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Rootstock-imposed alterations in nitrate reductase and glutamine synthetase activities in leaves of rose plants

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Abstract

The activities of nitrate reductase and glutamine synthetase in leaves of greenhouse grown rose plants (*Rosa hybrida* cvs. Ilseta and Mercedes) grafted on various rootstocks were compared with those in leaves of non-grafted, own-root plants of these cultivars. The results obtained showed that the enzymatic activities as well as nitrate content in the leaves were altered by the grafting and by type of the rootstock used. These rootstock-imposed alterations differed between the two cultivars used in the study.

Additional key words: grafting, nitrate content, nitrogen nutrition, *Rosa hybrida*, scion.

Introduction

Cultivars of greenhouse roses (*Rosa hybrida*) are characterized by recurrent, year round growth of lateral shoots with terminal flowers. Promotion of vegetative growth by nitrogen nutrition is one of the major factors affecting flower formation in greenhouse rose plants. It has been well established (*e.g.* Zieslin *et al.* 1973) that flower production of grafted rose plants is superior to that of non-grafted plants, propagated from cuttings. Consequently most of the greenhouse rose plants are propagated by grafting on appropriate rootstocks. It is therefore possible, that the superiority of the grafted rose plants may stem, among other reasons, from improved uptake and metabolism of nitrogen by the root system of the rootstock.

One of the possible means of investigation of the nitrogen metabolism is by measuring activities of nitrate reductase and glutamine synthetase (Beevers and Hageman 1983). Activities of these two enzymes were measured in leaves and roots

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Abbreviations: GAM - l-glutamic acid monohydroxymate; GS - glutamine synthetase; HSP - heat shock proteins; LSD - least significant difference; NR - nitrate reductase.
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of 4 to 6 weeks-old rose graftlings (concomitantly grafted and rooted cuttings) as compared to the activities in leaves and roots of non-grafted cuttings. The obtained results published elsewhere (Agbaria *et al.* 1996) showed that the activities of both enzymes in the leaves of the graftlings were altered by the grafting procedure as well by type of the rootstock used. Furthermore, it was also found that the activity of these two enzymes in the roots was differentially altered by the cultivar of the scion.

However, the plants used in the previous study (Agbaria *et al.* 1996) were propagated by one-leaf cuttings in an aeroponic fog generated from tap water containing only minute amounts of nitrates and in conditions of low irradiance (Zieslin and Abolitz 1994). Therefore, the data obtained from the small, young underdeveloped plants could represent only temporary, transitional phenomen due to limitations of nitrogen and limited supply of carbohydrates essential for nitrogen metabolism (Aslam and Huffaker 1984).

For clarification of this assumption, the activities of nitrate reductase and glutamine synthetase were also investigated in leaves of one-year-old rose plants grafted on various rootstocks as well as in leaves of non grafted 'own-root' plants. The results of this study are described in the present report.

Materials and methods

Plants: Following rooting in the aeroponic fogger (Agbaria *et al.* 1996) the 5-week-old own-root (non-grafted) *Rosa hybrida* plants of cvs. Mercedes and Ilseta were planted in rockwool slabs. As well as homografts of Mercedes on Ilseta or Ilseta on Mercedes and heterografts of Mercedes or Ilseta on *Rosa indica major* as a rootstock were employed as the scion-root combinations of the graftlings. In order to discriminate between the effects of the graftling procedure and the specific effects of the rootstocks, autographs of Mercedes on Mercedes, and of Ilseta on Ilseta were also used as the controls.

During one-year cultivation prior to the examination of enzymatic activity, the plants were irrigated with nutrient solutions containing nitrates, ammonium, and all other macro- and micro-elements. The irrigation regimes, plant nutrition formulas as well as the plant handling methods were according to established practices for greenhouse rose management in Israel.

Two uppermost, fully developed five-leaflet leaves used in the study were detached from the stems when sepals of the flower buds started to unfold and petals begin to liberate (marketing harvest stage).

Nitrate content: Samples of 100 mg dry leaf tissue were dispersed for 60 min in double-distilled water at 45 °C. After centrifugation for 15 min at 6 500 *g* (*Sorvall, RC-285*), 0.2 cm^3 of the supernatant were incubated for 20 min at room temperature with 0.8 cm^3 of 5 % salicylic acid in concentrated H_2SO_4. The pH of the solution was adjusted to 12 by the addition of 19 cm^3 of 2 M NaOH. Following cooling of the solution, the light absorption of the colour developed was measured spectrophotometrically (*Varian DMS-100*) at λ = 410 nm. The nitrate content was calculated

according to a standard curve obtained from solutions containing known concentrations of KNO_3 (Cataldo *et al.* 1975).

