Effect of 22(S), 23(S)-Homobrassinolide on Adventitious Root Formation in Grape Rootstocks

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In recent times, brassinosteroids have been identified as a group of hormones that regulate plant growth and development. They affect plant development from seed germination to senescence. The aim of this research was to study the effects of brassinosteroids on the rooting of three American grapevine rootstocks (1103 Paulsen, 110 Richter and 99 Richter) used frequently to produce grafted grapevines. Rootstock cuttings were dipped for 10 minutes into five different concentrations of 22(S), 23(S)-homobrassinolide and planted into a peat-perlite mixture. Data such as fresh and dry root weight, root number and development level were collected and assessed. Some shoot growth features were also determined. The results showed that the grapevine rootstocks with the most significant response in improved root and shoot growth were 1103 Paulsen, followed by 110 Richter. Root development level was influenced by both the rootstock and the concentrations of the substance. The lowest concentration, 0.05 ppm, induced more root numbers in 1103 Paulsen, while 0.15 ppm resulted in the highest number of roots in 99 Richter. A statistical analysis of the data revealed a significant difference between root development and shoot growth.

INTRODUCTION

Plant hormones have been classified into five groups: auxins, gibberellins, cytokinins, abscisic acid and ethylene. However, another group of chemical compounds called brassinosteroids have emerged as the sixth group of plant hormones. These compounds were first isolated from rape (*Brassica napus* L) pollens in 1979 (Rao *et al*., 2002), and subsequently in other plants (Sasse, 1997). Brassinosteroids collectively refer to naturally occurring 5α-cholestane steroids that elicit growth stimulation in nano- or micromolar concentrations (Clouse & Sasse, 1998; Choe, 2004).

Research conducted to study the effects of brassinosteroids on different developmental stages of various plant species have focused primarily on determining their impacts on seed germination and alleviating stress effects on development (Vardhini & Rao, 1997; Leubner-Metzger, 2001; Anuradha & Rao, 2003). However, the influence of brassinosteroids on the growth and development of perennial species has not been researched extensively. This study was carried out to test the effect of brassinosteroids on the rooting of woody species, mainly three American grapevine rootstock hybrids.

MATERIALS AND METHODS

One-year-old dormant cuttings of three American hybrid rootstocks (1103 Paulsen, 110 Richter and 99 Richter, the hybrids of *V. berlandieri* x *V. rupestris*) were obtained from the Viticultural Research Institute of Tekirdağ in Turkey. Cuttings were collected from the middle section of the canes intended for grafting (6 to 8 mm in diameter). Bundles of cuttings were kept in a black polyethylene bag in a cold storage room at 1 to 2°C and 80% relative humidity until the experiment was set.

Cuttings were prepared as two-bud sections with the lower bud removed. Five different concentrations (control, 0.05, 0.10, 0.15 and 0.25 ppm) of 22(S), 23(S)-homobrassinolide (Sigma H-1267) was prepared and the cuttings were dipped into the solutions for 10 min. The control group was dipped in distilled water. Plastic pots (13 x 20 x 7 cm), filled with perlite and peat moss (1:2 v/v), were used for growing the cuttings. The pots were then placed in a climatic chamber (24 to 26ºC and 80% relative humidity) under conditions of 16 hrs light and 8 hrs dark. The experiment was ended when sprouting plants no longer showed signs of growth (approximately after 10 weeks).

After finalising the experiments, the following measurements were taken according to the method described by Dardeniz *et al*. (2008): 1) root development level on a scale of 0 to 4 (0 = no root formation, 1 = one-sided weak root formation, 2 = two-sided root formation, 3 = three-sided root formation, and 4 = four-sided root formation); 2) root fresh and dry weights (g); 3) number of roots at least in 1 mm length; 4) primary shoot length (cm); 5) node number on the

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main shoot; 6) sprouted cuttings (%); 7) rooted cuttings (%); and 8) healthy plant (%) with live shoot and roots together.

The study was conducted in a randomised parcels trial design, with four replicates and 15 cuttings per replicate. The data obtained was evaluated with Minitab (Release 13.1, Minitab Inc.) for two-way ANOVA for the treatments and rootstocks, and the differences were tested with Duncan's multiple range test. Correlation and regression analyses were also performed on the characteristics using the same statistical program.

RESULTS

Clear effects of the 22(S), 23(S)-homobrassinolide were seen in the root development level of the cuttings (Table 1). The most significant development was observed in 1103 Paulsen cuttings treated with 0.05 ppm, while 99 Richter cuttings treated with 0.15 ppm had the highest root development. 110 Richter cuttings did not show any statistically important differences in relation to the concentrations applied.

