

THE EFFECT OF MYCORRHIZATION ON THE GROWTH, FLOWERING,
CONTENT OF CHLOROPLAST PIGMENTS, SACCHARIDES AND
PROTEIN IN THE LEAVES OF *SINNINGIA SPECIOSA* (LODD.) HIERN

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Abstract. The research was conducted on two cultivars of *Sinningia speciosa* (Lodd.) Hiern: 'Defiance' and 'Blanche de Meru'. Plants were cultivated with or without symbiosis with endomycorrhizal fungi. In order to evaluate the biochemical changes in the leaves of *Sinningia speciosa* at the vegetative growth stage the content of chlorophyll a+b, carotenoids, protein and saccharides was determined. Plant growth parameters, such as height, diameter, number of leaves and number of initiated flower buds, were determined when first flower was developed. Mycorrhizal plants of *Sinningia* 'Defiance' and 'Blanche de Meru' had more flower buds, 66.7 and 57.1%, respectively. The mycorrhization had a positive influence on the content of chlorophyll a+b in the leaves of *Sinningia speciosa* 'Defiance', whereas in the Blanche de Meru cultivar this dependence was observed only in the ninth and tenth week of cultivation. At the vegetative stage the mycorrhized plants had a higher content of carotenoids in their leaves, except for the tenth week of cultivation in the Defiance and the seventh and tenth weeks of cultivation in the Blanche de Meru cultivar. The mycorrhization did not influence the content of protein in the cultivars under investigation, except for the ninth week of cultivation. The highest content of saccharides in the leaves of Defiance and Blanche de Meru cultivars was noted at the beginning of vegetation and it was similar in the mycorrhized and non-mycorrhized plants.

Key words: ornamental plants, mycorrhizal fungi, biochemical changes in leaves

INTRODUCTION

The root-soil interface is a dynamic environment, a microcosm where microorganisms, plant roots and soil constituents interact (Lynch 1990) and develop what is known as the rhizosphere. Therefore, the rhizosphere, is the zone of influence of plant roots on the associated microbiota and soil components, characterised by an altered microbial diversity with increased activity and number of microorganisms (Kennedy 1998). It is clearly an environment which is physically, chemically and biologically different from the bulk soil (Bowen and Rovira 1998).

The association between soil fungi and plant roots is called mycorrhiza. The establishment of mycorrhiza implies profound morphological and physiological changes in the root, which operates in an integrated manner with the fungus, thus promoting gains in adaptability and survival of symbionts (Costa *et al.* 2002). According to Wang and Qiu (2006), out of a total of 3,617 species belonging to 263 families of terrestrial plants analysed, 80% of the species and 92% of the families are associated to mycorrhizae. Among the angiosperms, 85 and 94% of the species and families, respectively, are mycorrhizal.

Mycorrhizal fungi occur in most biomes on Earth and are a fundamental reason for the plant growth on the planet. The most common mycorrhiza is the one formed by arbuscular mycorrhizal fungi (AMF) which colonise the roots of over 80% of the plant kingdom (Falkowski *et al.* 2009). In recent years the mycorrhizal technology of micropropagated horticultural crops has enabled host plants to tolerate or withstand the impairing effect of abiotic and biotic stresses (Dodd 2000, Guillemin *et al.* 1992, Hooker *et al.* 1994).

Mycorrhizal fungi influence the development of a superior root system, enhance water conducting capacity, increase the uptake of macro-, micro- and immobile nutrients (El-Tahomy *et al.* 1999, Estrada-Luna *et al.* 2000, Auge 2001, Borkowska 2002). In the presence of mycorrhiza, higher photosynthetic rates develop as quantified carbon dioxide assimilation (El-Tahomy *et al.* 1999, Estrada-Luna 2000, Auge 2001, Borkowska 2002). While there are results reporting the involvement of mycorrhizal fungi in the dark phase of photosynthesis, the knowledge about the photochemical activity of plants growing in the presence of AMF is insufficient.

The aim of the study was to evaluate the influence of endomycorrhizal fungi on the growth and biochemical changes in the leaves of *Sinningia speciosa* during their vegetative growth.

MATERIAL AND METHODS

The research was conducted on two cultivars of *Sinningia speciosa* (Lodd.) Hiern: 'Defiance' and 'Blanche de Meru'. On 30th March, 2012, tubers with

a circumference of 15-18 cm were planted in pots with a diameter of 15 cm into a peat substrate with pH 6.2, enriched with a slow-release Osmocote Plus (3-4M) fertiliser, mixed with fresh, granulated pine bark at a volume ratio of 3:1 (v:v). The control treatments were those in which plants were grown in peat substrate, as opposed to the treatments in which the substrate was supplemented with mycorrhizal fungi. The inoculum, consisting of a mixture of *Glomus* species (Endorize-TA AMF, Biorize Sarl, France), was applied to the plants when their tubers were being planted. The amount applied was 100 propagating units per plant. One treatment consisted of 15 plants, five plants in three replications.

