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in

Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.).
New approaches for grassland research in a context of climate and socio-economic changes

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102

2012
pages 477-481

Root colonization of hybrid maize cultivars by mycorrhizal and pathogenic fungi at establishment in an acid soil with high phosphorus content

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Abstract. The formation of arbuscular mycorrhizas (AM), a symbiosis between roots and Glomeromycetes that improves P nutrition, health and stress tolerance of plants, can be important for establishment of maize in acid soils. In this work, a field experiment was set up in a limed acid soil with high Olsen-P content to study the colonization of roots by AM fungi and/or other soil borne fungi in 48 maize hybrid cultivars. Plants were under a severe drought at establishment. One month after sowing, AM fungi colonized over 40% of the root length of most cultivars, suggesting a mycorrhizal dependency of these plants to withstand water stress conditions. Ten cultivars presented less than 20% of AM root colonization. As determined by molecular methods, the most frequent AM fungi were *Gigaspora decipiens* and *Glomus* spp. Roots were also colonized by *Pythium* spp., *Fusarium solani*, and *F. oxysporum*.

Keywords. Arbuscular mycorrhizas – AM fungi – *Fusarium oxysporum* – *Pythium* sp. – *Zea mays*

Colonisation des racines des cultivars hybrides de maïs par les champignons mycorhiziens et pathogènes à l'établissement dans un sol acide avec haute teneur en phosphore

Résumé. *La formation des mycorhizes arbusculaires (MA), une symbiose entre les racines et Glomeromycetes qui améliore nutrition phosphorée, la santé et la tolérance au stress des plantes, peut être importante pour leur mise en place dans les sols acides. Dans ce travail, une expérience sur le terrain a été mis en place dans un sol acide chaulées à haute teneur en P Olsen-pour étudier la colonisation des racines par les champignons MA et/ou d'autres champignons du sol transmises dans 48 cultivars hybrides de maïs. Les plantes étaient en vertu d'une grave sécheresse à l'établissement. Un mois après le semis, les champignons AM colonisé plus de 40% de la longueur de la racine de la plupart des cultivars, ce qui suggère une dépendance mycorhizienne de ces plantes à résister à des conditions de stress hydrique. Dix cultivars présenté moins de 20% de colonisation MA des racines. Comme déterminé par des méthodes moléculaires, les champignons MA les plus fréquents étaient* Gigaspora decipiens *et* Glomus *spp. Les racines ont également été colonisés par* Pythium *spp.,* Fusarium solani*, et* F. oxysporum.

Mots-clés. *Mycorhizes arbusculaires – Champignons MA* – Fusarium oxysporum – Pythium sp. – Zea mays.

I – Introduction

In Galicia, a cattle-rearing region in NW Spain, 62,426 ha of maize are grown annually, producing 2,528,334 t of forage mainly used for silage, particularly in intensive dairy farms. The cultivated area and production have almost doubled in the last 10 years, in part as a result of the use of new hybrid cultivars with better productivity and quality.

Phosphorus promotes the initial development of maize, but maize hybrids in general are not efficient at extracting the nutrient (Kaeppler *et al.*, 2000). Also, differences in P uptake efficiency exists among maize genotypes, that can be explained by morphological and physiological plant traits, by environmental conditions, and by interactions of plants and microbes (Zhu and Lynch, 2004; Rosolem *et al.*, 2008; Gautam *et al.*, 2011).

The ability of maize hybrids to form arbuscular mycorrhizas (AM), a symbiosis between the roots and Glomeromycetes that improves P nutrition, health and biotic and abiotic stress tolerance of plants (Smith and Read, 1997), can be important to use more efficiently soil available P and to enhance plant establishment. Arbuscular mycorrhizas also alter the pattern of root exudates, resulting in differences in the microbial composition of the rhizosphere (Linderman, 1992) that could affect root invasion by fungal pathogens. The aim of this work was to study the colonization of roots by AM fungi and/or other soil borne fungi in 48 maize hybrids at establishment in an acid soil with high Olsen-P content.

II – Materials and methods

A field trial was carried out in a 0.3 ha plot located in Mazaricos (A Coruña, NW Spain). The soil was acidic (pH 4.8), and with high Olsen-P (46 mg/kg) and available K (220 mg/kg) contents. Forty eight commercial cultivars of forage maize hybrids were planted in a randomised block experiment with three replicates. In May 2011, after tillage, 150 kg N/ha, 175 kg P_2O_5/ha, and 250 kg K_2O/ha were applied. To prevent cutworms and wireworms, chlorpyrifos (5%) was applied in the furrow line at planting and, for the control of spontaneous vegetation, a pre-emergence herbicide consisting of acetochlor (45%) and terbuthylazine (21.5%). On May 18th, 2011, in each block, each forage maize cultivar was planted in three 4 m long rows. The planting density was 90,000 plants/ha.

