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The effect of benzyladenine and naphthalene acetic acid on rooting and subsequent growth of *Portulaca umbraticola* Kunth

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ABSTRACT

Portulaca umbraticola Kunth stem cuttings were treated with BA or BA and NAA in different concentrations. BA was applied by spraying or by the quick-dip method. Independent of application type, in almost all of the treatments BA and BA applied with NAA increased the percentage of rooted cuttings, but had no effect on the number of roots, apart from BA in the 0.5 g dm^{-3} concentration, which doubled the root number. Treatment with BA alone resulted in more intensive axillary shoot development, although only in one concentration (0.2 g dm^{-3}) did BA significantly influence the number and length of lateral shoots. BA applied together with NAA had no effect or inhibited lateral shoot development.

After measurement, rooted cuttings were planted in pots in order to assess subsequent plant growth. Our data indicated that the positive effect of BA on shoot development disappeared during the subsequent cultivation of the plants. BA alone applied by spraying did not influence the number and length of initial and secondary lateral shoots, whereas BA applied with NAA negatively influenced the length of initial lateral shoots as well as the number and length of secondary lateral shoots.

Key words: adventitious rooting, BA, cuttings, lateral shoots, NAA

Abbreviations:

BA – benzyladenine (6-benzylaminopurine)
NAA – naphthalene-1-acetic acid

INTRODUCTION

The economic success of ornamental horticulture depends on providing the highest quality plants in the shortest time of production. Commonly, growth regulators are used to achieve this purpose. The majority of flowerbed plants are propagated by stem cuttings. In this case, the rooting of cuttings and the cultivation of young plants are considered separately, although it is taken into account that a high quality of cuttings and a well-developed root system influence further plant production. In fact, such separation delays the final product and prolongs production. One of the reasons for this separation is the fact that in both of these stages, growth regulators from different groups are used. Auxins are the main promoters of the rooting of cuttings (Blakesley et al. 1991, Hilaire et al. 1996, Hartmann et al. 2002), whereas various types of growth regulators are used for cultivation, depending on the shape of the plant that is needed. For flowerbed plants, compact growth with a low height and dense shoots is desirable. To get compact plants, growth retardants are used, while plants are treated with cytokinins in order to obtain good branching (Nowak and Grzesik 1997). While growth retardants usually promote root initiation and development (Davis and Sankhla 1988), cytokinins are regarded to have a negative influence on this process. These conclusions were drawn after exogenous application of cytokinins as well as from indirect evidence, such as root development in plants with high natural cytokinin levels (Hartmann et al. 2002). Recent years have brought additional evidence to this approach. More intensive development of roots in *Arabidopsis* mutants compromised in cytokinin accumulation (Dello Ioio et al. 2007) or in the overexpression of cytokinin oxidase genes (Werner et al. 2003) corroborated the role of cytokinins in rhizogenesis. On the other hand, there are also reports that under specific conditions, cytokinins can promote rooting (Davies and Joiner 1980, Fabijan et al. 1981). Furthermore, recent studies emphasise the role of cytokinins in root meristem growth. Cytokinins, together with auxins and ethylene, are regarded as hormones with the strongest influence on root development (Schiefelbein and Benfey 1991, Aloni et al. 2006). They appear to regulate auxin transport (Pernisová et al. 2009, Růžička et al. 2009).

The fact that cytokinins are strongly involved in meristem development, and play the opposite role in root and shoot morphogenesis (Werner et al. 2003), has prompted us to undertake the experiment with the aim of determining the effect of the application of benzyladenine in different concentration levels on *Portulaca umbraticola* Kunth plants from the stage of propagation from cuttings as well as the evaluation of its cooperation with auxin. From a practical point of view, the most notable purpose of the study was the possibility of obtaining well-branched plants as a final product of cultivation.

