



Changes in free amino acid concentrations in mycorrhizal strawberry plants

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ARTICLE INFO

Article history:

Received 19 May 2008

Received in revised form 19 August 2008

Accepted 19 August 2008

Keywords:

Arbuscular mycorrhizal fungus

Free amino acid

Strawberry plants

Symbiosis

ABSTRACT

The influence of arbuscular mycorrhizal fungi (AMF) colonization on free amino acid concentrations in strawberry (*Fragaria × ananassa* Duch., cv. Nohime) plants was investigated using two AMF species [*Glomus mosseae* (Gm), *Gl. aggregatum* (Ga)] under phosphorus-supplemented (+P) or non-supplemented (−P) conditions. Ten weeks after AMF inoculation, mycorrhizal plants showed higher values in dry weight of both shoots and roots than did non-mycorrhizal ones among most of the treatments. Shoots and roots of mycorrhizal plants had greater phosphorus concentrations in −P plots, while in +P plots, P concentrations differed little among the inoculation treatments. AMF colonization was greater in plants inoculated with Gm than in plants inoculated with Ga. Total amino acid concentration was higher in most of the plants inoculated with Gm than in non-mycorrhizal ones regardless of P treatment. Serine, glutamic acid, glycine, alanine, leucine and GABA were higher in both mycorrhizal plants in −P plots than in non-inoculated plants. In +P plots, threonine and isoleucine concentrations were greater in both mycorrhizal plants than in non-inoculated ones. Asparagine, glutamic acid, glycine, citrulline, GABA and arginine were greater in plants inoculated with Gm than in non-mycorrhizal ones. These findings verified that inoculation with AMF increases total amino acid concentrations and concentrations of specific amino acids in strawberry plants with or without phosphorus supplementation, though the effects varied with species of mycorrhizal fungus.

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1. Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil inhabitants, and form a symbiotic relationship with the roots of most terrestrial plants. AMF can promote host plant growth by enhancing phosphorus uptake through symbiosis (Marschner and Dell, 1994). The use of mycorrhizal symbiosis may provide an alternative to high inputs of fertilizers and pesticides in sustainable crop production systems.

As for strawberry plants, growth enhancement through AMF inoculation was reported in several combinations of fungal species and strawberry cultivars (Robertson et al., 1988; Chavez and Ferrera, 1990; Williams et al., 1992; Niemi and Vestberg, 1992; Varma and Schuepp, 1994). In addition, trials to inoculate *Phytophthora fragariae* to strawberry plants colonized with AMF were reported to reduce disease; however, the effect differed with host cultivar and AMF species (Baath and Hayman, 1984; Mark and Cassells, 1996; Norman et al., 1996). Previously, we reported tolerance to fusarium wilt,

caused by *Fusarium oxysporum* f. sp. *fragariae*, in mycorrhizal strawberry plants (Matsubara et al., 2004). Also, Kobayashi (1988) and Singhet al. (2000) report that mycorrhizal plants improve disease tolerance in several crops. However, many points remain unclear about the mechanisms of disease tolerance in mycorrhizal plants.

Baltruschat and Schonbeck (1975) demonstrated that in tobacco plants, an increase in both arginine and citrulline occurred in mycorrhizal (*Glomus mosseae*) plants, which inhibited the propagation of *Thielaviopsis basicola*. Other reports also mention that free amino acid content in plants changed through AMF colonization. Sood (2003) and Fattah and Mohamedin (2000) reported an increase in free amino acid content in tomato colonized with *Glomus fasciculatum* and sorghum plants colonized with *Glomus intraradices*. While in leek plants, Rolin et al. (2001) reported a decrease in total amino acid content in roots of mycorrhizal (*Glomus etunicatum*) leek plants, and the relative proportions of different amino acids were not affected by colonization. However, it has been unclear whether free amino acid content changes through AMF symbiosis in strawberry plants and whether difference in amino acid content varies with AMF species in the same host. In this study, the influence of AMF colonization on free amino acid concentrations in strawberry

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plants was investigated using two AMF species under different phosphorus conditions.

2. Materials and methods

2.1. AMF inoculation and growing plants

Strawberry (*Fragaria × ananassa* Duch., cv. Nohime) plants were obtained by directly rooted runners from mother plants. Bedding soil, coconutshell medium (pH 5.5–6.0, autoclaved at 1.2 kg cm⁻² and 121 °C for 1 h), was packed in pots (10.5 cm diameter) and strawberry runner plants were inoculated with *G. mosseae* (Gm) (Nicol. and Gerd.) Gerdemann and Trappe, or *Gl. aggregatum* (Ga) Schenck and Smith, according to Matsubara et al. (2004), using commercial inocula provided by Idemitsu Kosan Co., Ltd. in Japan; spore densities were unknown. One-third of the plants were not inoculated (controls). Two weeks after AMF inoculation, the AMF-inoculated plants (M plants) and non-inoculated control plants (NM plants) were fertilized with a mixed fertilizer (N:P:K = 13:11:13, 1 g per pot); additional P (superphosphate, 3 g per pot; +P plot) was given to one-half of NM and M plants. Six plots, consisting of 30 plants per plot, were irrigated regularly by a capillary watering system in a bench culture and raised in a greenhouse [28 °C ± 3/23 °C ± 3 (day/night), natural light and daylength].

