic matter content was determined in each plot, in the 0 to 30 cm and 30 to 70 cm layers (results presented in appendix A). In both years, the soils had low organic matter contents (less than 2%), and a well developed plough pan at 30-40 cm.

3.3.2 Experimental design

The treatments were organized on the field site following a randomized complete block split-plot design. The treatments were comprised of factorial combinations of three factors: RDI level (recommended level, moderate deficit and severe deficit – see section 3.3.3 for more details), irrigation water distribution pattern (alternate and every furrow irrigation) and crop (common bean and mungbean). RDI treatment was the main plot factor and the combinations of furrow irrigation strategy and crop constituted the subplot factor. There were four blocks. Each subplot measured 15 x 12 meters with an additional 1.5 meter of buffer zone on each side of the irrigation ditch.

3.3.3 Irrigation scheduling

Levels of RDI were determined according to the concept of soil water depletion factors, as defined by FAO Water Report #56 (Table 22, Allen et al., 1998). Depletion factors are measures of soil water depletion as a

percentage of the total available soil water; the longer the interval of time between irrigation events the higher the depletion factor. For common bean, the depletion factors used were 0.45 as the recommended level (Allen et al., 1998), 0.6 and 0.7 as the moderate and severe stress levels, respectively. For mungbean, the recommended depletion factor was also 0.45 (Allen et al., 1998), but the moderate and severe stress levels were 0.65 and 0.8. Local growers informed us that only one irrigation event is necessary to successfully produce mungbean, suggesting greater tolerance to water stress than most crops, and this is why we used greater depletion factors for mungbean. In addition, we believed that these depletion factors would be good approximations of a moderate and a severe level of stress for each of the crops. Prior to our work, there was no information available regarding yield effects of RDI depletion factors for common bean or mungbean.

Daily ET values were computed from climatic data from our meteorological station (according to the FAO Penman-Monteith equation) and an ET gauge placed in a mungbean plot adjacent to our field (Allen et al., 1998). These were used in six water balances corresponding to six combinations of RDI and crop treatments, and soil water depletion was confirmed using soil moisture readings. Once the soil was depleted to the appropriate fraction of soil available water, the irrigation amount was determined from the water balance, and the treatment was irrigated. Soil water content was determined by the gravimetric method for depths of 0, 10, and 20 cm in duplicate samples, and with a neutron probe (Neutron, Moscow, Russia) for depths of 40 and 60 cm, again in duplicates. Soil moisture readings were taken two days before and two days after each irrigation event, and at five-day intervals between irrigation events.

For AFI, the head of every other furrow was blocked. Blocked furrows were alternated between irrigation events, when there was more than one

event. Alternate furrow irrigated plots were irrigated the same day as the corresponding every furrow irrigated plots, but only received 75% of the water applied. More details on the irrigation scheduling can be found in a companion paper (Webber et al., 2006) or in Webber (2008).

3.3.4 Cultural practices

Each field site produced winter wheat immediately prior to our experimentation. The wheat was harvested, the straw and stubble burned, and the field ploughed and levelled, all following standard practices in the region. Sixty-cm wide furrows were formed on the field site with a tractor drawn lister; a pre-irrigation of approximately $800 \text{ m}^3 \text{ ha}^{-1}$ was applied to every furrow to consolidate the resulting furrows and raised beds, and to bring the soil back to field capacity.

Seeds for the crops were purchased at a local market, and some of the seeds harvested in the first year were kept for planting in the second year. We have retained a sample of these seeds and they are available, upon request. Seeds were sown by hand, at a 5-cm depth, on both sides of the raised beds, at 10 cm intervals, to achieve a plant density of 300,000 plants ha^{-1} . Planting was done on July 14 and 15th in 2003, and July 9 to 12th in 2004. In 2004, based on experience acquired in 2003, an irrigation event of approximately $600 \text{ m}^3 \text{ ha}^{-1}$ applied to every furrow was included five days after seeding, to assist in seedling establishment.

Based on availability of agro-chemicals, fertilization in 2003 consisted in 115 kg ha^{-1} of superphosphate, applied immediately prior to the land levelling, plus a manual application of ammonium phosphate (100 kg ha^{-1}) and potassium (15 kg ha^{-1}) a week after planting. In 2004, a mix of phosphate and potassium was applied in the furrow immediately prior to the pre-irrigation at a rate of 150 and 75 kg ha^{-1} for P and K. Commercial

inoculants for common bean and mungbean were unavailable. Thus, nodule development reported in section 3.4.7 refers to symbioses formed with indigenous rhizobial strains. Weed control was conducted manually.

Multiple harvests were necessary as mungbean pods tend to shatter when dry. In 2003, there were two harvests for common bean (October 3rd and 14th), and six for mungbean (September 21st, 24th, 27th, 30th, October 7th and 13th). In 2004, there were also two harvests for common bean (September 20th, and October 3rd), and four for mungbean (September 21st, 26th, October 1st and 9th).

3.3.5 Common bean and mungbean seeds

As stated previously, seeds were purchased at the local market and not from a seed company or research institute. The local common bean used in this study had a bushy, and determinate growth habit. The local mungbean was also bushy, slightly prone to lodging and semi-determinate. Under the conditions of the field study, both crops were ready to harvest within 90 days. A second flush of flowering did not occur, although a few flowers were still to be seen at the end of the season on mungbean.

3.3.6 Measurements

An area of 5 m x 5 m, in the center of each plot, was used for sampling. Mature pods were harvested at regular intervals within the sampling area and threshed by hand. Grain yield was determined for each harvest, then combined across harvests and corrected for moisture content (to 0% moisture), and converted in kg ha⁻¹ from plant population estimates before statistical analysis. One hundred seeds were randomly selected from the harvested seeds of each plot and these were used to determine 100-seed

weight; this subset was then oven-dried at 65-70 °C for 24 h, to a constant weight, to determine seed moisture level. Plant population estimates were determined by counting the number of plants in three 2-m row sections per plot, within the sampling area. Number of seeds per pod was also determined at each harvest, from ten pods randomly picked within the sampling area of each subplot.

Stem water potential was measured on six plants per plot within the sampling area, one day before and two days after each irrigation event with a portable pump-up pressure bomb (PMS Instruments, Albany, USA). Stomatal conductance was measured on ten plants, at the same time as stem water potential, with a diffusion porometer LICOR-1600M (LICOR Biosciences, Lincoln, NE, USA).

For crop height and number of flowers and pods, six plants were marked at the beginning of the season and measurements were made on these same plants as the season progressed. In 2003, measurements were taken once a week after crop establishment, while in 2004, measurements were taken twice a week until the first pod harvest. Plants were also harvested at four growth stages during the season for determination of leaf area, above-ground biomass dry weight, and root dry weight (in 2004 only). Fifty-centimetre of row was harvested, plants were cut off at ground level, and roots were carefully dug out. Leaves were then separated and placed on a white sheet. A digital picture was taken of every individual plant, along with a standard of known area, and these images were later processed with the computer program SigmaScan Pro 5 (Systat Software, San Jose, CA, USA) to determine leaf area. Above-ground biomass was determined from the stem, leaves, and reproductive structures when appropriate, of plants harvested in the destructive samplings described above. Plants were dried at 70°C for at least 24 hours, to a constant weight. Similarly, root biomass was determined from dried roots

previously excavated. Finally, harvest index was calculated as the ratio of yield over total above-ground biomass at final harvest, all on a dry weight basis.

3.3.7 Statistical analyses

Statistical analyses were performed by analyses of variance (ANOVA) and repeated measures analysis by multivariate analyses of variance (MANOVA) using the general linear model (proc GLM) in SAS/STAT software (SAS, Cary, NC, USA). In general, treatment effects were considered significant only when they occurred at the 0.05 level of probability. However, in some cases, we consider relevant differences when the probability level is between 0.05 and 0.1; in these cases, the p value is given in the text and in tables when appropriate. If fixed main effects or a fixed interaction were found to be significant in the ANOVA, then means separations were carried using t-tests on least squares means (Milliken and Johnson, 1984). In 2003, when significant, the plant population density was used as a covariant. Because there were few nodules, the statistical analysis for nodule number was performed with a non parametric approach using the RANK procedure prior to proc GLM.

3.4 Results

3.4.1 Yield

Mungbean responded differently than common bean to the various levels of RDI imposed during this experiment (Figure 3.3), resulting in a crop by RDI interaction with p values of $p \leq 0.0913$ and $p \leq 0.0005$ for 2003 and 2004, respectively. While common bean yields decreased with increasing stress, mungbean yields were highest at the moderate water stress level (2003) and severe stress level (2004). Interestingly, common bean yields at the moderate stress level were only slightly lower than bean yields at

the recommended irrigation schedule, and not significantly so. In addition, AFI did not reduce yields (Table 3.1).

The yield difference between the two experimental years can be explained, at least in part, by the differences in the plant population density. In 2003, variation was relatively high, due to uneven emergence. In particular, plots in the severe stress treatment only reached 50 to 75% ground cover. This probably led to yield limitations from lower radiation interception, but might also have led to greater evaporation from the soil surface, and a lack of transpirational boundary layer. This would have resulted in higher evaporative demand and more stressful environmental conditions for these plants, contributing to lower yields.

3.4.2 Yield components

The number of seeds per pod responded to the RDI treatments differently between mungbean and common bean (Table 3.2). The number of seeds decreased with increasing water stress for both crops. However, this decrease was more important in mungbean in the severe stress treatment. This crop by RDI interaction was not as significant in 2003 as in 2004 (p values of 0.0585 and 0.0024 respectively). There was also a difference ($p \leq 0.0441$) between alternate and every furrow irrigation strategies (Table 3.1) in 2004, but not 2003. Since yields were not affected by AFI, it seems that other yield components must have compensated, but this compensation was not detectable statistically.

One-hundred seed weight was not affected by RDI or AFI, except for common bean seed weight in 2003 (Table 3.2). In addition, there was no crop by RDI interaction, or any other significant interaction in either year. Interestingly, seeds were smaller in 2004 than 2003 for both crops. Soil nitrogen content at the beginning of the season was less in 2004 than in

2003 (appendix A), and starter nitrogen fertilisers were not available in 2004. In addition, the higher plant density in 2004 might have led to greater competition among plants, and lower seed weights.

There was a strong crop by RDI interaction in both years for the number of pods per plant (Table 3.2). For mungbean, the number of pods per plant was lower in the severe stress treatment, as compared to the moderate stress and recommended levels. In contrast, common bean maintained the number of pods per plant with increasing water stress. The difference in the number of pods per plant between the two years could be partially explained by lower nitrogen levels in 2004. Again, the plant population density was relatively low especially for mungbean in 2003. As such, it seems that mungbean in the recommended and moderate stress irrigation schedule treatments had enough water to compensate for the low density by growing to their full potential, thus explaining large numbers of pods per plant in 2003.

3.4.3 Harvest index

Harvest index (HI) was affected by RDI but differently for the two crops in both years. In the severe stress treatment, HI decreased in common bean, but increased in mungbean (Figure 3.4). In addition, there were no differences between the recommended and moderate stress irrigation schedules within crops. Thus, it seems that mungbean has a greater capacity than common bean to allocate resources to seeds under conditions of severe stress.

3.4.4 Stem water potential

In both years, before irrigation events, mungbean maintained a higher (i.e. less negative) stem water potential (SWP) than common bean across RDI

and AFI treatments. In addition, SWP was not different between furrow irrigation strategies in either year (Table 3.1). The data for SWP showed a different response to RDI for each year (Table 3.3). In 2003, there was a strong crop by RDI interaction in which mungbean decreased its SWP with increasing stress, while common bean showed the lowest SWP at the moderate stress level. This might lead one to think that common bean has a capacity to osmotically adjust but only until a threshold of water stress. This interaction however was not significant in 2004 ($p < 0.1238$), and numerically, the lowest SWP in common bean occurred in the severe stress level, whereas for mungbean, all depletion levels showed the same SWP.

After irrigation events, the SWP was lower under AFI for both crops, and across RDI levels in both years (Table 3.1). Again, the crops responded differently to RDI in each year (Table 3.3). In 2003, mungbean had a lower SWP after irrigation events in the severe water stress. On the other hand, for common bean, SWP was not different among RDI levels, and thus had returned to a relatively high SWP following irrigation. In 2004, mungbean showed the highest SWP after irrigation events and no difference among RDI levels, while common bean had a lower SWP in the severe water stress treatment. In any case, it seems that both crops are generally able to return to a high SWP after irrigation, no matter how dry the soil was when irrigated.

3.4.5 Stomatal conductance

In both years, common bean maintained a higher stomatal conductance than mungbean across RDI and AFI levels before irrigation events. Stomatal conductance of both crops decreased as water stress increased, but decreased proportionally more in common bean than in mungbean, as shown in the crop by RDI interaction detected in both years (Table 3.3).

As before irrigation events, the stomatal conductance after irrigation events was higher in common bean than mungbean across RDI and AFI levels (Table 3.3). Stomatal conductance was then unaffected by RDI and values for stomatal conductance were higher than before irrigation events, indicating that gas exchange in all plants was able to recover from the stress, although in 2003, plants in the AFI plots showed a lower stomatal conductance than those in the conventional every furrow irrigation plots (Table 3.1). This effect however was not significant in 2004.

3.4.6 Crop development

While common bean showed high vigour at the beginning of the season, mungbean grew slowly early in the season, followed by a rapid increase in growth a few weeks after planting, and reached maximum height and biomass near the end of the season. This was clear in the data for above-ground biomass, leaf area, and crop height (Figure 3.5). Common bean also flowered earlier than mungbean (data not shown), and as such the statistical analysis was done separately for the two crops.

There was no effect of RDI treatment on above-ground biomass of common bean at any of the growth stages measured in either year. The above-ground biomass of mungbean however was lower in the harvest stage for the severe stress treatment in both years. There was also a substantial decrease in mungbean biomass in 2003 at the flowering stage in the severe stress treatment (Figure 3.5 A), again probably due to a plant density by RDI interaction in that year, as discussed earlier.

Similarly, there were no differences in common bean leaf area due to RDI levels, in either year, however, mungbean leaf area was lower under the severe stress treatment at flowering and harvest in 2003, but in none of the samplings in 2004, although the p value at harvest in 2004 was close

to the significance level at 0.0638 (Figure 3.5 B).

Similarly, there was no effect of RDI on crop height for common bean, but after flowering, mungbean plants in the severe stress treatment were shorter than the other two RDI treatments in both years (Figure 3.5 C).

In general, RDI had no effect on number of flowers, except for one date in each of the two crops in 2004 only (data not shown). In common bean, 42 days after seeding (DAS), the number of flowers was highest in the moderate stress treatment, but lowest in the severe stress treatment. This might be explained by the fact that the moderate stress treatment had been irrigated a few days before, potentially leading to a flush of flowers following the relief of stress. In mungbean, this difference in the number of flowers occurred 60 DAS, towards the end of flowering. The recommended and moderate stress treatments had more flowers than the severe stress treatment, indicating that water stress may have shortened the flowering period in mungbean.

In addition, RDI treatments had no effects on the number of pods m^{-2} in either crop in 2004 (Figure 3.5 D). However, mungbean was affected in 2003, as the number of pods was reduced in the severe stress treatment, again probably as a result of the lower population density.

Root biomass (only available in 2004) in common bean increased with decreasing water availability at the pod-filling and harvest stages, but mungbean root biomass was not affected by RDI treatments at any of the samplings (data not shown).

Finally, AFI had no effect on any of the crop development variables observed (data not shown).

3.4.7 Nodule development

Common bean failed to nodulate with the indigenous rhizobia in the experimental site soil. However, we found nodules in 87% of mungbean plots in 2003, and 100% of mungbean plots in 2004, when the plant density was higher. We found an average of 4.2 nodules per plant in 2003, and 6.4 nodules per plant in 2004. However, no differences were observed among RDI levels in either year for the number or dry weight (available only in 2004) of nodules (data not shown). The lack of inoculants, and the intensive winter-wheat and cotton rotation might have been detrimental to the indigenous rhizobial populations, which in controlled environment conditions, we found to nodulate mungbean successfully (data not shown).

3.4.8 Water use efficiency

In a related study, Webber et al. (2006) examined water use efficiency (WUE) defined as the seed yield produced per unit of water evapotranspired. This showed that the WUE of mungbean was almost double that of common bean in both years. In addition, while WUE remained relatively constant in common bean, in both years and across all treatment combinations, RDI and AFI improved (and sometimes considerably) mungbean WUE.

3.5 Discussion

Our results regarding the response of common bean to water stress are consistent with the findings of other researchers, who also measured a decrease in yield. Boutraa and Sanders (2001) withheld water during the flowering and pod-filling growth stages and found that yields were reduced, and that the yield component most affected was the number of

Pods per plant. Dapaah et al. (2000) showed a 50% increase in seed yield with irrigation. Nielsen and Nelson (1998) have shown that seed yield was reduced due to a reduction in the number of pods per plant and the number of seeds per pod. In a pot experiment, Wakrim et al. (2005) also showed a reduction in shoot and pod biomass from RDI and partial root drying. However, we have also shown that in both experimental years, the reduction in yield of common bean when irrigation events were reduced from the recommended irrigation schedule to the moderate level of stress was not significant, indicating that some level of stress can be tolerated without affecting yields, if the reduction in water is applied according to a RDI schedule that is appropriate.

Yet, yields were still relatively low compared to yields achieved in other areas, and the establishment of a genetic improvement program for common bean and collaborations with other international centers would be beneficial to the region. In addition, the use of appropriate inoculants would also increase nitrogen availability to plants, and thus improve yields.

Our yield results for mungbean contrast with some of previous experiments comparing the response to irrigation of mungbean with other crops, but are consistent with the experience of local growers. Irrigation schedules comprising four to six irrigation events following seedling establishment have been proposed (Poehlman, 1991). In addition, mungbean was found to be quite sensitive to water stress, when compared to a number of other crops, showing the greatest decrease in yield between the well-watered control and most severe stress treatments (Pandey et al., 1984; Senthong and Pandey, 1989).

However, these researchers used sprinkler irrigation, and irrigation water was decreased according to the distance of the experimental plot from the sprinkler. The resulting small irrigation depth (water penetration in the

soil) may be ineffective for mungbean, which seems to extend its roots deeper into the soil profile to extract water resources from greater depths (Haqqani and Pandey, 1994a). By filling the root zone during our single irrigation event, a few days before the onset of flowering, we appear to have provided mungbean with sufficient water at a critical time. The same irrigation amounts applied several times in the season, a few millimetres at a time, and applied on the surface, would not have the same effect. Other teams also found that withholding water at various growth stages, and particularly at flowering, decreased yields (De Costa et al., 1999; Thomas et al., 2004). However, Angus et al. (1983) found that mungbean was not responsive to irrigation, and Muchow (1985a) found that mungbean had the highest yields under water deficit conditions. In both cases, the dry treatment consisted in no irrigation at all after seedling establishment (and very limited rainfall if any). It is clear thus, despite population density differences between the two experimental years, that mungbean is able to sustain water deficit, and even seems to be stimulated by it, as illustrated by the occurrence of maximum yields in the moderate stress (2003) and the severe stress (2004) treatments. Finally, the production approach taken by local farmers suggests that mungbean grows well with little irrigation water.

It is also important to note that mungbean has not benefited from the same research and breeding efforts as many other legume crops. The genotype we worked with bore some characteristics of wild plants, such as relatively small seeds, a propensity for lodging and shattering pods, which are still somewhat present in commercial cultivars. The lack of response to irrigation, as exemplified in this study, suggests a similarity with the lack of response present in landraces in other species. Breeding programs in Thailand and Australia have selected against undesirable characteristics, and some of the developed genotypes might present interesting genetic material for a local breeding program. Improved germplasm, and

improved rhizobial inoculants, combined with improved distribution, again, would be helpful.

Interestingly, alternate furrow irrigation, which saves 25% of the water applied by not watering every second furrow, did not reduce yields, or most of the yield components measured, and did not affect crops negatively when combined with RDI treatments. Although stem water potential was reduced in the AFI treatment after irrigation events, this was not translated into yield differences. Other field experiments have confirmed these findings: Grimes et al. (1968) reported a 23% decrease in water use with no decrease in yields; Crabtree et al. (1985) reported an 'acceptable tradeoff' for soybean: water use decreased 40 to 50% while yields decreased 7 to 10%; Graterol et al. (1993), however, showed that AFI could require more irrigation events than conventional every furrow irrigation, but that net water applied was still less than the conventional every furrow irrigation, and WUE was improved. Therefore, AFI appears to be a simple yet effective way to increase WUE while maintaining yields. More importantly, this study is the first to evaluate the combination of AFI and RDI, and we have found that AFI does not negatively affect crops subjected to RDI, or vice versa.

Osmotic potential does not seem to be a good indicator of water stress in common bean and mungbean. Mungbean not only kept a higher SWP than common bean, but also showed no differences in water potential across RDI treatments. Thus, it seems that osmotic adjustment does not contribute meaningfully to water stress tolerance in these two crops, as opposed to the findings previously reported in common bean (Costa Franca et al., 2000) or in other crops (Hsiao et al., 1984 in rice; Blum 1989, in barley; Santakumari and Berkowitz, 1990 in wheat), although Markhart (1985) has also reported a lack of osmotic adjustment in common bean. Thus, the emphasis on osmotic adjustment and water

potential to describe water stress and/or to screen for drought tolerant legume crops might be counter-productive.

Water stress was better described by the stomatal conductance of the two crops. The lower stomatal conductance of mungbean compared to common bean, across RDI levels, indicates a greater intrinsic transpiration efficiency in mungbean, possibly the result of a lower number of stomata on leaves. This seems to be supported by a higher biomass and leaf area production per unit of water consumed, and greater biomass WUE (see Webber et al., 2006 for water consumption data). This seems related to its ability to yield better under water limited conditions than under conditions where abundant water is available, possibly by translocating more resources to seeds, as illustrated by its higher harvest index under severe stress. In fact, the general lack of differences between above-ground biomass weight, leaf area production, and number of flowers and pods m^{-2} across RDI treatments (more so in common bean than mungbean) indicates that one of the main factors allowing yield maintenance under water stress for these two crops is the translocation of resources to seeds late in the season. In addition, in mungbean, severe water stress seemed to have accelerated the end of flowering and induced pod drying, thus leading to higher yields in more stressful conditions.

In summary, it seems that mungbean has evolved for survival in water limited conditions (at least in Central Asia) by maintaining a low stomatal conductance regardless of water availability, and an ability to maintain or increase its harvest index under stress. Common bean, on the other hand, responds to water stress by closing stomata and increasing root biomass, yet still maintains the same above-ground biomass, leaf area, and number of flowers and pods. These responses seem to be too little too late, as yields were negatively affected under severe stress conditions.

It is very clear that legume production following the harvest of winter wheat is not only possible, but can also be done with relatively little water. Production of mungbean using a depletion factor of 0.80 (severe stress) and AFI resulted in the highest yields with the smallest use of applied water ($1,450 \text{ m}^3 \text{ ha}^{-1}$) in 2004, when plant density was higher (Webber et al., 2006 for water consumption data). A similar ability to produce well under the moderate stress level (depletion factor of 0.65 and applied water of $2,350 \text{ m}^3 \text{ ha}^{-1}$) was also observed in 2003. As a comparison, crops of winter wheat and cotton require averages of $4,790$ and $7,070 \text{ m}^3 \text{ ha}^{-1}$ of irrigation water, respectively, under Uzbekistan conditions (EC-IFAS, 1999). The ability of mungbean to yield better under conditions of water deficit was particularly demonstrated in 2004 by 36% higher yields and 60% lower water consumption compared to common bean in the recommended irrigation schedule. Not surprisingly, the production of mungbean under the severe stress treatment and AFI showed by far the highest seed WUE of all treatments (Webber et al., 2006).

