SCIENTIFIC NOTE

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

EFFECTS OF SOIL ALUMINUM ON EARLY ARBUSCULAR MYCORRHIZAL COLONIZATION OF WHEAT AND BARLEY CULTIVARS GROWING IN AN ANDISOL

Alex Seguel[1], Jorge Medina[1], Rosa Rubio[1], Pablo Cornejo[1], and Fernando Borie[1*]

Aluminum phytotoxicity in acid soils is an important environmental stress that negatively affects crop production, but arbuscular mycorrhizal (AM) fungi performance would allow plants to better withstand this environmental condition. This study aimed to analyze the effect of soil Al on early AM colonization of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) cultivars. Near-isogenic Crac, Invento, and Porfiado wheat cultivars and Sebastián and Aurora barley cultivars were sown in pots in an acid soil at three Al saturation levels (60, 34, and 11%). At 20 d after sowing (DAS) 'Crac' presented higher AM colonization (27%) than other cultivars. However, 'Invento' had the fastest colonization at 41 DAS, which was inhibited in short term at lower Al-saturation. Moreover, roots of 'Aurora' were colonized 28 and 51% at 20 and 66 DAS, respectively, and also decreased at lower Al-saturation. In soil with 60% Al-saturation a great spore production was observed at 41 DAS, 'Aurora' had the highest spore density at 66 DAS. At 20 DAS a negative relationship ($r = -0.37$; $p < 0.001$) was observed between the early root colonization and root weight. In addition, such relation was stronger ($r = -0.49$; $p < 0.001$) when plants were grown at high Al saturation. An early AM colonization was observed in all cultivars essayed when growing at high Al saturation being higher in cultivars apparently more Al tolerant, suggesting that an early AM colonization can be an important factor in Al tolerance for agricultural plants cropped in acid soils.

Key words: Acid soils, arbuscular mycorrhizal propagules, cereal crop, soil aluminum saturation.

Aluminum phytotoxicity in acid soils represents a major limitation to crop production. For overcoming such constraints, farmers usually apply liming for decreasing Al activity or the use of Al-tolerant cultivars. In this sense, plants differ greatly on their capacity to tolerate diverse chemical species, such as Al; and arbuscular mycorrizal (AM) fungi would play a very important role in the protection of colonized plants against Al toxicity. Arbuscular mycorrhizal symbiosis is an association established between specific soil fungi and host plant roots. The main function of AM is related to the acquisition of nutrients for the plant, such as P, Ca, Mg, NH_4^+, Cu, and Zn (Clark and Zeto, 2000; Jeffries *et al*., 2003; Cardoso *et al*., 2006; Cornejo *et al*., 2008b). In addition, AM is also known for its role such as protective agent of pathogens and enhancing some mechanisms of tolerance to several environmental stresses (Smith and Read, 1997; Finlay, 2008; Smith and Read, 2008). AM association plays an important role in alleviation of abiotic stresses in acid soils, specially with high levels of Al through the interaction Al-P in colonized roots (Marschner, 1995), an improvement of nutrient absorption, especially P, Ca^{2+}, and Mg^{2+} (Borie and Rubio, 1999; Clark and Zeto, 2000; Lux and Cumming, 2001) all of them antagonistic to Al damage, or even through the Al-sequestration by an enhancement of root organic acid excretion (Klugh and Cumming, 2009) and glomalin production by AM fungal structures (Aguilera *et al*., 2011).

In these conditions, there would a variation among Al tolerant AM fungal ecotypes in relation to others that probably have a lack of adaptations to this type of stress, providing a major ability to cope these conditions through an enhanced germination of spores, hyphal growth and/or root colonization intensity (Klugh and Cumming, 2007). The AM fungal ecotypes differ significantly in their external mycelium and these differences likely contribute to differences in their host root colonization strategies (Hart and Reader, 2005); however, the environmental factors could also affect the AM colonization pattern. In this sense, Klugh and Cumming (2009) reported that *Acaulospora morrowiae* and *Scutellospora heterogama* associated to *Andropogon virginicus* L. at high Al levels did not show effect on the AM colonization in the short term, which suggests that this element was not able to inhibit the formation of the AM association and its beneficial effects. Moreover, Goransson *et al*. (2008) showed that AM colonization was more common in soils

