

Araştırma Makalesi/Research Article (Original Paper)

The Effect of Jasmonic Acid on the Micropropagation of Potato (*Solanum tuberosum* L.) under Long Days Conditions

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Abstract: Meristem derived *in vitro* plantlets of potato (*Solanum tuberosum* L.) cultivars Pasinler, Granola, and Caspar were micropropagated on agar-solidified MS medium containing different concentrations of jasmonic acid (JA) using single-node stem cuttings. The results of the study showed that inclusion of 1.0 μ M JA in the MS medium provided a substantial and visible increase in most of the plantlet characteristics studied and the effect of this concentration was more pronounced compared to the control and all other JA concentrations. The highest number of shoots (8.00), nodes (19.00), leaves (19.00), and roots (15.75) on cv. Caspar and a maximum shoot length (15.13 cm) was observed on cv. Granola from 1.0 μ M JA concentration. This concentration of JA also helped to induce longest roots (14.95 cm) on cv. Pasinler. The highest plantlet fresh weight from cv. Pasinler (649.38 mg), and cv. Caspar (630.18 mg), and maximum dry weight of plantlets from cv. Pasinler (98.58 mg), cv. Caspar (93.89 mg) and cv. Granola (79.03 mg) were noted on MS medium containing 1.0 μ M of JA. Since the application of JA accelerates the multiplication of young plantlets throughout the year without depending on the season, this allows fast commercial propagation of new potato cultivars. These results may also serve as basis for the mass production of these economically important potato cultivars through *in vitro* micropropagation techniques and microtuberization studies.

Key words: *In vitro*, Jasmonic acid, Micropropagation, Potato, *Solanum tuberosum*, Tissue culture

Uzun Gün Şartlarında Patates (*Solanum tuberosum* L.)'in Mikroçoğaltımında Jasmonik Asidin Etkisi

Özet: Çalışmada *in vitro* şartlarda meristemden geliştirilen Pasinler, Granola ve Caspar çeşitlerinin tek-boğum kesimleri agarla katılaştırılmış MS ortamında farklı jasmonik asit (JA) konsantrasyonlarında mikroçoğaltıma tabi tutulmuşlardır. Araştırma sonuçları 1.0 μ M JA konsantrasyonunun incelenen çoğu bitki özellikleri üzerine önemli oranda bir artışa sebep olduğunu ve bu konsantrasyonun etkisinin kontrol ve diğer bütün JA konsantrasyonlarından daha belirgin olduğunu göstermiştir. En yüksek sürgün (8.00), boğum (19.00), yaprak (19.00) ve kök (15.75) sayıları Caspar çeşidinden ve en uzun sürgün uzunluğu (15.13 cm) ise Granola çeşidinden 1.0 μ M JA konsantrasyonundan elde edilmiştir. Bu JA konsantrasyonu Pasinler çeşidinde en uzun kökler (14.95 cm) meydana getirmiştir. En yüksek bitki yaş ağırlığı Pasinler (649.38 mg) ve Caspar (630.18 mg) çeşitlerinden, en fazla bitki kuru ağırlığı ise Pasinler (98.58 mg), Caspar (93.89 mg) ve Granola (79.03 mg) çeşitlerinden ve 1.0 μ M JA konsantrasyonundan elde edilmiştir. JA uygulaması mevsime bağlı kalmaksızın bütün yıl boyunca genç bitkiciklerin çoğaltımını teşvik ettiğinden, yeni ticari patates çeşitlerinin ticari olarak hızlı bir şekilde çoğaltımına imkan sağlar. Bu sonuçlar, ayrıca, ekonomik öneme sahip patates çeşitlerinin *in vitro* şartlarda mikroçoğaltımı ve mikro yumruların kütleli üretim çalışmalarına da temel teşkil edebilir.

Keywords: *in vitro*, Jasmonik asit, Mikroçoğaltım, Patates, *Solanum tuberosum*, Doku kültürü

Introduction

Potato (*Solanum tuberosum* L.) is traditionally propagated using tubers. Vegetative multiplication often contaminates tubers with different diseases, resulting in poor seed quality and low yield. Plant regeneration using a tissue culture system is generally desired to aid potato seed multiplication.