MATERIAL AND METHODS

The experiment of propagating *Portulaca umbraticola* Kunth from cuttings was carried out in the greenhouse of the Department of Horticulture at the Wrocław University of Environmental and Life Sciences, Poland. It was established in two cycles: in the spring and autumn of 2009. Apical stem cuttings, 4 cm in length, were treated with benzyladenine (BA) or benzyladenine with naphthalene-1-acetic acid (BA + NAA) in the following combinations:

BA: 0.1; 0.2; 0.5; 1.0 (g dm^{-3}),

BA + NAA: 0.1 + 1.0; 0.2 + 0.5; 0.5 + 1.0; 1.0 + 0.5 (g dm^{-3}).

The last combination consisted of control cuttings treated with water.

BA in 0.1 and 0.2 g dm^{-3} concentrations was applied by spraying on leaves after placing the cuttings in soil; the rest was applied by the quick-dip method: basal ends of the cuttings were dipped in solutions for five seconds before being placed in soil. The soil consisted of white peat, pine bark and perlit 3:1:1; v:v:v, pH 6.4. It was heated to the temperature of 21°C. Low plastic tunnels were installed over the cuttings.

The experiment was established in a one-factorial design in three replications, with 10 cuttings in each replication. The measurements of cuttings were taken after four weeks of rooting. Then, the rooted cuttings were planted into pots, in peat substrate of pH 6.47 containing (in mg dm^{-3}) N-NO_3^- 145, P 119, K 263, Mg 90, and Ca 1120, and placed in a non-heated greenhouse. The experiment was established in four replications, with five plants in each. After four weeks of cultivation, the initial and secondary axillary shoots were counted and their lengths were measured.

Data from the study were subjected to analysis of variance, and the least significant differences between means were calculated by the Tukey test at $p = 0.05$. Data concerning the percentage of rooted cuttings were formerly transformed according to the Bliss function.

RESULTS

The application of benzyladenine alone and together with naphthalene-1-acetic acid had a significant influence on the rooting and development of *Portulaca* cuttings in the first cycle of the experiment. Independent of the type of application, the three treatments with BA promoted rooting by increasing the percentage of rooted cuttings (Tab. 1). Only cuttings sprayed with BA in the 0.1 g dm^{-3} concentration rooted in the same percentage as the control cuttings. A similar effect was observed among cuttings treated with BA and NAA together: almost all combinations of the two regulators had a positive effect on the number of rooted cuttings. The exception was the combination with BA 0.1 and NAA 1.0 g dm^{-3}, in which the percentage of rooting decreased. In the second cycle of the experiment, none of the treatments influenced the percentage of rooting, as the cuttings from all of the combinations rooted in the maximum percentage. The treatments had also little effect on the number of roots. The only combination influencing root number was BA in the concentration of 0.5 g dm^{-3}. Compared to the

Table 1. The influence of BA and NAA application on rooting and the number of roots of *Portulaca umbraticola* Kunth cuttings

Feature	Cycle B	Treatments (concentrations in g dm^{-3}) A								
		Control	BA* 0.1	BA* 0.2	BA** 0.5	BA** 1.0[2]	BA* 0.1 + NAA 1.0	BA* 0.2 + NAA 0.5	BA** 0.5 + NAA 1.0	BA** 1.0 + NAA 0.5
Percentage of rooting	Spring	81.1	77.7	90.0	90.0	90.0	57.0	90.0	90.0	90.0
	Autumn	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
	Mean for A	85.6	83.6	90.0	90.0	90.0	73.5	90.0	90.0	90.0
		$LSD_{0.05}$ A = 5.4 $LSD_{0.05}$ A × B = 7.7								
Number of roots	Spring	20.3	18.5	19.5	24.6	14.8	18.1	16.7	19.3	16.3
	Autumn	11.8	7.7	14.0	25.9	14.8	15.5	13.5	13.0	9.2
	Mean for A	16.0	13.1	16.8	25.2	14.8	16.8	15.1	16.1	12.7
		$LSD_{0.05}$ A = 8.6 $LSD_{0.05}$ A × B = 12.9								

*applied by spraying
**quick-dip application

untreated cuttings, it doubled the number of roots in the second cycle.