[1]Universidad de La Frontera, Scientific and Technological Bioresource Nucleus (BIOREN-UFRO), casilla 54-D, Temuco, Chile. *Corresponding author (fborie@ufro.cl).
Received: 30 November 2011.
Accepted: 8 June 2012.

with high pH and relatively low Al concentrations, and that most of this association is explained by ecosystem biodiversity. On other hand, Nurlaeny (1995) concluded that root colonization of both maize (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.) by *Glomus intraradices* increased when pH increased from 4.7 to 6.4; Silva *et al*. (1994) showed that AM colonization of wheat was lower at more acid conditions, and Cavallazzi *et al*. (2007) concluded that AM colonization on apple plants was differentially influenced by fungal isolates, being an ecotype of *S. heterogama* the principal root colonizer (62%) at lowest soil pH (4.0), and consequently at the highest Al level. Based on the above, we hypothesized that the different AM colonization pattern observed under Al stress suggest that Al tolerance of AM host plants depends of the adaptability of the fungi to high Al levels and the specificity of the host plant to be colonized by a specific AM fungi ecotype; however, the AM colonization at different plant developing stages has yet not been considered in this analysis, being here only presented results from the early growing stages. For this reason, the aim of this work was to study the early effect of soil Al on AM fungal propagule density and root colonization of wheat and barley cultivars to correlate with plant growth and the overcoming of soil acidity constraints.

MATERIALS AND METHODS

We used an acid Andisol Gorbea series (medial, mesic, Typic Hapludands) collected at 0 to 20 cm deep (soil bulk density: 0.8 g cm^{-3}). The soil was air dried, sieved through a 5 mm mesh, amended or not with commercial lime (91% of $CaCO_3$, 5% of $Ca(OH)_2$ and 2% of S and Mg) at the equivalent to 1.25 and 2.50 g kg^{-1} soil, and incubated for 2 wk to obtain three Al-saturation levels corresponding to 60, 34, and 11%, respectively. Some other characteristics of natural and limed soil are described in Table 1. Each 1 L pot was filled with 800 g of the natural and limed soils, and seeds of three wheat (*Triticum aestivum* L.) cultivars and two barley (*Hordeum vulgare* L.) cultivars were sown. Near-isogenic Crac, Porfiado, and Invento wheat cultivars, and Aurora and Sebastian barley cultivars were provided by a local breeder (Semillas Baer™). Seeds were surface-sterilized with 2% Cloramin-T solution for 3 min and rinsed thoroughly. Fifty seeds per cultivar were germinated between wet tissue paper and then 30 seedlings were transplanted 7 d after seed germination. The pots were thinned to one plant after establishment.

Table 1. Selected chemical properties of the soil used[1].

	Natural soil	Limed soil 1.25 g kg^{-1}	Limed soil 2.5 g kg^{-1}
[a]Available P, mg kg^{-1}	17.00	31.00	26.00
[b]pH	4.91	5.06	5.45
[c]Organic matter, %	12.00	9.00	10.00
[d]K $cmol_{(+)}$ kg^{-1}	0.31	0.23	0.24
[d]Na $cmol_{(+)}$ kg^{-1}	0.04	0.03	0.03
[d]Ca, $cmol_{(+)}$ kg^{-1}	0.49	1.42	3.29
[d]Mg, $cmol_{(+)}$ kg^{-1}	0.03	0.15	0.23
[c]Al, $cmol_{(+)}$ kg^{-1}	1.32	0.96	0.45
[f]ECEC, $cmol_{(+)}$ kg^{-1}	2.19	2.79	4.24
Al sat, %	60.27	34.41	10.61
Bases sat, $cmol_{(+)}$ kg^{-1}	0.87	1.83	3.79

[a]Extractable by Olsen method; [b]Measured in H_2O; [c]Walkley and Black method; [d]Extracted by 1 M ammonium acetate; [e]Extracted by 1 M potassium chloride; [f]Effective cation exchange capacity.
[1]All the analytical techniques were according to the Normalization and Accreditation Commission of the Chilean Soil Science Society (Zagal and Sadzawka, 2007).

