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COMPARATIVE EVALUATION BETWEEN BULGARIAN AND ENGLISH ROSE VARIETIES BY DEGREE OF MULTIPLICATION AND ROOTING IN MICROPROPAGATION

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Abstract

The clonal micropropagation is fast and effective method for propagation of ornamental species with proven practical application. The aim of this study is by using biometrical methods to make a comparative evaluation between Bulgarian and English varieties of ornamental roses by degree of propagation ratio and rizogenesis.

The influence of two cytokinins BAP and TDZ with various levels (0 – 2 mg/l), added to the medium of multiplication (MS) and different doses of the two auxins IBA and IAA in rooting medium (½ MS) were tested for six cultivars of roses. The results show direct influence between the type and concentration of growth regulators, used genotypes and effectiveness of *in vitro* propagation. Statistically proven degree of multiplication and rooting were observed for cvs. V. fragrance and Anny compared to the control cv. Baccara. The provided information is useful for practical *in vitro* propagation of valuable breeding material of roses.

Key words: biometrical analysis, breeding, in vitro propagation; in vitro rooting, TDZ, auxins, *Rosa hybrida* L.

Micropropagation is an alternative method of vegetative propagation, which is well suited for the ornamental industry. It is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells, an unequivocally demonstrated, for the first time, by Steward et al. (1958). Rose is the most important cut flower culture as well as pot plant. Skirvin and Chu (1979) and Hasegawa (1979) first reported a rapid method for shoot multiplication and rooting of hybrid rose cultivars and several reviews have been written during the years (Skirvin et al., 1990; Short and Roberts, 1991; Rout et al., 2006). They have highlighted the role of growth regulators and physical factors on shoot multiplication and rooting of the different cultivars of hybrid roses and also illustrated the application of modern technology for improvement, conservation and documentation of roses. Carelli and Echeverrigaray (2002) developed an efficient protocol for propagation of hybrid roses by using MS medium amended with 3.0 mg/l BA and 0.5 mg/l NAA. The micropropagation is actual procedure today. An investigation for optimization of protocol of micropropagation of commercial cv. Black Baccara was presented by Bayanati and Mortazavi (2013). The nodal segments (1 – 1.5 cm) were cultured in VS media with various levels of BAP (0, 0.5, 1, 1.5 and 2 mg/l). Baig et al. (2011) developed an efficient *in vitro* protocol for culture establishment and multiplication of *R. gruss an teplitz* and *R. centifolia.* The aim of this study is application of biometrical methods to make a comparative evaluation between Bulgarian (Anny, Evmolpia and Trimontcium) and English (Velvet fragrance and Fragrant cloud) varieties of roses (*Rosa hybrid* L.) by degree of propagation ratio and rizogenesis compared to the control cv. Baccara.

MATERIAL AND METHODS

Virus-free plants from six cultivars of roses (*Rosa hybrid* L.) grown in pots, in a greenhouse condition at Fruit Growing Institute of Plovdiv, Bulgaria and Norman Borlaug Institute, Leicester, UK, were used. Shoot tips with partial reduced leaves were used as initial explants. They were thoroughly washed in running tap water for 20 minutes and immersed for 30 seconds with 70% (v/v) ethanol, followed by a 15 minute soak in a periodically shacked 2.5% (v/v) solution of sodium hypochlorite (NaOCl) supplemented with a few drops of Tween-20 as a wetting agent and after that rinsed three times with sterile distilled water. Explants were inoculated in jar/boxes at MS medium (macro, micro elements and vitamins) supplemented with various concentrations of BAP/TDZ (0; 0.5; 1.0; 1.5; 2.0) and NAA 0.001 mg/l. The media contained 30 g/l sucrose, the pH was adjusted to 5.8 before adding 6.8 g/l plant agar. For rooting was used ½ MS supplemented with varying concentrations of IBA/IAA (0.25; 0.5; 0.75; 1.0 mg/l) and 20 g/l sucrose. Media were autoclaved for 20 min at 121 °C and 1.2 kPa pressure. Cultures were placed in a culture room under cold white light (fluorescence lamps Philips) 16/8 hour light/dark cycle and maintained at 22 ± 2 °C. Shoot regeneration (average number of shoots per explant, average length of shoots) and average number of roots ≥ 10 mm were recorded after 4 weeks cultivation.

The experiments were designed with 5 observa-

tions and 3 replications and the data were analyzed with the means by LSD methods of SPSS 19.