Sinningia plants were grown in a greenhouse, and they began to be systematically nourished after five weeks of cultivation. As a nutrient solution 0.2% of the complete fertiliser Peters Professional (NPK, 15-11-29) was used every 10-14 days.

The quality parameters of the plants (height, diameter, number of leaves and initiated flower buds) was determined as the plants developed the first flower.

In order to evaluate the biochemical changes in the leaves of *Sinningia speciosa* at the vegetative growth stage, the content of chlorophyll a+b, carotenoids, protein and saccharides was determined. The samples were collected every seven days.

The level of pigments was determined following Hiscox and Israelstam (1997), after their extraction with dimethyl sulphoxide (DMSO) without tissue maceration. Weighed portions (100 mg) were treated with 5 cm³ DMSO and incubated in a water bath at 65°C for 60 minutes. In the extract obtained, the levels of the pigments were determined spectrophotometrically at a suitable wavelength. For chlorophyll a the absorbance of the extract was measured at a wavelength of 663 nm, for chlorophyll b – at 645 nm, and for carotenoids – at 470 nm. The content of the pigments was calculated by means of Arnon's (1949) formulae and given in mg per g of fresh weight.

Total saccharides were determined by means of the antron reagent (Björnsjö 1955). Under the effect of sulphuric acid saccharides transform into furfural derivatives which together with antron yield blue and green products. The intensity of the colour is proportional to their content. Weighed portions (0.5 g) were crushed in a mortar with 5 cm³ of distilled water and the homogenate was centrifuged at 10,000 g for 20 minutes. 1 cm³ of the supernatant was added to 2 cm³ of cooled antron reagent (0.02% in concentrated H₂SO₄), and then the content of the test tubes was heated, while slowly mixed, in a water bath at 90°C for 14 minutes. After the tubes were cooled, the absorbance of the solutions was measured in a spectrophotometer at a wavelength of 620 nm. The content of saccharides was read from a standard curve prepared for glucose. The final results, which were the means of four replications, were expressed in mg of glucose per g of fresh weight.

The content of protein in the leaves was determined by means of Bradford's (1976) method. 2 ml of a solution of Coomassie Brilliant Blue G-250 (CBB) in

85% orthophosphoric acid was added to 100 µl of a diluted extract, with the extraction in a phosphate-potassium buffer (pH 7.0). After 10 minutes the absorbance was measured at a wavelength of 595 nm. The protein content was determined from a curve plotted for albumin.

Root mycorrhizas were stained according to the method described by Phillips and Hayman (1970), and the root colonisation was expressed as the percentage of colonised root lengths versus observed root lengths. Soil hyphal length was determined according to the method described by Bethlenfalvay and Ames (1987).

The results were processed by means of analysis of variance. The means were grouped with Duncan's test and the significance level applied was $\alpha = 0.05$.

RESULTS

The results of the study showed that mycorrhization of *Syngia speciosa* cultivars affected only the number of flower buds. In both cultivars significantly more buds were observed in treatments with mycorrhization. Mycorrhizal plants of Defiance and Blanche de Meru cultivars produced 66.7 and 57% more buds, respectively, in comparison with the control plants (Tab. 1).

Table 1. The effect of mycorrhization on quality, flowering and on percentage of root colonisation of *Sinningia speciosa*

Treatment	Root colonisation by AMF (%)	Plant height	Plant diameter	Number of leaves	Number of initiated flower buds
AMF (control plants)					
Defiance	0.0 a	10.2 a	20.0 a	15.0 a	12.0 a
Blanche de Meru	0.0 a	9.9 a	22.0 a	16.0 a	14.0 a
AMF+					
Defiance	29.9 b	9.7 a	23.0 a	16.0 a	20.0 b
Blanche de Meru	32.1 b	9.8 a	21.0 a	14.0 a	22.0 b

Means followed by the same letter do not differ significantly at $\alpha = 0.05$

During the period before flowering changes in the content of chlorophyll a+b could be observed in the leaves of the Defiance cultivar (Fig. 1a). Both in the mycorrhized and non-mycorrhized plants the highest significant content of this pigment was observed in the eighth week of cultivation. The content gradually decreased, but it still remained at a higher level than at the initial stage of the research. Besides, mycorrhization proved to have a positive effect on the content of chlorophyll a+b in the leaves of the cultivar under investigation, because apart from the tenth week of cultivation it was higher than the content of chlorophyll a+b in the leaves of the non-mycorrhized plants.