On other hand, plants were grown under greenhouse conditions at temperature ranging from 25 ± 3 ºC day to 15 ± 3 ºC night, 16:8 h photoperiod, and a relative humidity of 80-90%. A photosynthetic photon flux density of 400-500 mmol m^{-2} s^{-1} as supplementary light was applied when necessary. The plants were irrigated manually with distilled water as needed during the experiment. Nitrogen (N) was supplied in two portions, at establishment (30% total N) and at 6 wk of cultivation (70% total N) to an equivalent amount of 0.113 g N kg^{-1} soil. The P was supplied with 0.016 g P kg^{-1} soil as NaH_2PO_4 and 0.063 g K kg^{-1} soil as KCl, respectively, both applied as solution. In general, nutrient doses were low to avoid inhibit the AM colonization by native propagules (Rubio *et al*., 2003). Three harvest stages were considered. The first stage was three leaves (20 d after sowing –DAS), the second stage was tillering (41 DAS) and the last stage was ear emergence (66 DAS).

The plants were separated into root and shoot and dried at 65 ºC in a forced-air oven for 48 h and then weighed. Before drying, a portion of roots was separated and AM colonization was measured, root samples were gently washed under tap water and stained in trypan blue after boiling in 10% KOH following the method of Phillips and Hayman (1970). The mycorrhizal colonization was determined by the gridline intersect method (Giovannetti and Mosse, 1980). Total and colonized root length was calculated by Tennant's gridline intersect method (Tennant, 1975). Arbuscular mycorrhizal spores were collected from soils by wet sieving and decanting according to the methodology described by Sieverding (1991). The spores were transferred to Petri dishes and counted under stereoscopic microscope at 50X.

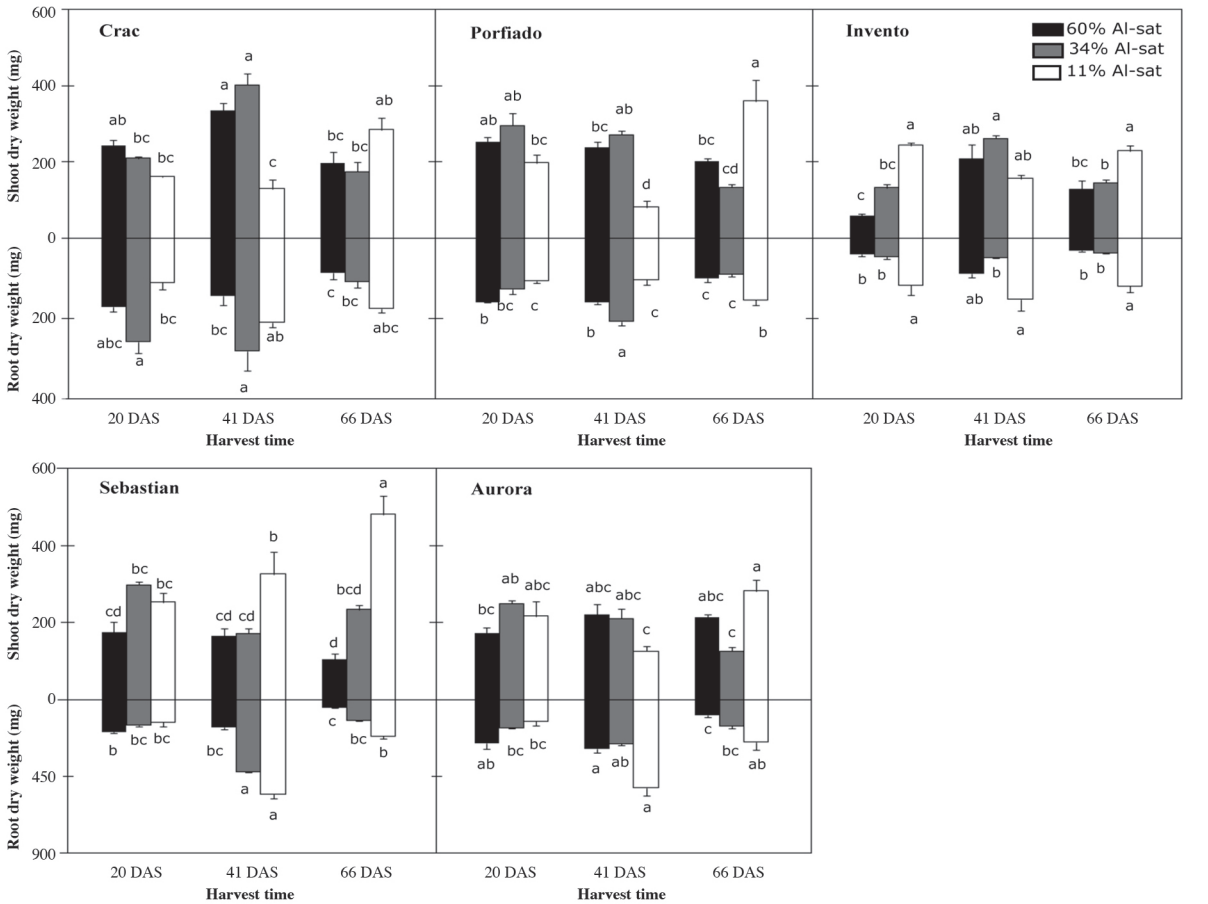
The experiment was established as a two way factorial design (three Al saturation levels × five cultivars at three harvest time), with four replicates per treatment (N = 180). Data were analyzed using ANOVA followed by orthogonal contrasts to identify significant differences among treatment means, and the correlation among the different variables obtained were analyzed using the Pearson correlation coefficient (r). All statistical analyses were carried out using SPSS software v. 10.0 (SPSS, Chicago, Illinois, USA).

