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Potential to breed for mycorrhizal association in durum wheat

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14

15 **Abstract**

16 The selection of genotypes under high soil fertility may have altered the effectiveness of mycorrhizal
17 symbioses naturally forming between crop plants and the mycorrhizal fungi residing in cultivated fields.
18 We tested the hypothesis that the mycorrhizal symbiosis of five landraces function better than the
19 mycorrhizal symbiosis of 27 cultivars of durum wheat bred after the development of the fertilizer
20 industry. We examined the development of mycorrhiza and the response of these genotypes to mycorrhiza
21 formation after four weeks of growth under high and low soil fertility levels in the greenhouse. The
22 durum wheat genotypes were seeded in an established extraradical hyphal network of *Rhizophagus*
23 *irregularis* and in a control soil free of mycorrhizal fungi. The percentage of root length colonized by
24 mycorrhizal fungi was lower in landraces (21%) than in cultivars (27%; $P = 0.04$) and in the most recent
25 releases (29%; $P = 0.02$), which were selected under high soil fertility levels. Plant growth response to
26 mycorrhiza varied from -36% to $+19\%$. Overall, durum wheat plant breeding in Canada has increased the
27 mycorrhizal development in wheat grown at low soil fertility level. However, breeding had inconsistent
28 effects on mycorrhizal development and has led to the production of cultivars with patterns of regulation
29 ranging from unimproved to inefficient.

30

31 **Keywords:** durum wheat, arbuscular mycorrhizal symbiosis, growth response.

32

33 **Introduction**

34 Climate variability threatens food security, the human population continues to grow, and the price of
35 fertilizers continues to rise. A paradigm shift in concepts about plant nutrition can address future
36 challenges by harnessing the potential of arbuscular mycorrhizal (AM) fungi. The AM fungi are
37 ubiquitous soil fungi that spontaneously develop a symbiotic organ called 'mycorrhiza' with the roots of
38 most land plant species, including wheat. These AM symbioses improve the efficiency of nutrients
39 ([Mäder et al. 2011](#)) and the tolerance of plants to stresses ([Ellouze et al. 2008](#)) by improving the ability of
40 plants to extract soil nutrients and water.

41 Mycorrhiza formation is generally beneficial to plants, but highly variable plant growth response to AM
42 fungi has shown that AM symbioses range from mutualistic to parasitic associations ([Johnson and](#)
43 [Graham 2013](#)). The genotype of the plant involved in an AM symbiosis influences the function of the
44 mycorrhiza. Wheat derives less benefit from AM relationships than other crop plants ([Plenchette et al.](#)
45 [1983](#); [Ryan and Kirkegaard 2012](#)). However, the genetic variability observed in the compatibility of
46 durum wheat (*Triticum turgidum* L. var. *durum* Desf.) with AM fungi ([Singh et al. 2012](#)) suggests the
47 potential for improvement of the mycorrhizal association of this important crop plant through
48 conventional breeding practices. Enhancement of mutualistic symbiosis in wheat would result in less
49 dependence on fertilizers, tolerance to environmental stress better than current cultivars, and thereby
50 improve the sustainability of cropping systems.

51 Wheat has been subjected to intensive selection since the Green Revolution, and this selection was largely
52 conducted in soil amended with inorganic fertilizers, which were developed after World War II
53 ([Paungfoo-Lonhienne et al. 2012](#)). High soil fertility renders the AM symbiosis unnecessary and
54 suppresses mycorrhiza formation ([Johnson and Graham 2013](#)). Thus, the genotypes selected in highly
55 fertile soil for high productivity may or may not have the ability to form an effective AM symbiosis. It
56 has been proposed that modern breeding practices produce wheat cultivars that are highly dependent on

fertilizer supply and are unresponsive to mycorrhiza ([Hetrick et al. 1992, 1993](#); [Zhu et al. 2001](#)), but this conclusion is not always supported ([Lehmann et al. 2012](#); [Singh et al. 2012](#)). Our objective was to clarify the influence of the selection of genotypes under high soil fertility on the regulation and response of durum wheat to mycorrhiza. We conducted a greenhouse experiment with five landraces brought into Canada prior to 1920 and 27 improved durum wheat cultivars released after 1957 in North America. We hypothesized that landraces, which were populations selected under in situ organic nutrient input conditions, respond and regulate their AM symbiosis better than modern wheat cultivars, which were selected under increasingly high inorganic soil fertility conditions. Changes in the above ground biomass and concentrations of N, P, and K were used to describe the response of the durum wheat genotypes to mycorrhiza, and secondly the change in the level of AM root colonization upon exposure to high and low soil fertility levels was used as an indicator of regulation of the symbiosis by the plants.