Nitrate reductase (NR, NADH:nitrate oxidoreductase, EC 1.6.6.1) activity in leaves was measured in preliminary experiments by both, *in vitro* and *in vivo* methods. Due to inconsistent results obtained by the *in vitro* method, the *in vivo* method according to Heuer and Plaut (1978) was employed throughout the entire study. Leaf disks, 9 mm in diameter, were infiltrated under vacuum in 10 cm^3 of 50 mM phosphate buffer, pH 7.5, containing 0.1 M KNO_3 and 0.1 % Triton X-100, at 0 - 2 °C. After 5 min the tissue samples were transferred into the same buffer solution, but without Triton X-100, for an additional 60 min at 28 °C. For determination of nitrite formed, 1 cm^3 of the solution was supplemented with 0.25 cm^3 of 1.5 M HCl containing 1 % (m/v) sulfanilamide and 0.25 cm^3 of 0.02 % (m/v) solution of N-[1-naphthyl-(ethylenediamine] dihydrochloride. The absorbance was measured at $\lambda = 540$ nm and the NR activity was calculated according to a standard curve obtained from known concentrations of KNO_2.

Glutamine synthetase (GS, EC 6.3.1.2) activity was examined by quantitative measurements of L-glutamic acid monohydroxymate (GAM) formed from glutamic acid by the action of GS (Brun *et al.* 1992). One g samples of leaf tissue were crushed in 10 cm^3 of 50 mM Tris-HCl buffer, pH 7.6, containing 2 % (m/v) polyvinylpyrrolidone (PVP-45), 10 % insoluble PVPP, 10 % glycerol, 14 mM β-mercaptoethanol, 2 mM EDTA, 5 mM $MgSO_4$ and 10 mM glutamate, at 0 - 2 °C. Following filtration of the crushed tissue through 4 layers of cheesecloth, the filtrate was homogenized with a glass-tephlon rod (*Elda*, Haifa, Israel) and centrifuged for 30 min at 40 000 *g*. For determination of GS activity, 0.1 cm^3 of the supernatant was supplemented with 1 cm^3 of 5 mM Tris-HCl buffer, pH 7.2, which contained 30 mM NH_2OH, 20 mM $MgSO_4$, 20 mM arsenate, 4 mM EDTA, 0.5 mM ADP and 125 mM glutamine. After 30 min, the reaction was terminated by addition of 1 cm^3 6 M HCl with 0.37 M $FeCl_3$ and 40 % (m/v) TCA. After 10 min of cooling, the absorbance was measured at $\lambda = 540$ nm. The activity of the enzyme was calculated from a standard absorption curve of GAM formed from glutamic acid and the reaction mixture.

Statistical analysis: Shoot material collected from individual plants was combined. Following mixing, the measurements in each treatment were repeated 9 times. Means, standard errors and least significant differences are presented in the table.

Results

The nitrate content in leaves of non-grafted, own-root plants of cv. Mercedes was higher than that in leaves of own-root plants of cv. Ilseta (Table 1). The data also show that, regardless of the rootstock used, the nitrate content in leaves of grafted plants of cv. Mercedes was higher than that in the leaves of non-grafted plants. In

contrast to cv. Mercedes, the leaves of cv. Ilseta contained an elevated content of nitrates only when the plants were grafted on the *Rosa indica* rootstock.

Similarly to the nitrate content, the nitrate reductase (NR) activity in leaves of own-root plants of cv. Mercedes was also higher than that in leaves of cv. Ilseta (Table 1). The effect of grafting on the NR activity in leaves of cv. Mercedes differed significantly from its effect on NR activity in leaves of cv. Ilseta (Table 1). NR activity in leaves of cv. Mercedes autografts was 72 % lower than the activity in leaves of non-grafted plants. On the other hand, the NR activity in leaves of Ilseta scions on Mercedes as an rootstock was 33 % higher than that in leaves of own root plants, whereas the NR activity in leaves of cv. Ilseta autografts was almost not affected by grafting (Table 1).

Table 1. Nitrate content [mg(NO_3^-) g^{-1}(d.m.)], nitrate reductase (NR) activity [nmol(NO_2^-) g^{-1}(d.m.) h^{-1}], and glutamine synthetase (GS) activity [μmol(GAM) g^{-1}(d.m.) h^{-1}] in leaves of one-year old rose plants (*Rosa hybrida*) cvs. Mercedes and Ilseta propagated from cuttings with own-roots or grafted on Mercedes, Ilseta or *Rosa indica* as rootstocks. Values are means of 9 replicates ± SE.

Rootstock	Nitrate content		NR activity		GS activity	
	Mercedes	Ilseta	Mercedes	Ilseta	Mercedes	Ilseta
Own-roots	7.08±0.43	5.78±0.67	547.4±27.2	235.2±11.3	754.4±133.5	752.9±144.5
Mercedes	7.94±0.31	6.30±0.32	156.6±27.2	313.4±14.7	584.9± 40.7	1598.7± 27.8
Ilseta	8.22±0.62	6.37±0.65	286.4±18.8	227.4±17.4	451.9± 42.4	1559.8± 10.0
R. indica	7.87±0.10	6.82±0.64	126.0±14.6	95.4±14.2	1061.2±125.4	1236.0± 86.5
$LSD_{0.05}$	0.77	0.89	37.64	28.03	200.6	164.6

The NR activity in leaves of reciprocal homografts of both cultivars, Mercedes on Ilseta and Ilseta on Mercedes, was higher than the activity in leaves of Mercedes and Ilseta autografts, 82 and 37 %, respectively, whereas the NR activity found in leaves of heterografts of both cultivars (especially in cv. Ilseta) has been markedly reduced by grafting on *Rosa indica* (Table 1).