In addition, because producers rarely monitor the inflow of irrigation water, and typically over-irrigate fields (EC-IFAS, 1999), it seems likely that water savings in a producer's fields will be much greater than would be suggested by our rigorously controlled experiment. In particular, although Webber et al. (2006) showed no improvement in WUE in any of the treatments tested in common bean, it seems likely that the reduction from four irrigation events to two in producers' fields, where large excesses of water are often applied, would improve the WUE substantially, so that the potential WUE benefits of RDI in common bean should not be dismissed.

Because there is such diversity in the methods used to impose water stress on crops, comparisons of results among studies can be difficult. In addition, prior to the application of irrigation water, soil water deficits might be quite different among experiments, depending on the rainfall received,

temperature, radiation intercepted and humidity, all factors that affect crop evapotranspiration (Allen et al., 1998). Unless soil moisture is monitored and serves as the basis of irrigation scheduling, the actual degree of water stress imposed might be quite variable. Given that the irrigation scheduling method utilized in this paper can be applied to all soils and all environments, we encourage researchers to work in interdisciplinary teams of crop physiologists and agricultural engineers in order to develop knowledge and technology that is useful to scientists and applicable for crop producers.

Further research is needed to assess the potential of sequential cropping of legumes (potentially instead of winter wheat or cotton) under deficit and alternate furrow irrigation. A wider range of crops should also be investigated, including cowpea, chickpea, pigeon pea, lentil, and relatively drought-tolerant cultivars of soybean, as these have shown good results under hot and dry conditions, and/or good economic viability on the world market. Development of new legume varieties, more drought tolerant, with earlier maturity, and day neutral (particularly relevant to mungbean) as well as better rhizobial inoculants (for improved N₂ fixation) would also be ways to improve agricultural production and food security in arid and semi-arid areas.

3.6 Conclusions

Overall, regulated deficit irrigation in combination with alternate furrow irrigation, as well as the cultivation of legumes following the harvest of winter wheat, are not only possible, but could have considerable positive effects on the economy, environment and national food security of Uzbekistan and nearby areas of Central Asia. We suggest that while both mungbean and common bean are possible crops, mungbean is better adapted to hot and dry conditions prevalent in semi-arid areas. A single,

but deep, irrigation event might be all that is necessary for mungbean to yield well, and to do so before the onset of rain and lower temperatures in the fall, given that the soil has a reasonable soil available water, and that the population density is high enough for the crop to reach full canopy. Our results show that regulated deficit irrigation is also possible with common bean, where yields were not substantially decreased by the moderate stress treatment. A reduction in irrigation events would also be desirable, as crop producers tend to over-irrigate to ensure even water distribution. Our results also suggest that the introduction of legumes in other agricultural systems in the semi-arid areas could be done with relatively little additional irrigation water.

Figure 3.1: Map of the Aral Sea Basin and location of experimental area (Fergana Valley).



The experimental field was situated in the Fergana Valley, Uzbekistan (40°23'N, 71°45'E).

Figure 3.2: Climatic data for the growing seasons of 2003 and 2004 in the Fergana Valley, Uzbekistan ($40^{\circ}23'N$, $71^{\circ}45'E$) from the beginning of July until the end of October.

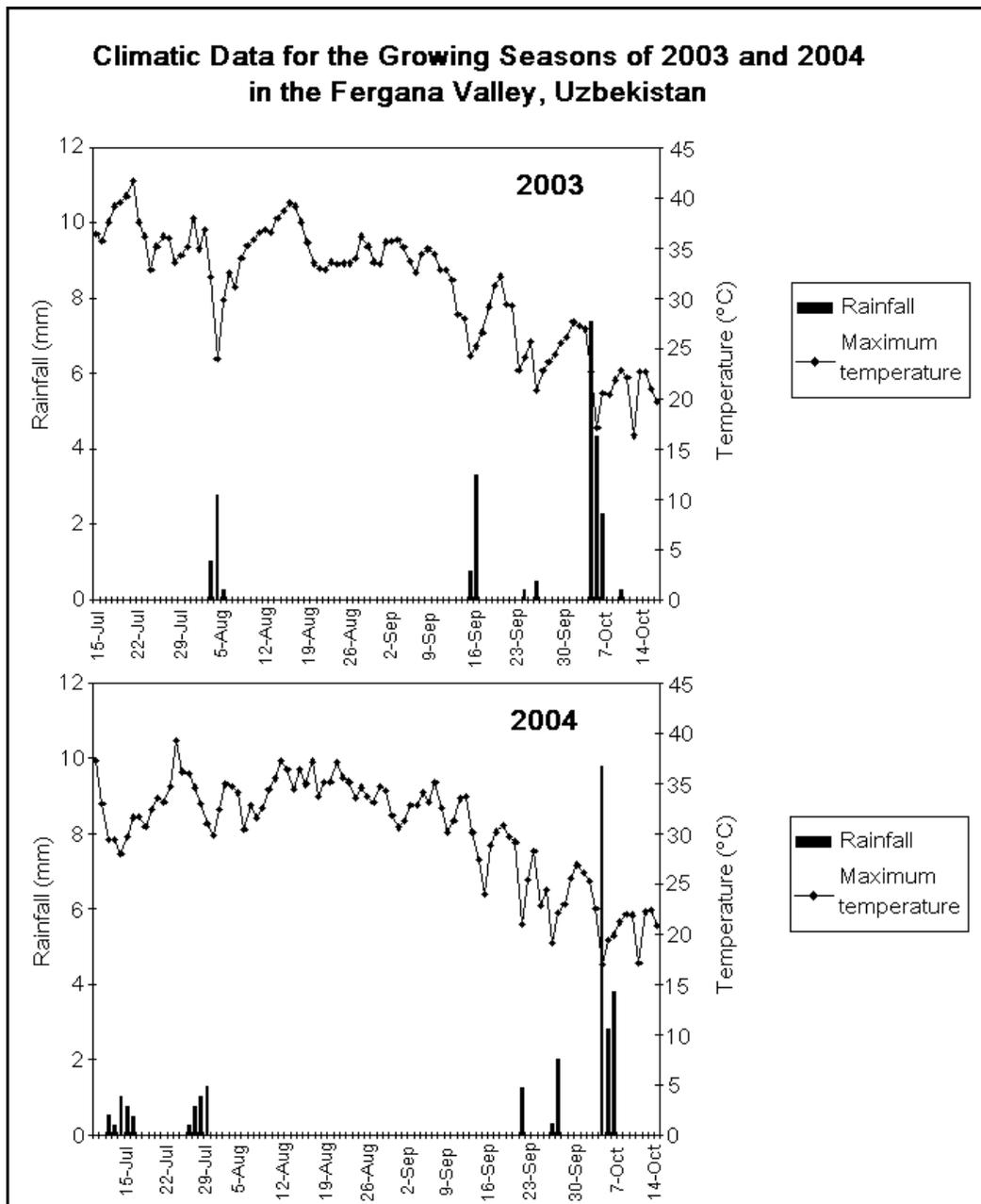
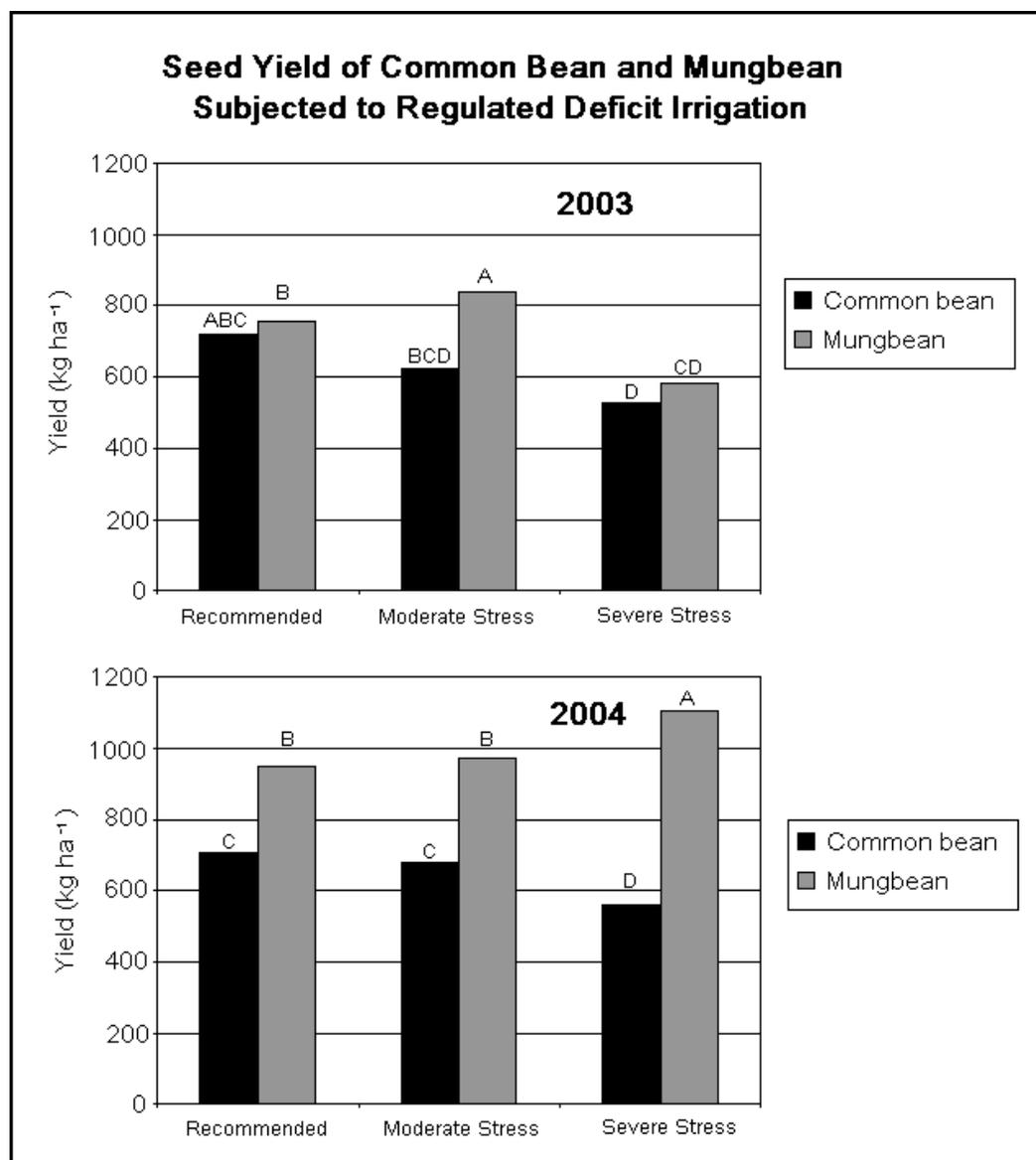
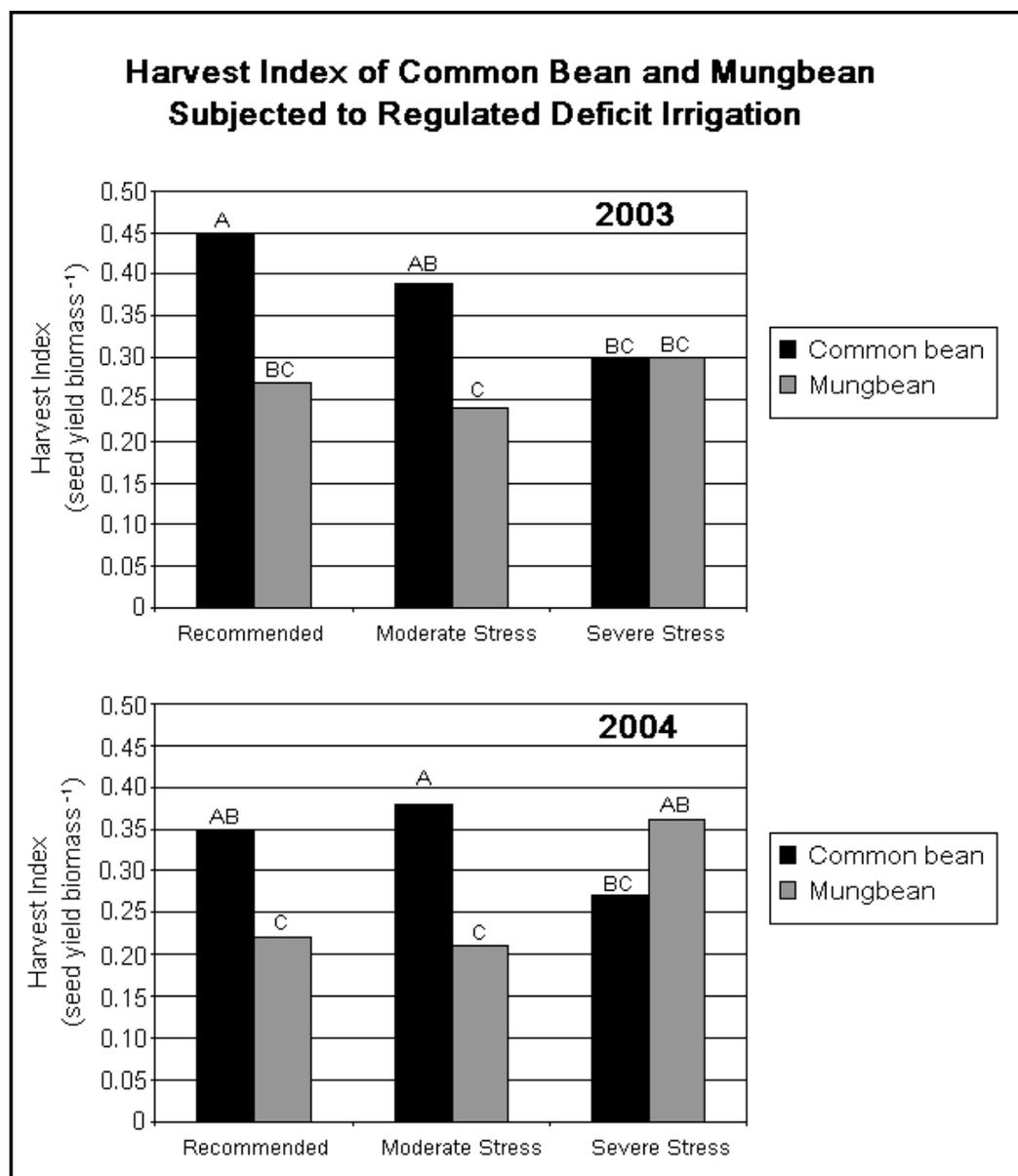


Figure 3.3: Seed yields of common bean and mungbean in 2003 and 2004 subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.



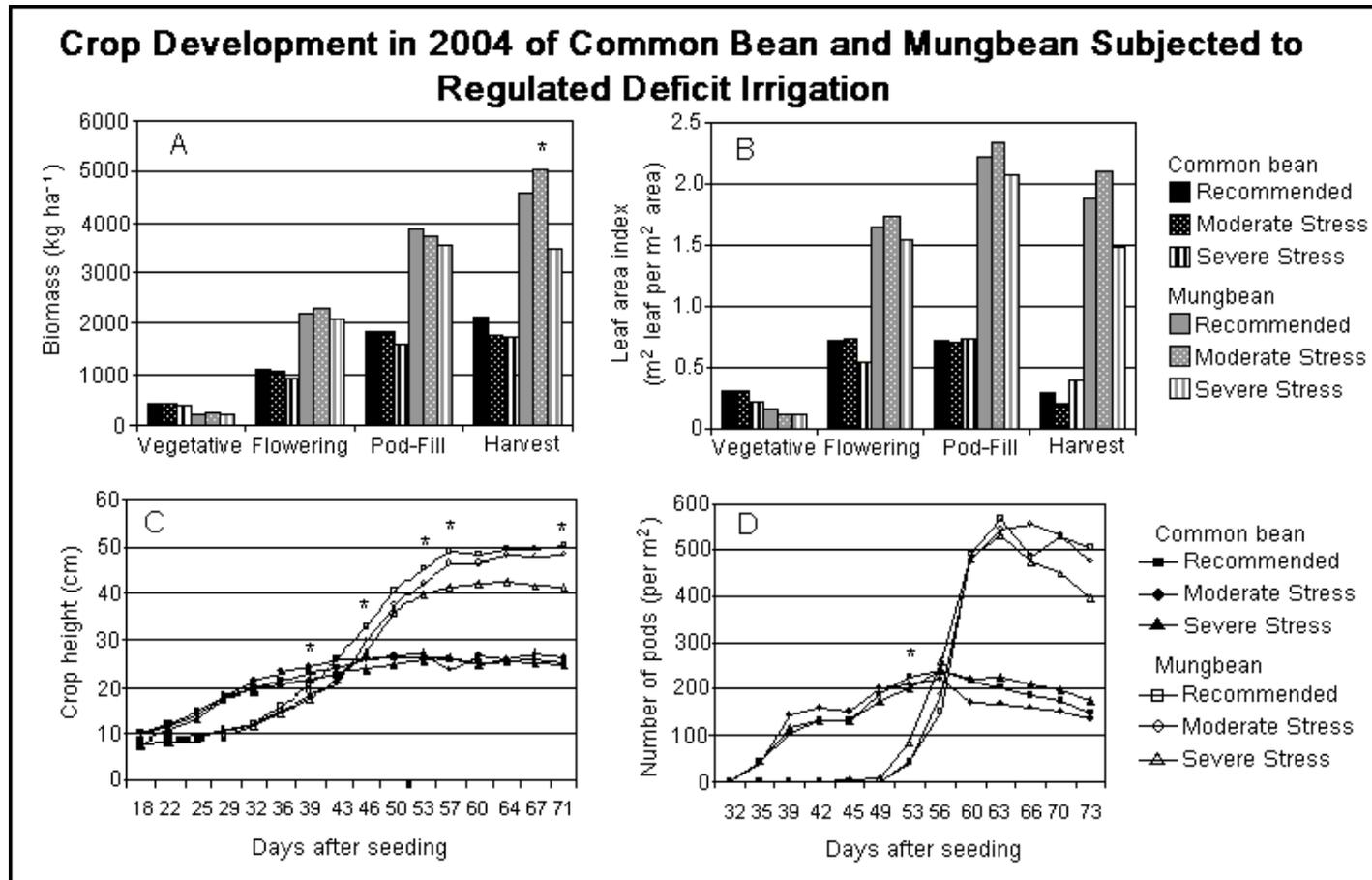
Histogram bars associated with the same letter are not different ($p \leq 0.05$) within the same year by t-tests on least squares means. Each bar represent an average of eight plots from four blocks and two AFI levels. The crop by RDI interaction was significant at $p \leq 0.0913$ in 2003, and $p \leq 0.0005$ in 2004.

Figure 3.4: Harvest index of common bean and mungbean in 2003 and 2004 subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.



Histogram bars associated with the same letter are not different ($p \leq 0.05$) within the same year by t-tests on least squares means. Each bar represents an average of eight plots from four blocks and two AFI levels. The crop by RDI interaction was significant at $p \leq 0.0243$ in 2003, and $p \leq 0.0001$ in 2004.

Figure 3.5: Crop development (biomass A, leaf area index B, crop height C, and number of pods D) of common bean and mungbean subjected to regulated deficit irrigation under field conditions in the Fergana Valley, Uzbekistan.



Values are averages of eight plots from four blocks and two AFI levels. Significant differences in mungbean are identified by an asterisk (*). No significant differences were observed in common bean.

Table 3.1: Alternate furrow irrigation as compared to every furrow irrigation on yield, yield components, and water relations of common bean and mungbean

Parameter of interest	2003			2004		
	Alternate furrow	Every furrow	P value	Alternate furrow	Every Furrow	P value
Yield (kg ha ⁻¹)	656	692	0.3113	832	826	0.8276
Number of seeds per pod	6.9	7.1	0.1023	6.6	6.8	0.0441
100 seed weight (g)	24.3	24.1	0.5972	19.1	20.0	0.0367
Pods per plant	25.2	28.3	0.1379	9.7	10.2	0.2198
Harvest index (%)	0.302	0.346	0.1339	0.306	0.284	0.2486
Stem Water Potential (MPa)						
Before irrigation events	-1.02	-1.00	0.5025	-0.81	-0.80	0.0554
After irrigation events	-0.89	-0.82	0.0027	-0.77	-0.75	0.0346
Stomatal conductance (mmol m ⁻² sec ⁻¹)						
Before irrigation events	221.0	233.5	0.1409	357.5	330.3	0.0947
After irrigation events	316.9	364.4	<0.0001	449.0	461.7	0.1410

Values are averages of twenty-four plots, from four blocks, two crops, and three levels of RDI treatments. There were no AFI level by crop or by RDI level interaction in any of the parameters presented.

Table 3.2: Yield components of common bean and mungbean under three levels of regulated deficit irrigation in the Fergana Valley, Uzbekistan

		Number of Seeds per Pod		100-seed weight (g)		Number of Pods per Plant	
		2003	2004	2003	2004	2003	2004
Crop	Regulated deficit irrigation treatment						
Common Bean	Recommended	3.7 c	3.3 c	41.4 b	34.6 a	11.7 c	5.0 c
	Moderate stress	3.2 c	3.1 c	42.4 ab	33.6 a	13.1 c	4.4 c
	Severe stress	3.3 c	2.9 c	44.0 a	34.2 a	16.0 c	5.1 c
Mungbean	Recommended	11.1 a	10.8 a	5.5 c	5.2 b	47.9 a	16.5 a
	Moderate stress	10.6 ab	10.5 a	5.8 c	4.8 b	45.0 a	15.3 ab
	Severe stress	10.1 b	9.5 b	6.2 c	5.0 b	27.0 b	13.3 b

Values are averages over eight plots from four blocks and two AFI levels.

Values with the same letter in the same parameter are not statistically different at $p \leq 0.05$ by t-tests on least squares means.

Table 3.3: Water relations of common bean and mungbean under three levels of regulated deficit irrigation

		Stem water potential (MPa)				Stomatal conductance (mmol m ⁻² s ⁻¹)			
		Average before irrigation events		Average after irrigation events		Average before irrigation events		Average after irrigation events	
Crop	Depletion fraction	2003	2004	2003	2004	2003	2004	2003	2004
Bean	Recommended	-1.00 bc	-0.91 b	-0.92 c	-0.86 b	317 a	449 a	394 a	569 a
	Moderate	-1.17 d	-0.88 b	-0.88 c	-0.90 b	273 b	463 a	388 a	544 a
	Severe stress	-1.06 bc	-0.86 b	-0.92 c	-0.87 b	214 c	317 b	389 a	520 a
Mungbean	Recommended	-0.82 a	-0.70 a	-0.78 b	-0.65 a	209 c	310 bc	282 b	406 b
	Moderate	-0.88 b	-0.67 a	-1.06 a	-0.65 a	187 cd	261 d	272 b	354 c
	Severe stress	-1.12 cd	-0.70 a	-0.73 c	-0.65 a	164 d	264 cd	300 b	338 c

Values are averages over eight plots from four blocks and two AFI levels. Values with the same letter in the same parameter are not statistically different at $p \leq 0.05$ by t-tests on least squares means.