2.2. Evaluation of AMF colonization level

Ten weeks after AMF inoculation, roots were sampled and stained according to Phillips and Hayman (1970) and the rate of AMF colonization in 1-cm segments of lateral roots (RFCSL) was calculated. Hence, RFCSL expresses the percentage of 1-cm AM fungus-colonized segments to the total 1-cm segments of all the lateral roots; the number of total segments was approximately 50 per a plant. The average colonization was calculated from the values of three plants.

2.3. Determination of phosphorus in plants

The P determination in plants was performed 10 weeks after AMF inoculation. Shoots and roots from five plants were sampled, dried for 2 days at 110 °C, and weighed. The samples were ground, wet-ashed, and their P concentrations determined, according to Matsubara and Harada (1996).

2.4. Determination of free amino acids in plants

Ten weeks after AMF inoculation, plants were sampled and partitioned into leaves, crowns and main roots characterized by no colonization, and lateral roots characterized by colonization, and all samplers were freeze-dried. The samples for free amino acid analysis were collected from 10 plants as follows: leaves (approximately 1-cm long from the base), tap roots (approximately 1 cm from the base) and lateral roots. Free amino acids in each 200 mg-weighted samples were extracted at 0 °C in 2 ml 0.2 N per chloric acid solution mixed with 1 ml 0.25 μM D,L-norleucine as an internal standard. Extracts were centrifuged at 14,000 rpm at 4 °C, and pH was adjusted to 4.0 with KHCO₃. Then the extracts (20 μl in each time) were filtrated by GL-chromatodisc. Free amino acid concentrations (41 constituents) were measured using an automatic amino acid analyzer (JLC-500) using Ninhydrin.

2.5. Statistical analysis

Mean values were separated by *t*-test at *P* < 0.05 and Tukey's multiple range test at *P* < 0.05.

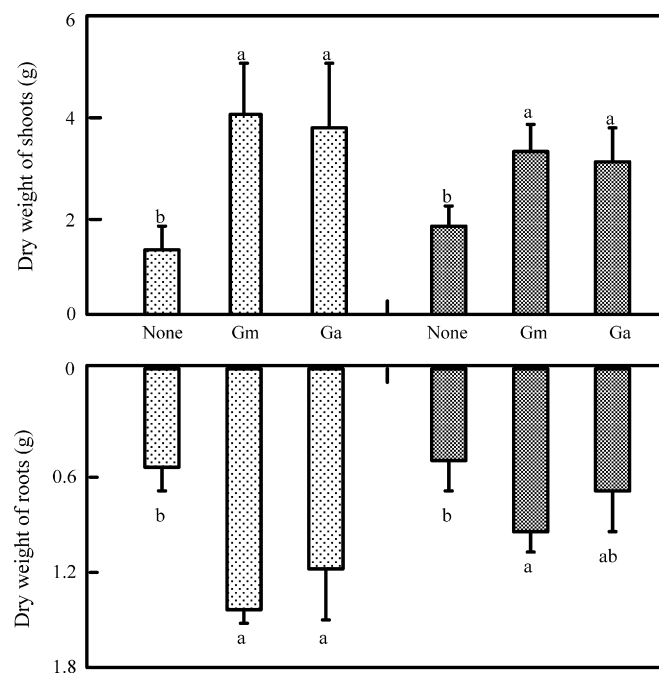


Fig. 1. Dry weight of shoots and roots in mycorrhizal strawberry plants 10 weeks after AMF inoculation. None, non-inoculated; Gm, *Glomus mosseae*; Ga, *Glomus aggregatum*. (□) Non-P supplemented; (■) P-supplemented. Bars represent standard errors. Columns denoted by different letters within each P treatment indicate significant difference according to Tukey's multiple range test (*P* = 0.05).

3. Results

Ten weeks after AMF inoculation, M plants had greater dry weight of both shoots and roots than did NM ones regardless of P supplementation, except in roots of Ga + P where no significant difference occurred between the plants inoculated with Ga and control plants (Fig. 1). Shoots and roots of M plants had greater P concentrations than those in NM ones in -P plots; plants

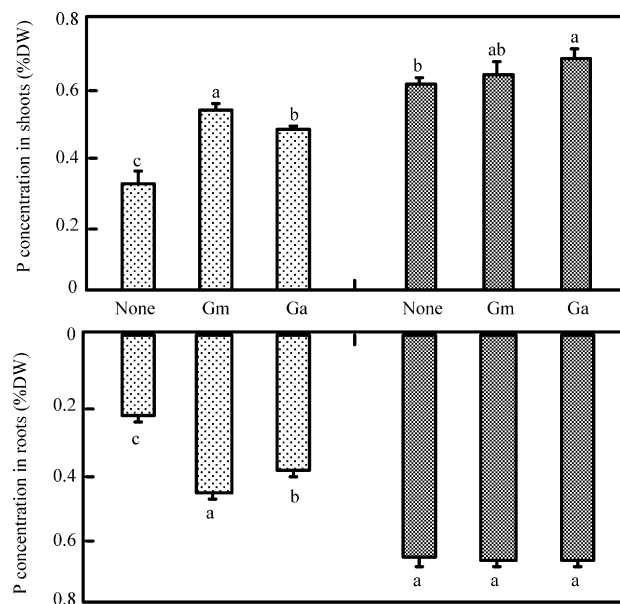


Fig. 2. P concentration in mycorrhizal strawberry plants 10 weeks after AMF inoculation. None, non-inoculated; Gm, *Glomus mosseae*; Ga, *Glomus aggregatum*. (□) Non-P supplemented; (■) P-supplemented. Bars represent standard errors. Columns denoted by different letters within each P treatment indicate significant difference according to Tukey's multiple range test (*P* = 0.05).

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