
Impact of Arbuscular Mycorrhizal Fungi on the Growth and Rhizobium Symbioses Development in Kabuli and Desi Chickpeas Grown Under Drought Stress Conditions

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Abstract

Arbuscular mycorrhizal fungi (AMF) have induced drought tolerance in several plants and could increase chickpea yield under semiarid climates. Desi chickpea are more drought tolerant than Kabuli suggesting a weak mycorrhizal symbiosis in this chickpea type. A greenhouse experiment was conducted to assess the extent and the impact of arbuscular mycorrhizal symbiosis on the growth and yield in Kabuli and Desi chickpea cultivars under well watered and drought stress conditions and on their association with *Mesorhizobium cicer*. The experiment had a split-plot design with two water levels, 30% of field capacity (deficient water) and 70% of field capacity (sufficient water) randomized in main plots. Two inoculation treatments, *M. cicer* + AMF and *M. cicer* only, were applied to chickpea (Kabuli, CDC Frontier and CDC Xena; Desi, CDC Anna, and CDC Nika). The factorial combinations of inoculation and cultivar were randomized in the subplots. There were four repetitions. One set of plants was harvested at the time of symbioses development and another set was harvested at seed maturation. The data was analyzed with ANOVA. Results indicated that the Kabuli and Desi chickpea mycorrhizal symbioses are not different, as indicated by the absence of a cultivar by inoculation interaction on shoot and root growth, nodulation and nitrogen fixation. AM Fungi inoculation delayed nodule development but had no effect on grain yield. CDC Frontier had 4 times more nodules and 6 times more nitrogenase activity than CDC Anna, Nika and Xena.

Introduction

Drought stress is a major constraint to agriculture in the semi-arid tropics (Quilambo, 2003). It may be the single most important stress in chickpea (Singh, 1993). Some researchers reported stress tolerance in nodulated legumes associated to arbuscular mycorrhizal fungi (AMF) (Sánchez-Díaz, 2001). AMF may increase drought tolerance of plants by means of several mechanisms. They can modify plant water relation and their hyphae can enhance plant water uptake capacity (Hardie, 1985; Faber et al., 1991; Ruiz-Lozano and Ázcón, 1995). On the other hand, the importance of AMF in legume plants has been attributed to the high P requirements of

the nodulation and N₂-fixation processes (Barea and Ázcón-Aguilar, 1983). Enhanced plant P nutrition is well known contribution of AMF to plant productivity.

There are physical differences between the Desi and Kabuli type of chickpea. Desi chickpea is more drought tolerant than the Kabuli (Anonymous, 2000). These observations suggest that the level of AMF roots colonization and symbiosis development with *Mesorhizobium cicer* may differ.

This work verifies the following hypotheses: (1) Desi and Kabuli chickpea respond differently to mycorrhizal inoculation. (2) Inoculation with AMF influence nodulation, nitrogenase activity and yield differently in Kabuli and Desi chickpea, particularly under drought stress conditions. The time of N₂-fixing symbiosis development was examined because competitive interactions between AMF and symbiotic N₂-fixers were reported at this stage (Zhang et al., 1995; Catford et al., 2003), and the sum of effects was obtained in a final harvest, 17 weeks after emergence.

Materials and Methods

The experiment was conducted in the greenhouse. It had a split-plot design with two water levels, 30% of field capacity (deficient water) and 70% of field capacity (sufficient water) randomized in main plots. Two inoculation treatments, *M. cicer* + AMF and *M. cicer* only, were applied to chickpea (Kabuli, CDC Frontier and CDC Xena; Desi, CDC Anna, and CDC Nika). The factorial combinations of inoculation and cultivar were randomized in the subplots. There were four repetitions. One set of plants was harvested at the time of symbioses development and another set was harvested at seed maturation. At the first harvest, early morning plant water potential was determined (Scholander et al., 1965) in a pressure chamber (Plant Water Status Console Model 3005, Soilmoisture Equipment Corp). The youngest part of the shoot tip cut just before the 5th leaf from the apex was used for this determination. Plant shoots water potential was measured immediately after cutting the shoot. Measures were taken early morning. Nitrogenase activity was estimated by the acetylene reduction assay (Carlsson and Huss-Danell, 2003). Measures of root length and surface area were determined using the root analysis system WinRhizo, after staining the roots (Costa et al., 2000). Plant shoots were dried and weighed. Seed number and seed weight per plant were determined at the second harvest. The data sets were analysed by ANOVA using Network JMP v3.2.6. A P value of 0.05 was used as threshold to accept the significance of effects, except when indicated otherwise. Treatment means were compared based on least significant differences, where significant treatment effects were found.