Preface to chapter 4

In the previous chapter, we demonstrated that short-season legume crops such as common bean and mungbean can be grown after the harvest of winter wheat. In a small experiment conducted in the Fergana valley, Uzbekistan, Central Asia, we evaluated the possibility of growing a short-season Canadian soybean cultivar as a second crop after the harvest of winter wheat. There is considerable interest in this crop in the region because of its high value and potential as a cash crop, but local cultivars do not mature in less than 120 days. We hypothesized that germplasm from Canada, although selected for much less water limited conditions, might still be able to complete its life cycle before the onset of cooler temperatures, and thus yield well under hot and dry climatic conditions in Uzbekistan. A secondary objective was to evaluate the yield benefit from inoculation, or alternatively, to determine the ability to which soybean can utilize the native rhizobial population for nitrogen fixation.

The results show that it is possible to grow a short-season Canadian soybean cultivar in Uzbekistan, after the harvest of winter wheat, and under climatic conditions that are drier and hotter than the Canadian conditions under which it was selected. This has interesting and practical implications, as soybean could be introduced in the current cropping systems in Uzbekistan without interfering with the government-prescribed growth of cotton and winter wheat. It also stresses the importance of inoculation with appropriate rhizobia, as very few nodules were found in the non-inoculated treatments. Further research to identify heat and drought tolerant soybean cultivars and rhizobial strains could probably improve yields in the region. Canadian cultivars could be interesting parents for use in a Central Asian breeding programme as they combined several traits of interest, including short time to maturity.

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Dr. Heidi Webber, a former graduate student from the Bioresources Engineering department, was involved in the execution of this experiment. Specifically, Dr. Webber was in charge of the tasks related to irrigation scheduling. Dean Chandra Madramootoo, who was also Heidi Webber's supervisor, contributed by meaningful advice in the planning and the execution of the experiment, and provided the research funds necessary. Mikhail Horst is an irrigation scientist at the Scientific Information Center of the InterState Coordination Water Commission (SIC ICWC) in Tashkent, Uzbekistan, and contributed his irrigation expertise to the experiment. Galina Stulina is soil scientist also at the SIC ICWC, and was instrumental in the establishment of the experiment in both years in the Fergana valley. Finally, Prof. Donald L. Smith, my supervisor, contributed to the paper by meaningful advice in the design stage of the experiment and provided constructive feedback and editorial assistance on the manuscript.

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4. EFFECT OF INOCULATION ON A SHORT-SEASON CANADIAN CULTIVAR OF SOYBEAN (*Glycine max* [L.] Merr.) GROWN IN UZBEKISTAN

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4.1 Abstract

An experiment was conducted in the Fergana valley, in Uzbekistan, Central Asia to determine the feasibility of growing a short-season Canadian soybean cultivar after the harvest of winter wheat in early July. An inoculated treatment was compared to a non-inoculated control in a randomized complete block design with four blocks. While the inoculation did not establish well in 2003, in 2004, the yield of inoculated soybean was twice that of the uninoculated control. Inoculation in 2004 increased seed weights, final pod number, and biomass dry weight. Nodules were, in general, only present in the inoculated treatments, which indicated that appropriate indigenous rhizobial strains were not present in these soils. Soybean production could be possible in Uzbekistan without competing with state prescribed crops such as cotton and winter wheat. This could provide farmers with a lucrative addition to their current production system.

4.2 Introduction

Agricultural policies in Uzbekistan emphasize the culture of cotton, an important component of the Uzbek economy, and to a lesser degree, winter wheat. Both are subject to state regulation through a system of quotas, and little agricultural land is left for other crops (M. Suleimenov, personal communication). To improve land productivity and food security in the region, we previously suggested the introduction of legumes as second crops after the harvest of winter wheat in late June, early July (chapter 2.2 and chapter 3). Food legumes can provide a good source of protein for human consumption, and their residues can improve the fertility and physical condition of the soil, due in great part to their ability to form a nitrogen-fixing symbiosis with rhizobia (Senthong and Pandey, 1989; Haqqani and Pandey, 1994a; Subbarao et al., 1995). In addition, legumes in rotation with other crops can break disease cycles and encourage the development of mycorrhizal associations (Subbarao et al., 1995). Unfortunately, the economics of their production often restricts them to less productive lands and/or periods of growth. As such, their productivity is limited in semi-arid and arid regions (Subbarao et al., 1995).

Soybean (*Glycine max* [L.] Merr.) is now the world's most important legume crop (Giller, 2001). Its annual production totalled 217 million tonnes in 2007 on almost 95 million hectares averaging about \$1000 tonne⁻¹ (FAOSTAT, 2009). Soybean is used both as a food crop and for its oil, and the meal resulting from oil extraction is an important protein source for livestock. International markets for soybean are well developed and easy to access, and its production has been growing in several dry areas including Australia, Brazil and the United States (FAOSTAT, 2008). While short-duration soybean cultivars have been developed in Canada to avoid cold temperatures, no such short-duration cultivars are available to Uzbek farmers. Local soybean cultivars generally mature in at least 120 days, and as such are in direct competition with government-prescribed culture of cotton and winter wheat.

In addition, in Australia, early maturity has been used as a drought escape mechanism to improve soybean production since the early 1980s (Rose et al., 1982). Drought escape refers to the ability of crops to complete their life cycle before the onset of water shortages (Subbarao et al., 1995). As such, we hypothesized that the soybean cultivar Costaud, which matures in 90 to 100 days (2600 to 2700 Corn Heat Units) under Canadian conditions, would represent a good candidate for production under the conditions of Uzbekistan.

In addition, the benefits of rhizobial inoculation are well established, and are generally important in semi-arid regions (Date, 2000; Egamberdiyeva et al., 2004; Ndakidemi et al., 2006). However, the ability of native rhizobial populations in the soils of Fergana valley, Uzbekistan, to form functional symbioses with soybean was not known prior to our work, and the performance of both the Canadian genotype and a Canadian inoculum had to be assessed before encouraging farmers to grow soybean.

Thus, the objective of this experiment was to determine the feasibility of growing a short-season Canadian soybean cultivar after the harvest of winter wheat in early July, and to evaluate the benefits from inoculation with *Bradyrhizobium japonicum* under these circumstances.

4.3 Materials and methods

The experiment was conducted in the Fergana valley, in Uzbekistan, Central Asia (40°23'N, 71°45'E) from mid-July to mid-October, in the growing seasons of 2003 and 2004. During this period, the climate is hot and dry, with typical daily high temperatures of 40°C and daily low temperatures of 20°C. Rain is very infrequent, except in early October. From July 15th to September 30th, 2003 and 2004, we recorded a total of 8.8 and 7.6 mm of rainfall, respectively, at our field sites. Based on

textural analyses, the soil at the experimental sites was a silt loam in both 2003 and 2004. The organic matter content was determined in an adjacent field (results presented in appendix A). In both years, the soils had low organic matter contents (less than 2%), and a well developed plough pan at 30-40 cm. Each field site produced winter wheat immediately prior to our experimentation. The wheat had been harvested, the straw and stubble burned, and the field ploughed and levelled, all following standard practices in the region. Sixty-cm wide furrows were formed on the field site with a tractor drawn lister.

The plots were organized on the field site following a randomized complete block design with four blocks and two treatments. The treatments consisted of inoculated plots, and control non-inoculated plots. Seeds of the Canadian soybean cultivar Costaud were chosen because of their short growing season. Seeds were covered with a slurry prepared from 10 g of commercial peat-based inoculant containing *Bradhyrhizobium japonicum* strain 532C (Nitragin, EMD Crop Bioscience, Milwaukee, WI, USA), and 15 mL of water, as directed on the package. Nitragin guarantees a minimum of 250 million viable bacterial cells per gram. The soybean seeds were left to dry, and then seeded by hand to a population density of 50 plants per m². Planting was done on July 22nd in 2003, and on July 13th in 2004. Weed control was done manually. Irrigation scheduling was performed using a water balance and evapotranspiration estimates from climatic data as described in Allen et al. (1998) and in Webber et al. (2006).

Plots consisted of 9 rows 5 m in length. Each plot was separated by a row of mutant non-nodulating soybeans. The two outside rows were not utilized for data collection. Yield was measured by harvesting all pods in three 2 m length sections of row in each plot. Pods were threshed by hand, and seed yield was corrected for moisture content (to 0% moisture),

and converted in kg ha^{-1} from plant population estimates before statistical analysis. The number of seeds per pod was evaluated on ten randomly selected pods from these areas, and seed weight was evaluated from one hundred seeds randomly selected from the harvested seeds from each plot. These were then oven-dried at 65-70 °C for 24 h, or until completely dry, to determine seed moisture level. Plant population estimates were determined by counting the number of plants in three 2-m row sections per plot.

To determine crop height and the number of flowers and pods, six plants were labelled at the beginning of the season and measurements were made on a weekly basis on these same plants until harvest. Above-ground biomass was also evaluated three times during the season at the flowering, pod-filling and harvest stages by sampling 0.5 m of row. Plants were dried at 70°C for at least 24 hours, until completely dry. The number of nodules was also determined in these destructive samplings.

Statistical analyses were performed by analysis of variance (ANOVA) using the SAS/STAT software and GLM procedure (SAS, Cary, NC, USA). In general, differences between the inoculated treatment and the control were considered significant only when they occurred at the 0.05 level of probability. However, in some cases, relevant differences are discussed when the probability level was between 0.1 and 0.05; in these cases the p values are given in the text. If fixed main effects or a fixed interaction were found to be significant in the ANOVA, then means separations were carried using the Student-Newman-Keuls procedure (Milliken and Johnson, 1984). Because there were few nodules on the control plants, the analysis for the number of nodules was performed with a non parametric approach using proc RANK prior to proc GLM.

4.4 Results and discussion

In both years, a soybean crop was successfully grown using a short-season Canadian variety after the harvest of winter wheat in the Fergana valley, in Uzbekistan, Central Asia. The cultivar Costaud, although originally developed for colder climate conditions prevalent in Canada, is able to grow and yield in the hot and dry conditions of Fergana valley, Uzbekistan, provided some irrigation is available. This is interesting as it provides farmers with a possible alternative crop that does not compete with the production of cotton and winter-wheat which are subject to state quotas. However, yields were relatively low (Table 4.1), and soybean production in the region would probably benefit from improved germplasm that combined higher heat and drought stress tolerance with early maturity. It also seems likely that better adapted rhizobial strains would improve the yield of soybean in the area.

The inoculation of soybean did not increase yields in 2003. In fact, very few nodules were observed that year. Because of this failure of soybean to nodulate in 2003, the following discussion will pertain to the 2004 results, where the average yield of inoculated soybean plants was doubled that of the non-inoculated control plants (Table 4.1). We suspect that in 2003 seeding conditions were too hot and dry for the survival of rhizobial cells in the commercial inoculant, leading to poor nodulation. The success of 2004 however seems to indicate that with proper care (seeding around dawn, and irrigating shortly after), this inoculant can perform relatively well.

The higher yield observed due to inoculation is mostly explained by higher seed weights, and higher final pod numbers, as compared to the control non-inoculated soybean plants (Table 4.1). The number of seeds per pod, however, was unaffected by inoculation. It is also interesting to note that

except for two instances, nodules were not found on plants in the non-inoculated control treatment plots (Table 4.2), which indicates that indigenous rhizobia were not able to nodulate soybean in this soil. The number of flowers was not affected by inoculation in either year on any of the days of observation, but the number of pods started to become greater in the inoculated treatment by mid-September (data not shown). The above-ground biomass dry weight (not shown) also began to show increasingly significant differences by the pod-filling stage ($p < 0.0533$), and showed very clear differences at the harvest stage ($p < 0.0098$). Our data suggest that the benefit of inoculation was greatest in the late stages of the plant development.

Environmental factors such as high temperature and drought affect nodulation and the ability of rhizobia to colonize plants (Hungria and Vargas, 2000). Research to develop heat and drought tolerant soybean cultivars and rhizobial strains should improve soybean yields in the region. Canadian cultivars could be interesting parents for use in a breeding programme because of their early maturity. Large variability in rhizobial strains has been documented in a number of semi-arid areas (Arun and Sridhar, 2005; Hungria et al., 2006; Giongo et al., 2008). Research with Brazilian inoculants and native rhizobia isolated from calcareous soils is being performed in the region (Egamberdiyeva et al., 2004). In addition, large genetic variability in nodulation sensitivity to water deficit stress among soybean cultivars has been demonstrated (Serraj and Sinclair, 1998), and low petiole ureide content has been associated with the maintenance of nitrogen fixation under water stress (Sinclair et al., 2000). This could provide a relatively simple method for screening soybean cultivars and rhizobial strain combinations for higher nitrogen fixation in drought-prone areas.

4.5 Conclusion

We have demonstrated that the Canadian soybean cultivar Costaud could be grown successfully in Uzbekistan, and that inoculation increases yields substantially, as long as the inoculant is not subjected to high temperatures at seeding. A short-season, drought tolerant soybean cultivar, associated with an equally heat and drought tolerant rhizobial strain, would be ideal in this semi-arid region to provide farmers with a new and potentially very profitable crop to include in their current production system.

Table 4.1: Yield and yield components of soybean (cv. Costaud) grown in the Fergana Valley, Uzbekistan

Parameters	2003		2004	
	Inoculated	Control	Inoculated	Control
Yield (kg/ha)	1047 A	968 A	1476 A	715 B
Seeds per Pod	na	na	2.45 A	2.34 A
100- Seed Weight (g)	na	na	16.5 A	12.7 B
Final number of Pods	41.2 A	39.6 A	33.6 A	26.0 B

Values given are means of three (2003) or four blocks (2004) with the same treatment. Values with the same letter within the same year are not significantly different by the Student-Newman-Keuls test.

Table 4.2: Average number (per 0.5 m row) and dry weight of nodules found in soybean (cv Costaud) grown in Uzbekistan in 2004

Treatment	Flowering		Pod-Filling		Harvest	
	Number	Dry weight (g)	Number	Dry weight (g)	Number	Dry weight (g)
Inoculated	Na	0.185 a	122.5 a	2.338 a	13.5 a	0.285 a
Control	0	0.000 b	0.75 b	.005 b	0 b	0.000 b

Values given are means of four blocks with the same treatment. Values with the same letter within the same year are not significantly different by the Student-Newman-Keuls test on ranks.

Preface to chapter 5

In chapter 3, we demonstrated that mungbean had the highest yields in the moderate stress treatment in 2003, and in the severe stress treatment in 2004 under field conditions. In the following chapters, experiments were performed to further characterize the response of both common bean and mungbean to water deficit stress in controlled environment conditions.

Two experiments are described in this chapter. The first experiment was conducted during the summer of 2006, and evaluated the effect of deficit irrigation on individual plants grown in PVC pipes of 15 cm diameter and 55 cm height. In the second experiment, conducted from March to May and June to September 2007, we tested the combined effect of salinity and deficit irrigation on the development and yield of crops grown in large plastic boxes. Soil salinity in Uzbekistan can be a serious problem, and this study explores the overlap of responses of crops to salinity and drought stresses on water relations and, potentially, the different responses in mungbean.

We have used the same germplasm as used in the field experiment (chapter 3), not only for consistency, but also because, as Uzbek landraces selected under a hot and dry environment, these common bean and mungbean lines are potentially exhibiting greater drought tolerance than most cultivars of their respective species.

It should be noted that data sets from this study was used by both myself and Dr Heidi Webber. Data and discussion that pertain specifically to salinity stress and, in particular, applications to modelling can be found in Dr Heidi Webber's thesis (Webber, 2008).

In this first study, contrary to our findings in the field, mungbean did not show high yields under water deficit stress, and showed lower leaf area and biomass in the severe salinity stress. Neither crop showed a capacity for osmotic adjustment, but differences were found in transpiration efficiency, particularly at higher soil water levels. In addition, mungbean maintained the same proportion of biomass in reproductive structures at the pod-filling stage, and maintained its harvest index under stress, whereas common bean showed a significant decrease. The lack of osmotic adjustment in both crops, and the maintenance of harvest index in mungbean observed in this study are consistent with the field data presented in chapter 3. Furthermore, the maintenance of harvest index could represent a relatively easy and inexpensive selection criterium for use in breeding programs if genetic variability in the trait exists.

The contrasting response of yield to water deficit stress treatments presented in chapter 3 and in this chapter point to the difficulty in conducting water deficit stress studies in controlled but artificial conditions. Whereas the severe deficit irrigation treatment was irrigated only once at flowering in the field, in columns and boxes in the greenhouse, this treatment was irrigated several times, and the root system might have been considerably restricted, thus contributing to lower yields. Alternatively, the stress might have been more severe due to the fast rate of water depletion in columns and boxes, so that the range of water availabilities might have been different than tested in the field. Therefore, an experiment was designed to characterize the responses of common bean and mungbean to a larger range of water treatments, from quasi-waterlogged conditions to extreme water stress from complete lack of watering after seedling establishment (chapter 6). In addition, early root growth morphology was observed and characterized for the two crops (chapter 7).

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Dr. Heidi Webber, a former graduate student from the Bioresources Engineering department, was involved in the execution of this experiment. In general, Dr. Webber was in charge of the tasks related to irrigation scheduling, while I was responsible for the crop measurements. On occasion, Dr. Webber helped me with the biomass samplings, while I helped with the soil samplings. In addition, close interaction with Dr. Webber has allowed thorough discussion of several key issues from this experiment. Dean Chandra Madramootoo, Mikhail Horst, and Galina Stulina provided feedback on the manuscript. Finally, Prof. Donald L. Smith, my supervisor, contributed to the paper by meaningful advice in the design stage of the experiment and provided constructive feedback and editorial assistance on the manuscript.

5. EFFECTS OF DEFICIT IRRIGATION AND SALINITY STRESS ON COMMON BEAN (*Phaseolus vulgaris* L.) AND MUNGBEAN (*Vigna radiata* (L.) Wilczek) GROWN IN A CONTROLLED ENVIRONMENT

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5.1 Abstract

As water for irrigation purposes becomes increasingly scarce due to climate change and population growth, there is growing interest in regulated deficit irrigation (RDI) as a way to improve water use efficiency and farm productivity in arid and semi-arid areas. Salinity is also becoming an important problem in these same regions. Experiments were performed to investigate the effects of RDI and salt stress on two legumes crops, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek); previous work showed contrasting responses to RDI by these two crops under field conditions. The seed and biomass yields of both crops were reduced as a result of increasing water deficit stress, however, mungbean was able to maintain the same proportion of its biomass in reproductive structures under stressed and well-watered conditions whereas common bean's proportion of biomass in reproductive structures decreased. In addition, photosynthesis in mungbean was consistently higher than in common bean and higher at the same levels of

transpiration. Finally, salinity stress decreased yield, yield components, and photosynthesis, but not differently between the two crops, and without interacting with the RDI levels.

5.2 Introduction

Climate change and population growth are the two most important challenges facing agriculture today (Wallace, 2000). Water scarcity is the most important factor limiting crop yields worldwide (Begg and Turner, 1976). An increased but sustainable use of irrigation water will be necessary to feed our growing population (Postel et al., 2001). Irrigated cropland accounts for about 17% of the total land area, yet produces more than 40% of the global agricultural supply (Postel et al., 2001).

Unfortunately, mismanagement of water resources in irrigated systems has led to substantial degradation of soils due to increased soil salinity due to waterlogging and the movement of salts upwards towards the soil surface (Szabolcs, 1994). As water (rather than area) becomes limiting, irrigation management options have increasingly emphasized the optimization of water use efficiency or water productivity in cropping systems (Pereira et al., 2002).

One of these options is the use of regulated deficit irrigation (RDI) whereby crop producers allow the crop to experience some water stress (English and Raja, 1996). If conducted correctly, the water saved allows an increase in the area irrigated, or could be put to more productive use elsewhere (Pereira et al., 2002). Under RDI, however, it is particularly important that farmers have precise information on factors such as the response and sensitivity of crops to water stress, critical stages of development when deficit irrigation should or should not be performed, and how much water can be conserved (Kijne et al., 2003).

Salinity stress and drought stress are often compared and viewed as two stresses that often occur together. As the soil dries, the soil water solution becomes more concentrated and the overall soil water potential becomes lower. The initial response of crops to drying soil and salinity stress are identical, and toxicity effects of the salts themselves are only manifest later in the crop development (Munns et al., 2002).

The benefit of legumes in cropping systems has long been recognized. Their residues can improve the fertility and physical condition of the soil, due in great part to their ability to form a nitrogen-fixing symbiosis with rhizobia; they can also break disease cycles and encourage mycorrhizae (Subbarao et al., 1995; Hedley, 2001). As the price of nitrogen fertilizer increases, they are considered increasingly profitable crops, because of the lower input requirements. Furthermore, in developing countries, diversification into more horticultural crops such as legumes and access to markets is seen as particularly effective in helping the poor break the poverty trap (Polak, 2005; Sachs, 2005; Sanchez et al., 2007).

The mechanisms of drought tolerance in legumes have not been as well characterized when compared to cereals, and the use of physiological and genetic markers for improved legume germplasm has been limited (Turner et al., 2003). This is unfortunate as legumes tend to be grown in more marginal environments, under various biotic and abiotic stresses, and as such their yield is often much below their potential (Subbarao et al., 1995; Turner et al., 2003). Information on mungbean is particularly scarce.

Common bean is a very diverse pulse crop, with approximately 500 varieties. It has been domesticated independently in the Andes and in Central America (Polhill and van der Maesen, 1985), and possibly from distinct wild progenitors within these same areas (Miklas and Singh, 2007). It is the most important pulse crop in the world, being eaten

directly more than any other legume crop (Hedley, 2001; Broughton et al., 2003). It is cultivated in a range of complex multiple-cropping systems and a range of environments, including relatively dry areas (Graham and Ranalli, 1997; Singh, 2007). Recent studies suggest that only 7% of the bean growing area receives adequate rainfall (Broughton et al., 2003), and that 60% of the production occurs under severe water stress (Graham and Ranalli, 2007).

Mungbean, also known as green gram, is a small-seeded crop less known in the Americas, but widely cultivated in Asia, particularly in India and Myanmar, where it is thought to have originated (Lawn and Ahn, 1985; Hafeez et al., 1991; Poehlman, 1991). Central Asia appears to be a secondary center of origin, and it is also cultivated to some degree in the United States and in Australia, and often consumed as sprouts (Lawn and Ahn, 1985; Poehlman, 1991). The crop is known as one that performs well under conditions of low soil moisture (Lawn and Ahn, 1985). Furthermore, mungbean demonstrated greater drought tolerance than common bean in a field study in Uzbekistan (chapter 3). Despite its long history in Asian agriculture, mungbean received very little research attention prior to 1970 (Lawn and Ahn, 1985), and the research effort has decreased since 1985.

The objective of this study was to investigate the effects of RDI and salt stress on two legumes crops, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek), which had previously shown contrasting responses to deficit RDI in field conditions. Because controlled environment studies are notoriously difficult to compare to field studies, this study should not be used to make recommendations regarding the use of RDI in the field, rather, our objective was to examine more closely the possible mechanisms by which each crop responds to water stress. In addition, since mungbean has demonstrated an ability to

yield well under relatively low osmotic potential (dry) soil conditions (chapter 3), we hypothesized that the same mechanisms would similarly allow it to be well adapted to soil with low osmotic potential due to salinity stress.

5.3 Materials and methods

5.3.1 Drought experiment

5.3.1.1 Experimental design

The experiment was organized following a factorial randomised complete block design with five blocks and five repetitions per treatment per block. The units were sufficiently large to allow for destructive samplings during the season. The two factors were crops (common bean and mungbean) and RDI levels (FAO-recommended, moderate stress and severe stress) as defined previously in Webber et al. (2006), and further described in section 5.3.1.3.