In the first cycle of the experiment, BA in the 0.2 g dm^{-3} concentration stimulated the growth of cuttings, whereas NAA and BA applied by the quick-dip method inhibited cutting elongation. The rest of the treatments had no effect on the cutting height. None of the treatments influenced the height of cuttings in the second cycle (Tab. 2).

Independent of the cycle, NAA in the 1.0 g dm^{-3} concentration and foliar application of BA in the 0.2 g dm^{-3} concentration had no effect on the number and length of axillary shoots, while other treatments with BA and NAA together inhibited lateral shoot formation and elongation (Tab. 2). On the other hand, treating with BA alone resulted in more intensive axillary shoot development, although only in the case of the 0.2 g dm^{-3} concentration

Table 2. The influence of BA and NAA application on height and lateral shoot development of *Portulaca umbraticola* Kunth cuttings

Feature	Cycle B	Treatments (concentrations in g dm^{-3}) A								
		Control	BA* 0.1	BA* 0.2	BA** 0.5	BA** 1.0[2]	BA* 0.1 + NAA 1.0	BA* 0.2 + NAA 0.5	BA** 0.5 + NAA 1.0	BA** 1.0 + NAA 0.5
Height of cuttings (mm)	Spring	125.1	123.0	145.0	130.2	111.4	101.1	107.8	90.2	94.9
	Autumn	97.5	89.3	105.5	98.1	109.9	112.4	102.4	112.1	103.3
	Mean for A	111.3	106.1	125.2	114.1	110.7	106.7	105.1	101.1	99.1
		$LSD_{0.05}$ A = 10.95 $LSD_{0.05}$ A × B = 15.5								
Number of lateral shoots	Spring	2.30	2.73	2.83	4.10	2.47	2.00	2.10	1.43	1.53
	Autumn	1.87	2.00	3.43	1.17	1.30	0.67	1.40	1.27	1.33
	Mean for A	2.08	2.37	3.13	2.63	1.88	1.33	1.75	1.35	1.43
		$LSD_{0.05}$ A = 0.50 $LSD_{0.05}$ A × B = 0.70								
Length of lateral shoots (mm)	Spring	42.6	40.0	63.8	39.3	23.9	19.2	23.2	10.5	9.8
	Autumn	4.4	17.8	17.5	26.4	21.0	1.7	13.5	6.0	5.3
	Mean for A	23.5	28.9	40.7	32.8	22.4	10.4	18.4	8.3	7.5
		$LSD_{0.05}$ A = 11.29 $LSD_{0.05}$ A × B = 15.9								

*,**Explanations: see Table 1

Table 3. The influence of BA and NAA applied on cuttings on the subsequent growth of young *Portulaca umbraticola* Kunth plants

Feature	Cycle B	Treatments (concentrations in g dm^{-3}) A								
		Control	BA* 0.1	BA* 0.2	BA** 0.5	BA** 1.0^2	BA* 0.1 + NAA 1.0	BA* 0.2 + NAA 0.5	BA** 0.5 + NAA 1.0	BA** 1.0 + NAA 0.5
Number of initial lateral shoots	Spring	2.75	3.55	3.40	3.50	3.45	1.90	2.85	2.15	2.70
	Autumn	9.35	6.95	8.55	9.45	9.80	9.40	8.30	10.30	8.75
	Mean for A	6.05	5.25	5.97	6.48	6.62	5.65	5.75	6.22	5.72
		$LSD_{0.05}$ A = 0.88					$LSD_{0.05}$ A × B = 1.25			
Length of initial lateral shoots	Spring	169.4	141.7	153.6	158.4	127.1	103.7	124.1	97.8	117.2
	Autumn	92.4	106.4	101.2	93.3	96.8	36.7	39.7	83.4	93.7
	Mean for A	130.9	124.0	127.4	125.8	112.0	70.2	81.9	90.6	105.4
		$LSD_{0.05}$ A = 9.83					$LSD_{0.05}$ A × B = 13.9			
Number of secondary lateral shoots	Spring	17.00	17.90	13.70	14.80	11.20	7.80	8.15	7.90	9.65
	Autumn	8.20	8.20	12.35	9.70	7.90	0.00	0.85	1.35	3.75
	Mean for A	12.60	13.05	13.02	10.75	9.55	3.90	4.50	4.62	6.70
		$LSD_{0.05}$ A = 1.79					$LSD_{0.05}$ A × B = 2.54			
Length of secondary lateral shoots	Spring	41.7	27.9	40.4	38.9	41.3	41.6	43.0	37.8	47.5
	Autumn	24.9	29.4	24.8	11.4	22.4	0.0	1.1	3.9	10.0
	Mean for A	33.3	28.7	32.6	25.2	31.8	20.8	22.1	20.8	28.8
		$LSD_{0.05}$ A = 5.6					$LSD_{0.05}$ A × B = 8.0			