One month after planting, in each block, three plants (shoot and roots) of each cultivar were carefully removed from soil in every replicate. The shoots were dried at 80ºC in an oven to determine dry weight. For each plant, a sample of the roots was cleared and stained with trypan blue (Phillips and Hayman, 1970) to estimate the percentage of root length colonized by AM fungi (Giovannetti and Mosse, 1980). Data on shoot dry weight and AM root colonization were subjected to a one way ANOVA. Means were compared by the Duncan test at $P<0.05$.

For detection and identification of pathogenic fungi, pieces of roots were placed in Petri dishes with Komada medium and in Petri dishes with V8 medium. The plates were incubated at 24ºC. *Fusarium* colonies were transferred to PDA medium (Potato Dextrose Agar), to obtain monosporic cultures, and *Pythium* colonies were subcultured in V8 medium at 22-24ºC. Both *Fusarium* and *Pythium* species were initially identified by observing macroscopic and microscopic characteristics.

Identification of AM and pathogenic fungi in roots was also carried out by molecular methods. Fungal DNA was extracted from pieces of roots with the EZNA Fungal DNA Mini Kit (Omega Bio-tek). For AM fungi, a region of approximately 550 bp in the SSU rDNA gene was amplified using primers NS31 and AM1 (Helgason *et al.,* 1998). For *Fusarium* species, the ITS region of rDNA and a portion of the gene sequence of the elongation factor 1 α (gene EF-1 α) were studied. DNA was extracted from mycelium of monosporic cultures and amplified with primers ITS1-ITS4 and EF1-EF2, following the methods of White *et al.* (1990) and O'Donnell *et al.* (2000), respectively. For *Pythium* isolates, DNA was amplified by a semi-nested PCR using primers DC6-ITS4 in the first round and ITS6-ITS4 in the second (Cooke *et al.*, 2000). The amplified products from all fungal species were purified and sequenced on a ABI PRISM 3130 (Applied Biosystems), comparing the obtained sequences with those deposited in GenBank and, for *Fusarium* species, in the *Fusarium* comparative database of the Broad Institute.

III – Results and discussion

At establishment, plants were under severe water stress. There was no rainfall after planting and the initial plant growth was supported by the soil water reservoir, which was soon depleted since the water balance in the first months growth was highly negative (-89 l/m^2 and -121 l/m^2 in May and June, respectively). Together with the acid pH, low soil moisture probably resulted in a low availability of nutrients in the rhizosphere, particularly phosphorus (Gahoonia *et al.,* 1994).

Maize cultivars significantly differed in growth, ranging from 240 mg DM/plant for 'Jumbo 48' to 950 mg DM/plant for 'Automat' (average 548 mg DM/plant) (data not shown). These differences might be partly explained by different morpho-physiological responses to deficient water and P availability, but also to the ability of cultivars to form arbuscular mycorrhizas (Boosma and Vyn, 2008). Modern maize genotypes have been selected under conditions of good soil P fertility, what explains that, when no other factors are limiting, under high soil P conditions the percentage of root length colonized by AM fungi (below 20%) and plant growth are lower than at low P (Kaeppler *et al.,* 2000). In our work, all cultivars formed the mycorrhizal symbiosis, although not with the same AM fungus. Molecular analysis showed that the most frequent AM fungi colonizing roots were *Gigaspora decipiens* and *Glomus* spp. (data not shown).

AM fungal colonization ranged from 3.8 % (cultivar Jumbo 48) to 75 % (cultivar DKC 4888) of total root length. Ten maize hybrids presented less than 20% of AM root colonization, but most of them (thirty one cultivars) showed over 40% of the root length colonized, suggesting a mycorrhizal dependency of these plants to obtain water and nutrients, particularly P, under soil moisture stress and acidic conditions. AM fungi have been shown to enhance early maize P nutrition and growth at low fertility conditions, but also in drought environments with high soil P levels (Sylvia *et al.,* 1993). Fungal contribution to water and nutrient uptake is done through mycorrhizal hyphae, that extend far beyond the rhizosphere and act as an extension of the root system (Ruiz-Lozano and Azcón, 1995; Smith and Read, 1997).

The major effect of the AM symbiosis is to enhance uptake of P, and also other scarcely mobile nutrients and water, but AM fungi can also play an important role in the control of root fungal pathogens (Linderman, 1992). In our work, morphological and molecular analysis demonstrated that roots from all maize cultivars were colonized by *Pythium* spp., *Pythium irregulare*, *Fusarium solani*, and *F. oxysporum* (Table 1), independently of the AM fungus found in their roots was *Glomus* spp. or *Gigaspora* spp. and of the percentage of AM root colonization. This is in contrast with the results of Maherali and Klironomos (2007), who concluded that fungi in the Glomeraceae colonize more extensively plant roots, form less external hyphae, and are more effective at reducing infection by either *F. oxysporum* or a *Pythium* sp. in *Plantago lanceolata* that fungi in the Gigasporaceae.