5.3.1.2 Plant growth

Plants were grown one each in PVC pipes of 15 cm diameter and 55 cm depth covered with cheese cloth, fitted with a custom pot #4 (5 L) at the bottom. The pipes were filled with a mix of medium coarse sand and quality topsoil (Fafard, Quebec) at a ratio of 1:1. The soil mix had a field capacity volumetric water content of about 23% and a permanent wilting point of about 7%, and each column had a plant available water content capacity of approximately 1.5 L. Pipes were perforated at 10, 30, and 50 cm from the top to allow the introduction of a probe for soil moisture measurements. These holes were covered with duct tape between measurements. Pipes were spaced 10 cm apart so that competition between plants did not occur. Temperature was controlled to be at 32/20°C day/night with a 16/8 h light/dark photoperiod. Humidity was not

controlled and varied from 75 to 90%. Light intensity was on average $800 \mu\text{mol m}^{-2}$, depending on outside conditions, but was minimally $600 \mu\text{mol m}^{-2}$. Pipes were seeded on July 28th, 2006. Seeds used were pure lines from common bean and mungbean varieties previously used in field work (chapter 3), and were surface-sterilized in 1:1 (v/v) distilled water and bleach, and then thoroughly rinsed in distilled water. Seeds were then soaked 2 h prior to seeding in order to improve the emergence rate.

5.3.1.3 Watering schedule

Levels of RDI were determined as described in Webber et al. (2006). Briefly, RDI levels were determined according to the concept of soil water depletion factors, as defined by the FAO Water Report #56 (Table 22, Allen et al., 1998). Depletion factors are measures of soil water depletion as a percentage of the total available soil water; the longer the interval of time between irrigation events the higher the depletion factor. For common bean, the depletion factors used were 0.45 as the recommended level (Allen et al., 1998), 0.6 and 0.7 as the moderate and severe stress levels, respectively. For mungbean, the recommended depletion factor was also 0.45 (Allen et al., 1998), but the moderate and severe stress levels were 0.65 and 0.8. Once the soil was depleted to the appropriate fraction of soil available water, the irrigation amount was determined from the water balance, and the treatment was irrigated. Soil moisture was monitored using a time-domain reflectometry (TDR) 300 field scout soil moisture meter (Spectrum Technologies, Plainfield, IL, USA) three times a week on two repetitions per treatment per block, at 10, 30 and 50 cm depth through holes in the side of the column.

5.3.1.4 Measurements

Photosynthesis and other gas exchange measurements (LICOR 6400, LICOR Biosciences, Lincoln, NE, USA) were taken on the topmost fully

expanded middle trifoliolate, at the same time and on the same plants as soil moisture measurements, namely approximately three times a week on two repetitions per treatment per block, from the day individual leaves were more than 6 cm² to the onset of plant senescence. Destructive samplings were conducted three times during the season: at the vegetative, flowering, and pod-filling stages. Leaf area (Delta-T Devices, Cambridge, UK), dry biomass (stem, leaf, and reproductive structures) and water potential (WP4-T water potentiometer, Decagon Devices, Pullman, WA, USA) were determined. Plants were dried at 70°C for at least 24 hours, until completely dry. The water potential was determined on two random central trifoliolates per plant. At harvest, pods were collected and threshed by hand, and seed yield and pod weights were determined.

5.3.2 Drought and Salinity experiment

5.3.2.1 Experimental design

The experiment was organized following a randomized complete block split-plot design with four blocks. The main plots consisted of crops, and sub-plots consisted of the combination of salt and RDI levels. There were two crops (common bean and mungbean), three levels of salinity stress (control, medium level and high level, described further in section 5.3.2.3), and three levels of RDI (FAO-recommended schedule, a moderate stress and a severe stress) as described above. The experiment was repeated twice.

5.3.2.2 Plant growth

Plants were grown in large plastic bins (160 L containers, 50 cm deep, 43 cm wide, and 70 cm long), equipped with a drainage pipe fixed in the bottom to prevent waterlogging, and filled with approximately 176 kg of a

fine sandy loam agricultural soil. The soil had a field capacity volumetric water content of 28%, and a permanent wilting point of 9%. Each box had a plant available water content capacity of 31 L.

Salts were mixed into the soil according to treatment prior to filling boxes. Each box also received an addition of 3.3 kg of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) to mimic the agricultural soil used in a previous field study (Webber et al., 2006). The addition of calcium is also believed to help maintain cell membrane integrity and adequate potassium content (Cramer et al., 1995). Plants were seeded sixteen per box to allow for destructive samplings. Experiments were started on March 21st, 2007, and again on June 28th, 2007. Plants were grown in a greenhouse chamber with temperatures set at 32/20°C day/night, and a 16/8 h light/dark photoperiod. Humidity was not controlled and varied between 75 and 90%. Light intensity was on average 800 $\mu\text{mol m}^{-2}$, depending on outside conditions, but was minimally 600 $\mu\text{mol m}^{-2}$. Seeds used were from the same lines as the previous experiment, and were sterilized, and soaked as described in section 5.3.2.2.

5.3.2.3 Salinity stress

Levels of salinity were determined using the saturated extract method and the 5:1 extract method (Rhoades, 1996). The control consisted of the agricultural soil with gypsum only, which had an electrical conductivity (EC_e) of 3.0 dS m^{-1} . Boxes with the medium level of salt received 82.5 g of sodium chloride (NaCl), to reach an EC_e of 5.4 dS m^{-1} , and boxes with the high level of salts received 165 g of NaCl to reach an EC_e of 7.8 dS m^{-1} .

5.3.2.4 Water scheduling

Levels of RDI were determined as described in section 5.3.1.3. Soil moisture was determined gravimetrically before and after watering events, and between events at least every 7 days, at 5, 20, and 40 cm depths.

5.3.2.5 Measurements

Destructive samplings were conducted four times during the season: at the vegetative, flowering, pod-filling and maturity stages. Leaf area and dry biomass were determined each time as described in section 5.3.1.4. At harvest, the seed yield, hundred-seed weight, number of pods per plant, and pod weights were determined. The harvest index (HI) was also calculated from the ratio of seed weight at 0% moisture to total dry biomass. Photosynthesis and other gas-exchange measurements (LICOR 6400, LI-COR Biosciences, Lincoln, NE, USA) were taken on two plants per box once a week at the vegetative and flowering stages of crop development in the spring of 2007 only. Water potential (WP4-T water potentiometer, Decagon Devices, Pullman, WA, USA) was also determined at this time as described in section 5.3.1.4.

5.3.3 Statistical analysis

For both experiments, the data were analysed with the SAS/STAT software (SAS, Cary, NC, USA) using the GLM procedure for regression analyses, analyses of variance (ANOVA), and multivariate analyses of variance (MANOVA) with repeated measures for photosynthesis and transpiration data. On occasion, when trends and values were similar for all experiments for the same parameter, data were pooled for the same statistical analysis. Treatment effects and interactions were considered statistically significant when they occurred at the 0.05 level of probability. If fixed main effects or a fixed interaction were found to be significant in the ANOVA, then means separations were carried using t-tests on least

squares means. To control the experimentwise error rate within the means separation t-tests, the Bonferroni correction was used (Miller, 1981; Milliken and Johnson, 1984).

5.4 Results and discussion

5.4.1 Yield and yield components under regulated deficit irrigation

Yield decreased in both crops with increasing water deficit in all experiments, although absolute values of yield for mungbean were generally greater than for common bean (Figure 5.1). In addition, for each experiment, there was a crop by RDI interaction suggesting that the two legumes respond differently to water deficit stress, and that mungbean is less sensitive than common bean.

Yield components observed in the drought and salinity experiment, in general, all account for the lower yield resulting from the increased water deficit stress. Number of pods per plant, number of seeds per pod and 100-seed weight all decreased significantly; only mungbean 100-seed weight was not affected (Table 5.1).

This decreasing trend in yield is consistent with previous field observations for common bean (Nielsen and Nelson, 1998; Dapaah et al., 2000; Boutraa and Sanders, 2001), although in our field experiment, this decrease was not statistically significant under the moderate stress level of RDI (chapter 3). In addition, the results of this study are inconsistent with our field observations for mungbean. The crop showed the highest yields at the moderate stress level in 2003 and severe stress level in 2004 under field conditions in Uzbekistan (chapter 3).

Mungbean's ability to maintain yields under arid conditions might be due to dehydration avoidance mechanisms rather than dehydration tolerance

mechanisms *per se* as defined by Jones et al. (1981). Measures of dehydration avoidance (also called dehydration postponement) include mechanisms to reduce water loss and/or maintain water uptake such as deep rooting habits, paraheliotrophy, low transpiration rates, etc. On the contrary, mechanisms of dehydration tolerance are defined as measures that enable the maintenance of turgor at low tissue water potentials, and these include osmotic adjustment and changed in cell wall elasticity (Jones et al., 1981). In this experiment, pot-grown mungbean would have been unable to reach into deeper soil profiles for wetter soil and, as such, would not have been able to maintain water uptake as it might have done in the field.

5.4.2 Leaf area, biomass, and harvest index under regulated deficit irrigation

In general, leaf area decreased with increasing water deficit stress in both crops, but there were sometimes crop by RDI interactions indicating that the decrease in leaf area was proportionally more important in mungbean than in common bean (Figure 5.2). Experience with common bean suggests that the interval of time between the last watering and the sampling tends to confound results. The leaf area seems to increase drastically in the few days following a watering event, and might become similar to the well-watered control.

Total above ground biomass followed a trend very similar to leaf area (data not shown), but an analysis of the separate parts of biomass revealed that mungbean was able to partition a greater proportion of its biomass to reproductive structures under water deficit. This was especially so at the pod-filling stage of development (Table 5.2), but was also confirmed by the HI in the drought and salinity experiment (Table 5.2), which was maintained across RDI levels in mungbean, but not by

common bean. This was also consistent with our previous findings (chapter 3). The maintenance of HI could be an interesting selection trait for improved productivity under drought-prone environments, as it is relatively simple to evaluate.

5.4.3 Water potential, photosynthesis, transpiration and specific leaf weight under regulated deficit irrigation

When water content was taken as a covariant of leaf water potential, there were no significant differences between the RDI levels in either crop (data not shown). Because osmotic adjustment (OA) is an active accumulation of solutes, and not just the passive accumulation of solutes from lower water content, this indicated neither crop was using OA as a means of drought tolerance. This lack of OA is consistent with our field results (chapter 3), and with other studies (Markhart, 1985 in common bean; Muchow, 1985b in mungbean).

For the same soil depletion levels, mungbean showed consistently higher photosynthesis values than common bean (Figure 5.3). Furthermore, analyses using repeated measures revealed highly significant ($p \leq 0.0001$) crop by RDI interactions in photosynthesis data in all three trials. However, transpiration was similar for both crops (data not shown). Moreover, mungbean showed a higher rate of photosynthesis with the same level of transpiration (Figure 5.4), which seems to indicate a better intrinsic transpiration efficiency, especially at higher photosynthetic levels.

Specific leaf weight (leaf weight per unit area or SLW) was greater for mungbean than bean at pod-fill (data not shown). Correlation has been demonstrated between SLW and transpiration efficiency in switchgrass (Byrd and May, 2000), and it is possible indeed that this exists in legumes as well. More specifically, greater SLW might translate into greater

photosynthetic capacity, or reduced transpiration. In this study, SLW was not usually different between RDI treatments, except in the summer of 2007. Then, there was a crop by RDI interaction which showed a decrease in SLW in common bean with increasing water deficit, but an increase in SLW for mungbean. This could, again, suggest a drought adaptation mechanism present in mungbean but not in common bean. De Costa and Shanmugathan (1999) have also observed a lower SLW in irrigated mungbean compared to the rainfed control. This higher SLW under water deficit stress may be related to the thickness or other characteristics of the leaf epidermis leading to higher transpiration efficiency.

5.4.4 Salinity stress

Yields of both crops were reduced by salinity stress, and this decrease was largely attributable to a decrease in the number of pods per plant, although, there was also a decrease in the number of seeds per pod for mungbean in the spring of 2007 (data not shown). This same experiment also shows a crop by salinity levels interaction which resulted in a proportionally greater decrease in yield for mungbean than for common bean (data not shown). Although this interaction is not present in the summer of 2007, the decrease in yield was proportionally greater in common bean (data not shown). Thus, it seems the two crops do not differ in their response to salinity stress.

While biomass and leaf area were generally decreased by increasing salinity, the proportions of biomass allocated to reproductive structures were not affected by the salinity stress, nor is the HI (data not shown).

Previous studies have also shown that growth is depressed by salinity stress for both common bean (Tejera et al., 2005; Gama et al., 2007) and

mungbean (Misra et al., 1996; Sumithra et al., 2006), although Bayuelo-Jimenez et al. (2002) found considerable variability in salt tolerance when comparing numerous *Phaseolus* species, and this was especially so in wild ones. Unfortunately, many studies have performed short-term experiments on seedlings subjected to sudden and severe salt stress administered in the nutrient solution. It has been demonstrated that such experiments are not reflective of the true tolerance to salinity in field experiments (Munns et al., 2002). The authors believe that mixing the salts into the soil, and growing plants to full maturity is, although more resource-intensive, a far superior method not only because it is more representative of salinity conditions due to waterlogging, but also because it integrates the response to salinity stress over the entire crop growth cycle.

Water potential was also not lowered by the applied salt treatments, which indicates that both crops were relatively effective at limiting the entry of salt into the leaf cytoplasm. One mechanism of tolerance is to accumulate salts in the upper part of the roots (Munns et al., 2002), so salts could still have been present in the stems or roots, and this might have been stressful and contributed to lower yields. It seems however that these two legumes are not using OA to compensate for higher salinity levels in the soil. This is contrary to conclusions drawn by Wignarajah (1990) and Zlatev (2005) who present evidence of OA in common bean in response to salinity stress, although there could be considerable variation among common bean cultivars in OA capacity.

Specific leaf weight was also not affected by salinity treatments (data not shown). On two of the four days when photosynthesis measurements were taken, there was a significant salt effect where salts decreased photosynthetic rates. Repeated measure analysis also detected an overall salt effect on photosynthesis, but no interaction with crops or RDI

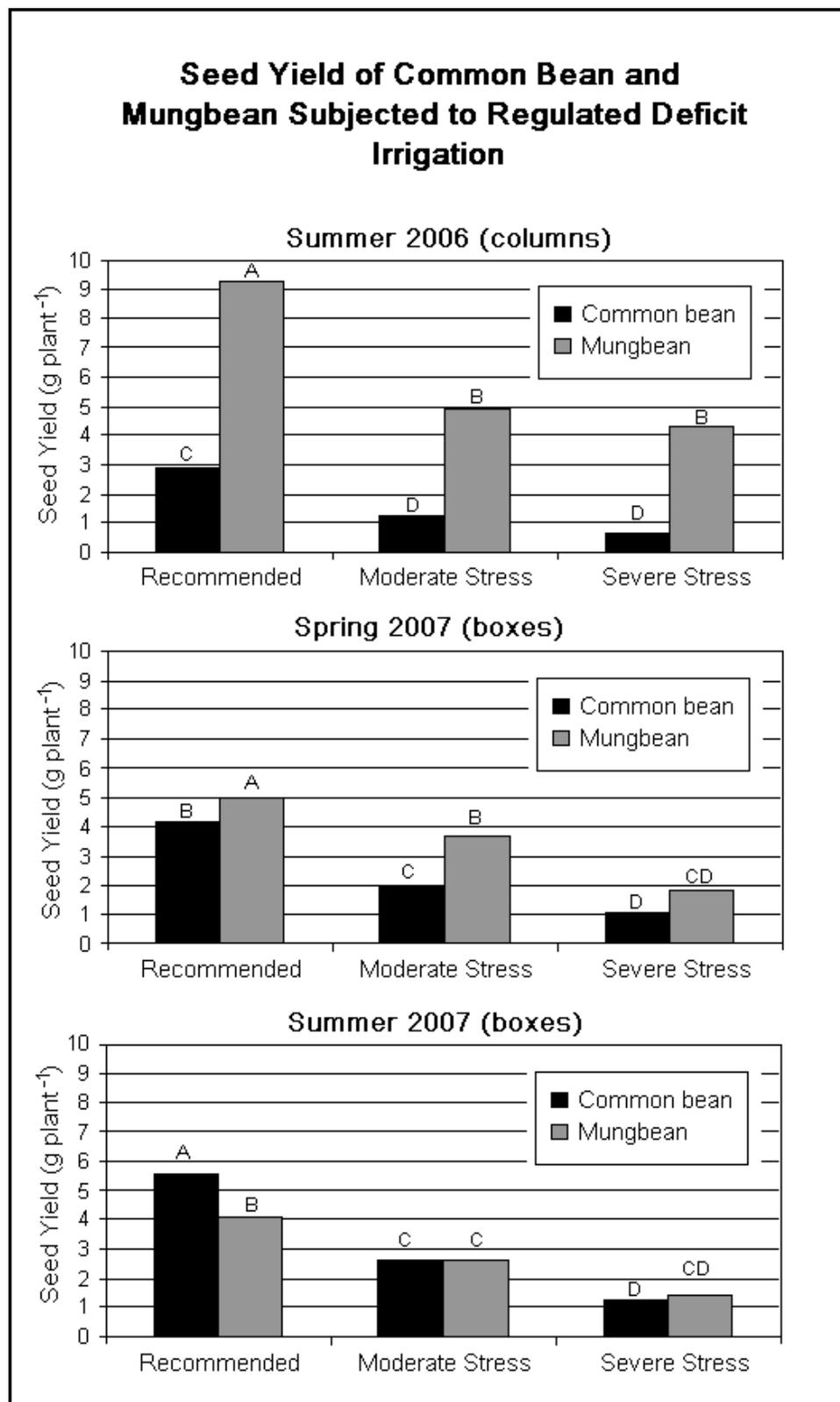
levels and, as such, both crops seem to be similarly affected by salts and RDI does not seem to affect this response to salt. As for transpiration, while univariate analyses of individual dates only detected a decrease in transpiration on May 8th, the repeated measures multivariate analysis detected significant salt effects, but again, no interactions.

While improvements in drought tolerance should, in theory, benefit crops growing in saline conditions, at least in the initial phase, our results seem to indicate that although salt and drought have similar effects, they may not trigger the same adaptive mechanisms in legumes.

5.5 Conclusions

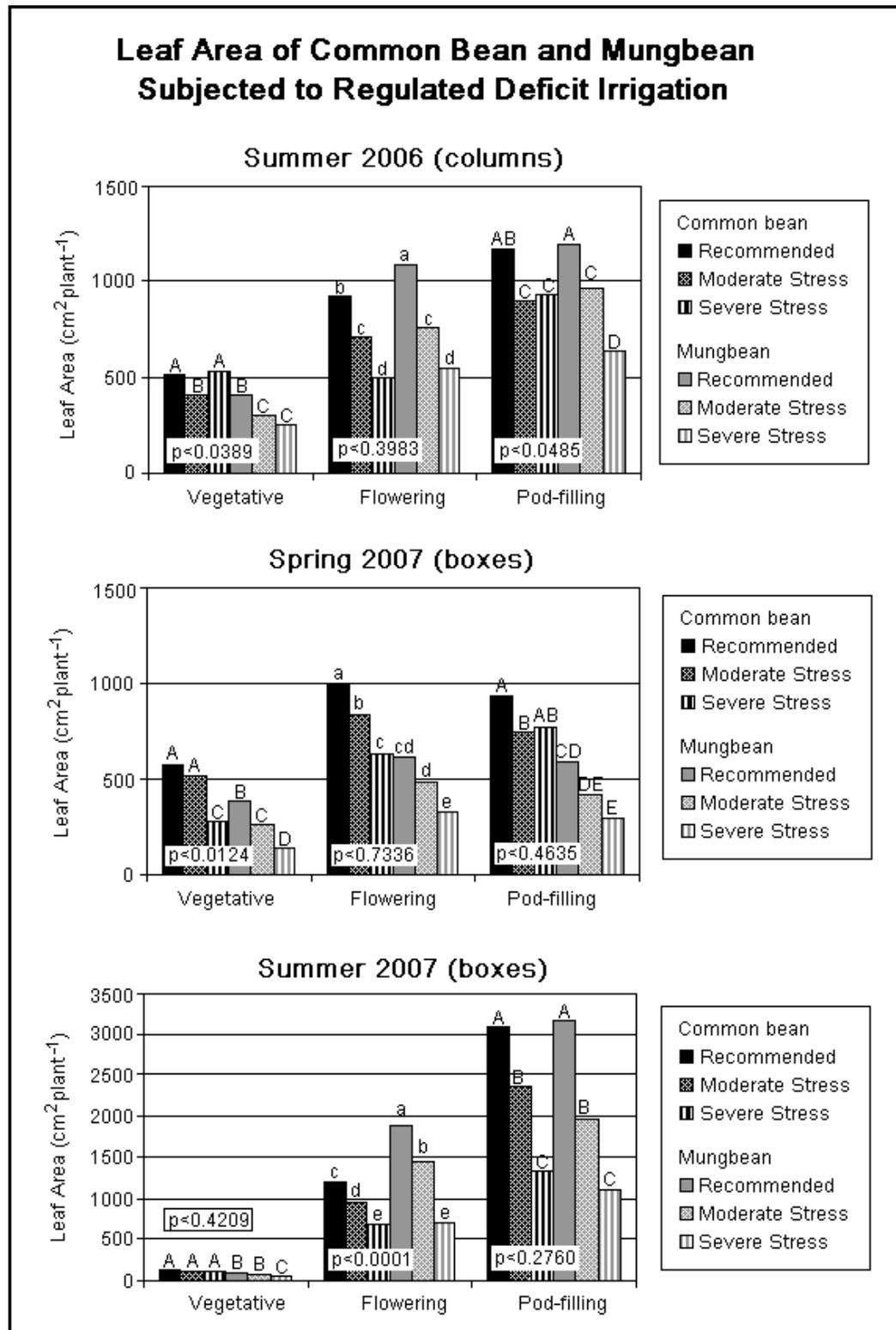
Our results demonstrated that mungbean is better adapted to semi-arid and arid climates. However, mungbean's reputation for drought resistance (Lawn and Ahn, 1985) has little to do with tolerance of low soil water potential. Instead, the response to water deficit stress might rely primarily on mechanisms that allow higher water uptake or that conserve water. In this study, we demonstrated that mungbean is able to maintain its harvest index under water deficit, whereas common bean is not able to do so. Mungbean also maintained higher photosynthetic rates than common bean at the same moisture level or the same transpiration rate. More research is needed to elucidate the higher photosynthetic capacity or higher transpiration efficiency exhibited by mungbean. At the same time, although the Uzbek common bean landrace potentially exhibits more drought tolerance than most cultivars of this species, there might be genetic variability in common bean germplasm, especially in landraces from dry areas, in the traits discussed above.

Figure 5.1: Yield of common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.



