EFECTUL ACIDULUI JASMONIC ASUPRA MULTIPLICĂRII IN VITRO A PORTALTOILOR DE PĂR ȘI CIREȘ DE VIGOARE MICĂ
EFFECT OF JASMONIC ACID ON *IN VITRO* MULTIPLICATION OF LOW VIGOROUS PEAR AND CHERRY ROOTSTOCKS

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Abstract

The capacity of Jasmonic acid (JA) to improve *in vitro* multiplication phase in micropropagated shoots of low vigorous pear and cherry rootstocks – Pyrodwarf and Gisela 6 respectively, was studied. The experiment was performed during the multiplication phase and it included 23 media types containing Murashige and Skoog (1962) macro, micro salts and organic complex supplemented with JA at four concentrations (1, 2.3, 5 and 10 µM) alone and in combination with benzyl adenine – BA (4.4 µM), indole-3-butyric acid – IBA (1, 2.30, 2.46, 5 and 10 µM), α-napthyl acetic acid –NAA (1, 2.30, 2.68, 5, 10 µM), and indole-3-acetic acid – IAA (1, 2.30, 2.85, 5, 10 µM). The multiplication parameters, such as multiplication index, length of axial and lateral shoots, FW and DW of shoots as well as quality of multiplied shoots were monitored. The highest multiplication rate in both genotypes was obtained on medium with BA (4.4 µM) in combination with IBA (1 and 2.3 µM). The longest axial and lateral shoots were also obtained on media with BA in combination with JA or other three auxins used. The main characteristics of the JA effect (used alone) were big shoots, large green leaves, small quantity of firm, nodular callus as well as occurrence of rooting. The obtained results suggest that JA used alone should not be used to improve the multiplication process, but only in combination with BA and IBA or NAA for the high quality plantlets which is important for the rooting phase, to skip elongation phase.

Cuvinte cheie: portaltoi, acid Jasmonic, *in vitro*, multimplicare
Keywords: rootstocks, Jasmonic acid, *in vitro*, multiplication

Abbreviations: MS – Murashige and Skoog (1962) medium; JA – Jasmonic acid; IBA – indole-3-butyric acid; NAA – α-napthyl acetic acid; indole-3-acetic acid – IAA; GA_3 – gibberellic acid.

1. Introduction

Jasmonic acid (JA) and related compounds seem to be involved in various morphogenic events of plants, being potent biological regulators in plant kingdom (Koda, 1997).

It has been established that JA can influence a number of aspects of plant growth and development. According to Koda (1997) the fundamental and remarkable roles of the jasmonates are in regulation of plant morphogenesis which is controlled mainly by the frequency and direction of cell division and cell expansion.

JA can also induce plant senescence and leaf abscission and inhibits seed and pollen germination, inhibits root growth, potato tuberization and induces expansion of cells in potato tubers. In addition, JA regulates water supply in the plant and plays a key role in plant defense responses against pathogens and pests (Koda, 1997; Vasyukova and Ozeretskovskaya, 2009; Shimasaki and Wang, 2009).

Pyrodwarf pear rootstock displayed very low multiplication index under *in vitro* conditions (Ružić et al., 2004), which we attempted to improve together with another genotype, cherry rootstock Gisela 6 (Ružić et al., 2009).

Therefore, the aim of this study was to continue with this investigation introducing the plant growth regulator such as JA, which has not been widely tested in multiplication phase of micropropagation, in order to establish a better multiplication rate or quality of shoots of low vigorous pear and cherry rootstocks – Pyrodwarf and Gisela 6, respectively.

2. Material and methods

2.1. Plant material

Very popular low vigorous pear rootstock Pyrodwarf (clone BU 5-18 singled out from the cross of Old Home x Bonne Louise d'Avranches) in Geisenheim Research Institute (Germany) was used as model plant for the purpose of this study. The patented name of this rootstock is Rhenus 1 (Jacob, 2002).

The Gisela rootstocks series was developed in Germany in the 1960s at Justus Leibig University in Giessen. Gisela 6 (*P. cerasus* x *P. canescens*), tested as Gi 148/1, is a very promising cherry rootstock.

