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A Short Season Canadian Soybean Cultivar Double Cropped After Winter Wheat in Uzbekistan With and Without Inoculation with *Bradyrhizobium*

M. Bourgault[1,3], C. A. Madramootoo[1], H. A. Webber[1,4], G. Stulina[2], M. G. Horst[2] & D. L. Smith[1]

[1] Faculty of Agricultural and Environmental Sciences, McGill University, Ste-Anne-de-Bellevue, Canada

[2] Central Asian Research Institute of Irrigation (SANIIRI), Tashkent, Republic of Uzbekistan

[3] Current address: Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Creswick, Australia

[4] Current address: University of Bonn, Crop Science Group, Bonn, Germany

Correspondence: Donald Smith, Department of Plant Science, Macdonald campus, Ste-Anne-de-Bellevue, QC, H9X 3V9, Canada. Tel: 1-514-398-7866. E-mail: donald.smith@mcgill.ca

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Abstract

Agricultural systems in Uzbekistan are dominated by the production of cotton and winter wheat as these crops are subject to state-prescribed quotas. An experiment was conducted in the Fergana valley, in Uzbekistan, Central Asia, to determine the feasibility of growing a short-season Canadian soybean (*Glycine max* [L.] Merr.) 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 non-inoculated control (106% increase). Inoculation in 2004 increased seed weights by 30%, final pod number by 29%, biomass dry weight at the pod-filling stage (56%) and at harvest (56%), as well as the harvest index by 22%. Nodules were, in general, only present in the inoculated treatments, which indicated that appropriate indigenous rhizobial strains were not present in these soils, but ineffective rhizobial competitors to commercial inoculants were also absent. Soybean production could be possible in Uzbekistan without competing with state prescribed crops such as cotton and winter wheat. Based on prices from 2004, this represents an additional income of more than 300$ ha^{-1}. More research is needed to determine the optimal conditions for inoculation success in hot and dry climates.

Keywords: double cropping, farming systems, *Glycine max*, inoculation, Uzbekistan

1. 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. A typical rotation starts with cotton being planted in April, and harvested from September to December. Winter wheat is planted in November to December, in between rows of cotton, and harvested late June to mid-July in the following year. The land is then kept fallow until April when cotton is planted once again. To improve land productivity and food security in the region, the introduction of food legumes as double crops after the harvest of winter wheat was previously suggested (Bourgault et al., 2013). In many systems, the intensification of agriculture with double cropping is a major tool to improve the efficiency of resource use, such as water and radiation use efficiency (Van Opstal et al., 2011; Fouli et al., 2012). In the Fergana valley, the period between July and mid-October represents over 40% of the total yearly radiation (data taken from Pereira et al., 2009; Table 2). In addition, legumes in rotation with other crops can break disease cycles, improve the fertility and structure of the soil and encourage the development of mycorrhizal associations (Subbarao et al., 1995).

Soybean (*Glycine max* [L.] Merr.) is now the world's most important legume crop (Giller, 2001). Its annual production totalled 276 million tonnes in 2013 on over 110 million hectares averaging producer prices of US

\$525 tonne^{-1} in 2012 (FAOSTAT, 2015). 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 including in several dry areas such as Australia, Brazil and the United States (FAOSTAT, 2015). 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 Uzbek soybean cultivars generally mature in at least 120 days, and are thus in direct competition with government-prescribed production of cotton and winter wheat. As such, we hypothesized that the soybean cultivar Costaud, which matures in 90 to 100 days under Canadian conditions, would represent a good candidate for production under the conditions of Uzbekistan.

While the benefits of inoculating soybean are well established, the ability of native rhizobial populations in the soils of Fergana valley to form functional symbioses with soybean was not known prior to our work, and the performance of both the Canadian cultivar and the 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.

2. Materials and Methods

2.1 Location and Field Preparation

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 was hot and dry, with typical daily high temperatures of 40°C and daily low temperatures of 20°C. Rain was 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 1) were collected using an on-site Vantage Pro Meteorological station (Davis Instruments Corp., Hayward, CA, USA), located approximately 200 m from the field site.