*,**Explanations: see Table 1

did the influence occurred to be significant. Such treated cuttings produced the longest and the most numerous axillary shoots (Tab. 2).

The influence of BA on subsequent development of young plants was not as explicit. Neither BA alone nor BA with NAA influenced the number of initial lateral shoots. Auxin with BA applied on leaves caused a decrease in the length of such shoots in the second cycle of the experiment. At the same time, all of the treatments with BA and NAA negatively influenced the number and length of secondary lateral shoots, but the effect of the treatments with cytokinins applied on leaves was stronger. Only BA in the 0.2 g dm^{-3} concentration applied by spraying significantly increased the number of secondary lateral shoots in the second cycle of the experiment, but inhibited their formation in the first cycle (Tab. 3).

DISCUSSION

Different groups of regulators often play opposite roles in plant development. Auxins, involved in adventitious rooting, usually negatively influence the branching of plants. On the other hand, cytokinins in most cases promote the development of lateral shoots but inhibit root formation. This inhibitory effect pertains to both lateral and adventitious roots (Biddington and Dearman 1982, Laplaze et al. 2007). There is still not enough knowledge about the direct influence of exogenous cytokinins on cuttings, because the application of cytokinins often gives uncertain results (Van Staden and Harty 1988). The results of our experiment, indicating a positive influence of BA on the rooting of *Portulaca* cuttings, contradict general knowledge about the influence of cytokinins on root initiation, but they are not unique. In some cases, predominantly in low concentrations, they can stimulate rhizogenesis (Davies and Joiner 1980, Fabijan et al. 1981). According to Biddington and Dearman (1982), zeatin in concentrations of 0.7×10^{-7} g dm^{-3} (3×10^{-10} mol dm^{-3}) and 2.2×10^{-7} g dm^{-3} (10^{-9} mol dm^{-3}) increased the number of lateral roots, while higher levels decreased it. Van Staden and Harty (1988) underlined the narrow concentration range that was optimal for root formation. Furthermore, the influence of cytokinins on rooting is very complex and depends not only on the concentration, but also on the place of application, the phase of rooting and the absence of auxins. Positive results were obtained after foliar spraying of cuttings, whereas its basal application was inhibitory (Eriksen 1974). Our data did not corroborate such observations. In the case of *Portulaca umbraticola* cuttings, foliar application in the lower concentration of 0.1 g dm^{-3} (5.5×10^{-4} mol dm^{-3}) inhibited adventitious root formation, while 0.2 g dm^{-3} (10^{-3} mol dm^{-3}) had no effect or increased it. The most advantageous combination appeared to be the basal application of BA in the concentration of 0.5 g dm^{-3}. Such treated cuttings rooted in higher percentages than untreated cuttings or those treated with other levels of BA; in addition, the roots were more numerous. At the same time, the influence of BA and NAA applied together enhanced the percentage of rooting but had no effect on the number of roots. In *in vitro* cultures, cytokinins used with auxins can stimulate rhizogenesis. Similar cooperation caused positive effect in the case of *Begonia* × *cheimantha* Everett leaf cuttings, which have to regenerate roots and shoots together (Heide 1965). A relatively high level of cytokinins stimulated adventitious shoot formation, while high levels of auxins promoted rooting and inhibited bud development.