2.2. Media

Upon the multiplication, a sufficient number of Pyrodwarf and Gisela 6 shoots in the Tissue culture lab of Fruit Research Institute (Čačak, R. Serbia) were placed on 23 media types containing Murashige and Skoog (1962) (MS) macro and micro salts, organic complex and supplemented with Jasmonic acid (JA) [(±)-Jasmonic acid (±)-1α,2β-3-Oxo-2-(cis-2-pentenyl) cyclopentaneacetic acid, $C_{12}H_{18}O_3$, SIGMA J 2500 Plant Cell Tissue Culture Tested, Liquid] at 4 concentrations of 1, 2.3, 5 and 10 μM and in combination with benzyl adenine – BA (4.4 μM), indole-3-butyric acid – IBA (1, 2.30, 2.46, 5 and 10 μM), α-napthyl acetic acid – NAA (1, 2.30, 2.68, 5, 10 μM), and indole-3-acetic acid – IAA (1, 2.30, 2.85, 5, 10 μM) (Table 1).

All the media contained agar at a concentration of 7 g/L and sucrose at 20 g/L. Prior to autoclaving, pH of all media was adjusted to 5.75 with 0.1 N KOH. The media were sterilized in an autoclave for 20 min at 120^{o}C. JA was added through filter-sterilization by Millipore filter of 0.22 μm upon media autoclaving.

2.3. Cultural conditions

Cultures were grown under a 16 h photoperiod, light intensity 41 mol $m^{-2}s^{-1}$ on culture surface (cool white fluorescent tubes 40 W, 6,500^o K), at temperature 25 ± 1^{o}C. Each variant of treatment involved ten culture vessels x 5 uniform shoots x 2 replications. The data were analyzed by ANOVA and individual Duncan's Multiple Range Test at 5% level of significance.

3. Results and discussions

It is a well-known fact that jasmonates are potent biological regulators in plant kingdom, playing diverse roles. Exogenous jasmonates perform different effects, either inhibitory or promoting. According to Martin et al. (2009) JA is usually present in the picomolar range per gram of fresh leaf tissue, and can quickly increase under external stimuli. Some organs and tissues had more than 10 times the level found in leaves, which suggests that these high levels have different functions in the regulation of particular processes of development (Wasternack and Hause, 2002).

Ružić et al. (2012) with the same genotypes have found that small concentrations of JA improved the rooting process as they produce good root system, vigorous and high quality plantlets. However, it was not the case with shoot multiplication.

Although there was no multiplication on media containing JA alone, basic characteristics of shoots cultivated on these media were: very big plants/shoots, large, dark green leaves, small firm callus, appearance of rizogeneze with small firm radially arranged roots up to 3 mm in lenght (Fig. 1 a, b; Fig 2 a). Roots phenomenon was present at Pyrodwarf rootstock up to 27–38%, however at Gisela 6 rootstock only up to 5.5%. The effect of JA (MS ½) on rooting of these rootstocks depends on JA concentration applied (Ružić et al., 2012). Ružić et al. (2012) obtained the highest rooting rate in both genotypes on medium with the lowest JA concentration, i.e. 0.2 mg/L (93.3% in Pyrodwarf and 40% in Gisela 6), with the dark green leaves, thick red pigmented radially arranged roots. In the studies of Martin-Closas et al. (2000), JA-treated potato plantlets had also larger leaf surface, thicker stems and stronger root system with thicker roots, which can be attributed to the typical effect of JA on cell expansion.

The highest multiplication index of Pyrodwarf rootstock was obtained on the media with BA + IBA (1 μM) and BA + IAA (1 μM), but the highest length of axial shoots on the medium with BA + IBA (1 and 2.3 μM), and for lateral shoots on media with BA + NAA or IBA (both at concentration of 2.3 μM) (Table 2). Shoots cultivated on medium with BA + IBA + JA were also big, with dark green leaves and small firm callus (Fig. 1 c), and shoots cultivated on medium with BA + IBA were similar but with appearance of multiplication (small shoots) at the base of axial shoots in 27–42% cases (Fig. 1 d).

Similar features of a strong JA effect are recorded for Gisela 6 rootstock (Fig. 2 a, b). JA present alone in the medium had no effect on multiplication and the best multiplication index was obtained on medium with BA + IBA (2,3 μM), and the highest length of both axial and lateral shoots on medium with BA + NAA (2.3 μM) (Table 3). On the mentioned media it was observed multiplication (small shoots) at the base of axial shoots in 72% cases (Fig. 2 c).

IAA had no great effect neither alone, nor in combination with JA (Tables 2, 3). IAA only effected the multiplication index (BA + 1 μM IAA) (Fig. 2 d) and DW of axial shoots in combination with JA (2.3 μM), both for Pyrodwarf rootstock.

Generally, the FW and DW of shoots in both genotypes were highest on the media with JA in combination with other growth regulators. Although, Gisela 6 rootstock had the highest DW of axial shoots on medium with JA alone at concentration of 5 μM (Tables 4, 5). Zhang et al. (2006) also reported the significant increase in shoot fresh weight in two potato (*Solanum tuberosum* L.) cultivars.