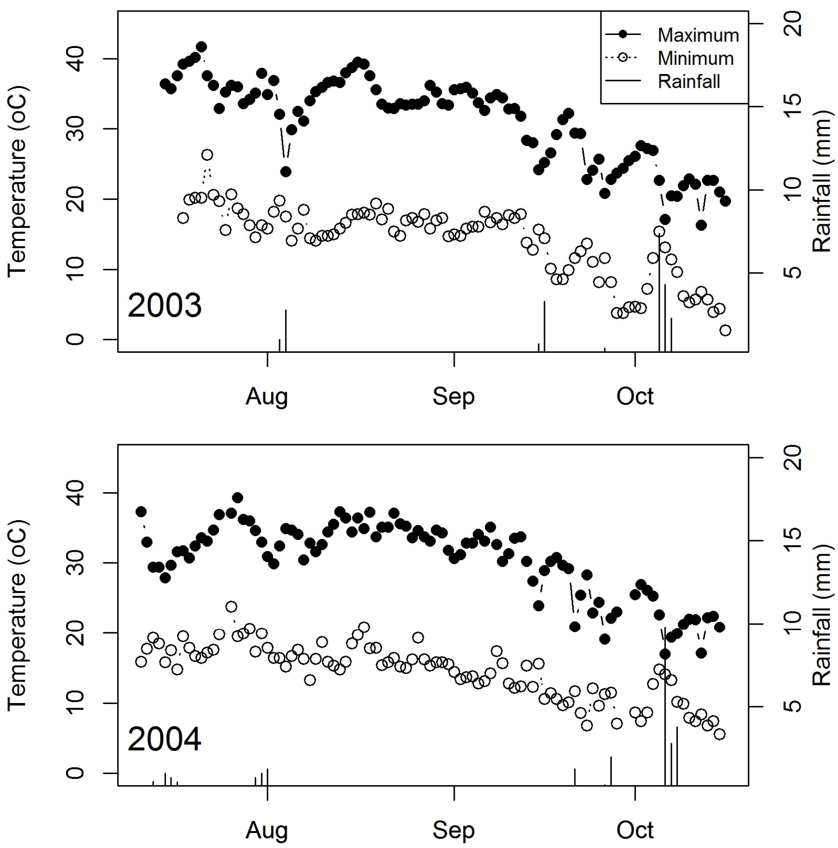

Figure 1. Climatic data for the growing seasons of 2003 and 2004 in the Fergana Valley, Uzbekistan (40°23'N, 71°45'E) from the beginning of July until the end of October

Based on textural analyses, soil at the experimental sites was silt loam. The available water content was 96 mm in 2003 and 75 mm in 2004, in the top 60 cm. The organic matter content was less than 2% and the soils had a well-developed plough pan at 30-40 cm depth.

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. Neither experimental site had any history of soybean production. Sixty-centimeter-wide furrows were formed on the field site with a tractor-drawn lister.

2.2 Irrigation Scheduling

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). There were 5 irrigation events in both years (including an irrigation just prior to planting), which brought back soil moisture to field capacity, for a total irrigation applied of approximately 3650 and 4000 m^3 ha^{-1} in 2003 and 2004 respectively.

2.3 Experimental design

Plots consisted of 9 raised beds 5 m in length. Each plot was separated by a double row of mutant non-nodulating soybean plants. 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.

2.4 Inoculation and Planting

The cultivar used was Costaud (Agrocentre Belcan, Ste-Marthe, QC, Canada), one of the shorter duration cultivar available in Canada at the time of the experiment. 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. Planting was done on July 22nd in 2003, and on July 13th in 2004. Seeds were sown by hand, with the non-inoculated control planted first to avoid contamination. Seeds were sown at 5 cm depth, on both sides of the raised bed to achieve a planting density of 50 plants m^{-2}. Weed control was done manually throughout the season.