RESULTS AND DISCUSSION

At 66 DAS all wheat and barley cultivars showed less biomass production at highest soil Al saturation, and a

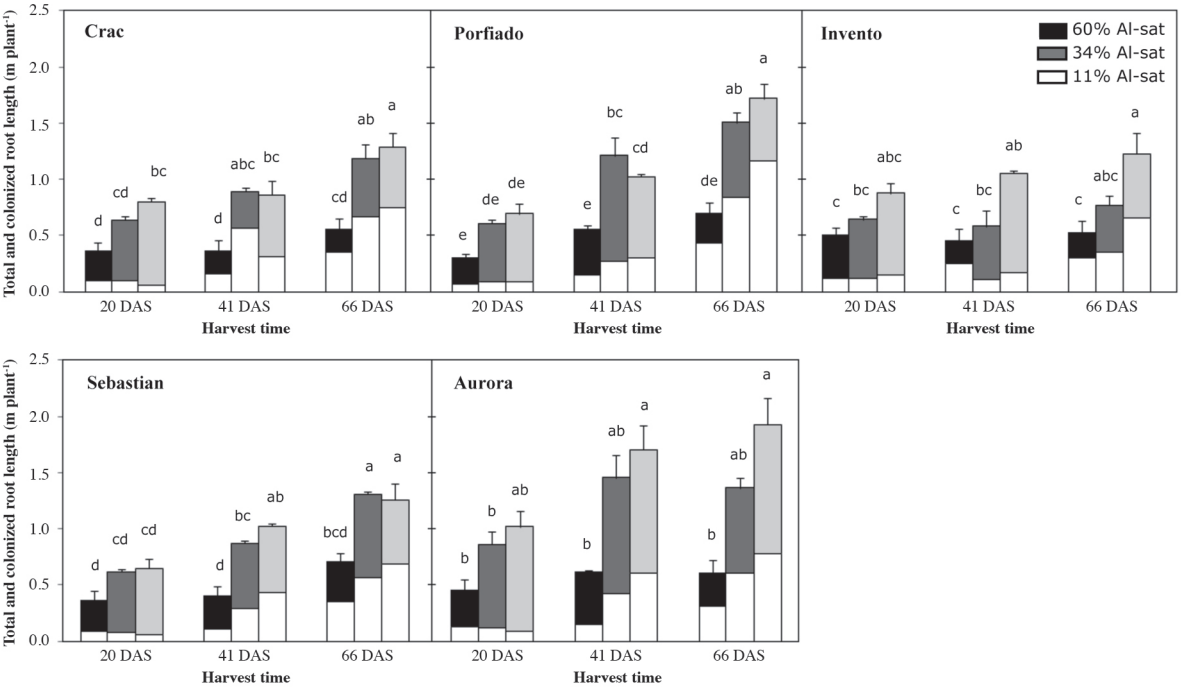
positive effect on plant growth was observed when the soil was limed, decreasing the Al levels. In these conditions, 'Porfiado' wheat and 'Sebastian' barley showed the higher increase by lime application (Figure 1). In short term, it was not observed significant lime effect on biomass production, probably due to Al presence produces alterations of root morphology including root thickening (Čiamporová, 2002), usually related to a higher root weight. For that reason, total root length was here used to analyze the Al effect in plant development. In this sense, 'Invento' wheat and 'Aurora' barley, in natural soil (60% Al-saturation), showed the greater total root length in the short term (Figure 2), which suggest that these cultivars are the most Al tolerant cultivars.

