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Selection of aluminum tolerant cereal genotypes strongly influences the arbuscular mycorrhizal fungal communities in an acidic Andosol

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ABSTRACT

In Chile, cereals cultivation is mainly in volcanic soils with pH values typically between 4.5–5.5 and high levels of exchangeable aluminum (Al) and low P availability. In this context, arbuscular mycorrhizal fungi (AMF) provide or enhance protection against this environmental stress. The aim of this study was to investigate the impact of the breeding process of Al-tolerant cereal plants on AMF community structure and diversity associated to cereals species. This breeding program has been developed since 1980 in our country and consists of obtaining cereal plants that can tolerate stress by Al. For this, we contrast cereals species and genotypes in which Al-stress has been included or not in this breeding program. Rhizosphere soils from Al-tolerant cereals recently developed (*Avena sativa, Hordeum vulgare, Triticum durum*, x. *Triticosecale Wittmack*, *Secale cereale* and *T. aestivum*) were collected from field plots in South-Central Chile. In addition, two cereals with recognized Al-tolerance (Crac wheat cultivar and rye) were also analyzed. AMF identification and taxonomy was performed based on spore morphological analyses. Colonization and glomalin related soil protein (GRSP) was also evaluated. In general, up to 80% of root colonization in all cereal was found. Extraradical mycelium reached levels close to 3 m g^{-1} of soil in the rhizosphere of *S. cereale*, *A. sativa* and *H. vulgare* selected under Al stress. While, GRSP values were statistically similar among selected or not selected genotypes under Al stress, this trend was not observed in *H. vulgare*, where a difference of 20 μg GRSP g^{-1} of soil was found. Moreover, large differences in AMF spore densities were observed, being 340 spores in 100 g soil the lowest and 1900 the highest one, in non Al tolerant *H. vulgare* and Al tolerant x. *Triticosecale Wittmack*, respectively. From a total of 10,000 AM fungal spores, 21 AMF species were identified, belonging to three classes, six orders, and eight families. The alpha diversity was higher in Al tolerant *T. durum* and almost similar to *T. aestivum*. Evenness index was significantly higher in Al tolerant *H. vulgare*. As conclusion, the use of target AMF species and cereals obtained under Al stress could be determinant factors for the appropriate AMF community establishment, potential inoculation assays and agricultural practices, especially oriented to soils with high Al levels.

1. Introduction

Worldwide, acid soils restrict agricultural production (von Uexküll and Mutert, 1995). In Chile, cereals cultivation is mainly carried out in Andosols characterized by pH values typically between 4.5 and 5.5 and low P availability. In general, these soils have undesirable properties, such as high P-adsorption and high levels of exchangeable aluminum (Al^{3+}), Mn^{2+} and H^{+} ions. These soil conditions create a significant decline in plant growth by a reduction of root length, which are limiting their capacity for absorbing water and nutrients (Seguel et al., 2013; Aguilera et al., 2015). It is recognized that phytotoxicity caused by the high levels of Al damages the cell membrane resulting in slower growth and root cell elongation (Magalhaes et al., 2007; Seguel et al., 2013; Aguilera et al., 2015).

The strategies that have allowed the cultivation of cereals in Chile, specifically on Andosols, are principally focused in the developing of genotypes differing in their capacity to tolerate Al phytotoxicity. The obtained cereal cultivars have been generated through breeding programs based on the introduction of cultivars which have been subjected to landraces adapted to local conditions from Southern-Central Chile. As a whole, this has caused an improvement on the germplasm characteristics, such as the tolerance to high Al levels (von Baer, 2007;

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Seguel et al., 2016a, 2016b). Additionally, these traits have been enhanced by associated soil indigenous microorganisms, which improve the plant protection against environmental stress (Meier et al., 2012; Seguel et al., 2013; Aguilera et al., 2011, 2015).

In the context of Al environmental stress, several studies have shown that arbuscular mycorrhizal fungi (AMF) favor biological adaptation of cereals living under abiotic stress conditions, such as low P bioavailability (Castillo et al., 2012) and Al phytotoxic levels (Cumming and Ning, 2003; Seguel et al., 2013, 2015, 2016b). Based on the above, among agricultural management alternatives of cereal growing under these conditions, it is considering the use of AMF in programs oriented to inoculants generation able to attenuate Al phytotoxicity and increasing P acquisition. In this way, it could increase plant productivity and grain production (Borie et al., 2010; Aguilera et al., 2015).

This work is part of a research line focused on elucidating the main mechanisms by which AMF help to reduce negative effects of high Al levels and to find efficient AMF strains able to be used as inoculants in crop production in acidic soils. According to previous studies (Castillo et al., 2006; Aguilera et al., 2014; Seguel et al., 2015, 2016a, 2016b; Marín et al., 2016) we hypothesized that genotypes developed under Al stress will positively influence on AMF diversity represented by the dominance of generalist AMF species for this soil conditions. Thus, few AMF species will be more functionally compatible with these cereals.

Generally, AMF propagules and glomalin related soil protein (GRSP) have been used as a tool for determining host-AMF interaction. Then, we also hypothesized that there will be higher AMF propagules volumes associated with cereals obtained under Al stress differentiate mass selection, because cereals could be depend in great magnitude of AMF symbiosis for overcoming this environmental stress condition. Consequently, the aims in this study were i) to determine the effect of Al-tolerance on AMF community structure and diversity in six cereals species, and ii) to compare AMF propagules interaction with these cereals in a long-term field assay under conventional farming.

