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Evaluation of hormonal balance influence on the micropropagation of some rootstocks plum

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Abstract Numerous researches support the importance of growth hormones for the *in vitro* propagation of various species included in *Prunus* genus. The influence of BAP, IBA and NAA hormones, in five variants of MS medium, on the micropropagation of four plum rootstocks was investigated, using two types of explants (shoot tip and node explant). The Murashige and Skoog culture medium (MS Sigma M 5519), to which 20 g of sucrose and 8 g of agar was used. Five experimental variants were studied: V1: 0.5 mg/l BAP, V2: 0.6 mg/l BAP, V3: 0.5 mg/l BAP + 1mg/l NAA, V4: 6 mg/l IBA, V5: 2 mg/l NAA. The best results were obtained with explants obtained from the shoots tips, grown on MS medium supplemented with concentrations of 0.5 and 0.6 mg/l of BAP and 2 mg/l of NAA.

Key words

Prunus, hormonal balance, micropropagation, rootstocks

Many researchers uphold the importance of growth hormones for the *in vitro* propagation of various species included in *Prunus* genus [12, 15, 16, 17]. The experimental results obtained by Ancu et al. (2015) reveal that, regardless of the composition of base medium, the best hormonal combination was composed of BAP (1.0 mg/L) and IBA (0.1 mg/L). Sabbadini et al. (2019) achieved good results when the rootstock 'Hansen 536 MB' was grown on WPM medium, enriched with 4,4 μM 6-Benzyladenine (BA), 0,1 μM 1-naphthaleneacetic acid (NAA) and 6.0 g/l agar. Nas et al. (2010) investigated the *in vitro* regeneration capacity of *Prunus microcarpa* rootstock on NRM medium and benzyladenine (BA), meta-Topolin (mT) and thidiazuron (TDZ) in various concentrations. The best rooting hormone reported by Vaez-Livari and Salehi-Soghadi (2005) was IBA (0.5 mg/L) with sucrose (30 g/L). Tricoli et al. (1985) studied the *in vitro* propagation of *Prunus serotina*, MS medium, supplemented with inositol, thiamine HCl, 6-benzylaminopurine, gibberellic acid, indole-3-butyric acid; multiple shoots were induced on the medium with 0.75 mg / l BAP, 0.2 mg/l GA3, 0.01 mg/l IBA and 3% sucrose. Fourth basal media (MS, LF, QL, WP) were compared by Calinescu et al. (2009) containing GA3 (0.1 g/l) and IBA (0.01 g/l) during initiation of apricot. Perez-Tornero et al. (2000) studied the effect of different media and cytokinin concentration on the proliferation of shoots of the cultivar 'Canino'; an IBA concentration between 0.5 and 0.6 mg/l produced an optimal number of shoots with a good length to be transferred to other subcultures. The rooting capacity expressed by the percentage of rooted plants was over 80%, but strongly depends on the concentration of IBA [5]. Arab and Shekafandeh (2016) reported that thidiazuron (TDZ) gave the highest percentage of adventitious shoots (68.8%) and the highest number of shoots per cotyledon (4.8) using Quoirin and Lepoivre (QL) medium. In the study undertaken by Jain and Babbar (2003), BA (0.5 or 1.0 mg/l) induced greening and opening of the incipient shoot buds, which however did not elongate; elongation of the shoot buds was facilitated on MS medium with 1.0 mg/l BA supplemented with casein hydrolyzate (1.5 g/l) or glutamine (200 mg/l). The successful shoot multiplication of *Prunus salicina* was achieved on WPM with 0.05–0.1 mg/l IBA, 0.2 mg/l BA, 0.3 mg/l KT and 1.0 g/l casein hydrolyzate [23]. In the present paper, the influence of BAP, IBA and NAA hormones, in five MS environment variants, on the micropropagation of four rootstocks for plums, using two types of explants, was investigated.

Material and Method

The study was conducted at the University of Craiova (INCESA - Plant Biotechnology Laboratory). The branches were harvested from the rootstock collection of the Horticulture Faculty, at the end of February and were placed in the laboratory for forcing. After 20 days, the shoots obtained were put under a sterilization protocol, by washing them with soap and water, followed by treatment with sodium hypochlorite (Domestos commercial product, diluted with distilled water, in a ratio of 2: 5), ethyl alcohol 70 % (15″), three consecutive washes with sterile distilled water.