Results

After 7 weeks of growth under greenhouse conditions, the root system of Kabuli chickpea was significantly larger than that of Desi. Based on plant dry mass, nodule number and nitrogenase activity, it appears that the differences between chickpeas are more related to cultivars than to classes. At symbiosis development, mycorrhizal inoculation induced a negative effect on all measured parameters except for nodule number at 30% of field capacity, where nodule number was very low and no difference was observed between mycorrhizal and control plants (Fig. 1).

As illustrated by Figure 2, inoculation reduced plant water potential ($P=0.03$) only in the unifoliate Kabuli chickpea CDC Xena.

The negative influence of mycorrhizal inoculation had disappeared at harvest (Fig. 3).

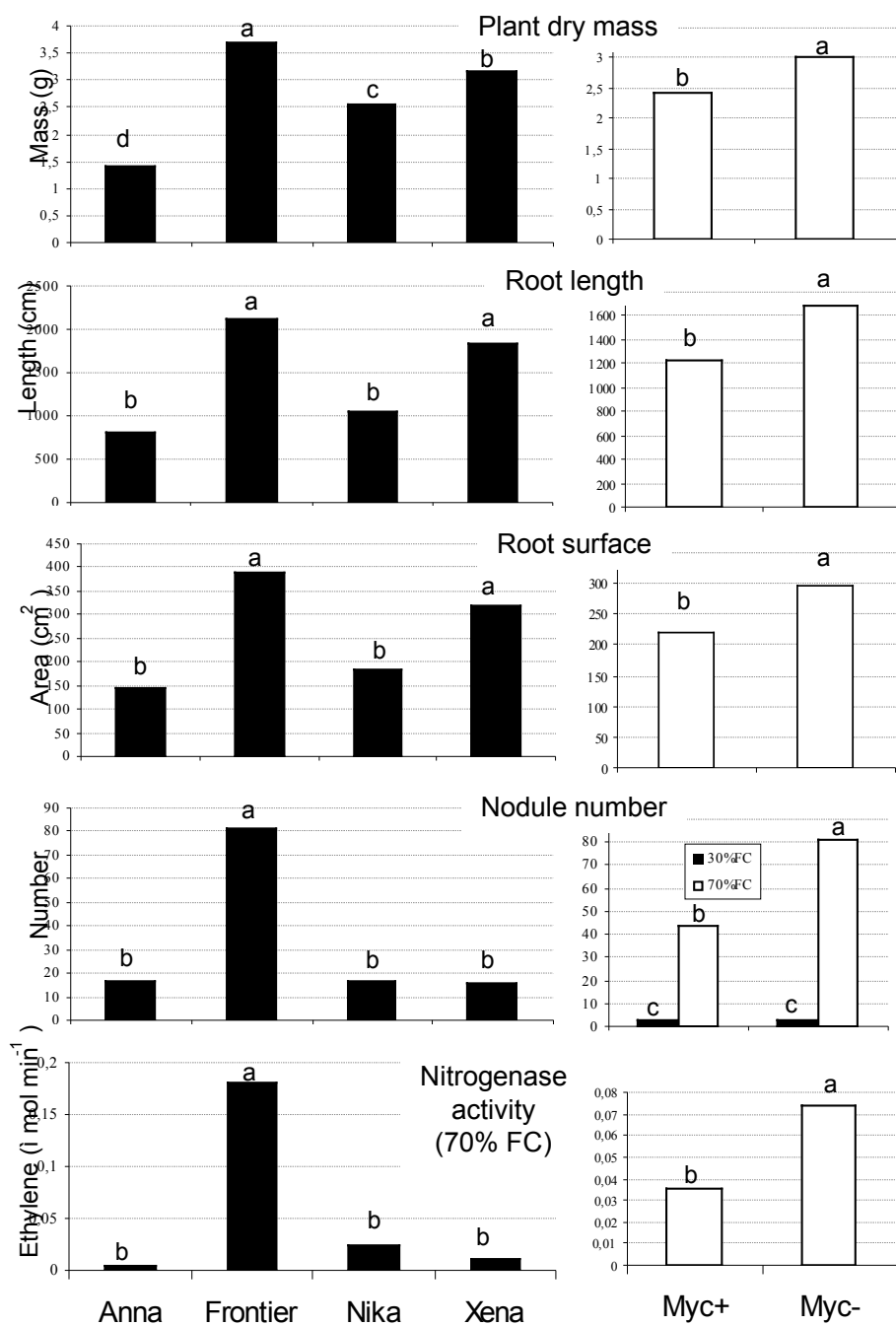


