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(RESEARCH ARTICLE)

Effect of *Glomus mosseae* inoculation on growth and flowering improvement of *Chamaecereus sylvestrii and Mammillaria laui*

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Abstract

The aim of the experiment was to study how *Glomus mosseae* influences the growth and flowering of cacti such as *Mammillaria* laui and *Chamaecereus sylvestrii*. The experimental theses have been: (1) soil inoculated with *Glomus mosseae* and fertilized; (2) soil without *Glomus mosseae*, irrigated with water and fertilizer (control). The test showed a significant increase in the agronomic parameters analysed in plants treated with *Glomus mosseae*. In fact, all cactus plants treated with endomycorriza showed a significant increase in the vegetative and radical part of the plants, in the number of flowers per plant and in the duration of flowering. This endomycorriza can therefore also have beneficial effects on plants that are slow to grow and prolong the life of their flowers, beautiful to see but that normally short-lived. Further studies will be done on the use of mycorrhiza on cacti and succulents and the influence on resistance to biotic and abiotic stress of different typologies.

Keywords: Cactus; Induction flowering; Mycorrhizae; Plant quality; Roots stimulation

1. Introduction

The association between fungal hyphae and the radical organs of plants is a symbiosis that in 1885 Frank called mycorrhiza, meaning by this term a sort of new organ with its own shape and a certain physiology. A wide range of relationships can be established between plant roots and fungi. In these reports the plant does not show pathological symptoms due to the presence of fungal organisms. The classification of mycorrhizae is based both on morphological aspects and on the location of the fungus. Three types of mycorrhizae are known: ectotrophic, endotropic, ectoendotropic, ectoendotropic. In ectotrophic mycorrhizae, the fungus remains mainly outside the root, forming a sheath of variable thickness, consistency and colour. The portion that penetrates the root is made up of intercellular filaments that form a mycelial structure called the Hartig network [1]. In the endotrophic mycorrhizae the fungus is localized in great prevalence inside the root. In addition to the intercellular mycelium, there is also intracellular penetration, often in the form of special austors. At a certain stage, there is the digestion of a part of the endophyte fungal structures. The endothelial mycorrhizae are classified according to the nature of the endocellular structures, to the fact that the mycelium is more or less fixed. The ectoendotropic mycorrhizae have characters intermediate to the two types mentioned above. Endotrophic mycorrhizae are widespread in a large number of spontaneous and cultivated herbaceous and arboreal plant species. Ectrophic mycorrhizae are mainly found in forest plants and natural soils. The ectotrophic mycorrhizae are widespread mainly in the Pinaceae among the gymnastics and in the Betulaceae, Fagaceae and few other families among the angiosperms. The mycorrhiza is mainly established on the lateral roots and in the ramifications. The mycorrhized roots remain shorter and tend to have a larger diameter. The external appearance varies depending on the type of fungus, the intensity of the infection and the way the plant's root system grows [2]. The intensity of mycorrhizal infection varies from soil to soil. The amount of roots is greater in acid soils of humus mor than in dormant soils. The formation of the mycorrhizal roots is favoured by conditions of nutrient