In a previous experiment, we have observed a greater AM colonization at 60 DAS in several wheat and barley Al tolerant cultivars at high Al saturation levels (unpublished data). For this reason, in this work we studied the early AM colonization, focusing in the wheat and barley cultivars that previously showed marked responses in parameters as plant and fungal growth. In general, AM fungal colonization was not inhibited by Al saturation and an early AM colonization was observed in all wheat and barley cultivars, growing in natural soil, at high Al saturation levels. 'Crac' wheat presented higher AM colonization (27%) at 20 DAS than other wheat cultivars. However, 'Invento' wheat had the fastest colonization at 41 DAS, reaching about 60% and a typical sigmoid colonization function according to Allen (2001). The fast AM colonization in some cultivars is probably due to the presence of more infective fungal structures and the difference in the architecture of the external mycelium (Hart and Reader, 2005). In addition, some studies have shown the close relationship between P uptake at early stages and its final yield (Elliott *et al*., 1997; Snyder *et al*., 2003). Other studies have observed the AM effect of increasing P uptake when plants grow at high Al levels (Clark, 1997; Siqueira and Moreira, 1997; Borie and Rubio, 1999), suggesting that an early colonization could be an important AM factor in Al tolerance. However, the effect of AM fungi in Al tolerance cannot be regarded as a single consequence of an improved P uptake. Moreover, roots of 'Aurora' barley were colonized in a 28 and 51% at 20 and 66 DAS, respectively; and it was inhibited in