RESULTS AND DISCUSSION

A variety Evmolpia concentration of 1.5 mg/l BAP is fairly most effective for obtaining the maximum number of shoots per explant (Table 1). For variety Baccara the highest value for the number of shoots was recorded at the same concentration. Statistically it was found that the maximum degree of multiplication (number and length of the shoots) was achieved at a concentration of 1 mg/l BAP (Figure 1).

BAP is the most effective plant growth regulator used to stimulate proliferation of adventitious buds of roses (Vijaya et al., 1991). Purine cytokinin N^6-benzyladenine (BA) is also successfully used for axillary proliferation in the *in vitro* culturing of *Rosa hybrida* L. (Yakimova et al., 2000). The effect of phenylurea cytokinins CPPU and TDZ is less studied (Barna 1995; Kapchina-Toteva et al., 2000, Georgieva et al., 2009). TDZ is urea derivative and does not contain a purine ring. Mok et al. (1982) agree that TDZ possesses higher cytokinine effect than purine cytokinins, but the mechanism of action is not fully understood. In our experiments, the

Table 1. Influence of different concentration of BAP and TDZ on shoot proliferation (average number of axillary shoots per explant) of rose cultivars

Cultivar	Variants (mg/l)									
	0		0.5		1.0		1.5		2.0	
	BAP	TDZ	BAP	TDZ	BAP	TDZ	BAP	TDZ	BAP	TDZ
Anny	0.26 ns	0.22 ns	1.2 ns	1.62 ns	**4.57 + + +**	3.6 ns	2.34 ns	2.22 ns	1.34 ns	1.21 ns
Evmolpia	0.43 ns	0.21 ns	1.5 ns	1.57 ns	**4.21 + +**	3.3 ns	2.4 ns	2.21 ns	1.47 ns	1.32 ns
Trimontcium	0.28 ns	0.36 ns	1.25 ns	1.45 ns	**4.19 + +**	3.74 ns	2.76 ns	1.98 ns	1.32 ns	1.11 ns
Velvet fragrance	0.31 ns	0.32 ns	1.47 ns	1.38 ns	**4.6 + + +**	**4.34 +**	3.01 ns	2.34 ns	1.42 ns	1.08 ns
Fragrant cloud	0.24 ns	0.38 ns	1.38 ns	1.23 ns	**3.97 + +**	**4.3 +**	2.87 ns	2.10 ns	1.24 ns	0.97 ns
Baccara	0.25	0.27	1.34	1.36	3.2	3.56	2.63	2.24	1.21	1.02
LSD p < 0.05 *p < 0.01* *p < 0.001*	*0.19*	*0.15*	*0.18*	*0.32*	*0.56* *0.76* *1.03*	*0.63* *0.86* *1.14*	*0.40*	*0.69*	*0.45*	*0.37*

Table 2. Influence of different concentration of BAP and TDZ the on average shoot length of rose cultivars

Cultivar	Variants (mg/l)									
	0		0.5		1.0		1.5		2.0	
	BAP	TDZ	BAP	TDZ	BAP	TDZ	BAP	TDZ	BAP	TDZ
Anny	1.3 ns	1.11 ns	2.13 ns	2.81 ns	**6.2 + + +**	4.3 ns	4.7 ns	3.9 ns	3.02 ns	3.11 ns
Evmolpia	1.12 ns	1.31 ns	2.45 ns	2.64 ns	3.7 ns	3.0 - -	**5.7 +**	2.92 ns	3.5 ns	2.41 - -
Trimontcium	1.5 ns	1.34 ns	2.15 ns	2.43 ns	**5.9 + +**	3.8 ns	4.52 ns	3.0 ns	3.13 ns	2.87 -
Velvet fragrance	1.6 ns	1.52 ns	2.31 ns	2.54 ns	**6.13 + + +**	3.21 - -	4.71 ns	3.53 ns	3.8 ns	3.0 ns
Fragrant cloud	1.28 ns	1.43 ns	2.71 ns	2.63 ns	**5.5 + +**	5.1 ns	5.0 ns	3.4 ns	3.13 ns	3.21 ns
Baccara	1.22	1.30	2.3	2.51	3.8	4.3	**4.93**	3.2	3.21	3.25
LSD p < 0.05 *p < 0.01* *p < 0.001*	*0.45*	*0.35*	*0.45*	*0.37*	*1.26* *1.71* *2.27*	*0.92* *1.24* *1.65*	*0.72* *0.97*	*0.65*	*0.63*	*0.58* *0.75* *0.99*