Figure 1. Dry weight, root length and surface area, and nodule number of chickpea, as influenced by cultivar ($N = 16$) and mycorrhizal inoculation ($N = 32$). There was no nitrogenase activity at 30% FC and this treatment was excluded from the ANOVA. $N = 8$ for cultivar effect and $N = 16$ for inoculation effect. Myc+, mycorrhizal inoculated; Myc-, control; 30% FC, soil moisture maintained at 30% of field capacity; 70% FC, soil moisture maintained at 70% of field capacity. Bars with the same letter are not significantly different according to LSD $P = 0.05$.

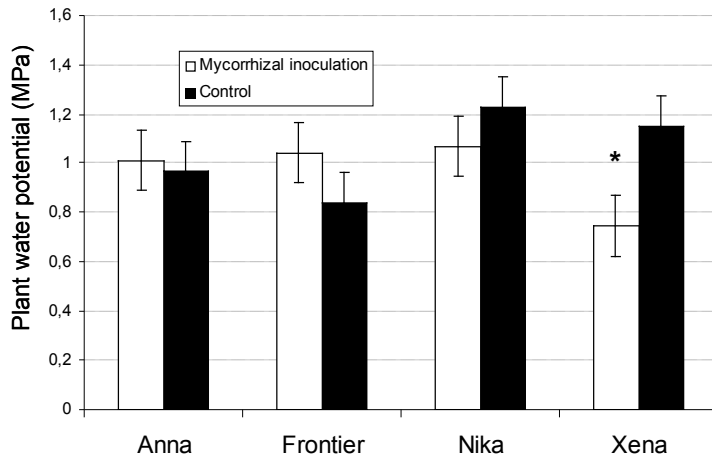


Figure 2. Plant water potential measured early morning 7 weeks after emergence. A star indicates inoculation by cultivar interaction ($P = 0.1$) and a significant ($P = 0.03$ according to contrast) negative effect of inoculation on the water potential of AC Xena plants. $N = 8$.