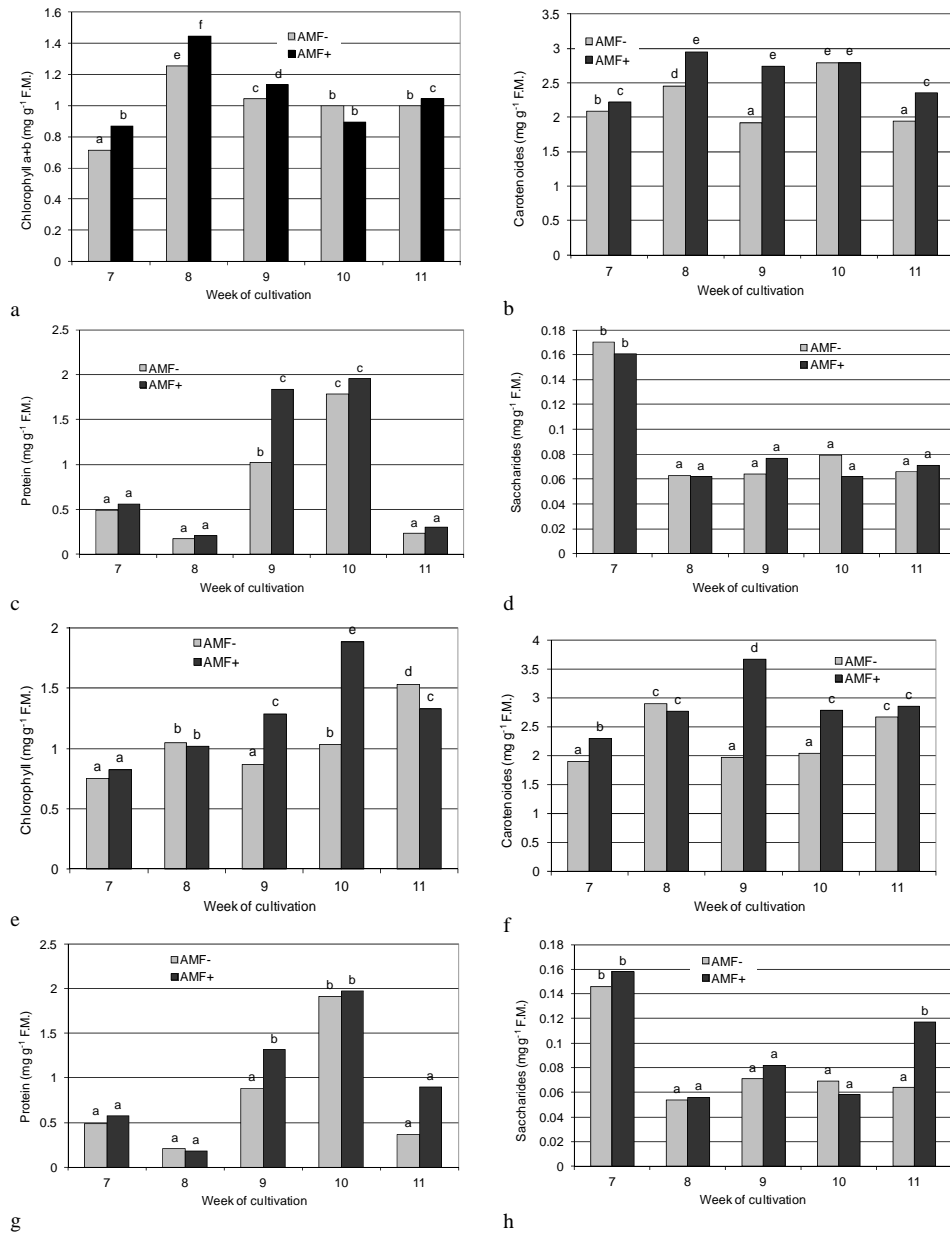


Fig. 1. The level of chloroplast pigments, protein and saccharides in the leaves of *Sinningia speciosa* 'Defiance' (a, b, c, d) and 'Blanche de Meru' (e, f, g, h) after mycorrhization. Means followed by the same letter do not differ significantly at $\alpha = 0.05$

The comparison of the content of chlorophyll a+b in the leaves of the Blanche de Meru cultivar in the non-mycorrhized plants revealed an increase in the eighth, tenth and eleventh week of cultivation, but the highest content of the pigment was noted in the last week of observation. In the mycorrhized plants of this cultivar the content of chlorophyll a+b increased in the consecutive weeks of cultivation, reaching the maximum in the tenth week of cultivation (Fig. 1e).