Materials and methods

Experimental design, plant material and plant growth conditions

The experiment was arranged in a randomized complete block with four replicates, in a greenhouse. The blocking factors were a strong airflow, running from the east to the west side of the greenhouse bench, and time. Blocks 1 and 2 were set up first, and blocks 3 and 4 were set up subsequently to distribute the workload. The 128 treatments were factorial combinations of 32 durum wheat genotypes, two symbiotic status (AM and non-AM), and two fertility conditions (low and high). The durum wheat varieties used were a set of five landraces brought to Canada prior to 1920 ([Knott 1995](#)) and a historical set of 27 cultivars produced using modern breeding practices i.e., through crossing and progeny selection. The modern cultivars were primarily developed in Canada ([Clarke et al. 2010](#)), except ‘Quilafen’, developed in Chile, and the cultivars ‘Ramsey’ and ‘Lakota’, developed at North Dakota Agricultural Research

Station. The seven recent releases (2004-2010) were chosen to represent cultivars selected under a high level of soil fertility for a dryland cropping system. These cultivars were selected under conditions of soil nutrient targets at planting of 100-120 kg ha⁻¹ nitrogen, 60 kg ha⁻¹ phosphorous and 20 kg ha⁻¹ sulphur (DePauw et al. 2011); potassium was not added as the soil test levels were higher than required for wheat production. The recommended level of fertilization was raised in the 1990's when the average protein content of the Canadian durum wheat crop had dropped and was affecting commercial acceptance. In the mid-1990's, the fertility levels used in the breeding nurseries increased from 60 kg N ha⁻¹ to 100 to 120 kg ha⁻¹ N (DePauw personal communication), and therefore, the cultivars released after 2003 would have been selected under much higher soil N status.

Six surface sterilized seeds of each durum wheat genotype were sown in 1-L pots. One week after emergence, plants were thinned to one per pot. A mycelial network is the functional element of AM fungi (Kabir 2005). In a western Canadian field situation, because no-till is practiced, wheat seeds grow within an AM mycelial network. Thus in this experiment, wheat was sown in a pre-established extraradical AM fungal mycelial network achieved by using lentil (*Lens culinaris*) as the mother plant to support the development of the extraradical AM fungal mycelial network in experimental pots. Lentil was chosen because it is the most common crop preceding durum wheat in Canadian. Lentil and durum wheat seeds were surface sterilized by soaking for five minutes in 10% bleach solution (final concentration 0.53% NaOCl) containing 0.1% Tween 20, and were rinsed with distilled water three times. Eleven weeks prior to seeding durum wheat, six surface sterilized seeds of lentil cultivar 'CDC Glamis' were sown in the 1-L pots filled with 0.95-L of pasteurized soil mix (90 °C for 3 h). The mixture of field soil and sand (1:1) contained 16.18 mg kg⁻¹ NO₃-N and 7.63 mg kg⁻¹ PO₄-P after pasteurization, as per the sodium bicarbonate extraction method (Olsen et al. 1954). Pasteurized soil was mixed with live or autoclaved (30 min at 130 °C, 138 kPa) AM inoculant in order to provide 165 spores per 100 g of soil and placed 3 cm below the lentil seeds in each pot. The AM inoculant was MYKE® PRO GR (Premier Tech Biotechnologies, Rivière-du-Loup, Quebec, Canada), a granular inoculant containing 110 axenically

produced infective propagules of *R. irregularis* (syn. *Glomus intraradices*) per gram of product. This commercial inoculant has long been used in horticultural crops and is used in field crops, including wheat, in the Canadian prairie since 2009. The bottom of the pots was perforated and lined with nylon window screen to prevent soil loss through drainage holes. Lentil plants were grown for eight weeks before watering was stopped. Pots were left to dry for three weeks before cutting the shoots at ground level and seeding wheat seeds as described previously.