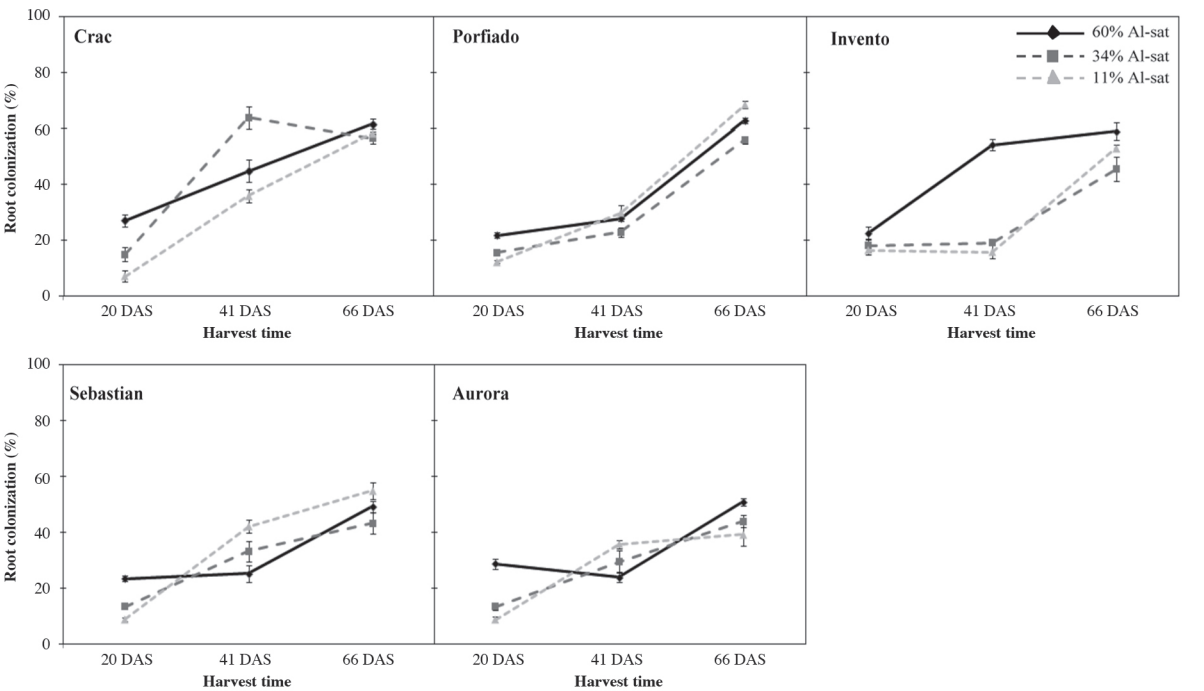
Bars denote means ± SE (n = 4) and different letter for each cultivar represent a mean difference between treatments by orthogonal contrasts test (P < 0.05).
Al-Sat: Aluminum Saturation; DAS: d after sowing

Figure 1. Shoot and root biomass production in three wheat ('Crac', 'Porfiado', and 'Invento') and two barley ('Sebastian' and 'Aurora') cultivars at three plant growth stages, under three Al saturation levels.



White bars means colonized root length. Bars for total root length denote means ± SE (n = 4) and different letter for each cultivar represent a mean difference between treatments by orthogonal contrasts test (P < 0.05); Al-Sat: Aluminum Saturation; DAS: d after sowing

Figure 2. Total and colonized root length in three wheat ('Crac', 'Porfiado', and 'Invento') and two barley ('Sebastian' and 'Aurora') cultivars at three plant growth stages, under three Al saturation levels.



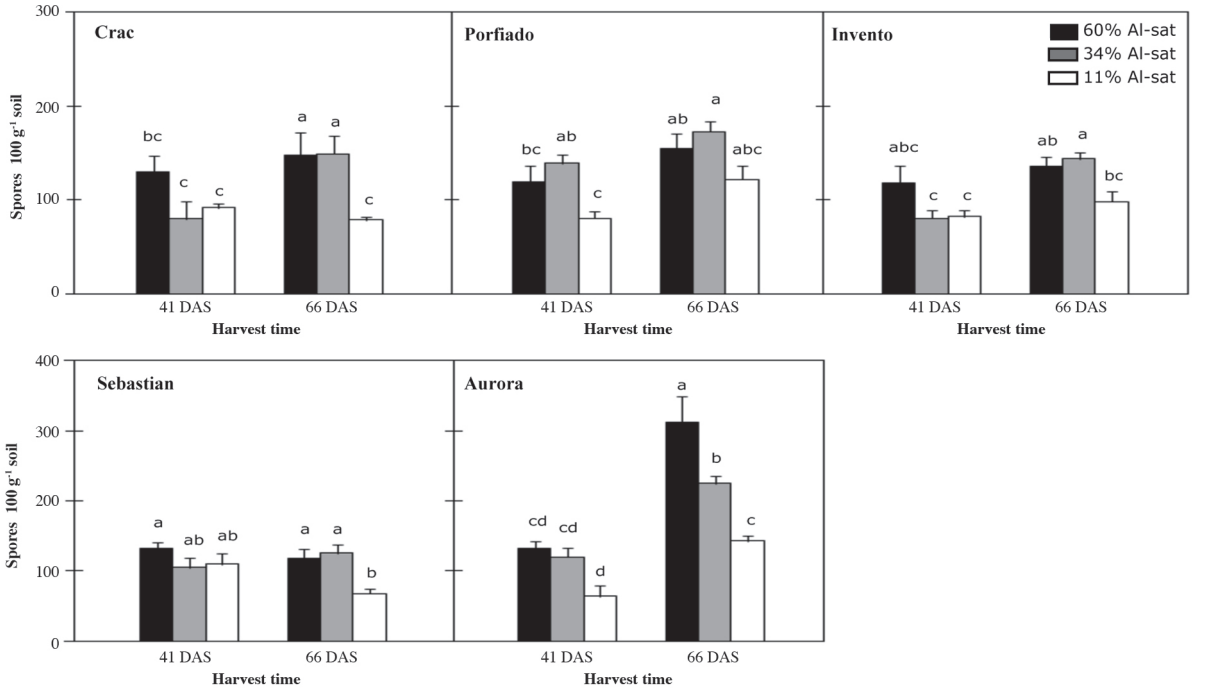
Bars denote ± SE (n = 4); Al-Sat: Aluminum Saturation; DAS: d after sowing