From the shoots were made top and nodal micro-cuttings, 5-6 mm long. The Murashige and Skoog culture medium (MS Sigma M 5519), to which 20 g of sucrose and 8 g of agar were added, was sterilized in an autoclave at 121°C and pressure of 1(2) atmospheres for 15-20 minutes. The pH was adjusted to 5.8 with NaOH solution before the agar was added. Five experimental variants were studied: V1: 0.5 mg/l BAP, V2: 0.6 mg/l BAP, V3: 0.5 mg/l BAP + 1mg/l NAA, V4: 6 mg/l IBA, V5: 2 mg/l NAA. The following conditions were ensured in the growth chamber: temperature of 25ºC (±1) and a lighting regime of 16 hours of light/8 hours of darkness. After 30 days, observations and measurements were performed, which focused on: diameter of vegetative mass developed from the explant, the height and number of leaves. All experiments were arranged in a completely randomized design. Data have been statistically processed using EXCEL, DATA ANALYSIS.

Results and Discussions

Plant growth regulators play an important role in high value horticultural crops to increase yield, increase crop quality and management [7, 10]. Bhagwat and Lane (2004) argue that the percentage of regeneration is influenced by plant growth hormones and the type, orientation and size of the explant. From the explants used for inoculation, a vegetative mass of shoots and calluses was obtained. The results on its average diameter for the shoots obtained from the top of the shoot and the nodal explant are presented in Table 1.

It varied depending on the concentration of hormones in the five environmental variants between 0.60 cm in 'Sel. Corcodus' rootstock (V3) and 3.20 cm in 'Fortival' (V1) rootstock. The vegetative mass obtained for the explants from 'Plamval' rootstock had an average diameter that varied between 0.80-3.00 cm. The results obtained for the diameter of explants obtained from nodal explant are lower: 0.40 cm for 'Sel . Corcodus' rootstock (V3) and 2.70 cm in 'Miroval' and 'Plamval' rootstock (V4), respectively in V5. Regarding the height of the vegetative mass, the highest value 2.20 cm was obtained for the explants from the top of the shoot (table 2) from 'Miroval' and 'Plamval' rootstock (V5) and 2.20 cm for the nodal explants come from 'Plamval' (V4) rootstock. V2 and V5 variants did not cause the elongation of the shoot and nodal explants of 'Sel. Corcodus' rootstock and those of the nodals in 'Miroval' rootstock.

Table 1. The diameter of vegetative mass obtained from top shoots and node explants (cm)

Rootstock / variant	Descriptive statistics	Hormonal balance and type of explant									
		V1		V2		V3		V4		V5	
		a*	b	a	b	a	b	a	b	a	b
Prunus cerasifera Selection	Mean	2.32	0.95	-	-	0.66	0.46	1.62	1.72	-	-
	SD	0.08	0.01	-	-	0.05	0.05	0.08	0.08	-	-
	Cv%	3.61	1.38	-	-	8.29	11.91	5.16	4.86	-	-
Miroval (sin. MVL 2)	Mean	2.44	-	-	-	1.12	1.16	2.72	2.62	1.92	2.54
	SD	0.09	-	-	-	0.08	0.11	0.08	0.08	0.08	0.11
	Cv%	3.67	-	-	-	7.47	9.83	3.08	3.19	4.36	4.49
Plamval (sin. H19-5-85; Rival)	Mean	2.90	1.02	2.80	1.72	1.12	0.82	0.92	2.12	1.72	1.68
	SD	0.10	0.08	0.16	0.01	0.08	0.01	0.08	0.08	0.08	0.13
	Cv%	3.45	8.20	5.65	0.52	7.47	1.59	9.09	3.95	4.86	7.76
Fortival (sin. H1-2V; Corval)	Medie	3.12	-	2.48	1.78	-	-	-	-	-	-
	SD	0.08	-	0.13	0.13	-	-	-	-	-	-
	Cv%	2.68	-	5.26	7.32	-	-	-	-	-	-

*a = shoot tip, b = node explant

Table 2. The height of vegetative mass obtained from shoots tip and nodal explants (cm)

Rootstock / variant	Descriptive statistics	Hormonal balance and type of explant									
		V1		V2		V3		V4		V5	
		a*	b	a	b	a	b	a	b	a	b
Prunus cerasifera Selection	Mean	1.12	1.14	-	-	0.72	-	0.90	1.50	-	-
	SD	0.08	0.05	-	-	0.08	-	0.10	0.16	-	-
	Cv%	7.47	4.80	-	-	11.62	-	11.11	10.54	-	-
Miroval (sin. MVL 2)	Mean	1.82	-	-	-	1.74	0.82	1.72	2.10	2.06	1.70
	SD	0.08	-	-	-	0.05	0.01	0.08	0.10	0.09	0.10
	Cv%	4.60	-	-	-	3.15	1.59	4.86	4.76	4.34	5.88
Plamval (sin. H19-5-85; Rival)	Mean	1.80	0.95	1.96	1.42	1.90	1.22	1.86	2.12	2.06	1.76
	SD	0.45	0.01	0.11	0.08	0.16	0.01	0.11	0.08	0.09	0.11
	Cv%	24.85	0.88	5.82	5.89	8.32	1.07	6.13	3.95	4.34	6.48
Fortival (sin. H1-2V; Corval)	Medie	1.54	-	1.74	0.99	-	-	-	-	-	-
	SD	0.09	-	0.11	0.01	-	-	-	-	-	-
	Cv%	5.81	-	6.55	0.84	-	-	-	-	-	-