Lentil and durum wheat plants were examined daily and watered as needed. Once lentil plants expressed signs of deficiency (yellowing of older leaves), usually on a weekly interval, they received 45 mL of Long Ashton nutrient solution ([Hewitt 1966](#)) containing (in mg L⁻¹) 750 KNO₃, 950 Ca(NO₃)₂·4 H₂O, 200 NaH₂PO₄·H₂O, 244 MgSO₄, 520 CaCl₂·H₂O, 1.7 MnSO₄, 0.25 CuSO₄·5H₂O, 0.30 ZnSO₄·7H₂O, 3.0 H₃O₃, 5.0 NaCl, 0.09 (NH₄)₆Mo₇O₂₄·4H₂O, and 32.9 NaFe-EDTA. All lentil plants received the same amount of nutrient solution. Wheat plants under the high fertility regime received 45 mL of the modified Long Ashton nutrient solution once per week and plants under the low fertility regime received 45 mL of a 25% Long Ashton nutrient solution. The day/night temperature was 19/15 ±2°C and the photoperiod was 16 h.

Harvest and data collection

Four weeks after seeding, durum wheat shoots were cut at ground level, and dried at 45°C until constant weight, and dry plant biomass was recorded. Dry shoot tissues were ground in a Thomas Wiley mill (model 4, Thomas Scientific, USA) and digested in H₂SO₄/Se/Na₂SO₄ ([Varley 1966](#)). Digests to determine N and P concentrations were measured on a segmented flow auto-analyzer (Technicon, AAIL System, Tarrytown, New York), and K concentrations were determined on an atomic absorption spectrophotometer (Z-8200 Polarized Zeeman, Hitachi, Japan).

Roots were washed thoroughly, cut into 1-cm fragments, and dispersed and mixed in water. One root sample was taken from each root system and placed in labeled plastic cassettes. Root samples were cleared in 10% KOH and stained using an ink and vinegar solution ([Vierheilig et al. 1998](#)). The percentage of root colonization by AM fungi was determined using the gridline-intersect method ([Giovannetti and Mosse 1980](#)) under a dissecting microscope. Essentially, zones of the root cortex defined by the intersection of 100 randomly distributed roots and the grid-lines of a gridded Petri dish were observed, and the number of zones where AM fungal structures were present was recorded.

Statistical analysis

The significance of treatment effects on shoot dry mass, nitrogen, phosphorus and potassium was assessed by analysis of variance using JMP v.6 (SAS Institute, Cary, NC, USA). The block effect was set as random, and durum wheat genotype, AM symbiotic status, and soil fertility treatments were set as fixed effects. The significance of the differences between treatment means was assessed using the LSMeans Student's *t* tests. Contrast analysis was used to compare the group of landraces (pre-1920) with each group of durum wheat cultivars i.e., all cultivars (1957-2010) and the most recent releases (2004-2010). Analysis of residuals was conducted on the data to identify and remove outliers; the data were tested for normality using the Shapiro-Wilk test. A Log transformation was applied to the values of N concentration to meet the requirement of normality of the test before analysis. The values of P concentration were normalized by square root transformation and the values of K concentration were normalized by square transformation. The transformed data were back transformed for data presentation. The significance of the genotype effect on AM root colonization was analyzed using the data from the mycorrhizal treatment only, because as expected all non-mycorrhizal plants were examined and exhibited zero percent mycorrhizal colonization.

The nutritional response of durum wheat to mycorrhiza was calculated as:

Response M = concentration of $M_{(AM)}$ - concentration of $M_{(non-AM)}$

where “response M ” is the nutritional response of a durum wheat genotype for the major nutrient M , “concentration of $M_{(AM)}$ ” is the concentration of major nutrient M in the shoot tissue of the AM plant and “concentration of $M_{(non-AM)}$ ” is the concentration of major nutrient M in the shoot tissue of the corresponding non-AM control.

The growth response of wheat genotypes to mycorrhiza was calculated as:

Growth response = shoot biomass_(AM) – shoot biomass_(non-AM)

where “shoot biomass_(AM)” is the biomass of the AM plant and “shoot biomass_(non-AM)” is the biomass of the corresponding control plant. Redundancy analysis (RDA) was conducted with the rdaTest application ([Legendre and Durand 2010](#)) within the R Project for Statistical Computing version 2.15.1 to test the relationship between the multivariate response variables: response-N, response-P, and response-K of durum wheat tissues, and the explanatory variables: growth response and AM root colonization level. The explanatory and response variables (growth response and AM root colonization level) were standardized using the Hellinger transformation in the function ‘decostand’ in the vegan-package prior to analysis because some of these variables were expressed in different units or had different order of magnitude.