4. Conclusions

Investigations of this kind add greatly to the general knowledge on how growth substances act, especially those that are not used by default in micropropagation.

At this level of investigation, it can be concluded that JA could be use in multiplication phase only in combination with BA, IBA or NAA.

The effect of JA could be used for improving the micropropagation process by producing higher quality plants which is important for the rooting phase, to skip elongation phase.

Acknowledgements

The present work has been supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (Projects No. TR-20013 and TR-31064).

References

1. Jacob H.B., 2002. New pear rootstocks from Geinsenheim, Germany. Acta Horticulturae 596: 337–344.
2. Koda Y., 1997. Possible involvement of jasmonates in various morphogenic events. Physiologia Plantarum 100: 639–646.
3. Martin M.T., Pedranzani H., García-Molinero P., Pando V., Sierra-de-Grado R., 2009. Inhibitory effect of jasmonic acid and ethylene on epicotyl growth and bud induction in the maritime pine, *Pinus pinaster* Soland. Ait. Biocell 33, 3: 1–11.
4. Martin-Closas L., Sol S., Pelacho A.M., 2000. Potential application of jasmonic acid for *Solanum tuberosum* micropropagation. Acta Horticulturae 520: 127–134.
5. Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473–479.
6. Ružić Dj., Lazić T., Kuzmanović M., 2004. Micropropagation *in vitro* of low vigorous pear rootstock Pyrodwarf (*Pyrus communis* L.). Proceedings of Research Papers 10, 3: 61–68.
7. Ružić Dj., Vujović T., Cerović R., Kuzmanović M., 2009. The influence of imidazole fungicides on multiplication *in vitro* of low vigorous pear and cherry rootstocks. Acta Horticulturae 839: 79–86.
8. Ružić Đ., Vujović T., Cerović R., 2012. Potential application of jasmonic acid in *in vitro* rooting of low vigorous pear and cherry rootstocks. Book of Abstracts of 2nd International Symposium on Horticulture in Europe - SHE2012, Angers (France), 61.
9. Shimasaki K., Wang T., 2009. Effect of chitosan and jasmonic acid derivates on shoot formation in cultures of *Cymbidium* protocorm – like bodies. Acta Horticulturae 812: 181–184.
10. Vasyukova N.I., Ozeretskovskaya O.L., 2009. Jasmonate–dependent defense signaling in plant tissues. Russian Journal of Plant Physiology 56, 5: 643–653.
11. Wasternack C., Hause B., 2002. Jasmonates and octadecanoids: signals in plant stress responses and development. Progress in Nucleic Acids Research and Molecular Biology 72: 165–221.
12. Zhang Z., Zhou W., Li H., Zhang G., Subrahmaniyan K., Yu J., 2006. Effect of jasmonic acid on *in vitro* explant growth and microtuberization in potato. Biologia Plantarum 50, 3: 453–456.

Tables

Table 1. Media used in the experiment

Media designation	Type of plant growth regulators (in µM)				
	BA	JA	NAA	IAA	IBA
JK1	-	1.00	-	-	-
JK2	-	2.30	-	-	-
JK3	-	5.00	-	-	-
JK4	-	10.00	-	-	-
J1	4.44	1.00	-	-	-
J2	4.44	2.30	-	-	-
J3	4.44	5.00	-	-	-
J4	4.44	10.00	-	-	-
J5	4.44	2.30	2.68	-	-
J6	4.44	2.30	-	2.85	-
J7	4.44	2.30	-	-	2.46
JN1	4.44	-	1.00	-	-
JN2	4.44	-	2.30	-	-
JN3	4.44	-	5.00	-	-
JN4	4.44	-	10.00	-	-
JA1	4.44	-	-	1.00	-
JA2	4.44	-	-	2.30	-
JA3	4.44	-	-	5.00	-
JA4	4.44	-	-	10.00	-
JB1	4.44	-	-	-	1.00
JB2	4.44	-	-	-	2.30
JB3	4.44	-	-	-	5.00
JB4	4.44	-	-	-	10.00

