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COMPARATIVE ANALYSIS OF DIFFERENT ROSE CULTIVARS (*ROSA HYBRIDA* L.) ROOTING USING CONVENTIONAL AND BIOTECHNOLOGY APPROACHES

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Abstract: The rapid and efficient propagation of plant material obtained is an important stage in the breeding of flower species. The purpose of this study is to make a comparative assessment of the significance of differences from the application of two of the widely used practical approaches for propagation of four ornamental roses cvs (*Rosa hybrida* L.) – Anny, Trimontium, Peace and Baccara. The auxin indole-3-butyric acid (IBA) at concentration of 0.5 mg/l was used as a growth regulator in both methods applied – conventional and biotechnological. The percentage of rooting recorded is over 50% in both types of propagation. This rate in the *in vitro* propagation is between 73-90% for the tested varieties of roses, while the rooting rate reached 64% when the conventional method of propagation was applied. Both approaches are used in the propagation of valuable material selection and the leading factor for implementation is the availability of financial sources.

INTRODUCTION

Roses are usually propagated by grafting methods (Park Y. and Jeong B., 2012) or less frequently by cutting and subsequently growing on their own roots (WC Welch, Manners MM., 2014). *In vitro* culture techniques, which are used for propagation of different species, are gaining importance for rose propagation in the last four decades. Multiplication of roses *in vitro* is based on the subdivision of plantlets into branches, terminal buds and nodal sections (Short and Roberts, 1991). The attractiveness of *in vitro* technology stems from the fact that it allows the production of sufficient elite planting material with a health certificate for export; high control of abiotic and biotic factors during the stages of multiplication

and rooting and a high percentage of rooted cuttings with viable root system and independence from environmental factors (Rout G. et al.1990; Khosravi P et al., 2007; Razavizadeh R and Ehsanpour A. 2008; Bayanati M. et al., 2013). Both methods of propagation are successfully applied in practice.

The purpose of this study is to make a comparative assessment of the degree of rooting of four rose cultivars using two propagation techniques – green cuttings and *in vitro*.

MATERIALS AND METHODS

Plant material

Four genotypes of cut roses were used in this research:

– cv Peace was selected by Francis Meilland, France (synonyms Gloria Dei, 'Madame A. Meilland'). The cultivar was hybridized in 1935, from the hybrid tea 'Margaret McGredy' and an unnamed seedling. The cv Peace has large flowers with light yellow to cream color, slightly flushed at the petal edges with crimson-pink. "Peace" is hardy, vigorous and relatively resistant to disease, making it popular in the floral trade.

– cv Baccara was selected by Francis Meilland in 1954, France (synonyms Jacqueline', 'Meiger'). The Baccara Rose has a mean diameter of about 8 cm, long stem and deep red flower color, which does not change after emergence. These properties make cv Baccara one of the most popular cut roses.

The two French varieties were kindly provided by an English nursery of roses – David Austin® Roses.

– Anny is a cross between "Baccara" and "Eiffel Tower". The bush has moderate upgrowth, almost without thorns, and the flower bud is highly elongated in a shape that also resembles the father parent. The bud has a dark red-violet color and fragrance.

– Trimontium is a cross between Anny and an unnamed seedling. The bud is elongated with color "ashes of roses" and emanates a pleasant scent. The variety is suitable for cut flowers and has an average resistance to powdery mildew.

Both cvs Anny and Trimontium were selected by Prof. Angeliev, Agricultural University, Plovdiv.

The donor plants were cultivated in a greenhouse of the Agricultural University, Plovdiv. Clonal propagation was performed in the laboratory of biotechnology at the Fruit Growing Institute. Propagation of green cuttings was performed at private roses' farm of P. Telbis in Dragomirovo village.

Green cutting propagation

After normal flowering, each healthy stem was cut into 15-18 cm sections with three leaves each. The stems were cut above the bud's top, in order to remove the shoot tip, and down at the base. All the leaves along the stem were removed except from the top, which was shortened in half. The diameter of the stems was

0.8 to 1.2 cm. The base of cuttings was immersed for 3 minutes into liquid rooting solution (IBA – 0.5 mg/l^{-1}) (Baig MM, et al 2011; Maurya RP, et al 2013). The cuts were rooted directly in soil on high beds, prepared beforehand, composed of 1:1 mixture of enriched potting soil and sand. The shoots were covered with plastic tubes and during the period of vegetation were constantly sprayed with automatic sprinkler equipment type Gardena (Classic Polo 220) during the period of vegetation.

The green cutting was carried out three times - in late May, June and July (indicated in Table 1 as period of betting I, II and III). The number of rooted cuttings was recorded after 7 months.