Results and discussion

AM root colonization was significantly affected by fertility but not by genotype (Table 1). There were significant differences among genotypes for dry mass, phosphorous and potassium concentrations in the plant tissue. AM status resulted in significant differences for dry mass and concentration of N, P, and K. There was only one significant interaction detected. An AM status $\times$ fertility interaction (Table 1, $P = 0.02$) for above ground tissue P concentration indicated that the AM symbiosis, as expected, increased plant tissue P concentration to a larger extent at low soil fertility (mycorrhizal plants: 4.1 mg g^{-1} ; control: 3.7 mg g^{-1}) than at high soil fertility level (mycorrhizal plants: 3.5 mg g^{-1} ; control: 3.3 mg g^{-1}).

177 Soil fertility levels influenced plants' AM root colonization ($P = 0.002$) from 12% to 43% of the root
178 length across genotypes, under low fertility conditions, and from 11% to 30%, under high fertility
179 conditions (Fig. 1 and Table 1). Under high soil fertility conditions, the level of AM root colonization of
180 the landraces (mean across landraces = 17%), cultivars (22%), and most recent releases (21%) was similar
181 ($P = 0.22$). However, under low soil fertility the landraces developed lower level of AM root colonization
182 (21%) than the modern (27%, $P = 0.04$) and most recent cultivars (29%, $P = 0.02$), which were
183 developed under conditions of high soil fertility. Overall, modern breeding methods influenced the
184 regulation of the AM symbiosis of wheat, but this influence was inconsistent, leading to the release of
185 cultivars with good, but unimproved regulation and those with impaired AM symbiotic regulation. Under
186 low soil fertility conditions, the cultivars, Commander and Pathfinder, had the highest levels of
187 colonization, while under high fertility conditions, their levels of colonization were reduced (Fig. 1).
188 Reducing investment in AM fungal growth is an appropriate response in eutrophic conditions where the
189 AM symbiosis provides no nutritional benefits. Hercules was the only cultivar to express significantly
190 higher root colonization at high fertility compared to low fertility (Fig 1), which is an inappropriate
191 response to the AM fungi ([Johnson and Graham 2013](#)) and is considered an impaired AM symbiotic
192 regulation.

193 Overall, mycorrhizal durum wheat had reduced ($P = 0.002$) shoot dry mass, as compared to the non-
194 inoculated controls (Table 2), and this reduction was significant for cultivars Hercules, Wascana and
195 Eurostar under low soil fertility conditions (Fig. 2). However, the level of AM root colonization and plant
196 growth response to mycorrhiza were unrelated (Fig. 3). The level of AM root colonization provides an
197 evaluation of the compatibility between the host and fungus components of different associations ([Utobo
198 et al. 2011](#)), but it is unrelated to the degree of benefit derived from the mycorrhiza ([Hassan et al. 2013b](#);
199 [Hetrick et al. 1993](#); [Powell et al. 2009](#)). The plant's benefit from the AM symbiosis results from a carbon-
200 efficient adjustment ([Johnson and Graham 2013](#)) of the complex interplay between the hyphal and the
201 direct pathways of plant P uptake ([Smith et al. 2011](#)). The growth response of durum genotypes to

mycorrhiza is a measure of this AM benefit ([Reinhart et al. 2012](#)). In this study, mycorrhiza generally had a negative effect on the growth of durum wheat. However, the response of durum wheat to the presence of mycorrhiza may have been underestimated. It is possible that mycorrhizal lentil plants may have drawn more nutrients from the soil than non-mycorrhizal lentil plants. But, the soil conditions were the same for all durum wheat genotypes within an AM status treatment and the possible underestimation to mycorrhizal symbiosis due to lower soil fertility in inoculated pots would have been comparable for all durum wheat genotypes. It was important to establish an AM mycelium network in the designated pots because mycelia are essential functional elements of mycorrhizal symbiosis ([Kabir 2005](#)).