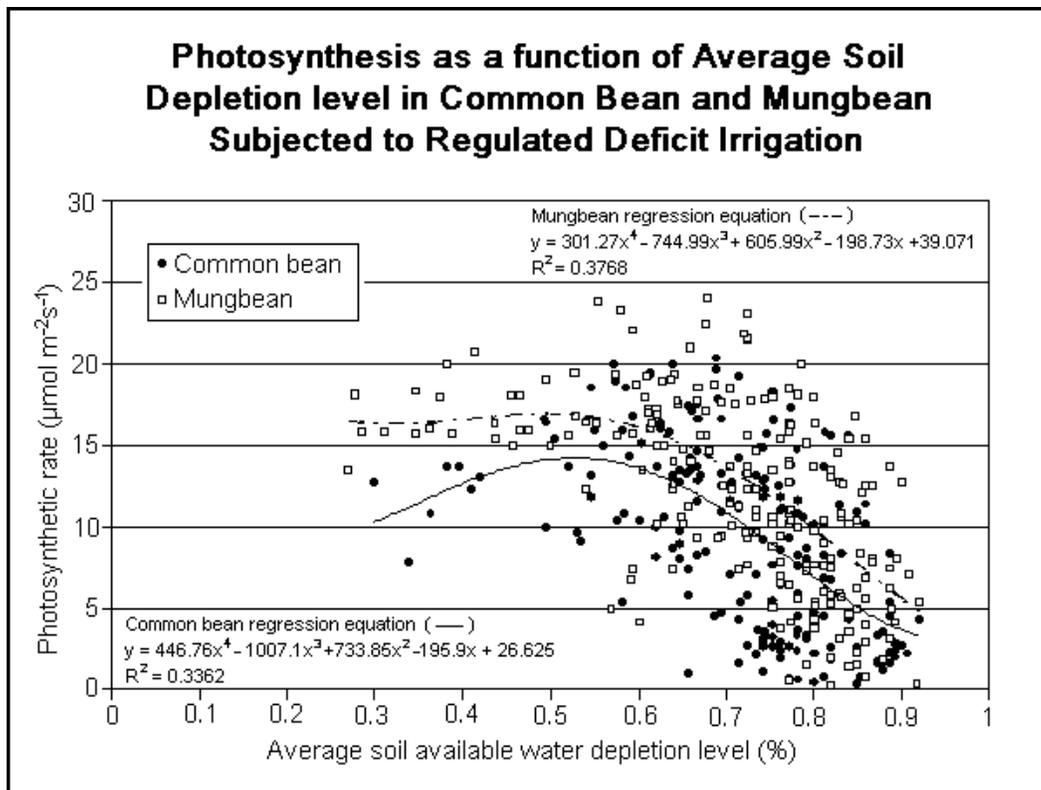
Histogram bars with the same letter are not significantly different from each other by t-tests of least squares means at $p \leq 0.0033$ (Bonferroni correction for 0.05 significance level) within each experiment. Values represent averages over at least four blocks.

Figure 5.2: Leaf area of common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.



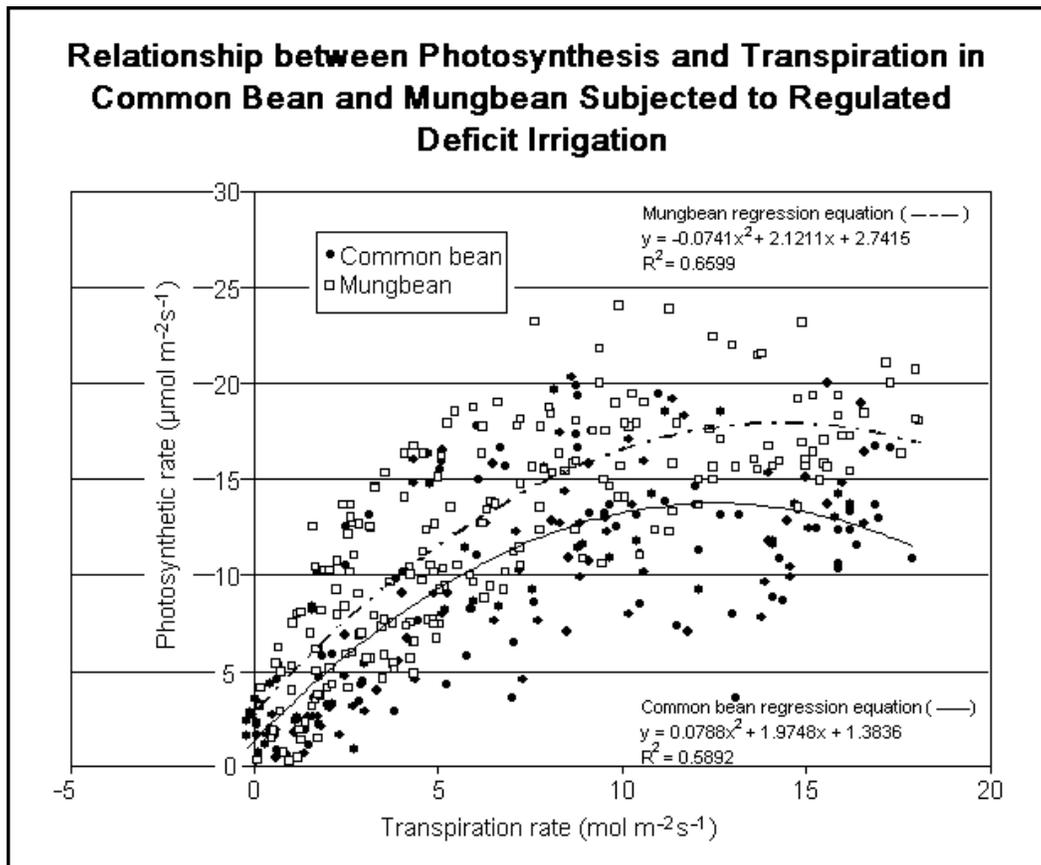
Histogram bars with the same letter in the same sampling are not significantly different from each other by t-tests on least squares means at $p \leq 0.0033$ (Bonferroni correction for 0.05 significance level). Values represent averages over at least four blocks. Crop by RDI interactions p-values are presented on the figure for each test. These are not always significant, but are presented for ease of comparison.

Figure 5.3: Photosynthesis as a function of average soil available water depletion level in common bean and mungbean subjected to regulated deficit irrigation in a controlled environment.



The repeated measures multivariate analysis detected a strong crop by RDI interaction ($p \leq 0.0001$) for photosynthesis.

Figure 5.4: Relationship between photosynthesis and transpiration in common bean and mungbean subjected to regulated deficit irrigation.



The repeated measures multivariate analysis detected a strong crop by RDI interaction ($p \leq 0.0001$) for transpiration efficiency (or photosynthesis rate divided by transpiration rate).

Table 5.1: Yield components of common bean and mungbean subjected to regulated deficit irrigation.

Crop	Regulated deficit irrigation (RDI) treatment	Number of pods per plant	Number of seeds per pod	100-seed weight (g)
Common bean	Recommended schedule	5.1 b	3.0 c	27.9 a
	Moderate deficit	3.1 c	2.6 c	25.0 ab
	Severe deficit	1.9 c	2.4 c	23.5 b
Mungbean	Recommended schedule	6.9 a	9.2 a	6.1 c
	Moderate deficit	5.0 b	8.4 ab	6.2 c
	Severe deficit	3.1 b	7.4 b	6.0 c
Significance of crop by RDI interaction		0.0532	0.0532	<0.0001

Values with the same letter in the same parameter are not significantly different from each other by t-tests of least squares means at $p \leq 0.0033$ (Bonferroni correction for 0.05 significance level). Values represent averages over the spring and summer 2007 experiments.

Table 5.2: Proportion of biomass allocated to reproductive structures and harvest index in common bean and mungbean subjected to water stress.

Crop	Regulated deficit irrigation (RDI) treatment	Proportion allocated to reproductive structures (%)			Harvest index
		Summer2006	Spring 2007	Summer 2007	
Common bean	Recommended schedule	32.1 a	38.5 a	50.4 a	48.2 a
	Moderate deficit	31.4 a	36.4 a	39.6 b	28.4 b
	Severe deficit	11.2 b	21.3 b	40.3 ab	18.2 b
Mungbean	Recommended schedule	40.2 a	40.2 a	26.0 c	33.4 ab
	Moderate deficit	42.3 a	30.1 ab	20.8 c	31.4 b
	Severe deficit	42.3 a	38.0 a	26.1 c	27.8 b
Significance of crop by RDI interaction		0.0188	0.0012	0.1997	0.0108

Values with the same letter in the same parameter are not significantly different from each other by t-tests of least squares means at $p \leq 0.0033$ (Bonferroni correction for 0.05 significance level). Values of harvest index represent averages over the spring and summer 2007 experiments.

Preface to chapter 6

As discussed previously (preface to chapter 5), water stress treatments in controlled-environment experiments are more difficult to impose with accuracy as the volume of soil is restricted and changes in soil moisture can occur quite rapidly. We hypothesized that the mungbean and common bean might have different optimum moisture regimes, but that this optimum was not obvious because of the limited number of water stress treatments imposed in the previous experiments. In order to acquire a greater understanding of mungbean and common bean responses to water stress, we performed an experiment with a wider range of water stress treatments, from quasi waterlogged conditions to a complete lack of watering after emergence.

The experiment was performed once at Macdonald campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada, and again at the Hermitage research station, Warwick, QLD, Australia. I was indeed fortunate enough to have the unique opportunity to meet and work with one of only two internationally recognized mungbean breeding programs. At the Macdonald campus, we used the same germplasm as used in the field study (chapter 3) and the previous controlled-environment study (chapter 5). Because of quarantine restrictions in Australia, these lines could not be used in the timeframe required for the repetition of this experiment at Hermitage. Therefore, local commercial cultivars were used. Both the Uzbek landraces and the commercial cultivars used in Australia have been selected under hot and dry environments, and thus, might exhibit greater drought tolerance than most cultivars of their respective species.

Differences at the flowering stage are clear between the two crops, in that common bean had greater biomass production (and greater water

consumption), and consequently a greater decrease from decreasing watering frequencies. This study highlighted the conservative water use of mungbean, and suggests there might be an intrinsic limitation to leaf area production that allows the crop to maintain sufficient soil water for use during pod-filling. In addition, mungbean demonstrated a limitation to maximum transpiration following watering events, which might translate into higher water use efficiency over the complete season.

The following manuscript was submitted to Crop Science in October 2008. Prof. Donald L. Smith, my supervisor contributed to the paper by meaningful advice in the design stage of the experiment and provided constructive feedback and editorial assistance on the manuscript.

6. COMPARATIVE STUDY OF COMMON BEAN (*Phaseolus vulgaris* L.) AND MUNGBEAN (*Vigna radiata* (L.) Wilczek) RESPONSE TO SEVEN WATERING REGIMES IN A CONTROLLED ENVIRONMENT

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6.1 Abstract

Legume crops are often grown in drought-prone areas, and subjected to water stress. Unfortunately, mechanisms of drought tolerance in legumes have not been as extensively researched as in cereal crops. An experiment was conducted to determine the response of two legume crops, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek), to watering regimes ranging from near waterlogging to extreme water stress. Soil moisture was determined before and after each watering event, and gas exchange measurements were collected before and after selected events. Plants were harvested at flowering for the determination of leaf area, biomass, relative water content, and water potential determination. Results demonstrate that mungbean is able to maintain higher relative water content when compared to common bean, especially at lower soil moisture levels, but not through a greater capacity for osmotic adjustment. Mungbean also showed lower transpiration rates the day after watering, and has an overall more conservative water use behaviour than common bean.

6.2 Introduction

Legume crops are often grown in drought-prone areas and subjected to water stress. Unfortunately, despite their importance in peoples' diets, especially in the developing world, legumes and their mechanisms of drought tolerance have not been as extensively researched as cereal crops (Turner et al., 2003). This is unfortunate as legumes play a vital role in sustainable agricultural systems because of their nitrogen-fixing symbiosis with rhizobia (Subbarao et al., 1995; Hedley, 2001). As importantly, legumes often fit in as second crops, and can contribute to poverty alleviation when smallholding farmers have access to markets (Sachs, 2005; Polak and Yoder, 2006; Sanchez et al., 2007). Thus, a greater understanding of their response to drought and investments in breeding programs for better drought tolerance in legumes would be likely to provide high returns.

Part of the challenge in adequately describing crops' responses to water deficit stress is the complexity of 'drought' as an environmental stress. An agricultural drought which decreases crop yields substantially is a combination of atmospheric conditions, soil moisture conditions, and crop conditions: high temperature and high vapour pressure deficit are combined with low soil moisture and increased soil hardness, and the stress experienced is further affected by the crop development stage and crop planting density (Begg and Turner, 1976; Thomas, 1997; Turner, 2003). In addition, rainfall in semi-arid areas is characteristically unpredictable (Erskine, 2003). Thus, drought is not only complex, but both timing and intensity of the water deficit stress experienced by the crop can be important and highly variable.

In irrigation research, this climate variability makes field results difficult to compare among studies and locations. Furthermore, the number of

irrigation treatments are often limited due to the cost of field studies, and the responses of crops can vary with the irrigation method used (sprinkler versus surface irrigation for example – see discussion in chapter 3). In breeding programs, this climate variability leads to significant and important genotype by environment interactions, which can slow down progress in developing drought tolerant cultivars (Chapman et al., 2002). All of these are challenges to the determination of the crop responses to water stress, and the identification of mechanisms of drought tolerance. Further, a universal measure of plant water status is not available; at this time, soil moisture and leaf relative water content seem to be the most appropriate measurements to describe the stress experienced by a crop (Jones et al., 2007).

Common bean is a very diverse pulse crop, with approximately 500 varieties. It has been domesticated independently in the Andes and in Central America (Polhill and van der Maesen, 1985), possibly from distinct wild progenitors within these same areas (Miklas and Singh, 2007). It is the most important pulse crop in the world, being eaten directly more than any other legume (Hedley, 2001; Broughton et al., 2003). It is particularly important in Latin America where it is a key part of national diets, but it is also widely cultivated throughout the world (Adams et al., 1985). Common bean is not noted for its drought tolerance, yet recent studies suggest that only 7% of the growing area receives adequate rainfall (Broughton et al., 2003), and 60% of the production occurs under severe drought stress (Graham and Ranalli, 1997). Mechanisms of drought tolerance identified from comparative studies include higher membrane stability (Costa Franca et al., 2000), higher transpiration efficiency (Costa Franca et al., 2000; Lizana et al., 2006), a more sensitive stomatal conductance response to soil drying (Markhart, 1985; Smith et al., 1988; Lizana et al., 2006) and osmotic adjustment (OA) (Costa Franca et al., 2000; Zlatev, 2005). However, the opposite (a lack of OA, and the maintenance of stomatal

conductance in tolerant cultivars) has also been documented (Markhart, 1985; Costa Franca et al., 2000).

Mungbean, also known as green gram, is a small-seeded crop less known in the Americas, but widely cultivated in Asia (Lawn and Ahn, 1985; Hafeez et al., 1991; Poehlman, 1991). It is also cultivated to some degree in the United States and in Australia, and is often consumed as sprouts (Lawn and Ahn, 1985; Poehlman, 1991). The crop is known as one that performs well under conditions of low soil moisture. It remains, however, one of the least researched and most under-exploited legume crops (DeCosta et al., 1999). It has been suggested that mungbean's reputation as a drought tolerant crop (Lawn and Ahn, 1985) is due to rapid maturity compared to other legumes, and thus that it manifests drought escape rather than dehydration avoidance or tolerance mechanisms (Pandey et al., 1984; Senthong and Pandey, 1989). However, others have suggested that OA and higher transpiration efficiency under water stress might be playing a role in mungbean's ability to cope with drought stress (Muchow, 1985b; Haqqani and Pandey, 1994b).

What is still lacking is a comprehensive investigation of the mechanisms of drought tolerance in legumes, and the determination of the relative importance of these mechanisms in sustaining yields under water stress (Ludlow and Muchow, 1990). Comparative studies between species allow the identification of possible adaptive traits that can then be further explored within species (Likoswe and Lawn, 2008). The objective of this work was to determine the response of common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek) plants to levels of water availability that ranged from quasi-waterlogged conditions, resulting from frequent watering to extreme water deficit stress, due to a complete lack of re-watering after seedling establishment. Particular emphasis was placed on determining the capacity for osmotic adjustment and gas exchange

responses to declining soil moisture.

6.3 Materials and methods

6.3.1 Experimental design

The experiment was organized according to a randomized complete block split-plot design with crops as main treatments, and seven watering regimes as sub-treatments. Each sub-treatment plot consisted of a row of five plants in individual pots. The experiment was performed in a greenhouse at the Macdonald campus of McGill University in Ste-Anne-de-Bellevue, Quebec, Canada from April 30th 2007 to May 28th 2007, and repeated at the Hermitage Research Station of the Department of Primary Industries and Fisheries at Warwick, Queensland, Australia from April 2nd to May 23rd, 2008.

6.3.2 Plant growth

In the experiment at McGill, pots of approximately 15 cm diameter and 20 cm depth, with an internal capacity of 7.5 L, were filled with a rich loam with a field capacity volumetric water content of approximately 50%, and a permanent wilting point of about 13%. Each pot had a plant available water content capacity of 2.8 L. Common bean and mungbean seeds used were pure landrace lines from Uzbekistan. Under field conditions, both crops were ready to harvest in 90 days (chapter 3). We have retained a sample of these seeds and they are available upon request. Pots were placed adjacent to each other with no space in between pots. Temperatures were set at 32/20°C day/night with a 16 h photoperiod. Humidity was not controlled and varied from 75 to 90%. Light intensity was on average 800 $\mu\text{mol m}^{-2}$, depending on outside conditions, but was minimally 600 $\mu\text{mol m}^{-2}$.

At Hermitage, pots of approximately 15 cm diameter and 18 cm depth, with an internal capacity of 6.4 L, were filled with a mix of sand, peat, and sandy loam at a ratio of 3:4:3. This mix had a field capacity volumetric water content of approximately 50%, and a permanent wilting point of about 9%. Each pot had a plant available water content capacity of 2.6 L. Common bean and mungbean seeds used were commercial cultivars 'Spearfelt' and 'White Gold' respectively. These exhibited a very similar growth habit to the Uzbek landraces, and all have evolved in semi-arid areas. During the day, temperatures were on average 32°C with a relative humidity of 40%, and during the night, there were on average 19°C with 63% relative humidity. The photoperiod was 12 h.

In both experiments, seeds were soaked in distilled water overnight and seeded directly into pots. Three seeds were placed in different holes of 4 cm depth per pot. The pots were then watered to field capacity. A week later, they were thinned to one plant per pot, and again watered to field capacity. Pots were subsequently watered according to their watering regimes.

6.3.3 Watering schedule

There were seven watering regimes: 1) watered five days a week (on week days); 2) watered three days a week (Monday, Wednesday, and Friday); 3) watered two days a week (Monday and Thursday); 4) watered once a week (Monday); 5) watered once every two weeks; 6) watered once every three weeks; and 7) never watered after watering at thinning. The pots were watered with 0.5 to 3.5 L depending on the water treatment, and stage of growth. This was to ensure containers were brought back to saturation, and the excess water was allowed to drain from pots.

6.3.4 Measurements

Evapotranspiration (ET) was calculated from soil moisture measurements taken before and after each watering event, or every seven days at most, with a FieldScout TDR 100 (Spectrum Technologies, Plainfield, IL, USA). Gas exchange measurements were taken with a LI-COR 6400 (LICOR Biosciences, Lincoln, NE, USA) on the topmost fully expanded trifoliolate central leaflet before and after selected watering events. At McGill, this was done on May 26th 2007, or 31 days after seeding DAS). At Hermitage, measurements were taken on April 28th and 29th, and May 5th, 6th, 19th and 20th 2008, or 21, 22, 35, 36, 42 and 43 DAS.

Plants were harvested 31-33 DAS at McGill, and 44-45 DAS at Hermitage. The experiment was lengthened the second time to allow for more data collection. Leaf area was determined on two out of five plants per plot, and the samples were dried at 60 °C for 72 hours, or until constant weight, for biomass dry weight determination. The center leaflet of the top-most trifoliolate of two remaining plants were used for water potential determination. Leaf samples were immediately frozen and stored at -20 °C until ready to be processed in the water potentialmeter (WP4-T, Decagon devices, Pullman, WA, USA). The other two leaflets of the trifoliolate used for water potential determination were harvested for the determination of relative water content (RWC) as described by Turner et al. (2007a). Briefly, leaf samples were collected and weighed, then placed on distilled water at 4°C for 8 hours, weighed again, and dried at 60°C for 48 h. The RWC was calculated according to the following formula:

$$\text{RWC} = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})}$$

The leaf water potential at full turgor, an indirect measure of the solute accumulation, was calculated by the product of the measured leaf water potential and the RWC (Turner et al., 2007a).

$$\text{LWP}_{\text{FT}} = \text{LWP} \times \text{RWC}$$

This removes the confounding effects of a lower water potential due to a passive concentration of solutes in tissues with lower water content. At Hermitage, we were not able to determine the leaf area, RWC, and water potential for the most severe water stress treatment (not re-watered after seedling establishment), as the plants had dried up and died by the time of sampling.

6.3.5 Statistical analyses

Results were analysed with the SAS/STAT software (SAS, Cary, NC, USA) using the GLM procedure for analyses of variance (ANOVA). Multivariate analyses of variance (MANOVA) with repeated measures were also performed for photosynthesis, transpiration and soil moisture data. When trends and values were similar for both repetitions of the experiment for the same variables, data were pooled into the same statistical analysis to be presented in figures. Treatment effects and interactions were considered statistically significant when they occurred at the 0.05 level of probability. If fixed main effects or a fixed interaction were found to be significant in the ANOVA, then means separations were carried using t-tests on least squares means. To control the experimentwise error rate within the means separation t-tests, the Tukey-Kramer's adjustment was used (Milliken and Johnson, 1984).

6.4 Results

6.4.1 Water consumption

The water consumption, or ET, shows that mungbean water use was consistently lower than common bean in the four most frequent watering regimes. The fact that plants watered five times a week did not consume as much water as those watered three times or twice a week suggests they experienced some level of stress from soil kept between saturation

and field capacity (Table 6.1).

6.4.2 Leaf area and biomass

At flowering, both leaf area and biomass data showed a highly significant ($p < 0.0001$) crop by watering regime interaction where common bean showed a proportionally greater decrease with decreasing watering frequencies. Mungbean, on the other hand, developed about half as much leaf area as common bean across watering regimes except for the more extreme water stress regimes (Table 6.1). Again, the stress of soil kept between saturation and field capacity was evident by the lower leaf area and biomass accumulation, and was proportionally more important in common bean.

The efficiency which each crop used water for leaf area development is different between common bean and mungbean ($p < 0.0001$; data not shown). Common bean had a higher efficiency when calculated as the ratio of leaf area to ET: $0.228 \text{ cm}^2 \text{ mL}^{-1}$ of water ET versus $0.136 \text{ cm}^2 \text{ mL}^{-1}$ of water ET in mungbean. However, when considering the efficiency of water use for biomass accumulation, both crops were equally efficient (data not shown). This, along with an analysis of the ratio of leaf area over total biomass ($p < 0.0001$), suggests that mungbean either had thicker leaves or had partitioned more biomass into stems or roots, rather than leaves.

6.4.3 Water potential and relative water content

Relative water content also showed a highly significant crop by watering regime interaction, and mungbean maintained a high RWC, especially in the severe water deficit stress treatments (Table 6.1). There were no differences in water potential at full turgor between the two crops (Table

6.1), but there was a highly significant watering regime effect ($p < 0.0001$). This suggests that both crops accumulate solutes in leaf cells during water deficit stress, but whether or not this process is an active one, as opposed to an accumulation due to lower growth rates, is not clear.