Clonal micropropagation

Nodal segments and tops (1-1.5 cm) were taken from the rose stems. They were washed thoroughly with running tap water for half an hour and had their surface sterilized for 30 seconds in 70% (v/v) ethanol, followed by a 15 min soak in 2.5% (v/v) sodium hypochlorite solution with a few drops of Tween-20 as a wetting agent. The segments and tops were then rinsed three times with sterile distilled water. MS (Murashige T. and Skoog F., 1962) basal medium, supplemented with BAP-0.7 mg/l^{-1}, IAA - 0.01mg/l^{-1} and 30 g/l^{-1} sucrose, was used for the *in vitro* multiplication. The pH of the medium was adjusted to 5.6 before adding 8 g/l^{-1} plant agar. Media were autoclaved for 15 min at 121^{o}C and 1.2 kPa pressure. Cultures were placed under a photosynthetic photon flux density of 45 μM m^{-2} s^{-1} and photoperiod of 16/8 hour light/dark in a cultivation chamber, maintaining a temperature of 21±2^{o}C. Shoots with length less than 2 cm were cultured on medium for prolongation (MS without hormones). Shoots longer than 2 cm and extended shoots were cultured in the rooting medium (1/2 MS macro, micro elements and vitamins) supplemented with 20 g/l^{-1} sucrose and 0.5 mg/l^{-1} IBA. The reporting of the number of rooted shoots was done 35-40 days after the cultivation. The experiment was repeated three times.

The experiment was performed via a completely random design with 6 replications of 5 cuttings each (30 cuttings). A non-standard scheme of betting variants was used for pair comparison. The analyses were regarded as significant at α=0.001.The experimental results were subjected to an analysis of variance (ANOVA) using the SPSS 19 program and LSD- test (criteria of Student).

RESULTS AND DISCUSSION

The results of cuttings of roses rooted by both methods are presented in Table 1. A higher number of rooted shoots were recorded in the third period of betting (III) for Bulgarian varieties propagated via *in vitro* techniques (30 and 29 rooted shoots).

Table 1. Number of rooted shoots of CVs rose, *Rosa hybrida* L. propagated by micropropagation and green cuttings.

Variants cvs	Period of betting	Number of shoots	Number rooted shoots	
			after 1 month (micropropagation)	after 7 months (green cuttings)
Anny	I	30	25	20
	II	30	26	23
	III	30	30	14
		n=90	$\overline{X}$ ±SE 27±0.13	$\overline{X}$ ±SE 19±0.22
Trimontium	I	30	13	16
	II	30	24	12
	III	30	29	15
		n=90	$\overline{X}$ ±SE 22.0±0.10	$\overline{X}$ ±SE 14.3±0.10
Average of two Bulg. cvs			$\overline{X}$ = 24,5	$\overline{X}$ = 16,65
Baccara	I	30	25	15
	II	30	27	16
	III	30	24	19
		n=90	$\overline{X}$ ±SE 25.3±0.07	$\overline{X}$ ±SE 16.7±0.10
Peace	I	30	25	10
	II	30	24	16
	III	30	26	18
		n=90	$\overline{X}$ ±SE 25±0.05	$\overline{X}$ ±SE 14.7±0.21
Average of two Fr. cvs			$\overline{X}$ = 25,15	$\overline{X}$ = 15,7
Total average			$\overline{X}$ = 24,83	$\overline{X}$ = 16,17

The same trend was observed in the results of the French cvs propagated by green cuttings (19 and 18 rooted shoots). The average number of rooted shoots for Bulgarian and French varieties, propagated by both methods, has fairly similar values – 24.5 and 25.15 for microclonal propagation and 16.65 and 15.7 for green cuttings. This does not mean that the areas necessary for rooting in both methods are identical. The sequential steps of rooting via green cuttings are presented in Figure 1: (1a) – Cuttings preparation; (1b) – Placed under a plastic tube; (1c) – Observations. Figure 2 shows some parts of the sequence of rooting *in vitro*: (2a) – Selecting appropriate segments of the donor plants; (2b) – Introduction in *in vitro* culture; (2c) – Adaptation of rooted shoots. The length of

the rooting period is about 7 times as long as that of green cutting methods. The areas used for rooting in both methods vary dramatically.

Figure 1. Some of the stages in the sequence of rooting of 4 cvs of roses by green cuttings.

Figure 2. Some steps of rooting through micropropagation.

The higher number of rooted shoots in micropropagation highlights the advantages of this method, which coincides with the findings of De Vries and Dubois (1994), who discuss the positive effect of micropropagation on six rose rootstocks.

The percentages of rooted roses for the four varieties are presented in Figure 3. The highest percentages of rooted shoots were reported for cvs Anny (90%) and

Baccara (84.4%), propagated *in vitro*. The same two varieties exhibit the highest values in % rooting propagated by green shoots – respectively Anny 63.4% and Baccara 55.5%. The lowest values, about 50%, were reported for cvs Trimontium and Peace, propagated by green cuttings. The rooting ability in representatives of the genus Rosa varies considerably. In his overview of the micropropagation in *Rose*, Norn (1992) reported successful rooting in sterile conditions from 55 to 100% and in pots - from 50 to 100% in different varieties of roses, quoting a number of authors. Therefore, this quality may be classified as genetically linked. Similar experiments related to *in vitro* rooting were carried out by researchers in the early 90s. Davies (1980) reported the development of well-branched roots in 20–100% of seven rose cultivars in liquid MS medium without plant growth regulators. Preliminary experiments with *in vitro* multiplication and rooting of five varieties of roses were conducted by Curir et al., (1986). They reported a percentage of rizogenesis from 70 to 80%; however the process could be further improved by taking into account the influence of many other environmental factors (temperature, photoperiod).