IV – Conclusions

Soil low pH and water shortage may have resulted in difficulties in uptake of P and other poorly mobile nutrients by commercial hybrid cultivars of forage maize in the high-P cultivated soil, which led to extensive colonization of roots by AM fungi. Differences in AM root colonization and plant growth at establishment suggest differences in mycorrhizal dependency of the maize hybrids under water and nutrient stress conditions. Arbuscular mycorrhizas did not prevent invasion of roots by species of *Fusarium* and *Pythium*, although it cannot be discarded an increased tolerance of maize hybrids to these pathogenic fungi mediated by the symbiosis.

Table 1. Co-occurrence of pathogenic fungal species in roots of 48 maize cultivars

Pythium sp.	*Pythium irregulare*	*Fusarium solani*	*Fusarium oxysporum*	Nº maize cultivars	Maize cultivars
■				11	Alexxandra, Amanatidis, Anjou 456, Atlético, DKC 4372, DKC 4608, Gladi, GW 001, Happy, Marcello, Organza
■			■	15	Altius, Bonpi, Columbia, Francisco, Ginko, Goldigest, Jennifer, Lucan, LG 33.85, Manacor, LG 32.76, Pesandor, Rulex, ZP 305, ZP 409
■		■	■	14	Automat, Bora, Brandy, Delli, Dixxmo, DK 315, DKC 4888, Stern, Fortim, ES Sigma, Es Fortress, Lemoro, LG 32.77, Mass 33.A
■		■		5	BC 244, ES Sensor, Josquin, Mamilla, Phileaxx
	■	■	■	1	Jumbo 48
	■	■		1	Castelli
	■		■	1	Mas 23.B

References

Boomsma C.R. and Vyn T.J., 2008. Maize drought tolerance: Potential improvements through arbuscular mycorrhizal symbiosis? In: *Field Crops Research* 108, p. 14-31.

Cooke D.E.L., Drenth A., Duncan J.M., Wagels G. and Brasier C.M., 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. In: *Fungal Genetics and Biology* 30, p. 17-32.

Giovannetti M. and Mosse B., 1980. An evaluation of techniques for measuring vesicular- arbuscular mycorrhizal infection in roots. In: *New Phytologist* 84, p. 489-500.

Gahoonia T.S., Raza S. and Nielsen N.E., 1994. Phosphorus depletion in the rhizosphere as influenced by soil moisture. In: *Plant and Soil* 159, p. 213-218.

Gautam P., Gustafson D.M. and Wicks III Z., 2011. Phosphorus concentration, uptake and dry matter yield of corn hybrids. In: *World Journal of Agricultural Sciences* 7(4), p. 418-424.

Helgason T., Daniell T.J., Husband R., Fitter A. and Young P., 1998. Ploughing up the wood-wide web? In: *Nature* 394, 431.

Kaeppler S.M., Parke J.L., Mueller S.M., Senior L., Stuber C. and Tracy W.F., 2000. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. In: *Crop Science* 40, p. 358-364.

Linderman R.G., 1992. Vesicular-arbuscular mycorrhizae and soil microbial interaclions. In: *Mycorrhizae in Sustainable Agriculture*. Eds. G.J. Beihlenfalvay, R.G. Linderman. Madison, USA: American Society of Agronomy Special Publication 54, p. 45-70.

Maherali H. and Klironomos J.N., 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. In: *Science*, 316, p. 1746-1748.

O'Donnell K., Kistler H.C., Tacke B.K. and Casper H.H., 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. In: *Proceedings of the National Academy of Sciences*, 97, p. 7905-7910.

Phillips J.M. and Hayman D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection In: *Transactions of the British Mycological Society*, 55, p. 158-161.

Rosolem C.A., Assis J.S. and Santiago A.D., 2008. Root growth and mineral nutrition of corn hybrids as affected by phosphorus and lime. In: *Communications in Soil Science and Plant Analysis*, 25(13-14), p. 2491-2499.

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. In: *Physiologia Plantarum*, 95, p. 472-478.

Sikes B.A., Cottenie K. and Klironomos J.N., 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. *Journal of Ecology*, 97, p. 1274-1280.
Smith S.E. and Read D.J., 1997. *Mycorrhizal Symbiosis.* Academic Press, Inc., San Diego, CA.
Sylvia D.E., Hammond L.C., Bennet J.M., Hass J.H. and Linda S.B., 1993. Field response of maize to a VAM fungus and water management. In: *Agronomy Journal*, 85, p. 193-198.
White T.J., Bruns T., Lee S. and Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M.A., Gelfand D.H., Sninsky J.J. and White T.J. (Eds). *PCR Protocols: A Guide to Methods and Applications*. Academic Press, Inc., San Diego, CA, p. 315-322.
Zhu J. and Lynch J.P., 2004. The contribution of lateral rooting to phosphorus acquisition efficiency in maize (*Zea mays* L.) seedlings. In: *Functional Plant Biology*, 31, p. 949-958.