Figure 3. Arbuscular mycorrhiza root colonization in three wheat ('Crac', 'Porfiado', and 'Invento') and two barley ('Sebastian' and 'Aurora') cultivars, at three plant growth stages, under three Al saturation levels.

lime treatments (Figure 3). Different AM colonization levels in different cultivars can be due to an increased Al tolerance across time by some cultivars that have a better adaptation to these conditions, or by the effect of different AM fungal species colonizing the plants. In this sense, Klugh and Cumming (2007) reported different AM

colonization in the same host (*Liriodendron tulipifera* L.) but colonized by different AM fungal species. In general, AM colonization levels in all cultivars, at 66 DAS, were similar to those reported for same wheat cultivars of this study, but at low Al saturation (Rubio *et al*., 2003; Cornejo *et al*., 2007; 2008a; Valarini *et al*., 2009). However, principal differences in AM colonization by Al presence were observed in the short term in the present study.

In this study, a significant and negative relationship was obtained between AM colonization and root dry biomass at 20 DAS (r = -0.37; $P < 0.01$). This correlation was stronger (r = -0.49; $P < 0.001$) in natural soil at high Al saturation; and lower (r = -0.15; $P < 0.01$) when plants growed at 10% Al saturation. In short term, the relationship between plant growth, expressed as the total root length, and AM colonization was greater (r = -0.64; $P < 0.001$), reinforcing the idea that in the short term the Al stress is highly related with the root thickening. As it was proved for other elements, in the case of Al, the strong negative relationship between the biomass production and/or total root length at high Al levels do not affect the colonization and their consequent contribution to tolerate Al phytotoxicity. Similar results were reported by Kelly *et al*. (2005), who concluded that AM fungi maintain high colonization levels when they are exposed to high Al concentration. Also, it was observed that all cultivars essayed presented the highest root colonization degree at the first growth stage (three leaves) and also at ear emergence stage, suggesting a positive relationship between root mycorrhizal colonization and Al activity in the soil. All cultivars showed an increased spore density when host plants were grown at high Al saturation. In natural soil great spore production was observed at 41 DAS and 'Aurora' barley presented the highest increase of spores with limed soil at 66 DAS (Figure 4). Regarding to AM fungal spores, the highest sporulation occurred in the natural soil with high Al saturation. Whereas some studies have shown that spores abundance is decreased by some stress factors as heavy metals (Del Val *et al*., 1999) or wastewater pollution (Ortega-Larrocea, 2001), other authors (Borie and Rubio, 1999) have observed that AM spores in the rhizosphere of Al-tolerant barley cultivar showed a higher number than the sensitive one and, in turn, the tolerant cultivar showed the greatest spore number in the soil with high Al saturation. This trend has also been observed in soils polluted with other metals as Cu, where the use of Cu-adapted AM fungal inoculum produced a significant increase in the spore density at the highest pollution levels (Meier *et al*., 2012). Therefore, other aspect to take into account is that one of the results of AM fungal adaptation to environmental stress conditions (as high Al or other metals levels) is the enhanced propagule production, which could ensure root colonization in other plants or in further annual crops. Moreover, spores number also were significant and negatively related (r = -0.25; $P < 0.01$) with total root length. This trend could suggest a response to extreme environmental stress level at which it is subjected. In barley, a major spore increase