Table 3. Influence of different concentration of IBA and IAA on the average number of roots (with length ≥10 mm) of rose cultivars on ½ MS rooting medium

Cultivars	Variants (mg/l)							
	0.25		0.5		0.75		1.0	
	IBA	IAA	IBA	IAA	IBA	IAA	IBA	IAA
Anny	2.9 ns	1.3 ns	**4.8** **+ + +**	4.12 ns	3.43 ns	3.10 ns	2.15 ns	1.85 ns
Evmolpia	2.3 ns	1.02 ns	**4.1** **+**	4.10 ns	3.25 ns	3.22 ns	2.24 ns	1.64 ns
Trimontcium	2.2 ns	1.04 ns	**3.8** **ns**	3.58 ns	3.15 ns	3.14 ns	2.15 ns	1.78 ns
Velvet fragrance	2.6 ns	1.1 ns	**4.5** **+ + +**	4.35 ns	3.45 ns	2.85 ns	2.10 ns	1.95 ns
Fragrant cloud	2.1 ns	1.3 ns	**4.3** **+ +**	3.76 ns	3.22 ns	2.65 ns	2.0 ns	1.73 ns
Baccara	2.4	1.05	3.45	**3.95**	3.10	2.95	1.98	1.88
LSD p < 0.05 *p < 0.01* *p < 0.001*	*0.6*	*0.32*	*0.49* *0.66* *0.88*	*0.45*	*0.35*	*0.41*	*0.29*	*0.27*

Fig. 1. Shoot proliferation of roses in vitro

Fig. 2. Rooted and acclimatized roses in greenhouse

applied concentration of 1 mg/l TDZ for English varieties Velvet fragrance and Fragrant cloud showed significant differences in the average number of shoots per explant compared to the control variant. For the other varieties of the same sign are considered non-essential differences as values obtained at a dose of 1 mg/l TDZ are highest compared to other concentrations (Table 1). At higher doses of TDZ callus in the area of cutting and water in the tissues were observed, which causes a problem in rooting of the shoots. For this reason high doses of TDZ are not recommended. At low concentrations of TDZ length of shoots was not significantly different between varieties, while at doses 1 – 2 mg/l for cvs. Evmolpia, Trimontcium and Velvet fragrance significant

differences were found, but with decreasing values of the control (Table 2). This makes those concentrations unsuitable for use in *in vitro* propagation of roses.

Maximum average number of roots were recorded at a concentration of 0.5 mg/l IBA followed by the same concentration of IAA for the studied varieties of roses (Table 3). However, a decreasing trend in the number of roots was observed after a certain level of both auxins as well as it is not reported evidence of a difference between the compared varieties and Baccara. Higher doses of auxin suppress the process rizogenezis, while the concentration of 0.25 mg/l is inadequate for the formation of the necessary number of roots. Therefore, we can conclude that for cvs. Anny, Evmolpiya and both English varieties the concentration of 0.5 mg/l IBA is most suitable for the formation of sufficient number of roots compared to Baccara and Trimontcium. In practice, the most commonly used auxin for formation of roots in plants is IBA in low concentrations. Probably the optimum dose of IBA is responsible for increasing the growth of cambial tissue in the area of micro cut at the base of the shoot and the subsequent differentiation of the root tip (Haq et al., 2009). Many researchers have reported the rooting of cuttings of 1/2 MS medium supplemented with low concentration of auxin in the range of 0.1 to 0.5 mg/l and with reduced carbohydrate content (Senapati and Rout, 2008). In our experiment, for the six cultivars of roses highest number of roots were obtained at concentration of 0.5 mg/l for both applied auxins, but significant differences at $p < 0.001$ were reported for cvs. Anny and Velvet fragrance at concentration of 0.5 mg/l IBA. All rooted explants of roses were acclimatized and established in the soil successfully. (Figure 2).

CONCLUSIONS

A system for *in vitro* propagation of six cultivars of roses (*Rosa hybrida* L.) was developed as an initial explants top cuttings were used.

Cultivar differences in *in vitro* multiplication were established in response to the applied two types of cytokinins (BAP and TDZ).

Efficient *in vitro* rooting was observed for six cultivars of roses on 1/2 MS medium supplemented with 0.5 mg/l IBA or IAA.

Regarding the average number of axillary shoots for explants, length and average number of roots, cvs. Anny and Velvet fragrance were significantly different at $p < 0.001$ compared to the same parameters for a cv. Baccara.

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