The poor response to mycorrhizal symbiosis measured by dry mass in durum wheat genotypes coincided with significantly elevated concentrations of N, P, and K in the aboveground tissue of the AM colonized plants compared to non-mycorrhizal controls (Table 2) indicating mycorrhiza function was repressed.

High soil fertility conditions resulted in higher concentrations of N and K in plant tissues, but in contrast decreased plant P concentration (Table 2). The AM symbiosis improves plant P uptake most particularly ([Hassan et al. 2013a](#); [Li et al. 2006](#)). This reduction in the concentration of P, accompanied with an increase of other nutrients in durum wheat grown in high fertility conditions is consistent with a dilution effect. The formation and function of the AM symbiosis is regulated by soil fertility. Less carbon is normally invested in mycorrhiza formation and maintenance when nutrients are easily available to roots ([Johnson and Graham 2013](#)). High assimilate partitioning to fungal sinks would be an inefficient strategy in rich soils where the AM symbiosis brings no additional benefit to plant nutrition.

The growth response and the N and K response of durum wheat genotypes to mycorrhiza were inversely related (Fig. 3). When a mycorrhizal treatment genotype grew larger, tissue N and P concentrations were diluted. Similar amounts of N, P, and higher amount of K were extracted by mycorrhizal and non-mycorrhizal plants, but mycorrhizal plants produced less biomass (Table 2), suggesting that mycorrhizal plant growth is carbon rather than nutrient limited. This possibility is supported by a recent report of carbon limitation in Canadian wheat fields with low soil fertility ([Dai et al. 2014](#)). The growth response to

mycorrhiza of durum wheat genotypes ranged from positive trends to significant growth depression, which is in agreement with the model of mutualism-parasitism continuum of mycorrhizal function proposed by Johnson and Graham (2013). This model explains that mycorrhiza can depress the growth of their host through the diversion of carbon assimilates from plant sinks (Johnson and Graham 2013). The plants that grow larger with mycorrhiza or to a size similar to that of non-mycorrhizal plants compensate their investment in the maintenance and activity of AM fungi by enhancing their photosynthetic rate (Koide and Elliott 1989). Apparently, the cultivars Hercules, Wascana and Eurostar, could not compensate for the carbon drain accompanying the AM symbiosis and therefore the growth was reduced in the presence of AM fungi compared to the control (Fig. 2). The productivity of cultivars with limited capacity for carbon dioxide assimilation may suffer from mycorrhiza formation, in contrast to cultivars with higher photosynthetic capacity. It might be important to ensure the good regulation and photosynthesis capacity of durum wheat prior to cultivar release, as AM fungi are ubiquitous in commercial fields and can reduce wheat productivity (Dai et al. 2013).

Wheat was reported to respond positively (Hetrick et al. 1992; Mäder et al. 2011) and negatively (Li et al. 2006; Ryan and Kirkegaard 2012) to the AM symbiosis. Growing durum wheat genotypes which support and respond positively to AM symbiosis could provide global benefits to cropping systems (Ellouze et al. 2014).

Conclusion

We found little evidence that landraces, which were selected in low soil fertility, respond and regulate their AM symbiosis better than modern wheat cultivars selected under high inorganic soil fertility conditions. Plant breeding had inconsistent effects on AM symbiosis and cultivars with a range of AM symbiosis efficiencies from unimproved to inefficient have been developed.