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deficiency, in particular nitrogen, as well as by intense photosynthetic activity. It therefore seems that the carbohydrate content of the roots is a decisive factor and that any condition that promotes the presence of an excess of carbohydrates stimulates the mycorrhizal infection. In recent years, particular importance has been given to mycorrhizal fungi, in particular shrubby mycorrhizas, which develop a complex and specialised structure that mainly contributes to the adaptation and development of plants [3], where over 90% of plant communities in the world can form mycorrhizal symbiosis. These fungi enter the cortical area of the plants and promote the absorption of less soluble and mobile elements such as phosphorus, ammonium, potassium, copper, iron and zinc. However, these effects have been observed above all in the annual plants [4-5-6-7]. Mycorrhizal association can improve the water and nutrient supply to the host plant [8]. The use of mycorrhizae has found particular interest in recent years, especially to reduce the negative effects of various environmental stresses (salt, water and thermal stress) [9-10-11-12-13]. Mycorrhization determines a series of physiological and biochemical manifestations, influenced also by environmental factors [14-15]. Mycorrhizae once colonized the root can promote: (I) nutrient uptake [16], (II) photosynthetic activity [17-18], (III) water efficiency [17], (IV) accumulation of compatible solutes [19-20], and (V) enzyme antioxidants [18, 21]. The symbiosis between mycorrhizal fungi and arboreal species in the natural populations is well documented [22-23-24]. Although information on the association of cacti with mycorrhiza in natural populations is scarce [24-25-26-27]. So the question that arises here is: what would happen if cactus plants such as Chamaecereus and Mammillaria, normally slow to grow, were treated with *Glomus Mosseae*? Can inoculation with *Glomus mosseae* improve flower production, plant growth and root development? Therefore, the purpose of this work was to determine the changes in growth characteristics, root colonization, production and duration of blooms and new suckers caused by inoculation with *G. mosseae*.

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments started at the beginning of June 2018, were carried out in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on plants of *Mammillaria laui* (Fig. 3A) and *Chamaecereus sylvestrii* (Fig. 3B). The plants were placed in pots ø 10 cm; 60 plants for thesis divided into 3 replicas of 20 plants each, for each type of cactus.All plants were fertilized with the same amount of nutrients supplied through a controlled release fertilizer (5 kg m-3 Osmocote Pro® 3 - 4 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplantation.

The 2 experimental groups in cultivation were:

- Group without *Glomus mosseae* (CTRL), irrigated with water and previously fertilized substrate;
- Group with *Glomus mosseae* (GM) and fertilized substrate. The spores of *Glomus mosseae* have been propagated by inoculating the root of Sorghum (*Sorghum biocolor L.*) planted in autoclaved soil. The plants have been watered daily and grown for 4 months.

One hundred grams of moist soil were sieved and the spores were separated according to the method of Brundett et al. (1996) [28]. The spores were counted and calculated for the fresh weight of the soil containing 50 spores of G. mosseae (100 g of soil) used as inoculum. On 4 July 2019 vegetative weight, suckers number (Fig. 3C), shoots number, roots weight, flowers number, flowering time on plants of *Chamaecereus sylvestrii* were recorded. And vegetative weight, shoots number (Fig. 3D), flowers number, roots weight, plants circumference, flowering time on plants of *Mammillaria laui.*

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

3.1. Plant growth

The test showed a significant increase in the agronomic parameters analysed in plants treated with *Glomus mosseae*. In fact, all the plants of your cactus types, treated with (GM), showed a significant increase in vegetative weight, plants circumference, suckers number, shoots number, roots weight, flowers number, and flowering time.

In *Chamaecereus sylvestrii*, the vegetative weight was 46,93 g in the thesis (GM), against 32,92 g of the thesis (CTRL) (Fig. 1A), 17,42 of the thesis treated with mycorrhizae against 7,25 g of the control thesis for the number of new suckers (Fig. 1B), 21,17 in the the thesis (GM) against 13,08 for the shoots number (Fig. 1C), 19,02 g in the thesis (GM) against 14,45 g for the weight of the roots (Fig. 1D) (Fig. 4B-4D). In addition, there was a significant increase in the number of flowers, 19.50 of the (GM) thesis compared to 13.08 of the control thesis (Fig. 1E) and the duration of the flower 3.33 days in the thesis treated compared to 1.91 days of the control thesis (Fig. 1F).

In *Mammillaria laui*, the vegetative weight was 158.18 g in the thesis (GM), against 130.54 g in the thesis (CTRL) (Fig. 2A) (Fig. 4A-4C), 26 g in the thesis treated with mycorrhiza against 17.50 g in the control thesis for the number of new branches (Fig. 2B), 25.75 g in the thesis (GM) against 12.17 g for the number of flowers (Fig. 2C), 54.81 g in the thesis (GM) against 35.75 g for the weight of the roots (Fig. 2D). In addition, there was a significant increase in flower duration, 6.83 days of the thesis (GM) compared with 4.17 days of the control thesis (Fig. 2E). There is also a significant increase in plant circumference of 36.97 cm in the thesis (GM) compared to 22.35 cm in the control group (Fig. 2F).

