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BRIEF COMMUNICATION

Development of seeded and seedless hypanthium of *Rosa canina* after application of growth substances

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Abstract

Dog rose (*Rosa canina* L.) plants in the bloom stages of flowering were sprayed by indole-3-acetic acid (IAA) in concentrations of 0.06 and 0.60 mM and gibberellic acid (GA_3) in concentrations of 0.60 and 1.50 mM. Ascorbic acid, total sugar, reducing sugar and carotenoid contents gradually increased, while the protein content remained unchanged and the content of phenolic substances decreased during hypanthium development. Ascorbic acid, total sugar, reducing sugar and carotenoid contents increased in hypanthium sprayed by GA_3 and IAA. However, IAA and GA_3 applications (except low concentrations) decreased contents of phenolic substances. IAA and GA applications might be a good way to produce the high quality hypanthium in *R. canina*.

Additional key words: rose, indole-3-acetic acid, gibberellic acid, parthenocarpy.

Rosa canina L. (dog rose) is a shrub plant which grows naturally. The hypanthium are globes (sometimes ovoid), 1 - 3 cm in diameter and yellowish red or pure red when mature. Hypanthium contains hard fruits and spins (Davis 1972).

Production of seedless (parthenocarpic) fruits has been investigated by using growth substances. For example, Prosser and Jackson (1959) reported that parthenocarpy in *R. rugosa*, *R. spinosissima* and *R. arvensis* was induced by α-naphthaleneacetic acid, α-naphthaleneacetamide, and 2,4,5-trichlorophenoxyacetic acid. In addition, it has been found that gibberellic acid (GA_3) induced parthenocarpic fruit set in *R. canina* but not indole-3-acetic acid (Kadioğlu and Atalay 1999). It is necessary to know biochemical changes in seeded and seedless fruits produced by application of growth substances. There are some studies about this topic (Knopp *et al.* 1970, Drake *et al.* 1978, Quesada *et al.* 1992). The activities of peroxidase and indole-3-acetic acid oxidase in the pericarp of both seeded and parthenocarpic fruits of peach induced by 1-(3-chlorophthalimide)-cyclohexane carboxamide were investigated by Quesada *et al.* (1992). They found that total peroxidase and IAA oxidase activities increased with development on both types of fruits, but higher values were found in seedless fruits. Drake *et al.* (1978) reported an increase in the content of ascorbic acid in seedless fruits of cherry. Total phenolic substances were decreased by GA_3 and IAA applications in fruits of peach and grapes, respectively (Knopp *et al.* 1970, Kidron *et al.* 1978).

This research has been initiated to evaluate the biochemical changes during development of seeded and seedless hypanthia produced by applications of IAA and GA_3.

The experiment was conducted in 1997 and 1998 in Giresun, Turkey (approx. 100 m above sea level). Ten uniform 10-year-old *R. canina* shrubs were used. Indole-3-acetic acid (IAA) in concentrations of 0.06 and 0.60 mM, and gibberellic acid (GA_3) in concentrations of

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Abbreviations: IAA - indole-3-acetic acid; GA_3 - gibberellic acid; PEG - polyethylene glycol.
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0.60 and 1.50 mM were applied to the plants in the bloom stage of flowering. The solution of growth substances containing 0.1 % Tween-80 was sprayed to about 50 flowers of uniform development on each plant. Distilled water containing 0.1 % Tween-80 was applied to flowers of control scrubs. The growth substance applications were repeated 3 times at 5-d intervals.

The phenolics in the hypanthium were extracted using a modified procedure of Walter *et al.* (1979). A 0.2 g sample of hypanthium was homogenised in a *Waring* blender with 20 cm^3 95 % ethanol for 2 min. Then, 3 cm^3 of the homogenate was evaporated and alcohol was removed. The residue was mixed with 15 cm^3 of 0.1 M sodium phosphate buffer (pH 6.3) and passed through four layers of cheese cloth. Absorbances were measured with and without *Dowex* (chloride form) at 323 nm (spectrophotometer *Shimadzu UV 120-01*, Japan).