6.4.4 Gas exchange measurements

Before watering events, photosynthetic rates in mungbean were generally higher than in common bean. This was supported by a crop by watering regime interaction ($p < 0.0001$) found on May 5th, 2008 (35 DAS), and by the multivariate repeated measure analyses performed on the data from the Hermitage experiment (data not shown). On May 5th, mungbean maintained higher photosynthetic rates than common bean in the treatments watered once a week, and once every two weeks (Figure 6.1). The significance level of differences between the two crops in the treatments watered once every three weeks, and not re-watered were $p \leq 0.0539$, and $p \leq 0.0748$ respectively. A similar trend was observed on May 26th, 2007 at McGill when mungbean had higher photosynthetic rates in the treatments watered every two weeks and every three weeks when compared with t-tests on least squares means using the Tukey-Kramer adjustment (data not shown). However, the crop by watering regime interaction was not quite significant ($p \leq 0.0662$) in this test.

Photosynthetic rates the day after watering events were generally not different between the two crops, or between watering regime treatments, suggesting that the photosynthetic apparatus had not been damaged by the water deficit experiences prior to re-watering (data not shown).

Transpiration rates data before watering events also showed significant crop by watering regime interactions with trends similar to photosynthesis data (data not shown). After watering events, transpiration rates tended to

be lower in mungbean compared to common bean, as illustrated by crop by watering regime interactions on April 29th ($p \leq 0.0006$), and May 6th ($p < 0.0001$), and the multivariate repeated measure analyses performed on the data from the Hermitage experiment. On May 6th, this was especially obvious in the more frequent watering regimes (Figure 6.2).

In some cases, measurements of instantaneous transpiration efficiency (TE; from the ratio of photosynthetic rate to transpiration rate on the same leaf) showed significant crop and/or watering regime effects. On May 5th, TE was higher in the treatment watered once every three weeks ($p \leq 0.0014$), which at this point had not been rewatered yet (data not shown). On May 6th, TE was higher in mungbean than in common bean ($p \leq 0.0154$; data not shown); TE was also the highest in the treatment watered once every two weeks, which had not been rewatered the day before, and second highest in the treatment watered once every three weeks ($p \leq 0.0003$), watered the day before (data not shown). This however is not consistent in all days of gas exchange measurements, and low values of both photosynthesis and transpiration rates in greater stress treatments might overestimate TE.

6.5 Discussion

The leaf area, biomass, and ET data suggest that it is not the availability of water that restricts mungbean growth, but rather that some intrinsic mechanisms might limit its leaf area expansion. This, in turn, would reduce transpiration losses, and thus ET, and might represent constitutive mechanisms of dehydration avoidance (as defined in Jones et al., 1981). Specifically, we suggest mungbean exhibits a more conservative water use when compared to common bean. If grown on residual soil moisture, this would allow the retention of enough water in the soil profile at flowering for use during pod-filling. In a field experiment, we have

demonstrated that while mungbean showed lower leaf area and biomass in the vegetative stage, it considerably outgrew common bean at the pod-filling and harvest stages (chapter 3). Thus, mungbean can develop a large leaf area and accumulate considerable biomass, but might only do so if it has enough water by flowering.

The argument for more conservative use of water by mungbean is further supported by the lower ratio of leaf area per amount of biomass accumulated when compared to common bean. We suggested earlier that this might translate into thicker leaves, or greater mobilization of resources to stems and roots.

Thicker leaves have been associated with higher TE in other species (Wright, 1993 in peanut; Brown and Byrd, 1997 in peanut and pearl millet; Byrd and May, 2000 in switchgrass). Results of previous experiments have shown that mungbean specific leaf weight tends to increase with increasing water deficit stress (De Costa and Shanmugathan, 1999; chapter 5). However in this study, crop differences in TE were only detected on one day (out of seven days of gas exchange measurements), and there were no crop by watering regime interactions. Therefore, we conclude that when TE increased with increasing water deficit stress (which again, did not occur consistently), it did so similarly in both common bean and mungbean.

Greater mobilization of resources to stems might allow for larger reserves, and greater remobilization to leaves or seeds, which ultimately might help in the maintenance of the harvest index observed for mungbean under field conditions (chapter 3). Greater mobilization of biomass to roots would have allowed greater water extraction if plants were not grown in pots, but would then have led to greater ET. A conservative water use might be associated with lower root hydraulic conductance, or a smaller

root system (Passouira, 1981; Passouira, 1983). However, given the comparatively low biomass and leaf area in mungbean during the vegetative stage, even a relatively small root system might translate into a greater root-to-shoot ratio. This would balance transpiration losses with a capacity for water extraction without exhausting the water stored in the field. All three mechanisms would be worth further investigation in cultivars of these two crops, but particularly in mungbean.

We have presented data that show mungbean maintains RWC in leaves under severe water stress. This appears to be common to relatively drought tolerant species of the genus *Vigna*: Cruz de Carvalho et al. (1998) showed that cowpea (*Vigna unguiculata*) maintained higher RWC when compared to common bean; Likoswe and Lawn (2008) compared cowpea, pigeon pea, and soybean, and also showed that cowpea maintained higher tissue water content for the longest time when grown with plants of the same species.

We also reported here a decrease in leaf water potential (LWP) with decreasing watering frequencies. This is contradictory to our previous results in the field (chapter 3), and in a controlled environment (chapter 5), where we failed to detect such decrease in LWP. It is unclear whether this was a result of a passive accumulation of solutes due to lower growth rates, or a result of a fundamental shift in metabolism involving the active accumulation of solutes. Either way, the lowering of LWP in common bean has not led to the maintenance of RWC; whether the decrease in LWP is sufficient to account for the maintenance of RWC in mungbean is doubtful.

For both legumes, it would indeed be interesting to determine the nature of the solutes accumulated, and their potential role in adaptation to water deficit stress. In addition, environmental conditions might affect the

capacity for OA, and these could be investigated further. Finally, unknown cellular mechanisms or cell wall characteristics, not related to the lower LWP *per se*, might contribute to the maintenance of RWC. This might be linked to the higher photosynthetic rates in mungbean under some levels of stress observed in this study, or might be associated with better recovery after stress.

We have demonstrated that mungbean exhibits lower transpiration rates compared to common bean after watering events, and this might be at least partly responsible for the lower ET observed. It did not, in this study, show a more responsive stomatal closure to declining soil moisture. A cap on maximum transpiration has recently been proposed as a water conservation mechanism in 'slow-wilting' soybean genotypes (Fletcher et al., 2007). Furthermore, the imposition of a maximum transpiration rate in a simulation study of sorghum showed that yields could be increased by 9 to 13% in low-yielding years (Sinclair et al., 2005). Such a mechanism would be beneficial in semi-arid environments, especially if crops are frequently subjected to terminal droughts.

We also reported one instance where mungbean has shown significantly greater instantaneous TE when compared to common bean. This is consistent with our previous data comparing these two crops (chapter 5), and with results presented in an independent study (Haqqani and Pandey, 1994a). As mentioned above, the very low levels of photosynthesis and transpiration observed in the more severe water deficit stress treatments make it difficult to properly evaluate differences between watering regimes and crops. The use of the carbon isotope discrimination method might have provided better estimates (Farquhar et al., 1982). Further, through this method, Ryan and Lambrides (personal communication) have found unprecedented genetic variability in mungbean germplasm.

Thus, mungbean appears to exhibit a conservative water use in order to retain enough water in the soil profile at flowering for use during pod-filling, which would be particularly suitable to crops grown on residual soil moisture without supplemental irrigation. This might be an indication of historical growing conditions in mungbean. Common bean, on the other hand, seems to consume water as long as it is available, and wilts quickly once it has exhausted water stored in the soil. In addition, common bean has historically been grown in intercropping systems (Adams et al., 1985), and rapid water extraction might have evolved to enable the crop to compete effectively for water.

Finally, our results also demonstrated the challenges associated with describing water stress and plant water status with plant-based indicators. For example, RWC was maintained for mungbean even for plants growing (or surviving) in soils at 80 to 90% depletion for most of their growth. We agree with Cruz de Carvalho et al. (1998) that RWC alone cannot be used to determine plant water deficit stress level. It might however serve as an integrative measurement of dehydration avoidance capacity. Leaf water potential has captured some of the treatment differences but has not proven accurate enough to detect differences between treatments which considerably affected leaf area and biomass accumulation. Relative water content requires destructive samplings, and LWP requires RWC measurements, so that regular monitoring is not possible without eventually affecting the growth of the plant. Under the conditions of this experiment, ET along with leaf area and biomass appear to be the best indicators of water deficit stress treatments. Gas exchange measurements were also relatively good indicators of plant water status, but also tend to be affected by other environmental conditions, and as such are not recommended to compare measurements taken on different days. In addition, instantaneous measurements of TE from the ratio of

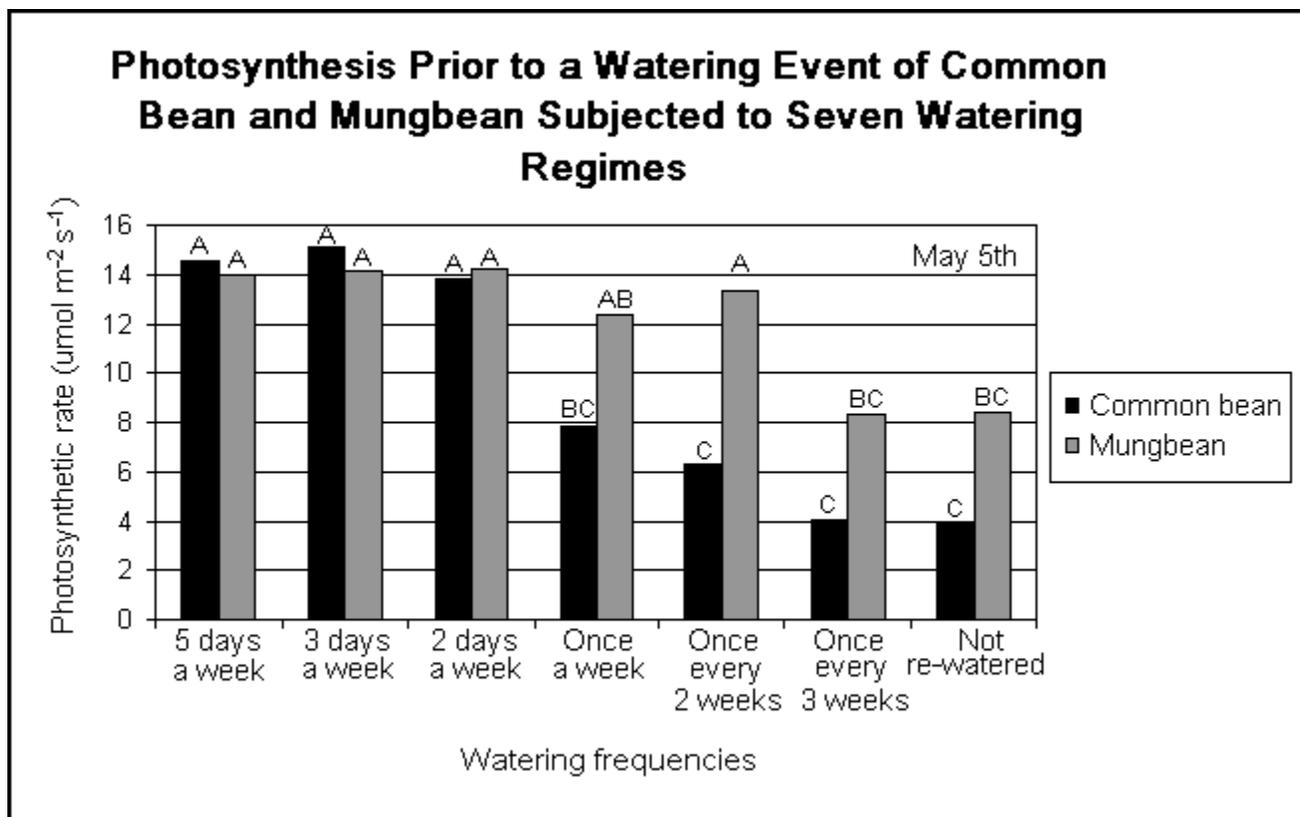
photosynthetic to transpiration rates in severe water deficit stress treatments might not be accurate.

We suggest that a minimum data set for studies trying to determine mechanisms of drought tolerance in crop plants under the conditions of this experiment should include the regular monitoring of soil moisture, gas exchange measurements before and after watering events, as well as a destructive harvest at flowering to determine leaf area, above-ground biomass, RWC and LWP. Ideally, root biomass and other characteristics should also be measured. This could also be used to screen various germplasm lines in a breeding program, although the number of lines might be limited by gas exchange measurement capacity.

6.6 Conclusion

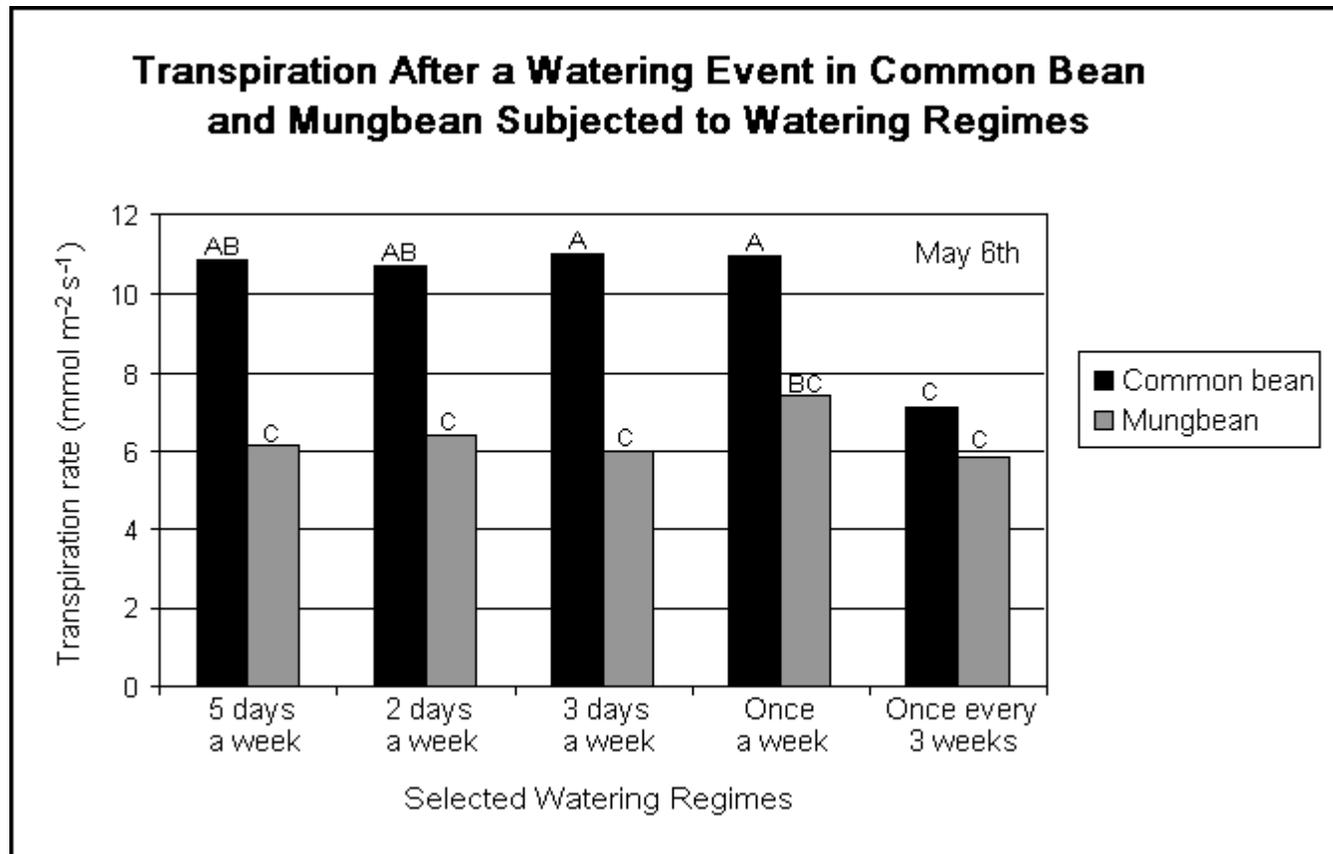
This comparative study between common bean and mungbean showed that mungbean exhibits a more conservative use of water by limiting leaf area development and biomass accumulation. Mungbean also showed a better dehydration avoidance capacity than common bean by maintaining higher relative water content, especially at lower soil moisture levels. Contrary to current theory regarding drought tolerance mechanisms, this does not seem to be achieved through more responsive stomatal closure to declining soil moisture. The contribution of osmotic adjustment is equally inconclusive. Rather, mungbean seems to limit maximum transpiration rates after watering events when compared to common bean, again suggesting a conservative water use.

Figure 6.1: Photosynthetic rates prior to a watering event in common bean and mungbean subjected to seven watering regimes at Hermitage Research Station, Warwick, Australia.



Histogram bars with the same letter are not significantly different from each other by t-tests on least squares means at the 0.05 level of significance using Tukey-Kramer's adjustment. Values represent averages over four blocks taken on May 5th (35 DAS). The treatment watered once every two weeks had been watered the previous week, and the treatment watered once every three weeks had not been watered yet, but was watered later this same day.

Figure 6.2: Transpiration rates after a watering event in common bean and mungbean subjected to seven watering regimes at Hermitage Research Station, Warwick, Australia.



Histogram bars with the same letter are not significantly different from each other by t-tests on least squares means at the 0.05 level of significance using Tukey-Kramer's adjustment. Values represent averages over four blocks taken on May 6th (36 DAS). The treatment watered once every three weeks had been watered the day before. The treatments watered once every two weeks and not re-watered are not presented because they were not watered the day before.

Table 6.1: Evapotranspiration, leaf area, dry biomass, relative water content, and osmotic potential of common bean and mungbean subjected to seven watering regimes.

Crop	Watering schedule	Evapotranspiration (mL)	Leaf area (cm ² plant ⁻¹)	Biomass (g plant ⁻¹)	Relative water content (%)	Water potential (MPa)
Common bean	5 days a week	6598 c	2191 bc	10.6 a	81.6 ab	-1.22 a
	3 days a week	9120 ab	2854 a	12.3 a	79.3 ab	-1.45 a
	2 days a week	10012 a	2375 b	10 a	78.3 ab	-1.55 a
	1 day a week	7769 bc	1905 c	7.9 ab	74.6 b	-1.54 a
	1 per 2 weeks	4669 d	976 de	4.6 cde	68.5 bc	-2.00 bc
	1 per 3 weeks	3728 de	641 def	3.3 def	63.3 c	-2.04 bc
	Not re-watered	2424 e	533 efg	2.4 ef	64.2 c	-2.28 bc
Mungbean	5 days a week	4189 d	846 def	5.8 bc	84.8 a	-1.56 a
	3 days a week	6364 c	1017 d	6.5 bc	85.4 a	-1.52 a
	2 days a	6509 c	974 de	6.0 bc	82.1ab	-1.60 ab

	week					
	1 day a week	6321 c	778 def	5.0 cd	81.0 ab	-1.65 ab
	1 per 2 weeks	4121 d	464 fg	3.3 def	80.0 ab	-2.30 bc
	1 per 3 weeks	3434 de	405 fg	3.1 def	80.4 ab	-2.57 c
	Not re-watered	2289 e	229 g	2.0 f	74.9 b	-2.28 bc
Significance of crop by watering regime interaction (p value)		<0.0001	< 0.0001	< 0.0001	0.0019	0.4644

Values with the same letter in the same parameter are not significantly different from each other by t-tests on least squares means at $p \leq 0.05$ after adjustment with Tukey-Kramer's method. Values represent averages of data pooled over the McGill and Hermitage experiments. Crop by watering regime interactions are not always significant, but are presented for ease of comparison.

Preface to Chapter 7

In chapter 3, we established that common bean and mungbean could be grown in semi-arid areas under moderate regulated deficit irrigation (RDI), and that mungbean particularly, yielded best under the moderate level of RDI in 2003, and the severe level of RDI in 2004. Controlled-environment studies were performed to determine the mechanisms of tolerance to water deficit stress, and the lack of consistency in yield responses between the field (chapter 3) and pot experiments (chapter 5) hinted at a potentially important role for root systems. The experiment described in the next chapter was performed in order to determine the early growth patterns of root development in common bean and mungbean. We hypothesized from field observations, and from data presented in chapter 6, that mungbean's lack of early vigour (but considerable leaf area development after flowering) might be attributable to an important allocation of photosynthates to roots prior to development of substantial leaf area.

The experiment was performed once at Macdonald campus of McGill University, Ste-Anne-de-Bellevue, QC, Canada, and an additional three times at the Hermitage research station, Warwick, QLD, Australia. As mentioned previously (preface to chapter 6), I was fortunate enough to have the unique opportunity to work with one of only two internationally recognized mungbean breeding programs. This has also allowed me to have access to germplasm that would not have been available otherwise. At Macdonald campus, we used the same germplasm as used in the field study (chapter 3) and controlled-environment studies (chapters 5 and 6). Because of quarantine restrictions in Australia, these lines could not be used in the timeframe required for the repetition of this experiment at Hermitage. Therefore, both local commercial cultivars and Central Asian lines from the Australian Plant Genetic Resource Information Service were

used. All of these lines have been selected under hot and dry environments, and thus, potentially exhibit greater drought tolerance than most cultivars of their respective species.

With the WinRHIZO system, it is now much faster and much easier to analyse entire root systems, from the total root length, to root diameter classes, to the number of tips. Measurements that would otherwise be very time consuming, can now be conducted rapidly with the help of a computer software, as long as a complete root system can be obtained.

In this study, crop differences were slightly complicated by the different lines used. There might be important genetic variability in root traits in mungbean and common bean. However, over the four replications of the experiment and several genotypes, mungbean consistently had a significantly higher root-to-shoot ratio. In two of the three lines tested, mungbean also showed a higher root length for the same weight of biomass, indicating a higher efficiency of soil exploration. This chapter further examines root characteristics that might help crop plants cope with intermittent or terminal droughts.

The following manuscript is under preparation for submission as a note to Field Crops Research. Prof. Donald L. Smith, my supervisor, contributed to the paper by meaningful advice in the design stage of the experiment and provided constructive feedback and editorial assistance on the manuscript.

7. COMPARATIVE STUDY OF THE EARLY ROOT MORPHOLOGY IN COMMON BEAN (*Phaseolus vulgaris*) AND MUNGBEAN (*Vigna radiata* (L.) Wilczek)

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7.1 Abstract

Few studies related to drought tolerance have systematically looked at below-ground biomass because of the inherent difficulty associated with observing roots *in situ*. Yet, root development and architecture are probably involved in some of the most important components of plant response to water deficit stress. The objective of this experiment was to examine the early root development of two legume species, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek) that are known to differ in their responses to water deficit. Plants were grown in columns filled with sand in a controlled environment with temperatures of 32/20°C or 28/19°C day/night for twenty days. Roots were harvested by carefully washing down the sand from the bottom of the columns. They were then scanned and analysed with the WinRHIZO system. Results show that common bean consistently exhibited a greater root biomass and a greater number of tips per unit of root length. Mungbean, on the other hand, consistently demonstrated a higher root-to-shoot ratio. These rooting patterns are consistent with the hypotheses that common bean exhibits a competitive behaviour of early water extraction to compete effectively for water, while mungbean is more conservative, retaining water in the soil profile at flowering for use during pod-filling. However, there appear to be large differences between lines within the same crop

species, which might point to considerable genetic variability for root characteristics in these two crops.