22(S), 23(S)-homobrassinolide did not have a specific influence on the root number at the base of the cuttings. However, the rootstocks manifested their inherent characteristics and showed significant variation in the number of roots, in the decreasing order of 110 Richter (4.89), 1103 Paulsen (3.84), and 99 Richter (2.80). Fresh root weight similarly responded to the change in the root number, being 0.655 g, 0.515 g and 0.301 g in the 110 Richter, 1103 Paulsen and 99 Richter respectively. However, the dry root weights of the 110 Richter (0.056 g) and 1103 Paulsen (0.050 g) were similar, and statistically higher than that of 99 Richter (0.031 g).

Shoot growth of the rootstock cuttings was not affected by the application. 1103 Paulsen showed a superior development in shoot length (11.60 cm) and node number per primary shoot (4.51), compared to 110 Richter (7.16 cm and 2.70) and 99 Richter (6.66 cm and 3.42). The percentage of cuttings that rooted and turned into healthy plants was highest in 1103 Paulsen (69.17%), followed by 110 Richter (53.50%) and 99 Richter (45%).

Correlation analyses showed some statistically important ($p = 0.000$) positive correlations between the root growth and shoot growth aspects (Table 2), depending on the rootstock cultivar. In general, these correlations were between root development level, root number, shoot length and node number. Similar significant and positive correlations were observed between rooted plants and shoot length and node number. The correlations were higher in the 99R and 1103P cuttings compared to those in the 110R cuttings.

Regression analyses carried out on the parameters indicating significant correlations (Table 3) showed that root number on a cutting was more sensitive to the node number than the shoot length. However, this changed with node number than the shoot length. Root development level was influenced by both changes in the shoot length and node number. It was more sensitive to the node number, with a slope of more than double compared to the shoot length. On the other hand, their cumulative effects resulted in a similar slope in the lines by the variables alone.

DISCUSSION

Information on the effects of brassinosteroids on the root growth of woody plants is scarce. This research was an attempt to report on the effects of 22(S), 23(S)-homobrassinolide on the root development and growth in grapevine rootstock cuttings. 1103 Paulsen, 110 Richter and 99 Richter have different inherent characteristics as far as the rooting ability of their cuttings is concerned. The former is easiest to root among the three, while 110R roots harder compared to 99R. The response of the rootstocks to the chemical applied were not clear enough to safely conclude that 22(S), 23(S)-homobrassinolide either promotes or inhibits the root growth and development of the cuttings. It has been reported in the literature that the growth-promoting activity of brassinosteroids generally occurs if the plants are treated in the appropriate phase of development, and within a certain concentration range (Khripach *et al*., 1999). In the present experiment, the minimum concentration seemed to improve the general development of the roots, while the higher concentrations did not support better rooting. From the inclination of the plants responding to lower concentrations, it could be deduced that even lower concentrations would have given better results. Rönsch *et al*. (1993) also found that the lowest concentration of 22(S), 23(S)-homobrassinolide they used (3 ppm) was near optimum in terms of rooting percentage in Norway spruce cuttings.

The type of chemical has also been reported to have an influence on rooting. Swamy and Rao (2006) reported a more marginal effect of 28-homobrassinolide than 24-epibrassinolide when applied to geranium stem cuttings. Guan and Roddick (1988) showed inhibition of adventitious root formation in mung bean hypocotyls by 24-epibrassinolide. The chemical used in this study might be a reason for the rootstock cuttings showing inconclusive reactions.

The close relationship between root number and development level with node number shows that the more node there are, and hence new expanding leaves borne on a shoot, the more auxin-producing sites are on the shoot. An interaction between brassinosteroid and auxin at the rooting site might have resulted in the promotion of root formation around the base of the cuttings. Auxin response has been found to be connected to brassinosteroid, acting in concert with auxin to induce root growth in maize (Kim *et al*., 2000). Brassinosteroid and auxin are significant growth regulators that have effects on cell division and expansion, and on root growth (Bao *et al*., 2004). Hardtke *et al*. (2007) showed collaboration between the two compounds to modulate cell expansion and proliferation with overlapping activities. Similarly, Mandava (1988) reported the promotion of cell elongation when brassinosteroid was supplied with auxin.