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References

- Clarke, J.M., Clarke, F.R., and Pozniak, C.J. 2010. Forty-six years of genetic improvement in Canadian durum wheat cultivars. *Can. J. Plant. Sci.* **90**(6): 791-801. doi: 10.4141/cjps10091.
- Dai, M., Bainard, L.D., Hamel, C., Gan, Y., and Lynch, D. 2013. Impact of land use on arbuscular mycorrhizal fungal communities in rural Canada. *Appl Environ Microbiol* **79**(21): 6719-6729. doi: 10.1128/aem.01333-13.
- Dai, M., Hamel, C., Bainard, L.D., Arnaud, M.S., Grant, C.A., Lupwayi, N.Z., Malhi, S.S., and Lemke, R. 2014. Negative and positive contributions of arbuscular mycorrhizal fungal taxa to wheat production and nutrient uptake efficiency in organic and conventional systems in the Canadian prairie. *Soil Biol Biochem* **74**: 156-166. doi: <http://dx.doi.org/10.1016/j.soilbio.2014.03.016>.
- DePauw, R.M., Malhi, S.S., Bullock, P.R., Gan, Y.T., McKenzie, R.H., Larney, F., Janzen, H.H., Cutforth, H.W., and Wang, H. 2011. Wheat Production in Northern High Latitudes – Canadian example. *In The World Wheat Book: A History of Wheat Breeding. Edited by W. Angus and A. Bonjean and M. Van Ginkel.* Lavoisier Publishing, Paris. pp. 607-651.
- Ellouze, W., Esmaeili Taheri, A., Bainard, L.D., Yang, C., Bazghaleh, N., Navarro-Borrell, A., Hanson, K., and Hamel, C. 2014. Soil Fungal Resources in Annual Cropping Systems and Their Potential for Management. *Biomed Res Int* **2014**: 15. doi: 10.1155/2014/531824.
- Ellouze, W., Hanson, K., Nayyar, A., Perez, J., and Hamel, C. 2008. Intertwined existence: The life of plant symbiotic fungi in agricultural soils. *In Mycorrhiza : State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics. Edited by A. Varma.* Springer Berlin Heidelberg. pp. 507-528.
- Giovannetti, M., and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* **84**(3): 489-500.

- 281 Hassan, S., Liu, A., Bittman, S., Forge, T., Hunt, D., Hijri, M., and St-Arnaud, M. 2013a. Impact of 12-
282 year field treatments with organic and inorganic fertilizers on crop productivity and mycorrhizal
283 community structure. *Biol Fertil Soils* **49**(8): 1109-1121. doi: 10.1007/s00374-013-0813-7.
- 284 Hassan, S.E., Hijri, M., and St-Arnaud, M. 2013b. Effect of arbuscular mycorrhizal fungi on trace metal
285 uptake by sunflower plants grown on cadmium contaminated soil. *New Biotechnol* **30**(6): 780-787. doi:
286 <http://dx.doi.org/10.1016/j.nbt.2013.07.002>.
- 287 Hetrick, B.A.D., Wilson, G.W.T., and Cox, T.S. 1992. Mycorrhizal dependence of modern wheat
288 varieties, landraces, and ancestors. *Can J Bot* **70**(10): 2032-2040. doi: 10.1139/b92-253.
- 289 Hetrick, B.A.D., Wilson, G.W.T., and Cox, T.S. 1993. Mycorrhizal dependence of modern wheat
290 cultivars and ancestors: a synthesis. *Can J Bot* **71**(3): 512-518. doi: 10.1139/b93-056.
- 291 Hewitt, E.J. 1966. Sand and water culture methods used in the study of plant nutrition. Commonwealth
292 Agricultural Bureau, Farnham Royal, Bucks, England. pp. 431-432.
- 293 Johnson, N.C., and Graham, J.H. 2013. The continuum concept remains a useful framework for studying
294 mycorrhizal functioning. *Plant Soil* **363**(1-2): 411-419. doi: 10.1007/s11104-012-1406-1.
- 295 Kabir, Z. 2005. Tillage or no-tillage: Impact on mycorrhizae. *Canadian Journal of Plant Science* **85**(1):
296 23-29. doi: 10.4141/p03-160.
- 297 Knott, D.R. 1995. Durum wheat. In *Harvest of gold: the history of field crop breeding in Canada*. Edited
298 by A.E. Slinkard and D.R. Knott. University Extension Press, University of Saskatchewan, Saskatoon,
299 SK, Canada. pp. 67-81.
- 300 Koide, R., and Elliott, G. 1989. Cost, benefit and efficiency of the vesicular-arbuscular mycorrhizal
301 symbiosis. *Funct Ecol* **3**(2): 252-255. doi: 10.2307/2389310.
- 302 Legendre, P., and Durand, S. 2010. rdaTest: Canonical redundancy analysis. R package version 1.7.
303 <http://adn.biol.umontreal.ca/~numeralecology/Rcode/>. Accessed 30 June 2012.
- 304 Lehmann, A., Barto, E., Powell, J., and Rillig, M. 2012. Mycorrhizal responsiveness trends in annual crop
305 plants and their wild relatives - a meta-analysis on studies from 1981 to 2010. *Plant Soil* **355**(1): 231-250.
306 doi: 10.1007/s11104-011-1095-1.
- 307 Li, H., Smith, S.E., Holloway, R.E., Zhu, Y., and Smith, F.A. 2006. Arbuscular mycorrhizal fungi
308 contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of
309 positive growth responses. *New Phytol* **172**(3): 536-543. doi: 10.1111/j.1469-8137.2006.01846.x.
- 310 Mäder, P., Kaiser, F., Adholeya, A., Singh, R., Uppal, H.S., Sharma, A.K., Srivastava, R., Sahai, V.,
311 Aragno, M., Wiemken, A., Johri, B.N., and Fried, P.M. 2011. Inoculation of root microorganisms for
312 sustainable wheat-rice and wheat-black gram rotations in India. *Soil Biol Biochem* **43**(3): 609-619. doi:
313 <http://dx.doi.org/10.1016/j.soilbio.2010.11.031>.