During the cultivation statistically significant changes were observed in the content of carotenoids in the leaves of the Defiance cultivar (Fig. 1b). In the non-mycorrhized plants the highest significant content of carotenoids was noted in the tenth week of cultivation and it was slightly lower in the eighth week of cultivation. On the other hand, in the ninth and eleventh weeks of cultivation a decrease in the content of carotenoids was observed. Right before the beginning of the generative stage their content dropped to the level noted at the beginning of the observation. Apart from the tenth week of cultivation, the content of carotenoids in the leaves of the mycorrhized plants was significantly higher than in the leaves of the non-mycorrhized plants. In the leaves of the non-mycorrhized Blanche de Meru cultivar the content of carotenoids was significantly higher in the eighth and eleventh week of cultivation. On the other hand, the content of carotenoids in the leaves of the mycorrhized plants increased during the cultivation, reaching its maximum in the ninth week (Fig. 1f).

The comparison of the content of protein in the leaves of the Defiance cultivar revealed that it was higher in the ninth and tenth week of cultivation, both in the mycorrhized and non-mycorrhized plants (Fig. 1c). As far as the Blanche de Meru cultivar is concerned, a significantly higher content of protein in the non-mycorrhized plants was noted only in the tenth week of cultivation, whereas in the non-mycorrhized plants – in the ninth and tenth week (Fig. 1g).

The content of saccharides in the first week of observation remained at a similar level both in the mycorrhized and non-mycorrhized plants of the Defiance cultivar. In the consecutive weeks of cultivation a decrease in the content of saccharides was observed, which remained at a similar level until the end of observation (Fig. 1d). A significant decrease in the content of saccharides was observed in the leaves of the Blanche de Meru cultivar. In the non-mycorrhized plants the reduced content of saccharides remained at a similar level until the plants started the generative stage. In the mycorrhized plants, before the beginning of floescence the content of saccharides increased to the level from the beginning of the vegetative stage (Fig. 1h).

DISCUSSION

The mycorrhization of vegetable crops and ornamental plants improves the yield, increases resistance to stress, enables better nutrition of plants and has enormous influence on the physiological changes taking place in them (Lovato *et al.* 1996, Janowska *et al.* 2013).

Studies showed that mycorrhizal plants of Defiance cultivar produced 66.7% more buds and the cultivar Blanche de Meru – 57% in comparison with the control plants. According to Lovato *et al.* (1996) and Janowska *et al.* (2013), mycorrhization considerably improves flowering and the quality of plants, it enhances stress resistance, provides better plant nutrition and has a profound effect on physiological changes occurring in plants (Porcel *et al.* 2003, Gaur and Adholeya 2005, Bolandnazar *et al.* 2007). Janowska *et al.* (2013) reported that the use of mycorrhization in *Zantedeschia albomaculata* ‘Albomaculata’ improves flowering and increases the quality of inflorescences. Nowak (2009) reports no effect of mycorrhization on the abundance of flowering in *Callistephus chinensis*, while Lovato *et al.* (1996) report that mycorrhized miniature roses and chrysanthemums are better-branched and, as a result, flower more abundantly.

The mycorrhization had a positive influence on the content of chlorophyll a+b in the leaves of *Sinningia speciosa* cv. ‘Defiance’, whereas in the Blanche de Meru cultivar this dependence was observed only in the ninth and tenth week of cultivation. As results from worldwide research, the mycorrhization of numerous plant species proved to have a positive influence on the content of chlorophyll (Panwar 1991, Morte *et al.* 2000, Borkowska 2002). An increased content of chlorophyll implies a more intensive course of the photosynthetic process, as was proved in the studies by Bethenfalvay *et al.* (1988). However, Dixon *et al.* (1994) reported that various fungal species have different influence on the intensity of photosynthesis.

At the vegetative stage the mycorrhized plants had a higher content of carotenoids in their leaves, except for the tenth week of cultivation in the Defiance cultivar and the seventh and tenth week of cultivation in the Blanche de Meru cultivar. Carotenoids, which are terpenoids, have single and double bonds in their structure, which make a system of conjugated bonds. Similarly to a chlorophyll particle, it enables light absorption. Apart from that, carotenoids have a protective function in the processes of photo-oxidation, to which the unsaturated fatty acids in the chloroplast membrane lipids are particularly endangered. Reports on increased content of carotenoids in the leaves of mycorrhized plants can be found in the publications by such authors as Panwar (1991) and Mathur and Vyas (1995).