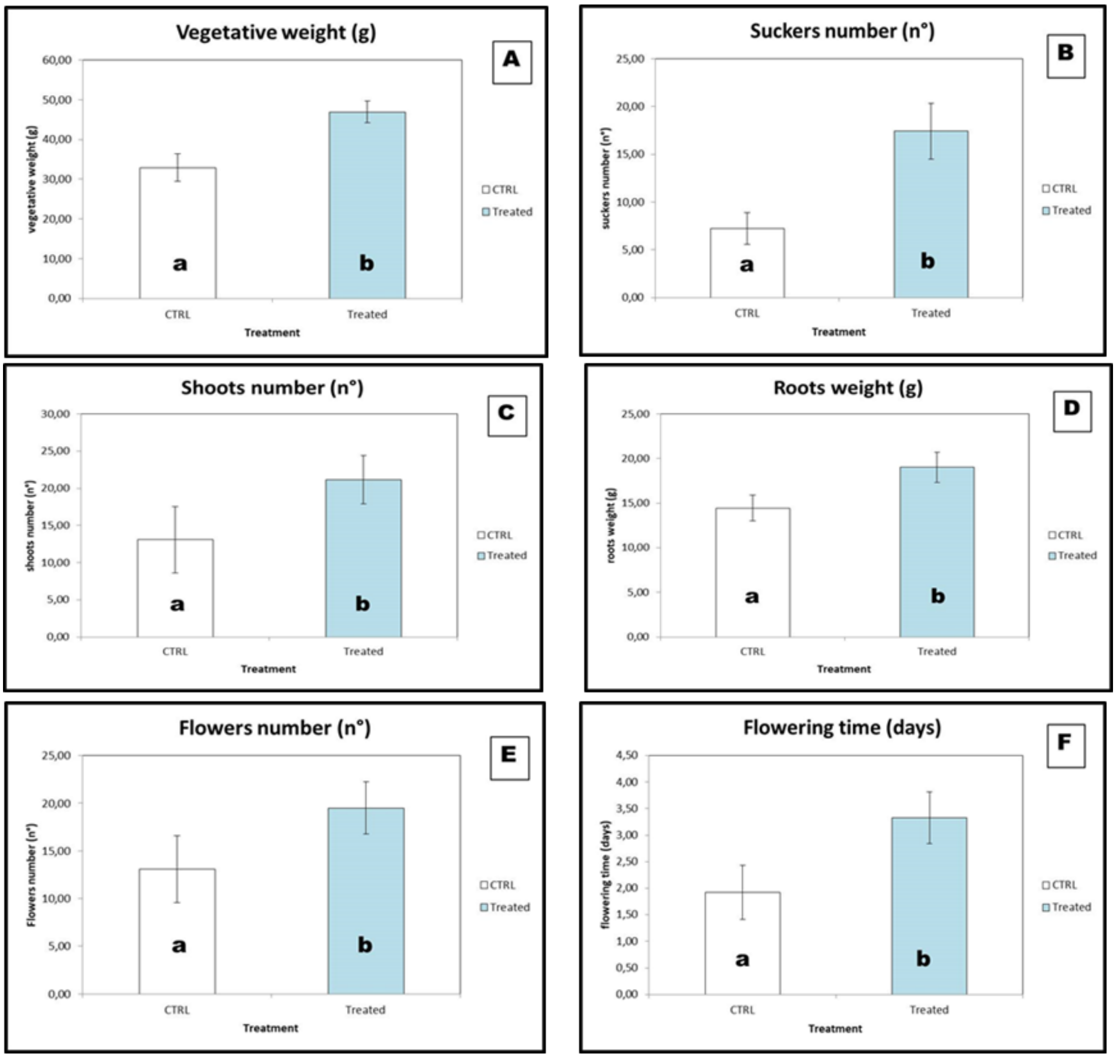

Figure 1 Effect of *Glomus mosseae* on growth and flowering improvement of *Chamaecereus sylvestrii*.
Legend: (A) vegetative weight; (B) suckers number; (C) shoots number; (D) roots weight; (E) flowers number; (F) flowering time. Each value reported in the graph is the mean of three replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