The determination of ascorbic acid was performed using the procedure of Shieh and Sweet (1979) with pure ascorbic acid as the standard. The samples were homogenised with 0.01 M phosphate-citric acid buffer at pH 3.0, filtered and centrifuged at 2 600 *g* for 5 min at 25 °C. The supernatant was used to determine the ascorbic acid content. The assay mixture consisted of 0.5 cm^3 of 0.01 M the buffer at pH 3.0, 2.4 cm^3 of 2,2'-Cu-biquinoline solution and 0.1 cm^3 of the extract. Ascorbic acid was determined spectrophotometrically at 540 nm.

Total content of sugar was determined by phenol-sulphuric acid method (Dubois *et al.* 1956). A standard curve was prepared to quantify hexoses and pentoses. Fresh samples (2 g) were extracted in distilled water and centrifuged at 1 000 *g* for 5 min. The fruit extracts were treated with pure sulphuric acid and phenol (5 %) and then their absorbances were measured at 480 nm for pentose and 488 nm for hexose. Reducing sugars were analysed as described by Ross (1959). A sample (1 g) was extracted in distilled water. The extract was filtered through *Whatman No. 1* filter paper. 2 cm^3 of filtrate was added to 6 cm^3 of dinitrophenol solute. The test tubes were incubated at 65 - 70 °C for 6 min and then cooled under running water. Absorbance was measured at 600 nm.

The carotenoids were extracted in 80 % acetone and centrifuged at 1 000 *g* for 10 min. Absorbances of the supernatant was measured spectrophotometrically at 450, 645 and 665 nm. The formula of Jaspars (1965) was used for the estimation of carotenoids.

Soluble proteins were extracted according to modified method of Park *et al.* (1985). Samples (5 g) were blended in 10 cm^3 of cold extraction buffer (10 mM ascorbic acid, 0.5 M phosphate buffer containing 0.5 % PEG, pH 7.3) and centrifuged for 20 min at 21 000 *g*. The amount of protein in supernatant was measured by the method of Bradford (1976).