The mycorrhization did not influence the content of protein in the cultivars under investigation, except for the ninth week of cultivation. In fact, different

results were expected due to the fact that being important components of plant cells, proteins regulate life processes, they are the building material of cellular structures and tissues and they are responsible for most biochemical reactions in living organisms. It is known that the content of protein in plants is stimulated by growth regulators from the group of gibberellins and cytokinins, which was proved by studies on vegetable crops (Klämbt 1976, Tagekami and Yoshiba 1997) and ornamental plants (Janowska 2013, Janowska and Stanecki 2013). According to Matysiak (2009), endomycorrhizal fungi are capable of producing growth regulators. Janowska *et al.* (2013) showed also that such fungi, added to the substrate with *Zantedeschia albomaculata*, stimulated the induction of flower buds in this species.

The highest content of saccharides in the Defiance and Blanche de Meru cultivars was noted at the beginning of vegetation, at a similar level in the mycorrhized and non-mycorrhized plants. The saccharides which are produced in the photosynthetic process are the chief structural and storage materials of plant organisms. Intensive photosynthesis favours the accumulation of larger amounts of carbohydrates. As results from the research by Panwar (1991), in mycorrhized plants the content of saccharides may be lower than in non-mycorrhized plants, because endomycorrhizal fungi use the saccharides produced by plants. According to Allen (1991), the fungi which live in symbiosis with plants use 10-20% of the photosynthetic products.

CONCLUSIONS

1. Mycorrhization stimulated the flowering of *Sinningia speciosa* 'Defiance' and 'Blanche de Meru' cultivars.

2. The mycorrhization had a positive influence on the content of chlorophyll a+b in the leaves of *Sinningia speciosa* 'Defiance', whereas in the Blanche de Meru cultivar this dependence was observed only in the ninth and tenth week of cultivation.

3. At the vegetative stage the mycorrhized plants had a higher content of carotenoids in their leaves, except for the tenth week of cultivation in the Defiance cultivar and the seventh and tenth week of cultivation in the Blanche de Meru cultivar.

4. The mycorrhization did not influence the content of protein in the cultivars under investigation, except for the ninth week of cultivation.

5. The highest content of saccharides in the 'Defiance' and 'Blanche de Meru' cultivars was noted at the beginning of vegetation; it was similar in the mycorrhized and non-mycorrhized plants.

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WPŁYW MIKORYZACJI NA WZROST, KWITNIENIE *SINNINGIA SPECIOSA* (LODD.) HIERN ORAZ ZAWARTOŚĆ BARWNIKÓW CHLOROPLASTOWYCH, CUKROWCÓW I BIAŁKA W LIŚCIACH

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Streszczenie. W badaniach oceniano wpływ grzybów endomikoryzowych na zmiany, w fazie wzrostu wegetatywnego, zawartości chlorofilu a+b, karotenoidów, białka i cukrowców w liściach *Sinningia speciosa* (Lodd.) Hiern 'Defiance' i 'Blanche de Meru'. Jakość roślin (wysokość, średnica, liczba liści i pąków kwiatowych) określono w fazie, gdy na roślinach rozwinął się pierwszy kwiat. Mikoryzacja siningii odmian Defiance i Blanche de Meru stymulowała tworzenie się pąków kwiatowych. Na roślinach mikoryzowanych rozwinęło się średnio o 66,7 i 57,1% więcej pąków kwiatowych, odpowiednio dla Defiance i Blanche de Meru. Mikoryzacja miała pozytywny wpływ na zawartość chlorofilu a+b w liściach odmiany Defiance. U odmiany Blanche de Meru zależność tą obserwowano tylko w 9 i 10 tygodniu uprawy. W fazie wzrostu wegetatywnego mikoryzowane rośliny miały większą zawartość karotenoidów w liściach, za wyjątkiem odmiany Defiance w 7. tygodniu uprawy i odmiany Blanche de Meru w 7. i 10. tygodniu uprawy. Mikoryzacja nie miała wpływu na zawartość białka w liściach badanych odmian, za wyjątkiem 9 tygodnia uprawy. Podwyższoną zawartość cukrowców u obu odmian odnotowano pod koniec fazy wegetatywnej, zarówno u mikoryzowanych jak niemikoryzowanych roślin.

Słowa kluczowe: rośliny ozdobne; grzyby mikoryzowe; biochemiczne zmiany w liściach