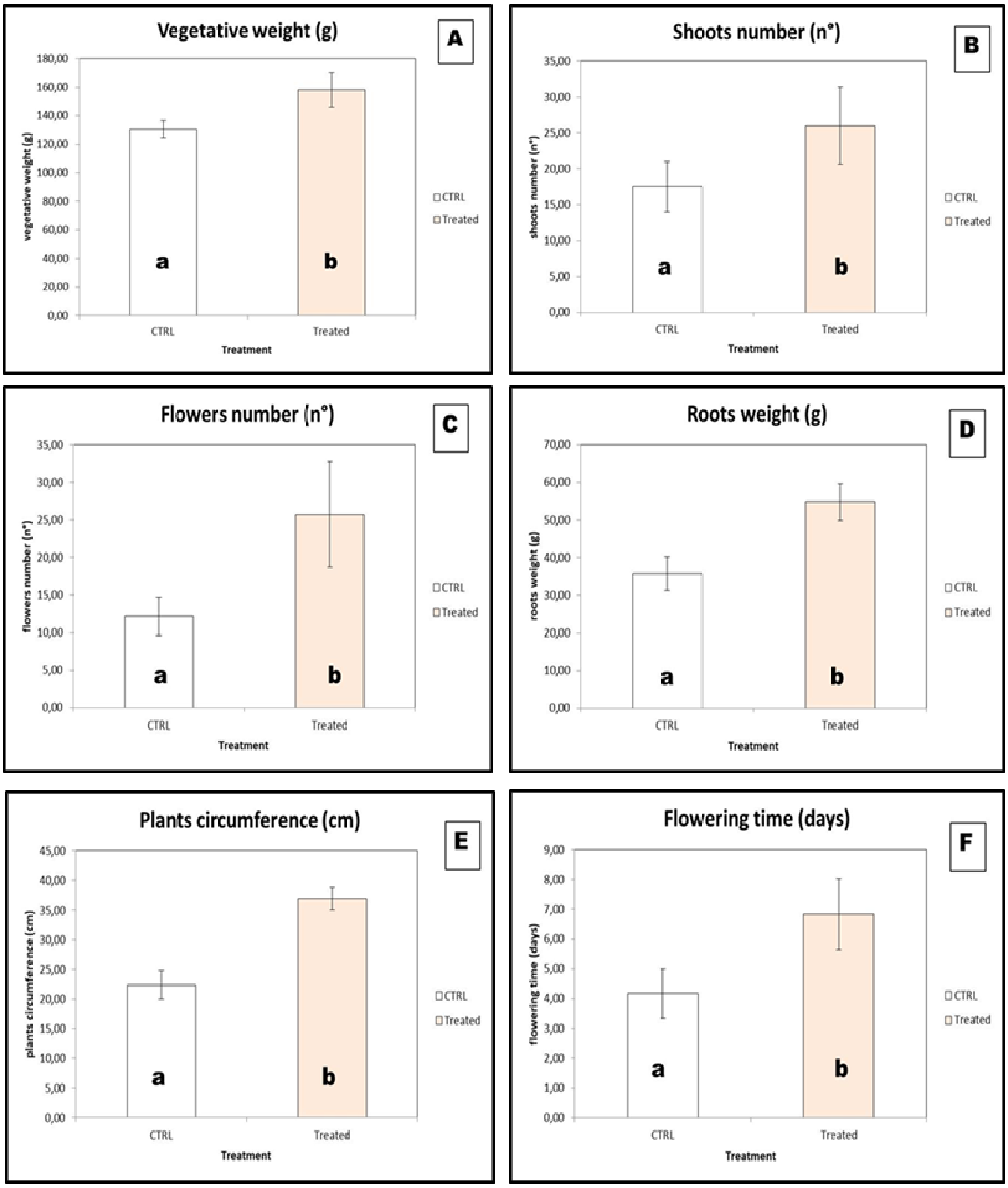

Figure 2 Effect of *Glomus mosseae* on growth and flowering improvement of *Mammillatia laui*.
Legend: (A) vegetative weight; (B) shoots number; (C) flowers number; (D) roots weight; (E) plants circumference; (F) flowering time. Each value reported in the graph is the mean of three replicates ± standard deviation. Statistical analysis performed through one-way ANOVA. Different letters for the same parameter indicate significant differences according to LSD test (P = 0.05).