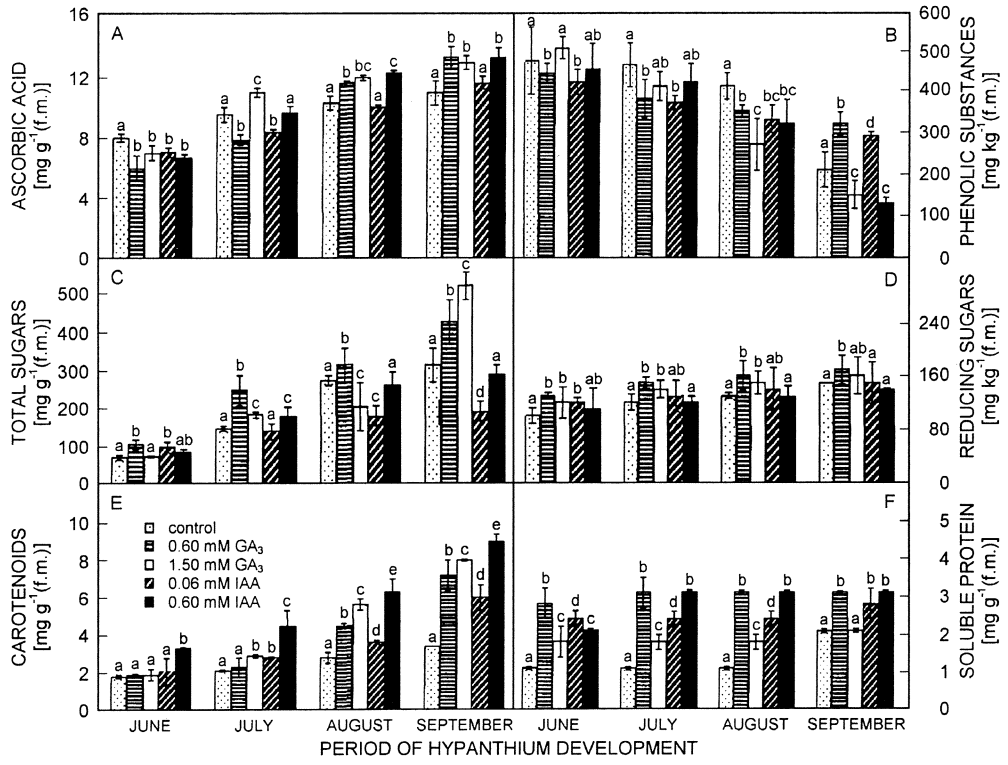

Fig. 1. The effects of growth substances on contents of ascorbic acid (*A*), phenolic substances (*B*), total sugars (*C*), reducing sugars (*D*), carotenoids (*E*), and soluble proteins (*F*) in hypanthium of *R. canina*. The *vertical bars* represent the standard deviation of the means. Within each month, the data followed by the same letter are not significantly different at 5 % level (Duncan's Multiple Range Test).

Treatment with 0.60 and 1.50 mM GA_3 gave 100 % parthenocarpic fruits, while IAA treated plants had the same seed number as controls. During hypanthium development from June to September contents of ascorbic acid, sugars and carotenoids increased, while in content of proteins and reducing sugars did not change and content of phenolics decreased. In June, the highest ascorbic acid content in hypanthium was found in control plants. In July, only 1.50 mM GA_3 application increased the content of ascorbic acid. In August and September, 0.60 mM and 1.50 mM GA and 0.60 mM IAA increased ascorbic acid content (Fig. 1*A*). Similar results were obtained in cherry (Drake *et al.* 1978). In earlier studies, it has been reported that ascorbic acid content decreased continuously during development and ripening of cherry laurel and kiwi fruits (Fuke and Matsuoka 1984, Kadioğlu and Yavru 1998).

In June and July, application of 0.60 mM GA_3 and 0.06 mM IAA decreased the content of total phenolic. In August and September 1.50 mM GA_3 and 0.60 mM IAA decreased the content of phenolic substances but in September 0.60 mM GA_3 and 0.06 mM IAA significantly increased the content (Fig. 1*B*). In the earlier studies, it has been reported that GA_3 and IAA decreased total phenolic substance in peaches and grapes, respectively (Knopp *et al.* 1970, Kidron *et al.* 1978).

In June, 0.60 mM GA_3 and 0.06 mM IAA applications increased total sugar in the hypanthium. In July and August, the highest increases were obtained in 0.06 mM GA_3 application. In September, both applications of GA_3 increased but 0.06 mM IAA application decreased content of sugars (Fig. 1*C*). All growth substances generally increased reducing sugar content in comparison with control in June. In July, August and September, 0.60 and 1.50 mM GA_3 applications increased the content of reducing sugars (Fig. 1*D*). It was generally reported an increase in soluble sugar content during the development and ripening of the fruits (Kaufman 1989, Kadioğlu and Yavru 1998). In addition IAA applications increased total sugar content in some plants (Abdul-Baki and Ray 1971, Mino *et al.* 1976).

In June and July, the highest increase of carotenoid content was found in 0.60 mM IAA application. In August and September, all growth substances also increased carotenoid content (Fig. 1*E*). The carotenoid content was gradually increased in the period of hypanthium development in all applications and control. Some workers showed that GA_3 and IAA applications generally increased carotenoid content in different plants (Sadowski and Sykut 1977, Kadioğlu 1992a,b).

In June the highest protein content was found after 0.60 mM GA_3 application. In July, August and September all growth substances increased the content of soluble protein in comparison with control (except 1.50 mM GA_3 in September) (Fig. 1*F*). Similar results have been obtained after application of IAA and GA_3 in other plants (Sharma *et al.* 1978, Brummell and Hall 1987, Salisbury and Ross 1991).

Consequently it has been found that the important changes in metabolism of hypanthium during parthenocarpic fruit set and the hypanthium which contains parthenocarpic fruits have a rich organic content. It has also been found that seeded and seedless hypanthium produced by applications of IAA and GA_3 have higher quality than the control hypanthium in *R. canina*.

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