Table 2. Multiplication of Pyrodwarf rootstock *in vitro*

Media designation	Multiplication index	Average length of axial shoot (cm)	Average length of lateral shoots (cm)
JK1	1.00 e*	1.47 fgh	-
JK2	1.00 e	1.58 efg	-
JK3	1.00 e	1.58 efg	-
JK4	1.00 e	1.66 def	-
J1	1.06 e	1.93 bcd	0.60 d
J2	1.06 e	1.72 cdef	1.00 cd
J3	1.00 e	1.54 efg	-
J4	1.00 e	1.45 fgh	-
J5	1.00 e	1.96 bc	-
J6	1.00 e	1.89 bcd	-
J7	1.39 de	2.44 a	1.17 bc
JN1	2.61 a	2.08 b	1.53 ab
JN2	2.61 a	1.63 defg	1.69 a
JN3	1.00 e	1.56 efg	-
JN4	1.00 e	1.81 bcde	-
JA1	2.39 a	1.73 cdef	0.90 cd
JA2	1.89 bc	1.69 cdef	1.05 cd
JA3	1.50 cd	1.90 bcd	0.91 cd
JA4	1.33 de	1.91 bcd	0.67 d
JB1	2.61 a	2.46 a	1.34 abc
JB2	1.94 b	2.46 a	1.67 a
JB3	1.50 cd	1.35 gh	1.02 cd
JB4	1.67 bcd	1.24 h	0.95 cd

*Means followed by the same letter in columns are not significantly different at 5% level of significance using Duncan's Multiple Range Test

Table 3. Multiplication of Gisela 6 rootstock *in vitro*

Media designation	Multiplication index	Average length of axial shoot (cm)	Average length of lateral shoots (cm)
JK1	1.00 e*	1.30 bcd	-
JK2	1.00 e	1.35 bc	-
JK3	1.00 e	1.39 bc	-
JK4	1.00 e	1.39 bc	-
J1	2.44 b	1.25 bcdef	0.73 de
J2	1.72 cd	1.00 fgh	0.59 defg
J3	1.50 cde	0.91 hi	0.62 defg
J4	1.22 cde	0.71 i	0.50 g
J5	1.28 cde	0.78 hi	0.54 fg
J6	1.28 cde	0.72 i	0.72 def
J7	1.61 cde	1.51 ab	0.77 cd
JN1	2.78 b	1.27 bcde	0.93 bc
JN2	2.72 b	1.66 a	1.14 a
JN3	1.17 cde	1.05 defgh	0.67 defg
JN4	1.17 cde	0.85 hi	0.50 g
JA1	1.56 cde	0.91 hi	0.68 defg
JA2	1.56 cde	1.01 efgh	0.77 cde
JA3	1.44 cde	0.93 hi	0.58 efg
JA4	1.39 cde	0.73 i	0.66 defg
JB1	3.06 ab	1.32 bc	0.75 de
JB2	3.44 a	1.39 bc	1.02 ab
JB3	1.06 de	0.96 ghi	0.60 defg
JB4	1.78 c	1.20 cdefg	0.61 defg

*Means followed by the same letter in columns are not significantly different at 5% level of significance using Duncan's Multiple Range Test

Table 4. Fresh weight (FW) and dry weight (DW) of Pyrodwarf shoots (in mg)

Medium designation	FW (mg)			DW (mg)		
	Callus	Shoot		Callus	Shoot	
		Axial shoot	Lateral shoots		Axial shoot	Lateral shoots
JK1	6.2 f*	198.4 bcd	-	1.7 gh	59.0 bcde	-
JK2	8.8 f	198.8 bcd	-	2.5 gh	65.0 abc	-
JK3	4.3 f	174.7 bcde	-	1.3 gh	66.6 abc	-
JK4	0.0 f	143.8 ef	-	0.0 h	50.6 defg	-
J1	15.1 ef	218.1 b	40.4 b	3.5 g	62.6 bc	9.4 ab
J2	13.8 ef	191.7 bcde	34.4 bc	3.4 g	54.9 cdefg	8.2 bc
J3	5.4 f	153.2 def	-	1.3 gh	44.1 gh	-
J4	5.5 f	172.2 bcdef	-	1.6 gh	48.8 efg	-
J5	49.3 d	206.7 bc	-	11.9 d	57.3 bcdef	-
J6	9.3 f	262.7 b	-	2.7 gh	76.0 a	-
J7	27.7 e	307.3 a	32.0 bc	6.9 f	67.4 ab	8.1 bc
JN1	57.3 d	209.2 b	42.5 b	10.2 de	44.6 gh	6.7 cde
JN2	99.3 c	156.7 cdef	41.7 b	16.9 c	34.9 hi	6.6 cde
JN3	95.0 c	180.1 bcde	-	19.1 c	47.8 efg	-
JN4	98.0 c	178.3 bcde	-	17.7 c	47.1 fg	-
JA1	55.3 d	189.3 bcde	35.3 bc	11.2 de	46.5 fg	6.1 de
JA2	46.3 d	158.3 bcde	43.3 b	8.6 ef	44.3 gh	8.1 bc
JA3	84.0 c	273.3 a	42.0 b	18.3 c	64.9 abc	8.0 bcd
JA4	54.8 d	272.4 a	26.7 c	12.4 d	61.5 bcd	4.2 e
JB1	100.6 c	213.6 b	39.7 b	19.8 c	50.0 defg	6.6 cde
JB2	124.7 b	277.9 a	60.8 a	25.0 b	61.1 bcd	11.1 a
JB3	166.0 a	146.9 ef	36.7 bc	27.8 a	32.7 i	6.0 e
JB4	139.3 b	123.6 f	39.6 b	23.7 b	26.0 i	5.2 e