2.5 Data Collected

Yield was measured by harvesting all pods in three randomly selected 2-m length sections of row in each plot, but at least 1 m away from the edge of the plot, and two outside rows were not utilized for data collection. 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 length 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 randomly 0.5 m of row. Plants were dried at 70°C for at least 24 h, until completely dry. The number of nodules and their dry weight were also determined in these destructive samplings. The nitrogen content and carbon to nitrogen ratio of the above-ground biomass was also determined with an elemental analyser (NC 2500 Elemental Analyzer, CE Instrument Inc., Milan, Italy). Non-nodulating soybean nitrogen contents were also looked at separately to ensure there was no spatial variability within the field.

2.6 Statistical Analyses

Statistical analyses were performed by analysis of variance (ANOVA) using the SAS/STAT software and GLM procedure (SAS, Cary, NC, USA). 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.

3. Results

In both years, a soybean crop was grown to maturity using a short-season Canadian variety after the harvest of winter wheat in the Fergana valley, in Uzbekistan, Central Asia. Soybean yields averaged 1.0 and 1.4 t ha^{-1} in 2003 and 2004, respectively (Table 1), for inoculated soybean. Inoculation increased yields by 106% in 2004, but did not significantly increase yields in 2003. In fact, very few nodules were observed in 2003 (Table 1).

The higher yield observed due to inoculation in 2004 is mostly explained by higher seed weights (30% increase) and higher final pod numbers (29% increase), as compared to the control non-inoculated soybean plants (Table 1). The number of seeds per pod, however, was unaffected by inoculation. Nodules were not generally found on plants in the non-inoculated control treatment plots (Table 2).

Table 1. Yield and yield components of soybean grown in Uzbekistan

	2003		2004	
Parameters	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
Above ground biomass (kg/ha)	2573 a	1887 a	3151 A	2020 B
Harvest index (%)	41.4 a	57.1 a	47.6 A	39.1 B

Values given are means of three (2003) or four blocks (2004) with the same treatment. Values associated with the same letter within the same year are not significantly different at p<0.05. Equipment malfunction in 2003 resulted in grains being lost before being counted.

*NA = "Not available". Due to oven malfunction, data on seeds per pod and 100-sed weight were not available in 2003.

Table 2. Average number (per 0.5 m row) and dry weight of nodules found in soybean grown in Uzbekistan in 2004

	Flowering		Pod-Filling		Harvest	
Treatment	Number	Dry weight (g)	Number	Dry weight (g)	Number	Dry weight (g)
2003						
Inoculated	2.3 a	NA*	8.7 a	NA*	16.0 a	NA*
Control	0 b	0	0 b	0	0 b	0
2004						
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	0.005 b	0 b	0.000 a

Values given are the means of four blocks with the same treatment. Values associated with the same letter within the same sampling are not significantly different at $p < 0.05$.

*NA = "Not available". Dry weights for the 2003 season were not available.

**The probability of significance between the inoculated and control treatments was $p = 0.0505$.

In 2004, the above-ground biomass dry weight began to show increasingly significant differences by the pod-filling stage ($p=0.0533$; not shown), and showed very clear differences at the harvest stage ($p = 0.0098$; Table 1). 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). Similarly, no differences were found in nitrogen content or nitrogen-carbon ratio at the flowering sampling. However, clear differences were detected for above-ground biomass at pod-fill and for grains at harvest (Table 3). The lack of differences in nitrogen content in leaves at the harvest stage suggests that most of the nitrogen from the symbiosis was translocated to grains. Our data suggest that the benefit of inoculation was greatest in the late stages of plant development.

Table 3. Nitrogen content and Nitrogen-Carbon Ratio of Soybean Grown in Uzbekistan in 2004

Treatment	Flowering		Pod-Filling		Harvest		Grains	
	N content (%)	N-C ratio	N content (%)	N-C ratio	N content (%)	N-C ratio	N content (%)	N-C ratio
Inoculated	3.98 a	0.0374 a	3.06 a	0.0245 a	2.65 a	0.0178 a	6.07 a	0.0491 a
Control	4.12 a	0.0352 a	2.41 b	0.0187 b	2.62 a	0.0148 b	5.27 b	0.0399 b

Values given are the means of four blocks with the same treatment. Values associated with the same letter within the same sampling are not significantly different at $p<0.05$.