- 314 Olsen, S.R., Cole, C.V., Watanabe, F.S., and Dean, L.A. 1954. Estimation of available phosphorus in
315 soils by extraction with sodium bicarbonate. U. S. Department of Agriculture, Washington, DC.
- 316 Paungfoo-Lonhienne, C., Visser, J., Lonhienne, T.A., and Schmidt, S. 2012. Past, present and future of
317 organic nutrients. *Plant Soil* **359**(1-2): 1-18. doi: 10.1007/s11104-012-1357-6.
- 318 Plenchette, C., Fortin, J.A., and Furlan, V. 1983. Growth responses of several plant species to
319 mycorrhizae in a soil of moderate P-fertility. *Plant Soil* **70**(2): 199-209. doi: 10.1007/bf02374780.
- 320 Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C., and Maherali, H. 2009.
321 Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi.
322 *Proc. R. Soc. B* **276**(1676): 4237-4245. doi: 10.1098/rspb.2009.1015.
- 323 Reinhart, K.O., Wilson, G.W.T., and Rinella, M.J. 2012. Predicting plant responses to mycorrhizae:
324 integrating evolutionary history and plant traits. *Ecol. Lett.* **15**(7): 689-695. doi: 10.1111/j.1461-
325 0248.2012.01786.x.
- 326 Ryan, M.H., and Kirkegaard, J.A. 2012. The agronomic relevance of arbuscular mycorrhizas in the
327 fertility of Australian extensive cropping systems. *Agric. Ecosyst. Environ.* **163**(0): 37-53. doi:
328 <http://dx.doi.org/10.1016/j.agee.2012.03.011>.
- 329 Singh, A.K., Hamel, C., DePauw, R.M., and Knox, R.E. 2012. Genetic variability in arbuscular
330 mycorrhizal fungi compatibility supports the selection of durum wheat genotypes for enhancing soil
331 ecological services and cropping systems in Canada. *Can. J. Microbiol.* **58**(3): 293-302. doi:
332 10.1139/w11-140.
- 333 Smith, S.E., Jakobsen, I., Grønlund, M., and Smith, F.A. 2011. Roles of arbuscular mycorrhizas in plant
334 phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal
335 roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant*
336 *Physiol* **156**(3): 1050-1057. doi: 10.1104/pp.111.174581.
- 337 Utobo, E.B., Ogbodo, E.N., and Nwogbaga, A.C. 2011. Techniques for extraction and quantification of
338 arbuscular mycorrhizal fungi. *LARCJI* **2**(2): 68-78.
- 339 Varley, J.A. 1966. Automatic methods for the determination of nitrogen, phosphorus and potassium in
340 plant material. *Analyst* **91**(1079): 119-126.
- 341 Vierheilig, H., Coughlan, A.P., Wyss, U., and Piche, Y. 1998. Ink and vinegar, a simple staining
342 technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol.* **64**(12): 5004-5007.
- 343 Zhu, Y.G., Smith, S.E., Barritt, A.R., and Smith, F.A. 2001. Phosphorus (P) efficiencies and mycorrhizal
344 responsiveness of old and modern wheat cultivars. *Plant Soil* **237**(2): 249-255. doi:
345 10.1023/a:1013343811110.