Similarly to adventitious bud initiation, cytokinins are involved in lateral shoot formation (Borkowska 1997, Hartmann et al. 2002, Li and Bangerth 2003). They act as regulators releasing from apical dominance, although auxins are required for axillary meristem initiation (McSteen 2009). Shoot development requires high levels of cytokinins (Doerner 2007). Furthermore, the effect of cytokinins is stronger with their increasing concentration (Li and Bangerth 2003). In commercial production, BA is often used to enhance branching. It is applied through the media or by leaf spraying. The most beneficial effect was observed in the case of cuttings treated by spraying with BA in a higher (0.2 g dm^{-3}) concentration. This positive influence disappeared during the subsequent growth of *Portulaca* plants. It might have resulted from the short-term cytokinin stimulation of lateral bud outgrowth (Tucker 1977), as well as a too-low concentration of cytokinins in the stems. The solution to this problem may lie in treating the cuttings or plants repeatedly (Li and Bangerth 2003). Our data demonstrated a negative influence of auxin and cytokinin cooperation on lateral shoot development and it was independent of the form of cytokinin application. All of our ambiguous results support the opinion that the role of cytokinins in rhizogenesis and shoot development deserves further investigation (Arteca 1996).

CONCLUSIONS

1. BA and BA applied with NAA in almost all of the treatments increased the percentage of rooted *Portulaca umbraticola* cuttings, but had no effect on the number of roots.
2. Treating with BA alone resulted in more intensive axillary shoot development, although only in one concentration (0.2 g dm^{-3}) did BA significantly influence the number and length of lateral shoots. BA applied together with NAA had no effect or inhibited lateral shoot development.
3. Independent of the cycle, BA applied by spraying did not influence the number and length of initial and secondary lateral shoots of *Portulaca* plants grown from treated cutting, whereas BA applied with NAA negatively influenced the elongation of initial lateral shoots as well as the number and length of secondary lateral shoots.

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WPŁYW BENZYLOADENINY I KWASU NAFTYLOOCTOWEGO NA UKORZENIANIE I DALSZY WZROST PORTULAKI CIENIOLUBNEJ *PORTULACA UMBRATICOLA* KUNTH

Streszczenie: Sadzonki pędowe portulaki cieniolubnej *Portulaca umbraticola* Kunth traktowano benzyloadeniną lub benzyloadeniną i kwasem naftylooctowym łącznie. BA aplikowano przez opryskiwanie lub metodę quick-dip. Niezależnie od sposobu traktowania, traktowanie sadzonek BA oraz BA i NAA zwiększyło procent ukorzenionych sadzonek, ale nie wywarło wpływu na liczbę korzeni na sadzonkach. Tylko traktowanie BA w stężeniu 0,5 g dm^{-3} zwiększyło liczbę korzeni dwukrotnie. Traktowanie samą benzyloadeniną wywołało intensywniejszy rozwój pędów na sadzonkach, ale tylko w przypadku BA w stężeniu 0,2 g dm^{-3} istotnie wpłynęło na ich liczbę i długość. Traktowanie BA zastosowaną z NAA nie miało wpływu lub wpływało hamująco na rozwój pędów bocznych.

Po wykonaniu pomiarów ukorzenione sadzonki posadzono do doniczek, aby ocenić ich dalszy wzrost. Stwierdzono, że u roślin uprawianych w doniczkach pozytywny efekt BA na rozwój pędów bocznych zanikł. Dolistne traktowanie samą BA nie wpłynęło na liczbę i długość pędów bocznych I i II rzędu, podczas gdy traktowanie BA łącznie z NAA negatywnie wpłynęło na długość pędów bocznych I rzędu oraz liczbę i długość pędów II rzędu.

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