Bars denote means ± SE (n = 4) and difference between treatments by orthogonal contrasts test (P < 0.05); Al-Sat: Aluminum Saturation; DAS: d after sowing

Figure 4. Spores number in 100 g of dry soil in three wheat ('Crac', 'Porfiado', and 'Invento') and two barley ('Sebastian' and 'Aurora') cultivars at three plant growth stages, under three Al saturation levels.

by high Al saturation was observed in both treatments, with and without lime, showing the degree of Al tolerance, principally, in 'Aurora'. Supported in the recent findings reported by Aguilera *et al*. (2011), it is possible that an early eclosion of AM fungal structures may produce a decrease in the activity of toxic Al.

CONCLUSIONS

This work showed that arbuscular mycorrhizae colonization was not inhibited at high Al saturation levels, suggesting that an early colonization can be an important factor in Al tolerance and, consequently, to be beneficial against Al toxicity effects. In addition, high Al saturation increased the presence of AM propagules in soil. This spores increase together with an early AM root colonization could produce an improved nutritional status of wheat and barley cultivars in soils with high Al levels, representing a feasible indirect mechanism of Al tolerance showed by mycorrhizal plants, which could in part explain the plant Al tolerance; aspect to be considered by farmers in crop cereals under soils with high Al levels.

ACKNOWLEDGEMENTS

This study was supported by FONDECYT 1100642 grant (F. Borie), from Comisión Nacional de Investigación Científica y Tecnológica (CONICYT), Chile. Alex Seguel also acknowledges the financial support of CONICYT through Doctoral Fellowship Program and Project 24100181. The authors of this study thank to Mr. Erick Von Baer (Semillas Baer) for providing the seeds of wheat and barley cultivars.

Efecto del aluminio del suelo en la colonización temprana por micorrizas arbusculares en cultivares de trigo y cebada creciendo en un Andisol. La fitotoxicidad por Al en suelos ácidos es un importante estrés que afecta negativamente la producción de cultivos, pero la actividad de hongos micorrícicos arbusculares (MA) permitiría que las plantas soporten mejor esta condición ambiental. Este estudio tuvo como objetivo analizar el efecto del Al en la colonización MA temprana de cultivares de trigo (*Triticum aestivum* L.) y cebada (*Hordeum vulgare* L.). Cultivares de trigo Crac, Invento, y Porfiado, y de cebada Sebastián y Aurora fueron sembrados en macetas en un suelo ácido con tres niveles de saturación de Al (60, 34, y 11%). A los 20 días después de la siembra (DDS) 'Crac' presentó la mayor colonización MA (27%); sin embargo, 'Invento' tuvo la más rápida colonización a los 41 DDS, la cual fue inhibida a corto plazo a una menor saturación de Al. Por otra parte, las raíces de 'Aurora' fueron colonizadas 28 y 51% a los 20 y 66 DDS, respectivamente, y también se redujo a una menor saturación de Al. En suelos con 60% de saturación de Al se observó una gran producción de esporas a los 41 DDS, siendo 'Aurora' el de más alta densidad de esporas a los 66 DDS. A los 20 DDS se observó una relación negativa ($r = -0.37$, $p < 0{,}001$) entre la colonización MA temprana y la masa de las raíces. Además, dicha relación fue más fuerte ($r = -0.49$, $p < 0{,}001$) cuando las plantas fueron cultivadas en alta saturación de Al. Una temprana colonización MA se observó en todos los cultivares ensayados cuando se cultivaron en alta saturación de Al y fue superior en las variedades aparentemente más tolerantes a Al, sugiriendo que una temprana colonización MA puede ser un factor importante en la tolerancia a Al para plantas agrícolas cultivadas en suelos ácidos.

Palabras clave: Suelos ácidos, propágulos de micorrizas arbusculares, cultivo de cereales, saturación de aluminio en el suelo.

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