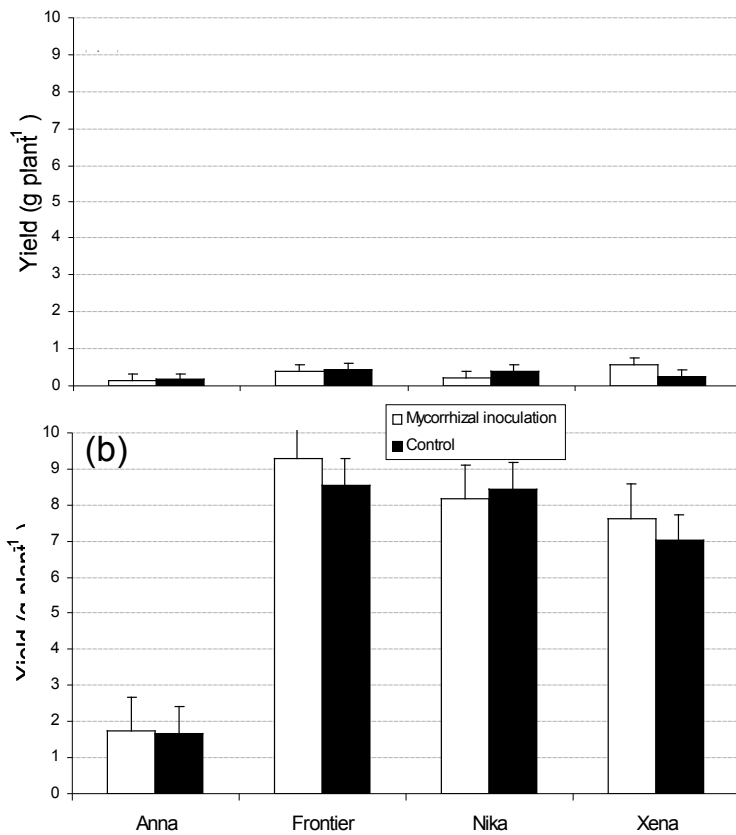


Figure 3. Grain yield of the different chickpea cultivars grown at 30% (a) and 70% (b) of field capacity. The negative influence of mycorrhizal inoculation had disappeared at harvest. $N = 4$.

Conclusions

Kabuli and Desi chickpea mycorrhizal symbioses are not different, as indicated by the absence of a cultivar by inoculation interaction on shoot and root growth, nodulation and nitrogenase activity.

Arbuscular mycorrhizal fungi inoculation delayed nodule development, but had no effect on grain yield.

CDC Frontier had 4 times more nodules and 6 times more nitrogenase activity than CDC Anna, Nika and Xena.

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References:

- Anonymous, 2000. Development of agronomic practices for chickpea production in Alberta. On line: <http://www.ppi-far.org/far/farguide.nsf>
- Barea, J.M., Azcon-Aguilar, C. 1983. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. *Advances in Agronomy* 36:1-54.
- Carlsson, G. and Huss-Danell, K. 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* 253: 353-372.
- Catford, J.G., Staehelin, C., Lerat, S., Piché, Y., Vierheilig, H. 2003. Suppression of arbuscular mycorrhizal colonization and nodulation in split-root systems of alfalfa after pre-inoculation and treatment with Nod factors. *Journal of Experimental Botany* 54:1481-1487.
- Costa, C., L.M. Dwyer, C. Hamel, D.F. Muamba, X.L. Wang, L. Nantais, and D.L. Smith. 2001. Root contrast enhancement for measurement with optical scanner-based image analysis. *Canadian Journal of Botany* 79: 23-29.
- Faber, B.A., Zasoski, R.J., Munns, D.N., Schakel, K. 1991. A method for measuring hyphal uptake in mycorrhizal plants. *Canadian Journal of Botany* 69:87-94.
- Hardie, K. 1985. The effect of removal of extraradical hyphae on water uptake by vesicular-arbuscular mycorrhizal plants. *New Phytologist* 101:677-684.
- Quilambo, O.A. 2003. The vesicular-arbuscular mycorrhizal symbiosis. *African Journal of Biotechnology* 2: 539-546.
- Ruiz-Lozano, J.M. and Azcón, R. 1995. Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. *Physiologia Plantarum* 95: 472-478.
- Sánchez-Díaz, M. 2001. Adaptation of legumes to multiple stresses in Mediterranean-type environments. *Options Méditerranéennes* 45: 145-151.
- Scholander, P.F., Hammel, H.T., Bradstreet, E.D. and Hemingsen, E.A. 1965. Sap pressure in vascular plants. *Science* 148:339-346.
- Singh, K.B. 1993. Problems and prospects of stress resistance breeding in chickpea. Pages 17-35 *in: Breeding for stress tolerance in cool-season food legumes*, K.B. Singh and M.C. Saxena (eds). ICARDA.
- Zhang, F., Hamel, C., Kianmehr, H., and Smith, D.L. 1995. Root-zone temperature and soybean (*Glycine max.* (L.) Merr.) vesicular-arbuscular mycorrhizae: Development and interactions

with the nitrogen fixing symbiosis. *Environmental and Experimental Botany* 35: 287-298.