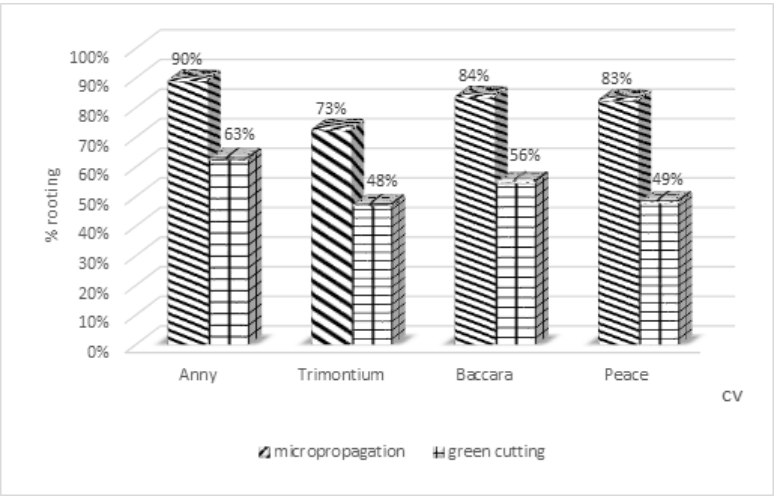

Figure 3. The average percentage of rooted shoots (by micropropagation and green cuttings) of cvs rose *Rosa hybrida* L.

The results were statistically analyzed using analysis of variance (ANOVA). The ANOVA results show significant differences between the two methods of propagation according to the percentage of rooted cuttings (empirical value F_{em}=30.11 was less than the critical value F_{cr}=4.30).

Comparative evaluation (cultivars in pairs) of the variants was performed using LSD test to determine the confidence level of the differences between the pairs.

The results from the LSD test and the respective confidence levels of the compared variants are presented in Table 2.

Table 2. Comparative evaluation with LSD between four cvs roses of % rooted shoots via micropropagation and green cuttings at significant level α=0.001.

Cultivars (in pairs)	Micropropagation		Green cuttings	
	Value of differences	Estimation with LSD and confidence	Value of differences	Estimation with LSD and confidence
1. Anny with Trimontium	**16.7**	4.845 +++	**15.6**	2.852 +++
2. Anny with Baccara	**5.6**	1.723 +++	**7.9**	2.852 +++
3. Anny with Peace	**6.7**	3.393 +++	**14.5**	3.501 +++
4.Trimontium with Baccara	**11.1**	4.689 +++	**7.7**	1.664 +++
5. Trimontium with Peace	**10.0**	5.528 +++	**1.1**	2,634 ns
6. Baccara with Peace	**1.1**	3.171 ns	**6.6**	2.634 +++

There are six possible comparison combinations of tested rose genotypes (Table 2). The first four comparative pairs were recorded at the highest level of confidence. Thus, it could be argued that the compared varieties of roses have proven differences in the percentage rooted shoots with both methods of propagation. In Table 2 the levels of confidence are marked with (+++). Cv Anny has a leading position to other varieties regarding rooting percentage. The highest level of confidence differences was reported between two Bulgarian cvs – Anny and Trimontium. From preliminary observations on the growth and development under field conditions, both cvs showed varying growth parameters and type of habitats (personal observations of the author), which explains the results obtained under controlled and field conditions. Cv Trimontium, in comparison to cv Baccara (with both methods of rooting) and cv Peace (by micropropagation), also exhibits the same high levels of confidence (+++).

In the last two pairs of cultivars, the comparative assessments have opposite results. Under controlled conditions in the *in vitro* rooting procedure, difference of 1% between both French cvs was insignificant, probably due to close genotype relations. In this sense, it could be argued that the reason for these results is a close genetic relation between the two varieties (their common pedigree).

In the comparative analysis between Baccara and Peace, high levels of confidence (+++) were also reported, i.e. there are significant differences in the percentage rooted from green cuttings. The difference of 6.6% proved significant for both varieties in outdoor conditions.

CONCLUSION

The conducted comparative analysis shows that the Bulgarian rose cvs have a statistically significant, higher percentage of rooted cuttings and shoots than the French cvs. No significant differences were reported in the percentage of rooted

roses by green cuttings when comparing cvs Trimontium and Peace. The rooting of green cuttings for three of the tested cvs (Trimontium, Baccara and Peace) showed values around 50%. The rooting percent was over 50% (63.4%) only in cv Anny's. All of this defines the above method as less efficient, more labor intensive, largely dependent on environmental factors and with greater genotype dependence.

The application of biotechnological approaches in ornamental roses rooting is used in order to speed up the propagation process, to maintain a smaller production area, to control the environmental factors on rooting and to increase the efficiency of the process. The data analyzed shows that the percentage of rooted shoots of four cvs ranges from 73 to 90% (83 and 84% for French cvs and 73 and 90% for Bulgarian cvs), which is in line with the empirical results of the cited researchers.

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