4. Discussion

4.1 It is Feasible to Grow Soybean in Uzbekistan as a Double Crop

The cultivar Costaud, although originally developed for colder climate conditions prevalent in Canada, is able to grow and yield as a double crop in the hot and dry conditions of Fergana valley, Uzbekistan, provided some irrigation is available. It averaged 1.0 and 1.4 t ha^{-1} in 2003 and 2004, respectively after the harvest of winter wheat. This 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 low compared to the world averages of 2.3 and 2.2 t ha^{-1} for 2003 and 2004 respectively (FAO, 2015), which suggest that genetic improvement for better adapted germplasm and inoculants could lead to greater yields. While the crop was irrigated, transient water stress during the day (with temperatures as high as 40 °C) were likely.

The production of an additional crop in the cotton-wheat rotation does require additional water. However, excessive water is often applied to furrow irrigated cotton and wheat in the region, and various strategies have been put forward to reduce irrigation amounts applied while maintaining yields (Horst et al., 2007; Pereira et al., 2009). For example, Horst et al. (2007) show that by irrigating cotton with surge-flow irrigation in alternate furrows, the irrigation water could be decreased by 44% (3891 m^3 ha^{-1}), which is about the amount of water necessary to irrigate soybean. Therefore water savings in cotton could compensate for the irrigation water necessary for soybean production. In 2004, the price of soybean was US \$211 t^{-1} (FAO, 2015), so this additional crop would increase income by approximately US \$300 ha^{-1}.

4.2 Importance of Environmental Conditions at Planting for Successful Nodulation

In 2003, the inoculation of seeds at planting was done in the afternoon, but due to technical difficulties in getting irrigation water, the plots remained dry until the next morning. In 2004, the inoculation and planting were done early in the morning and irrigation water was applied immediately afterwards, such that all operations were completed by midday. We suspect that in 2003 the soil was 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, this inoculant can perform relatively well.

Environmental factors such as high temperature and drought affect nodulation and the ability of rhizobia to colonize plants (Hungria & Vargas, 2000). Our findings indicate that best management practices for soybean inoculation need to be developed for Uzbekistan as the benefits can be substantial. The manufacturer’s instructions on the package (and quality assurance) are not taking into account environmental conditions that might be prevalent outside of Canada and the United States. In Australia, where the summer climate is similar, the current extension message to farmers is to inoculate and plant within 24 h (GRDC, 2013). Our experience suggests that even this might be too challenging for rhizobia survival. There is a real need to investigate genetic and agronomic solutions to this problem. Large variability in rhizobial strains has been documented in a number of semi-arid areas (Arun & Sridhar, 2005; Hungria et al., 2006; Giongo et al., 2008) and are potential genetic resources. Research with Brazilian inoculants and native rhizobia isolated from calcareous soils (prevalent in the region) is also being performed in the region (Egamberdiyeva et al., 2004).

To some extent, it is advantageous that no native bacteria were found in the soils of the Fergana Valley, as this indicates that there are no ineffective competitors to compete with introduced rhizobia.

4.3 Soybean Breeding Objectives for the Region Should Include Short Duration, Heat and Drought Tolerance

While Canadian, northern U.S. and northern Chinese cultivars could be genetic resources of short duration traits, Brazilian and Australian germplasm could be used as source of heat and drought tolerance. In addition, large genetic variability in nodulation sensitivity to water deficit stress among soybean cultivars has been demonstrated (Serraj & 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.

5. Conclusion

Soybean production is possible in Uzbekistan without competing with state prescribed crops such as cotton and winter wheat. This could provide farmers with an additional income to their current production systems. More research is needed to determine the optimal conditions for inoculation success in hot and dry climates and heat and drought tolerance should be breeding objectives in the region.

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