*a= shoot tip, b= nodal explant

The highest average number of leaves (17) was obtained for shoots from the shoot tip (table 3) in 'Plamval' (V2) rootstock, and the lowest number of leaves (2) in 'Sel. Corcodus' (V3). The highest average number of leaves (13) in nodal explants was in 'Plamval' rootstock in V2, while the smallest (3) was also in 'Plamval' rootstock, but in V4.

Table 3. Number of leaves / vegetative mass in shoot tip and nodal explants

Rootstock / variant	Descriptive statistics	Hormonal balance and type of explant									
		V1		V2		V3		V4		V5	
		a*	b	a	b	a	b	a	b	a	b
Prunus cerasifera Selection	Mean	8.40	3.60	-	-	2.80	-	3.80	5.80	-	-
	SD	1.14	0.55	-	-	0.84	-	0.84	0.45	-	-
	Cv%	13.57	15.21	-	-	29.88	-	22.02	7.71	-	-
Miroval (sin. MVL 2)	Mean	11.00	-	-	-	7.80	7.00	8.20	7.60	6.20	8.60
	SD	1.00	-	-	-	1.30	0.71	1.30	0.55	1.30	0.55
	Cv%	9.09	-	-	-	16.72	10.10	15.90	7.21	21.03	6.37
Plamval (sin. H19-5-85; Rival)	Mean	13.60	8.60	15.80	10.80	8.20	10.40	3.60	4.40	3.20	8.20
	SD	0.89	0.55	1.10	1.48	0.84	1.14	0.55	1.14	0.45	1.30
	Cv%	6.58	6.37	6.93	13.73	10.20	10.96	15.21	25.91	13.98	15.90
Fortival (sin. H1-2V; Corval)	Medie	8.00	-	13.60	9.40	-	-	-	-	-	
	SD	1.00	-	0.89	1.14	-	-	-	-	-	
	Cv%	12.50	-	6.58	12.13	-	-	-	-	-	

*a= shoot tip, b= nodal explant

In the explants from the shoot tip, the best results were given by the medium variants 1, 2 and 5, respectively, concentrations of 0.5 and 0.6 mg/l of BAP and 2 mg/l of NAA. In nodal explants the best results were obtained in variants 2, 4 and 5, which contain concentrations of 0.6 mg/l BAP, 6 mg/l IBA and 2 mg/l NAA. The results obtained are comparable to those in the literature. Abbasi et al. (2019) state that MS culture medium supplemented with 2.00 mg/l TDZ and ½ MS culture medium supplemented with 0.50 mg/l IBA are suggested for *in vitro* proliferation and, respectively, rooting in *P. scoparia*. Tsafouros and Roussos (2019) state that 6-benzyladenine was the most effective, for the number of shoots produced (3.5 shoots at 9.6 µM) and the number of nodes per explant (10 nodes at 9.6 µM), while meta-topoline, induced the highest number of nodes per cm. Zong et al. (2019) used the MS and WPM culture medium supplemented with various plant growth regulators; optimal regeneration with a frequency of 32.3% to 36.1% occurred with a WPM medium containing 8.88 µM 6-benzylamino-purine (BAP) and 0.98 to 3.94 µM indole-3-butyric (IBA). Kalinina and Brown (2007) experimented micropropagation for nine *Prunus* species; the formation of multiple shoots was obtained by using 1 mg/l 6-benzyladenine. The results show that micropropagation can be used to maintain the clonal properties for *Prunus* spp. Liu and Pijut (2008) obtained the best results on the medium with 9.0 pM TDZ and 1.07 pM NAA; the highest average number of shoots (8.2) was obtained on the medium containing 9.0 pM TDZ and 0.54 pM NAA

Conclusions

The best results were obtained with explants obtained from the shoots tips. The different concentrations of growth hormones do influence the development of explants (their diameter, height and number of leaves) in both the shoots obtained from the shoot tip and the nodal ones. The best results were obtained in the explants from the shoot tip, with concentrations of 0.5 and 0.6 mg/l BAP and 2 mg/l NAA. In nodal explants the best results were obtained on media containing concentrations of 0.6 mg/l BAP, 6 mg/l IBA and 2 mg/l NAA.

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