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Table 1 Summary of the probabilities of effects of durum wheat genotype, AM symbiotic status, soil fertility and their interactions on AM root colonization (Col), dry mass, and nitrogen (N), phosphorus (P) and potassium (K) concentrations in plant tissues, according to ANOVA ($n = 4$)

Source of variance	<i>df</i>	Col (%)	Dry mass (g)	N ($\mu\text{g}\cdot\text{g}^{-1}$)	P ($\mu\text{g}\cdot\text{g}^{-1}$)	K ($\mu\text{g}\cdot\text{g}^{-1}$)
Genotype	31	0.8272 ^{ns}	0.0373*	0.8040 ^{ns}	< 0.0001***	0.0103*
AM status	1	-	0.0015**	0.0146*	< 0.0001***	0.0022**
Fertility	1	0.0016**	< 0.0001***	< 0.0001***	< 0.0001***	< 0.0001***
Genotype $\times$ AM status	31	-	0.7409 ^{ns}	0.9109 ^{ns}	0.6483 ^{ns}	0.6250 ^{ns}
Genotype $\times$ Fertility	31	0.2592 ^{ns}	0.3772 ^{ns}	0.8483 ^{ns}	0.2000 ^{ns}	0.9603 ^{ns}
AM status $\times$ Fertility	1	-	0.4801 ^{ns}	0.6385 ^{ns}	0.0215*	0.8057 ^{ns}
Genotype $\times$ AM status $\times$ Fertility	31	-	0.7433 ^{ns}	0.9841 ^{ns}	0.7531 ^{ns}	0.5602 ^{ns}

ns: non-significant; * significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level.

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Table 2 Shoot nitrogen, phosphorus and potassium concentrations and above grown biomass production of 4-week old durum wheat plants, as influenced by arbuscular mycorrhiza and soil fertility

	N	N uptake (mg	P	P uptake	K	K uptake (mg	Dry mass
Symbiotic status	(mg g ⁻¹)	plant ⁻¹)	(mg g ⁻¹)	(mg plant ⁻¹)	(mg g ⁻¹)	plant ⁻¹)	(mg plant ⁻¹)
Mycorrhizal	26.8	18.0	3.8	2.4	44.8	31.8	680
Non-mycorrhizal	25.7	18.5	3.5	2.3	43.2	30.7	720
<i>P</i> value	0.0146*	0.1050 ^{ns}	< 0.0001***	0.2058 ^{ns}	0.0022**	0.0422*	0.0015**
Fertility ^z							
Higher	29.1	23.3	3.4	2.8	46.2	37.7	810
Lower	23.4	13.1	3.9	2.3	41.8	24.7	580
<i>P</i> value	< 0.0001***	< 0.0047**	< 0.0001***	0.0424*	< 0.0001***	< 0.0001***	< 0.0001***

ns: non-significant; * significant at the 5% level; **significant at the 1% level; ***significant at the 0.1% level.

^z All comparisons between higher and lower fertility levels were made under mycorrhiza treatment.

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Fig. 1 The level of AM root colonization (colonized root sections / 100 root sections) of landraces and modern durum wheat cultivars grown in the greenhouse under low or high soil fertility conditions. The most recent cultivars were selected under high soil fertility conditions. Stars indicate a statistically significant difference in colonization levels between low and high fertility conditions for the cultivars Commander, Pathfinder, and Hercules, according to LSMeans Student's t set at $\alpha = 0.05$ ($n = 4$). Modern cultivars have higher levels of AM root colonization than landrace, when grown in low fertility conditions ($P = 0.04$).

Fig. 2 Shoot dry mass of landraces and modern wheat genotypes grown in symbiosis with *R. irregularis* and of non-mycorrhizal controls grown under low soil fertility conditions. Overall, the biomass of symbiotic durum wheat was reduced under low fertility conditions ($P = 0.04$). Stars indicate that the difference between the mycorrhizal and non-mycorrhizal treatments was significant only for the cultivars Eurostar, Wascana, and Hercules, according to LSMeans Student's t set at $\alpha = 0.05$ ($n = 4$). The most recent cultivars were selected under high soil fertility conditions.

Fig. 3 Redundancy analysis biplot of the multivariate relationship ($P = 0.001$) between the response of leaf tissues N, P, and K concentrations to mycorrhiza, and the level of AM root colonization and growth response to mycorrhiza, in durum wheat (Axes 1 and 2 explain respectively 20.1% and 1.2% of the variance). The biplot shows that the P response of the plants is related with the level of AM root colonization, but unrelated to plant growth response. The biplots also shows a negative relationship between the nutritional responses and the growth response of durum wheat.