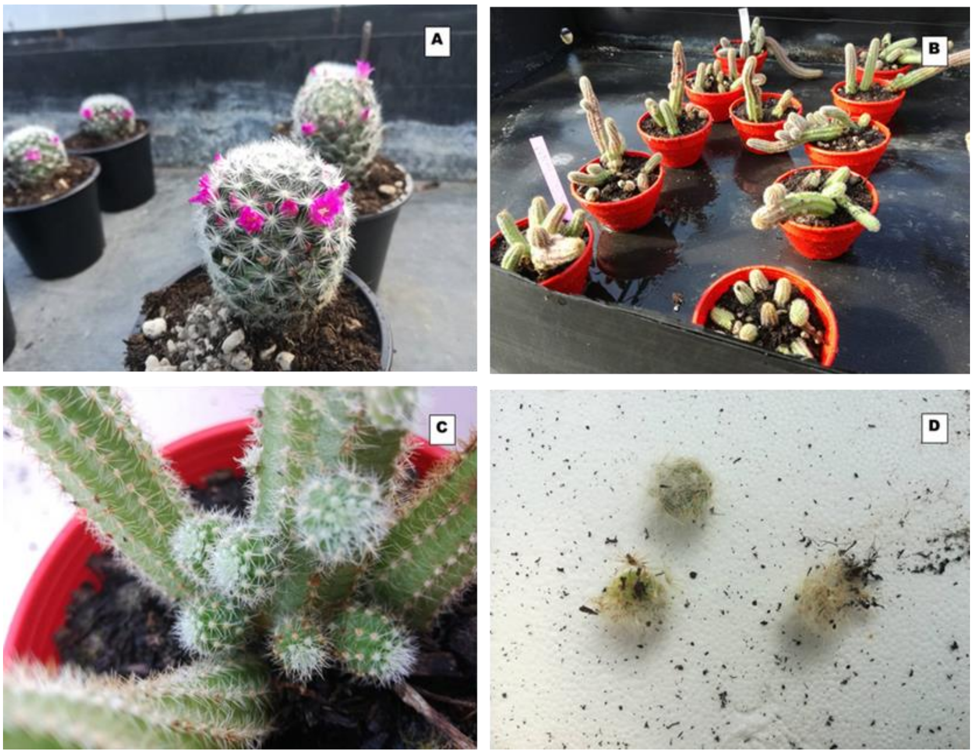

Figure 3 View of *Mammillatia laui* and *Chamaecereus silvestrii* in greenhouse cultivation and detail of their suckers. Legend: (A) *Mammillatia laui* plants; (B) *Chamaecereus sylvestrii* plants; (C) *Chamaecereus sylvestrii* suckers; (D) *Mammillaria laui* shoots

Figure 4 Effect of *Glomus mosseae* on growth and flowering improvement of *Mammillaria laui* and *Chamaecereus silvestrii*.