7.2 Introduction

Approximately one billion people live in semi-arid and arid regions, and of these about 40% live below the poverty line (Erskine, 2003). The benefits of legumes in cropping systems have long been recognized. Their residues can improve the fertility and physical condition of the soil, due in great part to their ability to form a nitrogen-fixing symbiosis with rhizobia. In addition, they can also break disease cycles and encourage mycorrhizal associations (Subbarao et al., 1995). As the price of nitrogen fertilizer increases, they are considered increasingly profitable crops, because of the lower input requirements. Furthermore, in developing countries, diversification into so-called minor food crops such as legumes and access to markets is seen as particularly effective in helping the poor break the poverty trap (Polak, 2005; Sachs, 2005; Sanchez et al., 2007). Their productivity is however often limited in semi-arid and arid areas by intermittent or terminal drought (Subbarao et al., 1995), a situation that is likely to be exacerbated by increasing global water scarcity due to population growth and climate change, especially in developing nations (Ragab and Prud'homme, 2002). It thus becomes imperative that drought tolerance in legumes be better understood.

Some of the most important and yet least researched components of plant response to drought relate to root development and architecture (Passouira, 1983; Sponchiado et al., 1989; White and Castillo, 1989). A grafting experiment with common bean suggested that most of the variability in drought tolerance is attributable to root stocks (White and Castillo, 1989). Several studies observing root systems of legumes have shown that drought tolerant cultivars often have a greater root biomass, a

greater root-to-shoot ratio and deeper root profiles (Grzesiak et al., 1997 in common bean and pea; Matsui and Singh, 2003 in cowpea; Benjamin and Nielsen, 2006 in soybean, pea and chickpea; Kashiwagi et al., 2006 in chickpea). Root growth has also been shown to be responsive to water deficit. For example, De Costa and Shanmugathan (1999) showed that root weight and root-to-shoot ratio in mungbean were highest in the rainfed treatment and lowest in the fully irrigated treatment.

However, Passouira (1983) suggested the optimal resource allocation might not be towards the maximum root biomass, but rather to maximize the return on investment on carbon allocated to roots rather than leaves. Crops grown on residual moisture might deplete the soil water early and thus suffer from severe water stress at the reproductive stage (Huang, 2000). Indeed, a breeding retrospective in tropical maize showed that selection for high grain yield in eight cycles of selection resulted in a reduction of the root biomass in the top 50 cm of the soil profile (Bolanos et al., 1993).

In other studies comparing the response of common bean and mungbean to water deficit stress under field (chapter 3) and controlled-environment conditions (chapters 5 and 6), mungbean has exhibited greater drought tolerance than common bean, partly due to a more conservative use of water prior to flowering. We hypothesized that the lower ratio of leaf area to above-ground biomass in mungbean when compared to common bean might be the result of higher relocation of resources to roots (chapter 6).

Thus, the objective of this experiment was to examine early root development of two legume species, common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* (L.) Wilczek) that are known to differ in their response to water deficit.

7.3 Materials and methods

7.3.1 Experimental design

The experiment was organized following a completely randomized design with eight repetitions per crop per sampling. There were four sampling times: at 9, 13, 16 and 20 days after seeding (DAS). The experiment was performed once in a greenhouse at the Macdonald campus of McGill University in Ste-Anne-de-Bellevue, Quebec, Canada from July 25th to August 14th 2007, and three times at the Hermitage Research Station of the Department of Primary Industries and Fisheries at Warwick, Queensland, Australia from May 14th to June 3rd, June 11th to July 1st, and July 9th to 29th, 2008.

7.3.2 Plant growth and measurements

In the experiment at McGill, common bean and mungbean seeds used were pure landrace lines from Uzbekistan. We have retained a sample of these seeds and they are available upon request. Plants were grown in a greenhouse chamber set at 32/20°C day/night temperatures and a 16 h photoperiod. Humidity was not controlled and varied from 75 to 90%. In the experiments at Hermitage, one experiment was done using seeds from the Australian Plant Genetic Resource Information Service lines AusTRCF 306674 (common bean line from Kazakhstan) and AusTRCF 307920 (mungbean line from Uzbekistan). The experiment was also repeated twice with commercial common bean cultivar, 'Spearfelt', and mungbean cultivar, 'White Gold'. All lines used have been selected under hot and dry environments, and thus, might exhibit greater drought tolerance than most cultivars of their respective crop species. At Hermitage, temperatures were on average 28°C with a relative humidity of 45% during the day, and 19°C and 61% during the night.

In each experiment, one seed of the appropriate crop was placed in each plastic column (15 cm diameter and 55 cm depth, with cheese cloth at the bottom) which had been previously filled with fine sand. The columns were then watered to field capacity with water containing 3 g 20-20-20 fertilizer L⁻¹. Roots were harvested by removing the cheese cloth, and carefully washing down the sand with water from the bottom of the column. Above-ground biomass was cut from roots at the point where the stem changes color (about at the soil line). Roots were further washed to remove as much sand as possible and were subsequently scanned and analysed with WinRHIZO (Regent Instruments, Ste-Foy, QC, Canada). Images of typical scanned root systems are included in Figure 7.1.

Above-ground biomass and root biomass were determined from fresh weights because dry weights are too small to be measured with accuracy. Data obtained with the WinRHIZO system and reported here include total root length, total number of tips, as well as the length of root in diameter classes 0-0.2, 0.2-0.4, 0.4-0.6, 0.6-0.8, 0.8-1.0, 1.0-1.5, 1.5-2.0, 2.0-2.5, 2.5-3.0, 3.0-3.5, 3.5-4.0, 4.0-5.0, and greater than 5.0 mm. Diameter classes 0-0.2 and 0.2-0.4 were considered 'fine roots'.

7.3.3 Statistical analyses

Statistical analyses were performed by t-tests using proc TTEST in the SAS/STAT software (SAS, Cary, NC, USA). Differences between crops were considered significant only when they occurred at the 0.05 level of probability. In addition, correlation analyses were performed on root characteristics for each line using proc CORR.

7.4 Results

Except for the experiment performed at McGill, common bean exhibited a

greater total root length, and a higher proportion of fine roots compared with mungbean. It also consistently exhibited a greater root biomass, and a greater number of tips per unit of root length. Mungbean, on the other hand, consistently showed a higher root-to-shoot ratio. This was consistent at all sampling times. Due to space restrictions, however, only 13 DAS is presented in Table 7.1. In one case, at McGill, mungbean showed superior values to common bean in root biomass and the proportion of fine roots.

In most cases, and in both crops, larger root systems (represented by a higher root biomass and a higher root length) were positively correlated with a large root-to-shoot ratio, a large proportion of fine roots and a high length per unit of biomass (data not shown). Thus, in the first twenty days, both crops were allocating proportionally more resources to the root system than to the shoot, and producing a higher proportion of small roots as the system developed. However, the number of tips per unit length did not generally correlate with any of the above parameters, suggesting that if tips were particularly important for water uptake, then water uptake might not be a primary determinant of early root development.

Exceptions to this situation are found in the mungbean landrace tested at McGill where root biomass and total root length were, respectively, not correlated, and negatively correlated with the root-to-shoot ratio, suggesting that proportionally more resources were allocated to the shoot as the root system expanded. This particular line also showed a very high proportion of roots in the 0 to 0.4 mm diameter classes compared to the common bean landrace and to the other mungbean lines (Table 7.1). In addition, the common bean line AusTRCF 306674 showed a negative correlation between the number of tips per unit length and both total root length and the proportion of fine roots (not shown), suggesting the plant produced numerous thick branches, then extended them. Indeed, this

particular common bean line had a peculiar rooting pattern consisting of a deep taproot, combined with dense lateral roots that grew about one centimetre under the soil surface, until they reached the edge of the columns, and then grew downwards.

7.5 Discussion

The observed rooting patterns are consistent with the hypotheses previously advanced based on historical growing patterns (chapter 6): common bean exhibits an aggressive root development patterns that suggests strong early competition for available water, while mungbean is more conservative, retaining water in the soil profile at flowering for use during pod-filling. The aggressive production of lateral roots near the surface of the soil in the common bean line AusTRCF 306674, in particular, suggests that this particular line might have been selected under conditions where rapid extraction of the soil water at the surface was beneficial. This could be under irrigated conditions, in dry areas where rainfall might be rare but heavy, or under highly competitive cropping conditions. By contrast, mungbean may have been grown for centuries on residual soil moisture, or limited irrigation. The extensive early root system and high proportion of fine roots in the Uzbek landrace might allow this line to explore a large volume of soil with a relatively high efficiency. The greater root-to-shoot ratio in mungbean compared to common bean is also consistent with the lower leaf area per unit of water consumed observed in a previous study (chapter 6). The lower transpirational losses resulting from lower leaf area, and the proportionally greater allocation of resources to roots, are two mechanisms by which mungbean seems to balance water conservation and extraction.

Although the lines were tested separately, the magnitude of the variation suggests that there might be substantial genetic variability in root

characteristics within these two species. Further, in breeding programs for improved drought tolerance, selection for ideal root traits might depend heavily on the cropping system used. For example, the common bean line AusTRCF 306674 grown as a sole crop in a semi-arid area with unpredictable rainfall might deplete the soil water too quickly and suffer severe water deficit stress during the yield formation stage. Selection for a smaller root system, as seen in the commercial cultivar 'Spearfelt' for example, might be more suitable in most dry areas.

On the other hand, while low above-ground biomass development and a high root-to-shoot ratio might allow a crop to establish access to substantial soil water resources before high transpiration losses, low early vigour can be problematic in other ways. From a grower's perspective, this translates into a need for more aggressive weeding as the crop tends not be competitive in the early stages (Lawn and Ahn, 1985). Selection for greater early vigour and a larger early root system might be beneficial in cultivated systems. In addition, recent studies in cowpea, chickpea and lentil suggest that present cultivars are not consuming all of the available water, and that selection for larger root systems lead to higher yields (Matsui and Singh, 2003; Serraj et al., 2004; Ali et al., 2005; Sarker et al., 2005; Kashiwagi et al., 2006). This could be the case in mungbean as well, especially if supplemental irrigation is available.

Two traits worth investigating further are the ratio of total root length to root biomass, and the proportion of fine roots. Both seem to be indicators of the efficiency of soil volume exploration: high values would indicate a greater volume of soil accessible for the same amount of fixed carbon allocated to roots. It would be interesting to determine the genetic variability in these traits and determine whether they are linked to the root-to-shoot ratio or early vigour. Yield gains could potentially be obtained if

efficiency soil volume exploration could be combined with high root-to-shoot ratio and early vigour.

7.6 Conclusion

The objective of this experiment was to examine early root development of two legume species (common bean *Phaseolus vulgaris* and mungbean *Vigna radiata* (L.) Wilczek) that are known to differ in their response to water deficit. Results show that common bean consistently exhibited a greater root biomass and a greater number of tips per unit of root length. Mungbean, on the other hand, consistently demonstrated a higher root-to-shoot ratio. These rooting patterns are consistent with the hypotheses that common bean exhibits an aggressive behaviour of early water extraction to compete effectively for water, while mungbean is more conservative, retaining water in the soil profile at flowering for use during pod-filling. The lower transpirational losses resulting from lower leaf area, and the proportionally greater allocation of resources to roots, are two mechanisms by which mungbean seems to balance water conservation and extraction. However, large differences were found between cultivars within the same species, which suggest large genetic variability with regards to root characteristics. We suggest ideal root traits depend on the cropping system and environment, and that increasing the root system might not always be beneficial. Finally, we suggest that the ratio of total root length to root biomass and the proportion of fine roots should be investigated further.

Figure 7.1: Typical root systems of mungbean (top row) and common bean (bottom row) scanned with the WinRHIZO system 13 days after seeding.

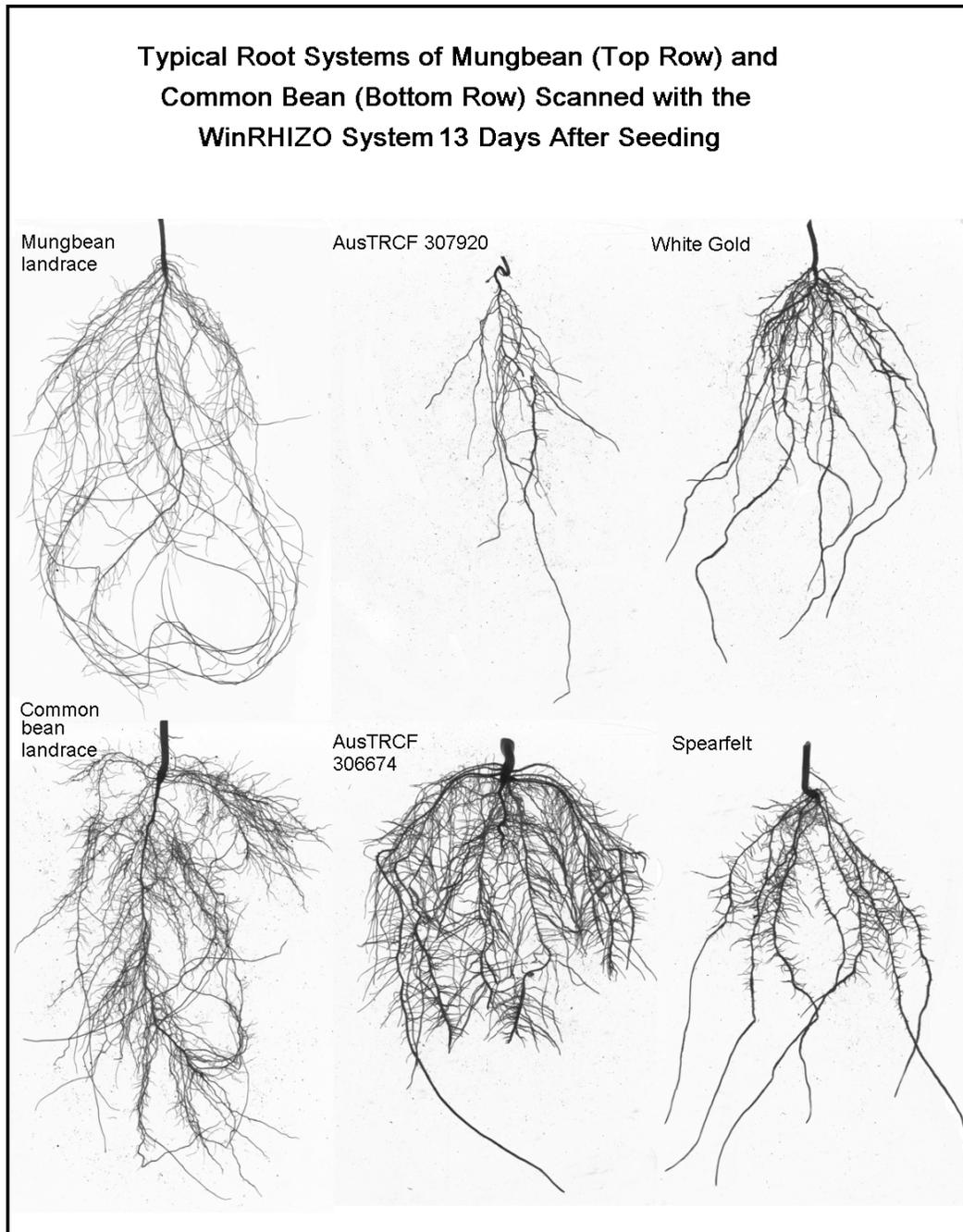


Table 7.1: Root characteristics of common bean and mungbean 13 days after seeding.

Germplasm			
Common bean	Landrace	AusTRCF306674	Spearfelt
Mungbean	Landrace	AusTRCF307920	White Gold
Total length (cm)			
Common bean	529.2	970.0	375.9
Mungbean	948.3	185.1	131.9
P value	0.0230	<0.0001	<0.0001
Root biomass (g fresh weight)			
Common bean	1.59	1.59	0.986
Mungbean	0.88	0.25	0.516
P value	0.0510	<0.0001	<0.0001
Root-to-shoot ratio			
Common bean	0.793	0.616	1.439
Mungbean	1.141	1.238	1.797
P value	0.1671	0.0012	0.0060
Number of tips per cm root length			
Common bean	3.14	3.99	4.64
Mungbean	2.27	3.42	3.07
P value	0.4018	0.0770	0.0001
Proportion roots in 0 to 0.4 mm diameter			
Common bean	0.444	0.509	0.560
Mungbean	0.713	0.357	0.358
P value	0.0003	0.0005	<0.0001
Ratio of total root length to root biomass (cm/g)			
Common bean	335.8	170.4	381.6
Mungbean	1095.6	307.8	252.6
P value	0.0003	0.0028	0.0039

Values are presented as averages per plant. Values presented for the landraces and AusTRCF lines are averages over 8 plants. Values for the 'Spearfelt' and 'White Gold' cultivars are averages of 16 plants, from 8 repetitions and two repeats of the experiment. P values represent the probability of crop differences by t-tests on means.

8. GENERAL DISCUSSION

8.1 Role of R&D in poverty alleviation

Extreme poverty is afflicting 1.1 billion people worldwide, a disproportionate number of which live in rural areas and depend on agriculture for a living (IFAD, 2007). Agricultural research and development programs and growth in agricultural productivity are unanimously viewed as necessary (although not sufficient) for poverty alleviation (Johnston and Mellor, 1961; Adelman, 1984; Alexandrato, 1999; Rola-Rubzen et al., 2001; Pingali, 2007; Thirtle and Piesse, 2007). Further, it is essential that research projects, and particularly international development projects, consider how the socio-politico-economic context will affect the adoption of the introduced technologies.

The findings presented in chapters 3 and 4 in this thesis are contributing tools to improve food security, overall agricultural water productivity and sustainability of cropping systems in Central Asia. We demonstrated that it is not only possible to grow short-season legumes after the harvest of winter wheat, but also that it can be done with relatively little water. In addition, we demonstrated that a short-season Canadian soybean cultivar could be grown as a second crop and could provide farmers with a lucrative option.

Growing legumes after the harvest of winter wheat is particularly relevant in the region because the Uzbek government exerts a high degree of control over the production of cotton and winter wheat. We showed that common bean, mungbean and soybean could all be grown without competing with the government-prescribed agricultural production.

8.2 On-farm water management: importance of monitoring inflows

In chapter 3, we demonstrated that two irrigation events in common bean, and one irrigation event in mungbean are sufficient to obtain maximum yields. We have discussed in chapter 2.2 how our approach, using regulated deficit irrigation (RDI) based on an evapotranspiration (ET) balance (as proposed by Allen et al., 1998, and used in Panda et al., 2003, as well as in a companion paper, Webber et al., 2006), and increased depletion fractions, or increased time intervals between irrigation events, is a superior method to applying RDI as a fraction of ET applied to the control treatment, but otherwise using the same schedule (as used by Pandey et al., 1984; Shani and Dudley, 2001; de Souza et al., 2003; Oktem et al., 2003; Oweis et al., 2004; Chaves et al., 2007), or withholding irrigation at particular growth stages (as used by Nielson and Nelson, 1998; Calvache and Reichardt, 1999; Pandey et al., 2000; De Costa et al., 1999; Boutraa and Sanders, 2001; Xue et al., 2003; Karam et al., 2005; Karam et al., 2007). Even if rainfall is negligible, air temperature, wind speed, irradiation, relative humidity, crop ground cover, soil water holding capacity and fertility conditions are all factors that affect ET (Allen et al., 1998), and that might vary considerably from year to year. The water stress imposed by withholding irrigation at a particular stage is thus hardly reproducible.

Reducing the number of irrigation events also has several implications. First, fewer irrigation events are easier to manage, and potentially less costly than the same amount of water applied in several events (Fereres and Soriano, 2007). Second, in Uzbekistan, the inflow of water in furrows is not monitored and excessive amounts of water are often applied (Horst et al., 2005). In these conditions, reducing the number of irrigation events by half would indeed reduce water use by half, thus reducing deep percolation, waterlogging and risks of salinization. This also highlights the

potential gains from proper monitoring of soil moisture and irrigation inflows to reduce water use in all crops. We suggest that proper training of irrigators could achieve much in terms of water use efficiency and overall water management in the region.

8.3 Challenges in applying water stress in field and controlled-environment experiments

As discussed in chapter 2.3, drought is a complex environmental stress with considerable variability in timing and intensity (Begg and Turner, 1976; Erskine, 2003; Turner, 2003). Thus, comparisons between field studies are difficult to interpret. In particular, a number of field studies have used sprinkler irrigation line designs where the treatments are imposed according to the distance to the irrigation source (Pandey et al., 1984; Senthong and Pandey, 1989; Haqqani and Pandey, 1994b). While this might be convenient to assess the response of various crops to water deficits, the small irrigation depth provided to the most severe stress treatments is far from ideal in terms of irrigation scheduling. Small irrigation depths do not bring the soil profile back to field capacity, but rather wet the upper layers, and result in a soil depletion that is increasing over time. This could potentially lead to severe damage at the yield formation stage.

On the other hand, controlled-environment studies often exhibit environmental conditions considerably different from field studies: they tend to have low solar irradiation, high relative humidity, stable temperatures, and plants are grown in pots with limited room for root development, and limited water holding capacity. Each of these factors imposes its own limitations, and could interact, and influence the response of a plant to the water stress imposed, including in particular transpiration rates. The theory presented by Jones (1990) suggests that a closed

canopy creates a layer of still air, the boundary layer. This layer can decrease the plant's control over transpiration rates substantially, but might also increase transpiration efficiency by increasing relative humidity around the leaf. Light, relative humidity, wind speed, and plant density all influence this boundary layer, and are often quite different between controlled-environment studies and field conditions (Jones, 1998). As such, transpiration rates observed in each environment might be considerably different numerically, and trends of both transpiration rates, and transpiration efficiency in response to water stress might also be different depending on the level of control plants might have over transpiration rates. In addition, the water stress often develops quite quickly in controlled-environment studies as a result of the small size of pots, and the adaptive response might not have time to fully respond to the stress (Tardieu, 1996).

8.4 Mechanisms of drought tolerance in legumes: osmotic adjustment

Osmotic adjustment (OA) is defined as the active accumulation of solutes and consequent decrease in osmotic potential. According to theory, OA helps maintain water extraction, turgor and growth under dehydration and salt stress (Hsiao et al., 1976, Turner and Jones, 1980; Morgan, 1984; Munns, 1988; Blum et al., 1996), and could enable plants to keep stomata open, and thus continue to take up carbon dioxide (Hopkins, 1995). While it is now doubtful that OA can maintain water extraction, turgor or growth (Munns, 1988, Tardieu, 1996, Serraj and Sinclair, 2002), compatible solutes might play important roles in gene signalling, in the protection of cell membranes, proteins, and the photosynthetic apparatus (Munns, 1988; Smirnoff and Cumbes, 1989; Bray, 2002; Shabala and Lew, 2002).