CONCLUSIONS

Variations in responses to the application of brassinosteroid seem to indicate that the correct concentrations might differ with rootstock cultivar. It would be worthwhile to study this further in order to find the right concentrations, timing and type of the compounds that result in better root development, especially in harder-to-root cultivars.

TABLE 1
Effect of 22(S), 23(S)-homobrassinolide on the root and shoot development of grapevine rootstocks (*Vitis* sp.).

	22(S), 23(S)-homobrassinolide (ppm)	Root number	Root development (0-4 scale)	Fresh root weight (g)	Dry root weight (g)	Shoot length (cm)	Node number on main shoot	Cuttings rooted (%)	Cuttings sprouted (%)	Healthy plant (%)
1103 Paulsen	0.05	4.23	2.48 a	0.689	0.050	12.67	4.55	85.33	93.34	75.83
	0.10	3.64	1.59 b	0.483	0.048	12.11	4.72	76.67	90.83	73.33
	0.15	3.63	1.88 b	0.531	0.054	11.65	4.77	80.00	97.50	66.67
	0.25	3.99	2.02 ab	0.513	0.048	12.81	4.78	82.50	93.33	71.67
	Control	3.70	1.66 b	0.360	0.049	8.79	3.74	70.83	90.00	58.34
	Mean	*3.84* B	1.92	0.515 B	0.050 A	11.60 A	4.51 A	79.17 A	93.00 A	69.17 A
110 Richter	0.05	5.01	1.79 a	0.739	0.055	7.79	2.78	57.50	55.83	50.83
	0.10	5.56	1.88 a	0.505	0.072	7.73	2.77	65.00	64.17	56.67
	0.15	3.76	1.66 a	0.605	0.042	5.90	2.35	61.67	59.17	55.83
	0.25	5.06	1.71 a	0.719	0.052	6.33	2.51	55.83	59.17	52.50
	Control	5.08	1.76 a	0.707	0.058	8.06	3.11	56.67	65.83	51.67
	Mean	*4.89* A	1.76	0.655 A	0.056 A	7.16 B	2.70 C	59.33 B	60.83 B	53.50 B

TABLE 2
Correlation analysis results of the grapevine rootstocks treated with 22(S), 23(S)-homobrassinolide.

Rootstocks	**Parameters**		**Correlation (r)**	***P* value**
1103 Paulsen	Shoot length (cm)	Root number (n)	0.729	0.000
		Root development (0-4 scale)	0.707	0.000
		Rooted plant (%)	0.833	0.000
	Node number	Root number (n)	0.645	0.000
		Root development (0-4 scale)	0.601	0.000
		Rooted plant (%)	0.817	0.000
110 Richter	Shoot length	Root development (0-4 scale)	0.436	0.040
	Node number		0.528	0.017
99 Richter	Shoot length (cm)	Root number (n)	0.710	0.000
		Root development (0-4 scale)	0.768	0.000
		Rooted plant (%)	0.444	0.050
	Node number	Root number (n)	0.709	0.000
		Root development (0-4 scale)	0.790	0.000
		Rooted plant (%)	0.547	0.013

TABLE 3
One-variable regression models of the individual rootstocks to describe the relationships between root number/root development level or rooted plant percentage and shoot length and node number of the rootstocks, independent of the concentrations of the substance. x = shoot length, z = node number.

Rootstock	**Parameter**	**Regression model**	**R^2**	**P value**
1103 Paulsen	Root number (n)	$y = 0.192x + 1.61$ $y = 0.540z + 1.40$	53.1 41.6	0.000 0.002
	Root development (0-4 scale)	$y = 0.124x + 0.483$ $y = 0.335z + 0.412$	50.0 36.1 41.0	0.000 0.005 0.000
	Rooted plant (%)	$Y = 3.21x + 41.9$ $Y = 9.99z + 34.1$	69.5 66.7	0.000 0.000
110 Richter	Root development (0-4 scale)	$y = 0.0143x + 1.46$ $y = 0.164z + 1.32$	21.4 27.8	0.040 0.017

TABLE 3 (CONTINUED)

Rootstocks	Parameters		Correlation (r)	*P* value
99 Richter	Root number (n)	y = 0.339x + 0.539	50.4	0.000
		y = 0.786z + 0.108	50.3	0.000
	Root development (0-4 scale)	y = 0.160x + 0.651	58.9	0.000
		y = 0.381z + 0.410	62.5	0.000
	Rooted plant (%)	Y = 1.32x + 43.7	19.7	0.050
		Y = 3.77z + 39.6	30.0	0.013

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