Legend: (A) *Mammillatia laui* flowering: plants treated with (GM) and control; (B) *Chamaecereus sylvestrii* flowering: plants treated with (GM) and control; (C) *Mammillaria laui*: plants circumference; (D) *Chamaecereus sylvestrii*: vegetative growth

4. Discussion

The relationship between the mycorrhizal plant and the fungus brings considerable advantages to both. In the mycorrhized plant, better water absorption and more efficient intake of the mineral elements, thanks to the development of the fungal iles that guarantee the roots to be able to explore and absorb water and mineral salts from a volume of soil greatly greater, reaching areas that normally the root cannot reach. Mycorrhizae are also able to solubilize and absorb for the plant mineral forms normally and thus modify the concentration of really available mineral elements [29]. Mycorrhization results in a return of organic matter to the soil and promotes greater release of nitrogen, phosphorus and potassium. The best mineral nutrition (especially phosphate) favours a better growth of the plant that can be seen especially in soils that lack minerals. The mycorrhized plants also have the ability to better tolerate environmental stresses [30].

The plant, in turn yield to the fungus simple sugars produced through photosynthesis and organic compounds processed by the plant cell. The fungus, thanks to the symbiosis, is able to complete its life cycle, and in the case of ectomycorrhizae, to form the fruiting bodies. From mìcorrìze then new ile develop that go to colonize the surrounding land and also the new rootlets emitted by the plant or those of other neighbouring plants. The cycle of activity of the mìcorrize follows that of the plant: in spring, with the vegetative recovery, the mycorrhizae resume to grow and continue throughout the summer if soil conditions remain favorable [31].The mycorrhizal plant has the ability to develop optimally even in soils poor in nutrients or unfavourable situations. Symbiosis can provide several benefits: (a) increased uptake by organic nitrogen roots [32]; (b) reduction of thermal, water, saline and transplant stresses; (c) improvement of soil structure; (d) increased plant resistance to nematode attack; (e) increased phosphorus uptake by plants; (f) completion of the life cycle of some fungi; (g) increased nutraceutical properties of products and aromatic substances; (h) increased root development of plants and increased resistance to diseases of fungal and bacterial origin; (i) increased availability of vitamins and carbon for microorganisms; (l) abatement of toxic metals present in the soil; (m) communication between plants thanks to the hyphae network in the soil; (n) defense from pathogenic fungi such as: Armillaria, Sclerotinia, Fusarium and Pythium. In this test, the plants of *Mammillaria laui* and *Chamaecereus sylvestrii* inoculated with *Glomus mosseae* showed a significant increase in vegetative weight, suckers number, shoots number, plants circumference, roots weight, flower number and flowering time. This effect is due to a higher intake of water and nutrients, in particular nitrogen and phosphorus, but also to an increase in photosynthetic efficiency mediated by the activity of radical stimulation of the mycorrhiza, in this case Glomus mosseae. The use of mycorrhizae in cacti can be of interest, especially as there are not many studies dealing with this subject, particularly the symbiosis between mycorrhizae and root systems of cacti and succulent non cactaceae. Also studying microbial colonization involving plants living in extreme environments, could be important in view of climate change. Select bacteria and fungi normally present in the roots of cacti, then suitable for extreme environments (thermal stress, water and salt) and inoculate them in plants of our climate zones (where, however, temperatures are increasingly increasing) could be used to stimulate resistance and metabolic efficiency and reduce mortality accordingly.

5. Conclusion

The test has shown that the use of *Glomus mosseae* can improve the growth and flowering of plants of *Mammillaria laui* and *Chamaecereus sylvestrii*, in particular by significantly increasing the vegetative and radical part of the plants, the number of flowers per plant and the duration of flowering. This endomycorriza can therefore also have beneficial effects on plants that are slow to grow and prolong their flower life, on plants whose flowers are beautiful but which do not last long. Further studies will be done on the use of mycorrhiza on cacti and succulents and the influence on resistance to biotic and abiotic stress of different typologies.

Compliance with ethical standards

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Disclosure of conflict of interest

Disclosure of conflict of interest. None to declare.

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