The activity of glutamine synthetase (GS) in leaves of all grafting combinations was much higher as compared with that of NR. However, the GS activity in leaves of non-grafted plants was similar in both cultivars (Table 1). The effects of grafting on the activity of GS in leaves of cv. Mercedes differed from these in leaves of cv. Ilseta. The GS activity in leaves of Mercedes autografts as well as in the homografts was markedly lower by 22 % and 40 %, respectively, as compared with the activity in leaves of own-root plants. On the other hand, the GS activity was profoundly promoted in leaves of Mercedes heterographs when grafted on *Rosa indica*. The promoted activity in the heterograft exceded 40 % as compared with the Mercedes own root plants and over 81 % as compared with the Mercedes autografts.

In contrast to Mercedes plants, the GS activity in leaves of all grafting combinations of cv. Ilseta was markedly higher than that in leaves of Ilseta own-root plants, more than two fold in leaves of Ilseta autografts and homografts and 64 % higher in the heterografts on *Rosa indica*.

Discussion

The data obtained in the present study showed that when a soilless (rockwool) medium with an excess of nutrients including nitrogen was employed, the content of NO_3^- in leaves of the one-year old plants of both cultivars was not markedly affected neither by the grafting procedure nor by the type of the rootstock (Table 1). Moreover, similarly to previously published data (Campbell 1988, Mohr *et al.* 1992) no direct relationship between the nitrate content and the NR activity in the leaves (Table 1) has been found as well as no relationship was present between NR and GS activities.

In all grafting combinations of cv. Mercedes, including the autografts, the activity of NR was lower as compared with the activity in own-root plants of this cultivar (Table 1). However, in contrast to cv. Mercedes, the NR activity in leaves of cv. Ilseta was not affected by the grafting procedure. There is a possibility that the reduced activity of NR in grafted plants of cv. Mercedes might be derived from cultivar specific compounds or factors as probably formed by Mercedes following grafting rather than to anatomical obstacles resulting from the grafting surgery.

All those data as well as the suppressed activity of NR in leaves of Ilseta heterografts on *R. indica* and the difference of the grafting effects on the activity of GS in the leaves indicated that roots, in addition to the function in water and nutrients uptake, originated compounds involved in regulation of metabolic processes in the leaves, and consequently, in growth and development of plants. Nevertheless, the nature and function of these hypothetical compounds still require a more detailed investigation.

In contrast to cv. Mercedes the GS activity in leaves of cv. Ilseta, regardless of the rootstock was promoted by grafting and was by two-fold higher than that in the non-grafted plants. High variation in GS activity in non-grafted Mercedes and Ilseta plants could stem from great variability of rose plants originated from cuttings (Zieslin *et al.* 1976).

It has been previously observed (Zieslin and Kool 1986, unpublished) that in contrast to other plant species (Barker and Mills 1980, Marschner 1986) the uptake of ammonium ions by rose roots precedes the uptake of nitrate ions. Moreover, it has also been shown (Feigin *et al.* 1986, Hyndman *et al.* 1982, White and Richter 1973) that flower formation in rose plants *in situ* and of the rose roots *in vitro* were promoted when ammonium ions were incorporated as part of the nitrogen nutrition. Thus, the elevated activity of GS in leaves of grafted Ilseta may indicate preference of ammonium ions as part of the nitrogen nutrition plants as compared with plants of cv. Mercedes.

In spite of the differences in activities of NR and GS enzymes imposed by the various rootstocks, no differences in number of formed flowers have been found between all scion : root combinations of cvs. Mercedes and Ilseta grown in soilless growth medium (Agbaria *et al.* 1995). It might be therefore possible that the effects of rose rootstocks associated with alterations in activity of the enzyme of nitrogen metabolism are beneficial only when rose plants are cultivated in soils or soil-based mixtures. At these conditions the root function might be restricted due to the higher

sensitivity of the own root plants to various types of stress conditions in the root environment (Zieslin and Snir 1989). On the other hand, in soilless growth media such as rockwool, which imposed to stress conditions are less prevalent, the beneficial effects of the rootstocks are less pronounced.

Results of gel-electrophoretic separation of proteins from leaves of cv. Mercedes grafted on various rootstocks (Agbaria *et al.* 1997) exhibited presence of new bands of proteins in the leaves which could be considered as heat-shock proteins (HSP). These proteins which could be beneficial in soil grown plants were absent in leaves of the own-root plants. The recent expansion of own root rose plant cultivation in soilless growth media which is accompanied by increased flower production, is in support of the above hypothesis.

The significance of the grafting and rootstock-imposed alterations in nitrate reductase and glutamine synthetase activities described in the present report, and the association of these alterations with development and productivity of rose plants grown in soilless media are still not clear and require further investigation.

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