*Means followed by the same letter in columns are not significantly different at 5% level of significance using Duncan's Multiple Range Test

Table 5. Fresh weight (FW) and dry weight (DW) of Gisela 6 shoots (in mg)

Medium designation	FW (mg)			DW (mg)		
	Callus	Shoot		Callus	Shoot	
		Axial shoot	Lateral shoots		Axial shoot	Lateral shoots
JK1	16.3 h*	208.1 cdef	-	4.4 i	61.9 ab	-
JK2	18.1 h	166.0 f	-	4.6 i	52.6 abcdef	-
JK3	19.0 h	198.2 def	-	5.1 hi	63.3 a	-
JK4	22.3 h	196.2 ef	-	5.4 hi	55.7 abcd	-
J1	75.3 efgh	279.3 ab	45.3 abcd	13.3 fghi	52.5 abcdef	7.2 bcdef
J2	68.0 fgh	165.3 f	52.7 ab	14.3 fgh	40.8 fg	8.8 abc
J3	48.0 gh	214.8 cdef	38.5 abcd	9.6 ghi	44.4 defg	6.7 bcdef
J4	68.7 fgh	192.7 ef	37.7 abcd	14.4 fgh	45.9 cdefg	7.2 bcdef
J5	178.2 c	212.2 cdef	57.1 a	31.3 bc	51.1 abcdef	11.1 a
J6	134.7 cde	166.2 f	45.6 abcd	24.6 bcde	41.4 efg	6.7 bcdef
J7	162.2 cd	269.1 abc	27.6 d	28.1 bcd	58.5 abc	3.6 f
JN1	131.8 cdef	224.6 bcdef	36.7 abcd	20.8 def	48.6 bcdef	5.7 cdef
JN2	265.3 b	261.3 abcd	31.3 bcd	34.3 b	54.4 abcde	4.8 def
JN3	125.5 cdef	194.0 ef	53.1 ab	21.9 cdef	50.8 abcdef	8.0 abcd
JN4	163.5 cd	177.3 f	28.7 cd	25.9 bcde	43.2 defg	4.0 ef
JA1	145.0 cd	218.5 bcdef	39.2 abcd	28.4 bcd	56.5 abcd	8.7 abc
JA2	136.0 cde	214.7 cdef	52.5 ab	25.6 bcde	50.2 abcdef	9.6 ab
JA3	170.0 cd	212.4 cdef	36.5 abcd	28.3 bcd	46.7 cdef	6.5 bcdef
JA4	140.0 cde	183.8 f	47.0 abcd	24.5 bcde	41.0 efg	7.7 abcde
JB1	155.3 cd	253.3 abcde	44.0 abcd	27.0 bcde	51.6 abcdef	8.0 abcd
JB2	447.3 a	288.2 a	51.7 abc	56.1 a	53.7 abcdef	7.6 abcde
JB3	125.0 cdef	165.8 f	44.0 abcd	20.4 def	33.2 g	7.9 abcd
JB4	109.0 defg	272.2 abc	43.0 abcd	17.6 efg	51.1 abcdef	7.5 abcde

*Means followed by the same letter in columns are not significantly different at 5% level of significance using Duncan's Multiple Range Test

Figures

a)

b)

c)

d)

Fig. 1. Multiplication phase of Pyrodwarf rootstock on MS medium supplemented with: 1.0 μM JA (a); 2.3 μM JA (b); 4.44 μM BAP + 2.3 μM JA + 2.46 μM IBA (c); 4.44 μM BAP + 5.0 IBA (d)

a)

b)

c)

d)

Fig. 2. Multiplication phase of Gisela 6 rootstock on MS medium supplemented with: 5.0 µM JA (a); 4.44 µM BAP + 1.0 µM JA (b); 4.44 µM BAP + 2.3 NAA (c); Pyrodwarf rootstock multiplication on MS medium supplemented with 4.44 µM BAP + 1 IAA (d)