2. Materials and methods

2.1. Study site and cereal species

For this study, 6 plots located in the South-Central Chile that belong to an Experimental Station dedicated to cereals breeding oriented to develop Al-tolerant cereal genotypes, were selected (39°06′14″S and 72°41′16″W). Genotypes of four cereal species (*Avena sativa* L., *Hordeum vulgare L*, *Triticum durum*, x. *T. Wittmack*) have been developed recently by means of breeding process based on mass selection including Al-stress as principal factor (see Table 1). Moreover, two genotypes of cereal species with a recognized Al-tolerance (*Secale cereale* L. and *Triticum aestivum* L. cv. Crac) were also included in our study.

Table 1
Cereal species and genotypes selected in a long-term assay in field conditions in an Andosol from south-central Chile in a breeding process under Al-stress and the respective cereal code.

Cereal species	Cultivar selection under Al-stress[a]	Cereal code
Avena sativa	−	AS1
Avena sativa	+	AS2
Hordeum vulgare	−	HV1
Hordeum vulgare	+	HV2
Triticum durum	−	TD1
Triticum durum	+	TD2
x. *Triticosecale Wittmack*	−	TW1
x. *Triticosecale Wittmack*	+	TW2
Secale cereale	+	SC
Triticum aestivum	+	TA

[a] (+) indicates genotypes selected under Al-stress and (−) indicates the genotypes without Al-stress. *A. sativa*, *H. vulgare*, *T. durum*, x *Triticosecale Wittm.* have been recently developed; whereas, *S. cereale* and *T. aestivum* correspond to proved Al-tolerant genotypes widely used in acidic soils from southern Chile, also included in this study.

2.2. Soil sampling

The soil present in the plots was a Dystric Andosol (pH 4.5, -SOM-12.2%, Al-Sat. 25%). Rhizosphere soil was considered the soil adhered to the roots of cereal plants obtained at 0–20 cm depth. Soil sampling was performed in three replicates plots, six rhizosphere soil sub-samples were obtained from each plot and then combined, air dried and sieved through a 2 mm mesh and analyzed as one individual sample per plot. Soil samples were taken at two weeks post-harvest (March, 2015), because previous studies by Cornejo et al. (2007, 2008b, 2008c) have demonstrated that in cereals as wheat growing in acidic Andosols the time between harvest and three months post-harvest represents the maximum densities of AMF spores. In November, 2014, trap cultures were established using field samples (0–20 cm soil depth) according to the methodology proposed by Oehl et al. (2003) in order to improve detection of the whole AMF diversity. Trap cultures of AMF were maintained for 1 year prior to analysis.

2.3. Morphological identification

Spores were extracted from soils using wet sieving and sucrose density gradient centrifugation (Sieverding, 1991). Briefly, 25 g of air-dried field soil were passed through sieves of 500, 125 and 32 μm and thoroughly washed with distilled water. The last soil portion collected in 32 μm mesh and the fraction between 500 and 125 μm were distributed into plastic tubes of 50 mL. To each fraction is added distilled water up to 25 mL. Then, 25 mL of a 70% sucrose solution were inserted at the bottom of the tubes and centrifuged at 2000 rpm for 2 min. Samples were decanted after centrifugation, washed and transferred to *Petri* dishes. Spores were carefully counted under the compound microscope (CX31, Olympus) at up to 400-fold magnification. The number of AMF spores was expressed as spores in 100 g of dry soil. Finally, all spores found in each sample were mounted on microscope slides in polyvinyl alcohol-lactic acid glycerol (PVLG) medium or PVLG mixed 1:1 (v/v) with Melzer's reagent (Sieverding, 1991; Oehl et al., 2003) for their taxonomic identification. The spores were classified based on Glomeromycota system of Oehl et al. (2011a, 2011b). Identification reports (Błaszkowski, 2012; Oehl et al., 2011a, 2011b) and the homepage of the Swiss collection for AMF (SAF; http://www.agroscope.ch/saf) were also used.

2.4. Mycorrhization analyses

Root colonization levels were determined by the gridline intersect method (Giovannetti and Mosse, 1980) after clearing the roots with 2.5% KOH solution (w/v) and staining with a solution of 0.05% trypan blue in lactic acid (Phillips and Hayman, 1970). The extraradical mycelium (ERM) was determined as hyphal length by an adaptation of the filtration-gridline method described by Rubio et al. (2003). Briefly, substrate samples (1 g) were mixed with 4 mL of a solution containing glycerol/12 M HCl/distilled H_2O (12:1:7) and 0.05% trypan blue. Then, the samples were shaken overnight. This suspension was washed thoroughly in 32 μm mesh, suspended in 20 mL distilled H_2O and filtered. An aliquot (1 mL) was taken from suspension that was transferred to a membrane filter of 0.45 μm pore size. To quantify the total hyphal density expressed as ERM the Newman (1966) intersect gridline method was used.

Glomalin-related soil protein was extracted according to Wright et al. (1996), with some minor modifications (Cornejo et al., 2017). Briefly, 1 g substrate in 8 mL of 50 mM citrate buffer pH 8.0 was autoclaved for 1 h at 121 °C. This procedure was repeated until no dark color was obtained in the supernatant. Then, the supernatant was