While OA has been correlated with higher yields in several cereal crops

(Ludlow and Muchow, 1990; Fukai and Copper, 1995 in rice), and some legume crops (Flower and Ludlow, 1987 in pigeon pea; Lecoeur et al., 1992 in chickpea; Baigorri et al., 1999 in semi-leafless pea), in this thesis, the maintenance of relatively high water potentials under water stress in both common bean and mungbean in the experiments presented in chapters 3 and 5 suggest that OA does not contribute meaningfully to water stress tolerance in these two crops. In addition, in chapter 6, we demonstrated the maintenance of higher leaf relative water content (RWC) in severe stress treatments in mungbean compared to common bean, while the decrease in water potential was the same for the two crops. This suggests other factors than OA are contributing to turgor maintenance in mungbean. Changes in cell wall elasticity, through the accumulation of new proteins in cell walls, for example, might allow cell division under full turgor pressure (Bray, 2002). Interestingly, such proteins were identified in common bean, and were found to be rich in proline (Garcia-Gomez et al., 2000). This has however never been investigated in mungbean. Instead of measuring leaf water potential, further investigation of the metabolism and catabolism of compatible solutes in response to declining soil water availability might be more worthwhile.

In addition, it has been shown that OA might be beneficial to sorghum yields under severe terminal stress, but not necessarily under intermittent stress (Chapman et al., 2002). The dynamics of the accumulation of compatible solutes should be investigated under various drought patterns.

Finally, there might be considerable genetic variability for OA capacity, or in the capacity to maintain high RWC, under water deficit stress in common bean and mungbean. If we could identify germplasm that maintained high RWC, or that demonstrated high capacity for OA, we might be able to identify the mechanism(s) through genomics approaches.

However, we would also need to investigate how and when such mechanism(s) could benefit yields.

8.5 Mechanisms of drought tolerance in legumes: transpiration efficiency

Stomatal closure is one of the most universal responses of plants to water deficit stress, and transpiration efficiency is one trait that can potentially increase yields under limited water supply. This has been a strategy in breeding for drought tolerant wheat (Richards, 2006) and sunflower (C. Lambrides, personal communication). In this thesis, some of the results presented support the hypothesis that mungbean has a greater intrinsic transpiration efficiency (TE) than common bean: in chapter 3, mungbean's stomatal conductance was consistently lower, but its final biomass was greater than in common bean; water use efficiency calculated from the total biomass divided by the water evapotranspired from a related study (Webber et al., 2006) also showed higher values for mungbean, and instantaneous measurements of gas exchanges showed a higher TE in mungbean in chapter 5. However, in chapter 6, the same analysis resulted in no difference between mungbean and common bean in TE over a range of water availabilities, although mungbean demonstrated a limitation to maximum transpiration efficiency after watering events. Thus, it appears that improvements in TE might not be consistently expressed, but might be specific to periods of growth, or ranges of water availability.

Lambrides et al. (2004) also found, while working on genetic variability of TE in sunflower, that genotypes did not all exhibit the same TE, or similar rankings of TE in the field and in controlled-environment studies. There might be considerable genetic variability in TE in common bean and mungbean, and indeed preliminary reports suggest there is an unprecedented level of genetic variability in mungbean (Ryan and

Lambrides, unpublished). There might also be considerable interaction of TE with climatic conditions.

Instantaneous measurements of TE with gas exchange analysers are relatively lengthy and only a limited number of plants can be assessed. Their value is limited by the short time period they cover. On the other hand, an integrative measurement of TE, such as the carbon isotope discrimination, is of limited value for physiological studies. Neither is fully appropriate to consider differences in TE between irrigation treatments and/or cultivars on a time scale. The Laboratoire d'Ecophysiologie des plantes sous stresses environnementaux, in Montpellier, France, is being followed by other crop physiology laboratories worldwide in the design of large-scale, controlled-environment experiments with computerized monitoring of various parameters such as soil moisture and leaf elongation rate in addition to climatic parameters. This set-up can monitor 360 plants every 15 minutes (K. Chenu, personal communication).

The High Resolution Plant Phenomics Center, in Canberra, Australia is working on modifying a similar design to monitor photosynthetic and transpiration rates in addition to soil moisture and leaf area development. We might then have access to time-dependent dynamics of transpiration efficiency, and this might lead to breakthroughs in understanding whole-plant responses to water stress.

8.6 Improvement of mungbean germplasm

The mechanisms of drought tolerance have not been as well characterized in legumes as in cereals (Turner et al., 2003). In addition, mungbean is poorly characterized among the legumes. Despite its long history and importance in Asian subsistence agriculture, and except for a short spike of research efforts in the 1970s, little research is being

conducted on mungbean (Lawn and Ahn, 1985), and less still on its mechanisms of drought tolerance. This is surprising given that the crop has a reputation for being quite drought tolerant, somewhat similar to sorghum in cereals. Mungbean is still a relatively unimproved crop in many respects: the seeds are small, pods shatter easily, it is very photoperiod sensitive (which makes it difficult to work with at higher latitudes), and it is easily prone to lodging (Lawn and Ahn, 1985; Ellis et al., 1994; Lambrides and Godwin, 2007).

Much of the effort in breeding to date has concentrated on seed quality and crop stand to make it fit better into mechanized agriculture (Lambrides and Godwin, 2007). Mungbean also has a relatively low harvest index (HI) (chapters 3 and 5), and the maintenance of HI under stress is one of the main mechanisms of drought tolerance identified in this study. To our knowledge, Bushby and Lawn (1992) is the only study that investigated translocations of resources in mungbean. They examined a wild accession, a landrace, and a commercial cultivar of mungbean and concluded that HI was a primary determinant of seed yield, more so than total biomass or total nitrogen accumulation. They also found that the commercial cultivar derived most of its nitrogen and carbon from fixation and photosynthesis, while the landrace derived most of its resources from translocation of accumulated resources in stems. Further investigations with a larger number of lines, including modern cultivars, and on the actual mechanisms of translocation of photosynthates to seeds under water deficit stress in mungbean might lead to further advances in other legumes as well.

In addition, although the mungbean genome is not likely to be sequenced soon, there are a number of molecular techniques available that could improve our understanding of mungbean response to water deficit stress. These include the analysis of quantitative trait loci (QTLs) (Tuberosa et al.,

2002), the characterization of expressed sequence tags (ESTs) from cDNA libraries (Lim et al., 2007), the analysis of transcript profiles (Poroyko et al., 2007), and the analysis of diversity array data (Mace et al., 2008).

9. CONCLUSIONS

The main conclusions of this research project are the following:

1. Short-season legumes crops such as common bean, mungbean, or an early maturity Canadian soybean cultivar can be grown after the harvest of winter wheat in the Aral Sea basin;
2. Alternate furrow irrigation does not decrease yields of legumes grown in the Aral Sea basin, even when used in combination with deficit irrigation;
3. Deficit irrigation with legumes grown in the Aral Sea basin is a viable water management strategy for both species: as little as one or two irrigation events around flowering for mungbean and common bean respectively might be enough to obtain maximum yields and maximum water use efficiency;
4. Inoculation with rhizobia increases yields of a short-season Canadian soybean cultivar in the Aral Sea basin, but precautions need to be taken to minimize the detrimental effects of high temperatures on the inoculant when seeding;
5. Mungbean is not more tolerant to salinity stress than common bean, and therefore, its higher yields in the field are probably not attributable to tolerance of low osmotic potential, but possibly to drought avoidance strategies;
6. In controlled-environment studies, mungbean exhibited higher relative water contents at low soil moisture levels, and a more conservative water use in the vegetative stage, but did not exhibit superior capacity for osmotic adjustment, or a stomatal behaviour more responsive to water stress, when compared to common bean. Mungbean might also show higher transpiration efficiency, especially at lower soil moisture levels, and showed lower transpiration rates than common bean after watering events.
7. Mungbean consistently exhibited a smaller root biomass than

common bean, but a higher root-to-shoot ratio in the first twenty days of development. In two of the three germplasm lines tested, it also exhibited a higher ratio of root length to root biomass, which suggests a higher efficiency for soil exploration. However, large differences were observed between lines within the same species, which suggests large genetic variability for root characteristics.

10. CONTRIBUTIONS TO KNOWLEDGE

The field experiment presented in chapter 3 is the first to evaluate the yield, water relation and development of common bean and mungbean subjected to regulated deficit irrigation (RDI) and alternate furrow irrigation (AFI), and the first to evaluate these in the context of crops grown after the harvest of winter wheat in Central Asia. The possibility of growing a short-season legume crop is particularly interesting in Uzbekistan as this land allocation does not compete with government-prescribed production of cotton and winter wheat. This should allow greater food self-sufficiency, and greater overall water productivity in agriculture in the region. We also hope that this may encourage the Uzbek government to lessen its dependency to cotton by diversifying its agricultural production and developing agricultural export markets for high-value commodities with high water use efficiency.

This study, with its companion paper by Webber et al. (2006), was also the first to demonstrate that the number of irrigation events could be reduced from the recommended schedule to a moderate stress schedule without significantly affecting the yields of common bean. Contrasting responses of mungbean to irrigation are also reported in the literature (Angus, 1983; Pandey et al., 1984; Muchow, 1985a; Senthong and Pandey, 1989; Haqqani and Pandey, 1994a; De Costa et al., 1999; Thomas et al., 2004). In this study, we obtained highest mungbean yields in the moderate stress treatment in 2003, and the severe stress treatment in 2004. In chapters 2.2 and 3, we also discussed why using RDI using increased time intervals between irrigation events, based on the water balance method for irrigation scheduling and greater depletion factors (as proposed by Allen et al., 1998, and also used by Panda et al., 2003), is a better approach. In addition, there is no previous report of the combined effects of RDI and AFI under field conditions in these two crops. Thus, in

addition to our report that neither crop is affected by AFI, we are the first to report that AFI does not interact with RDI to further decrease yields. On the contrary, the combination of RDI and AFI can allow legume production with reduced water input.

Combined with the findings of Webber et al. (2006) that water use efficiency, defined as the ratio of seed yield to water evapotranspired, is higher in mungbean than common bean, our yield results suggest that mungbean is better adapted to hot and dry conditions. Our interspecific comparative study has allowed us to suggest that better tolerance to water deficit stress is achieved in mungbean by maintaining a low stomatal conductance regardless of water availability, and an ability to maintain or increase its harvest index (HI) under water deficit stress.

The field experiment presented in chapter 4 is the first to evaluate the possibility of growing a short-season Canadian soybean cultivar as a second crop after the harvest of winter wheat. There is considerable interest in this crop in the region because of its high value and potential as a cash crop, but local cultivars do not mature in less than 120 days (Egamberdiyeva et al., 2004). In addition, the extent of the ability of native rhizobial populations in the soils of Fergana valley, Uzbekistan, to form functional symbioses with soybean was not known prior to our work, and the performance of both the Canadian genotype and a Canadian inoculum had to be assessed before encouraging farmers to grow soybean.

Our results show that it is possible to grow a short-season Canadian soybean cultivar in Uzbekistan after the harvest of winter wheat. This, again, has the interesting and practical implication that soybean could be introduced in the current cropping systems in Uzbekistan without interfering with the government-prescribed growth of cotton and winter wheat. Our data also stress the importance of inoculation with appropriate

rhizobia, as very few nodules were found in the non-inoculated treatments. We also suggested in chapter 4 that further research to identify heat and drought tolerant soybean cultivars and rhizobial strains could probably improve yields in the region. Canadian cultivars could be interesting parents for use in a Central Asian breeding programme as they combined several traits of interest, including short times to maturity.

The mechanisms of drought tolerance have not been as well characterized in legumes as in cereals (Turner et al., 2003). In addition, mungbean is poorly characterized among the legumes. This is surprising given that the crop has a reputation for being quite drought tolerant among the legumes, somewhat like sorghum in cereals (Lawn and Ahn, 1985). In addition, salinity stress and drought stress are often compared and thought of as two stresses that generally occur together. As the soil dries, the soil water solution becomes more concentrated and the overall soil water potential becomes lower (Munns et al., 2002). In chapter 5, we examined more closely the possible mechanisms by which each crop responds to water stress. Since mungbean demonstrated an ability to yield well under relatively low osmotic potential (dry) soil conditions (chapter 3), we hypothesized that the same mechanisms would similarly allow it to be well adapted to soil with low osmotic potential due to salinity stress.

Again, our interspecific comparative study has allowed us to confirm that mungbean is able to maintain the same proportion of reproductive structures under stressed and well-watered conditions whereas common bean is not able to do so. In addition, we showed that photosynthesis in mungbean was consistently higher than in common bean and higher at the same levels of transpiration. Finally, salinity stress did decrease the yield, yield components, and photosynthesis of both common bean and mungbean, but not differently between the two crops, and without

interacting with the RDI levels. This suggests mungbean's reputation for drought resistance has little to do with tolerance of low soil water potential. Instead, the response to water deficit stress might rely primarily on mechanisms that allow higher water uptake or that conserve water. There is no previous report on the combined effects of RDI and salinity stress in mungbean.

In chapter 6, we presented an experiment with a large range of water stress treatments, from quasi waterlogged conditions to a complete lack of watering after emergence. Our interspecific approach highlighted the conservative use of water in mungbean. We suggested there might exist an intrinsic limitation to leaf area production in order for the crop to maintain soil water availability during pod-filling. Mungbean has also shown a better dehydration avoidance capacity than common bean by maintaining higher relative water content, especially at lower soil moisture levels. In addition, mungbean demonstrated a limitation to maximum transpiration following watering events, which might translate into higher water use efficiency over the complete season. We are the first to report such limitation to maximum transpiration in mungbean. In fact, the only other report in legume species is Fletcher et al., (2007) for soybean. Contrary to current theory regarding drought tolerance mechanisms, mungbean's maintenance of high relative water content under water deficit stress does not seem to be achieved through more responsive stomatal closure to declining soil moisture; the contribution of osmotic adjustment is equally inconclusive.

The lack of consistency in yield responses between the field (chapter 3) and pot experiments (chapter 5) hinted at a potentially important role for root systems. The experiment described in chapter 7 was performed in order to determine the early growth patterns of root development in common bean and mungbean. We hypothesized from field observations,

and from data from chapter 6, that mungbean's lack of early vigour (but considerable leaf area development after flowering) might be attributable to an important allocation of photosynthates to roots prior to development of substantial leaf area. Our results showed that common bean consistently exhibited a greater root biomass and a greater number of tips per unit of root length, which might be related to the initial larger size of the seed. Mungbean, on the other hand, consistently demonstrated a higher root-to-shoot ratio. These rooting patterns are consistent with the hypotheses that common bean exhibits a competitive behaviour of early water extraction to compete effectively for water, while mungbean is more conservative, in order to retain water in the soil profile at flowering for use during pod-filling. However, there appear to be large differences between genotypes within the same species, which might point to considerable genetic variability for root characteristics in these two crops. This is the first report describing common bean and mungbean early root development.

11. DIRECTIONS FOR FUTURE RESEARCH

While this research project achieved considerable progress in the physiological characterization of the response of common bean and mungbean to deficit irrigation and water stress, it has also raised a number of interesting questions:

1. What other legumes crops could be grown with limited irrigation water (using deficit and alternate furrow irrigation) after the harvest of winter wheat in Uzbekistan? Depending on market conditions, other crops such as pigeon pea, chickpea, cowpea and even groundnut might be more profitable, and might help improve food self-sufficiency without interfering with the government-prescribed production of cotton and winter wheat;
2. Could early maturity, heat and water stress tolerance be combined to produce soybean germplasm better adapted to Uzbek summers? In addition, could better rhizobial inoculants be developed that withstand high summer temperatures and maintain nitrogen fixation under both heat and water deficit stress?
3. How much genetic variability is there in legumes (and in Uzbek legume landraces particularly) for drought tolerance traits such as osmotic adjustment, transpiration efficiency, root-to-shoot ratio, deep rooting, high root length per unit biomass, and high proportion of fine roots? Could such landrace germplasm be useful in legume breeding programs in semi-arid regions elsewhere in the world?
4. Which constitutive and adaptive traits might contribute meaningfully to higher yields in dry environments and to higher yield potential in legumes?

In addition, while some biochemical characterization of response to drought has been achieved in cereal crops, much less progress has occurred in legume crops, and a comprehensive biochemical

characterization of changes in ABA, proline, glycine betaine, antioxidant enzymes, total soluble carbohydrates, aquaporin activity, etc, in legumes as drought progresses would improve our understanding of the mechanisms of response to water stress.

Furthermore, genomics approaches that have been used in cereals, such as the identification and the analysis of quantitative trait loci (QTLs) (Tuberosa et al., 2002), the characterization of expressed sequence tags (ESTs) from cDNA libraries (Lim et al., 2007), the analysis of transcript profiles (Poroyko et al., 2007), and the analysis of diversity array data (Mace et al., 2008), could be developed on a regular basis for legumes. These would be particularly appropriate for example, in specific investigations of the metabolism and catabolism of certain compatible solutes or antioxidants, of signalling pathways, or of changes in translocation capacity of resources to seeds in response to water deficit stress. The development of genetic markers related to specific root traits would also ease the screening and incorporation of such traits in breeding programs.

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APPENDIX A: SOIL ANALYSIS RESULTS

Soil organic content and nitrogen content, Uzbekistan field, 2003

Plot ID	Depth (cm)	Organic matter content (%)	Content N-NH ₄ (mg kg ⁻¹)
1A	0-30	1.63	24.3
	30-70	1.12	27.2
1B	0-30	1.79	21.2
	30-70	1.70	20.5
1C	0-30	1.76	25.6
	30-70	1.34	22.4
1D	0-30	1.60	23.1
	30-70	1.15	31.6
2A	0-30	1.79	30.1
	30-70	1.18	36.0
2B	0-30	1.50	30.9
	30-70	1.06	21.8
2C	0-30	1.60	28.1
	30-70	1.31	26.5
2D	0-30	1.57	24.9
	30-70	0.86	25.0
3A	0-30	1.76	25.7
	30-70	0.96	33.8
3B	0-30	1.25	23.1
	30-70	0.70	28.7
3C	0-30	1.79	29.4
	30-70	0.99	35.3
3D	0-30	1.22	31.6
	30-70	0.80	37.5
4A	0-30	1.47	34.6
	30-70	1.25	39.7
4B	0-30	1.63	30.1

	30-70	1.09	36.8
4C	0-30	1.54	33.1
	30-70	0.86	41.2
4D	0-30	1.50	26.3
	30-70	0.64	21.2
5A	0-30	1.63	30.9
	30-70	0.85	33.8
5B	0-30	1.50	32.4
	30-70	0.61	27.2
5C	0-30	1.39	16.3
	30-70	1.12	13.7
5D	0-30	1.70	19.3
	30-70	0.75	14.9
6A	0-30	1.56	11.2
	30-70	0.44	16.3
6B	0-30	1.05	13.1
	30-70	0.58	19.8
6C	0-30	1.67	18.1
	30-70	0.68	22.4
6D	0-30	1.77	9.8
	30-70	0.82	12.5
7A	0-30	1.39	18.7
	30-70	0.54	15.6
7B	0-30	0.92	11.8
	30-70	0.41	16.8
7C	0-30	1.43	12.5
	30-70	0.78	21.2
7D	0-30	1.29	17.4
	30-70	0.65	25.7
8A	0-30	0.85	19.8
	30-70	0.34	14.9
8B	0-30	0.88	23.1

	30-70	0.71	20.5
8C	0-30	1.46	26.5
	30-70	0.98	21.8
8D	0-30	1.22	29.4
	30-70	0.92	36.0
9A	0-30	1.71	16.3
	30-70	0.72	19.3
9B	0-30	1.59	16.8
	30-70	0.72	20.5
9C	0-30	1.53	17.4
	30-70	0.81	18.7
9D	0-30	1.65	18.1
	30-70	0.60	23.1
10A	0-30	1.59	14.9
	30-70	0.60	17.4
10B	0-30	1.56	15.6
	30-70	0.78	19.8
10C	0-30	1.77	22.4
	30-70	0.78	17.4
10D	0-30	1.80	20.5
	30-70	1.20	16.3
11A	0-30	1.59	21.2
	30-70	0.63	15.6
11B	0-30	1.35	14.3
	30-70	0.75	16.8
11C	0-30	1.65	13.7
	30-70	1.20	16.3
11D	0-30	1.78	18.1
	30-70	1.74	19.8
12A	0-30	1.53	26.5
	30-70	1.08	21.8
12B	0-30	1.44	18.7

	30-70	0.66	14.9
12C	0-30	1.60	14.3
	30-70	1.65	16.8
12D	0-30	1.63	13.1
	30-70	1.50	15.6

Soil organic matter and nitrogen content, Uzbekistan field, 2004

Plot ID	Depth (cm)	Organic matter content (%)	Content N-NH ₄ (mg kg ⁻¹)
1A	0-30	1.19	18.1
	30-70	0.92	20.5
1B	0-30	1.26	16.3
	30-70	0.82	13.7
1C	0-30	1.57	11.8
	30-70	1.02	13.1
1D	0-30	1.50	17.4
	30-70	0.95	19.8
2A	0-30	1.33	9.8
	30-70	0.50	11.8
2B	0-30	1.64	14.3
	30-70	1.09	12.5
2C	0-30	1.16	21.8
	30-70	0.71	16.8
2D	0-30	1.22	29.6
	30-70	0.78	25.7
3A	0-30	1.20	21.8
	30-70	0.88	27.2
3B	0-30	1.68	24.3
	30-70	0.95	19.8
3C	0-30	1.29	21.4
	30-70	0.78	28.7
3D	0-30	0.88	18.7

	30-70	0.58	20.5
4A	0-30	1.39	29.1
	30-70	0.92	25.0
4B	0-30	1.48	23.5
	30-70	0.82	27.9
4C	0-30	1.53	17.4
	30-70	0.95	21.2
4D	0-30	1.67	18.7
	30-70	1.05	15.6
5A	0-30	1.26	22.4
	30-70	0.71	18.1
5B	0-30	1.63	27.9
	30-70	0.65	20.5
5C	0-30	1.19	16.3
	30-70	0.92	19.3
5D	0-30	1.56	14.3
	30-70	0.99	16.8
6A	0-30	1.60	13.1
	30-70	0.88	15.6
6B	0-30	1.46	18.1
	30-70	0.75	13.7
6C	0-30	1.22	16.8
	30-70	0.85	14.9
6D	0-30	1.29	19.8
	30-70	0.68	15.6
7A	0-30	1.65	24.3
	30-70	1.22	16.8
7B	0-30	1.78	19.3
	30-70	1.35	11.2
7C	0-30	1.45	16.3
	30-70	0.96	20.5
7D	0-30	1.39	18.7

	30-70	1.09	13.7
8A	0-30	1.49	11.8
	30-70	1.19	8.6
8B	0-30	1.58	21.8
	30-70	1.29	26.5
8C	0-30	1.79	27.9
	30-70	1.39	19.8
8D	0-30	1.42	17.4
	30-70	0.99	21.2
9A	0-30	1.32	14.3
	30-70	0.59	12.5
9B	0-30	1.52	18.1
	30-70	0.63	14.9
9C	0-30	1.35	23.1
	30-70	0.53	19.3
9D	0-30	1.29	13.7
	30-70	0.56	16.8
10A	0-30	1.25	12.5
	30-70	0.76	10.6
10B	0-30	1.36	19.8
	30-70	1.19	16.3
10C	0-30	1.47	8.8
	30-70	1.24	11.8
10D	0-30	1.42	11.2
	30-70	0.96	13.1
11A	0-30	1.29	10.0
	30-70	0.89	12.5
11B	0-30	1.39	16.8
	30-70	1.02	13.7
11C	0-30	1.49	15.6
	30-70	1.12	20.5
11D	0-30	1.22	8.6

	30-70	0.86	10.6
12A	0-30	1.25	6.3
	30-70	0.97	8.8
12B	0-30	1.13	13.1
	30-70	0.73	9.8
12C	0-30	1.19	10.0
	30-70	0.79	8.6
12D	0-30	1.52	7.6
	30-70	1.06	9.8