Biotechnological approaches make it possible to reduce both contamination through field exposures and enhance rate of multiplication many folds. Micropropagation of potato by *in vitro* culture has been commonly used for production of disease free plantlets, germplasm exchange, and seed tuber production (Hussain et al. 2005; Pruski, 2007). Recently, Ahmad et al. (2012) reported that the micropropagation of potato depends on the genotype, nutrients in the culture medium, and plant growth regulators (PGRs). Adding exogenous PGRs significantly reduce multiplication time and enhance number of plantlets for the *in vitro* micropropagation of potato (Saker et al. 2012).

Jasmonic acid (JA) is a class of endogenous PGRs that are widely distributed within the plant kingdom (Ulloa et al. 2002). Initially, JA was associated with senescence promotion (Koda et al. 1991; Shan et al. 2011) and microtuber formation (Koda and Kikuta, 2001), however, recent studies have shown that JA has unique and potentially useful properties that affect plant growth and development when applied exogenously (Pelacho et al. 1997; van den Berg and Ewing, 1991; Rohwer and Erwin, 2008). JA is known *in vitro* microtuber promoting agent (van den Berg, 1991) that also affects vegetative growth and root development (Martin-Closas et al. 2000). There are only a few reports on the *in vitro* effects of JA on the micropropagation of potato. Ravnikar and Gogala (1990) showed that meristem growth induction medium supplemented with 0.5 – 10 μM JA increased the number of potato meristems, which developed buds without symptoms of senescence. Addition of JA to the growth medium affected the vegetative development of plantlets (Ravnikar et al. 1990) and stimulated root formation of the *in vitro* cultured potato explants (Ravnikar et al. 1992). Zhang et al. (2006) showed that the application of 0.2–2.0 $\text{mg}\cdot\text{dm}^{-3}$ JA resulted in a significant increase in shoot fresh mass, as well as the number and the root length of plantlets. Pruski et al. (2002) and Zhang et al. (2006) also pointed out inhibitory effect of high concentrations of JA on explant growth. The present study was conducted to determine the effect of various concentrations of JA on micropropagation of Pasinler, Granola, and Caspar potato cultivars by using meristem derived nodal stem explants by promoting growth activity due to JA.

Materials and Methods

Preparation and Concentrations of Plant Growth Regulators

Murashige and Skoog (MS) medium (1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar was used in the study. The pH was adjusted to 5.7 ± 0.1 with 1 N HCl or 1 N NaOH after adding all medium components except the agar. The concentrations of PGRs used in the study were prepared as follows: no PGRs (control); 0.5 μM JA; 1.0 μM JA; and 2.0 μM JA. All constituents were sterilized by autoclaving at 120°C for 15 min.

Plant materials and micropropagation of explants

This study was carried out at the Tissue Culture Laboratory of Eastern Anatolia Agricultural Research Institute, Erzurum, Turkey. Three potato cultivars, namely Pasinler (locally improved and registered mid-early maturing cultivar), Granola (mid-late maturing), and Caspar (late maturing) were used in study. Single stem node cuttings were aseptically cultured on $1.0 \times \text{MS}$ medium using 40 explants per treatment and 10 explants per replicate that were replicated 4 times. Cultures were incubated at 2,000 lux light intensity with 16 h day light (at of $24 \pm 2^\circ\text{C}$) photoperiod for 6 weeks to allow regeneration.

Statistical Analysis

Average shoot length (cm), number of shoots per explant, number of nodes per shoot, number of leaves per shoot, number of roots per shoot, root length (cm), fresh weight (FW) and dry weight (DW) of plantlets (mg) were recorded. A completely randomized design (CRD) was used to evaluate three cultivars, four PGRs, and four replicates. Data were subjected to analysis of variance and the means were separated by Duncan's multiple range test using SPSS software.

Results and Discussion

JA and its related compounds are an interesting class of plant hormones. Exogenous application of JA stimulates leaf senescence and controls the expression of a series of senescence-related plant genes (van den Berg and Ewing, 1991). JAs can also alter physiological processes in plants, stimulate ageing, and

induce faster plant propagation (Rohwer and Erwin, 2008). Martin-Closas et al. (2000) showed that since JA might affect vegetative and root development in potato, *in vitro* effects of JA on the micropropagation of potato and its potential use in enhancing potato micropropagation are important. Regenerated plantlets from JA supplemented medium were morphologically uniform in terms of shoot and root type, leaf shape, and growth pattern. The results showed that the addition of JA to MS medium resulted in a visible increase in most of the studied plantlet characters compared to the control. The effect of PGR combinations on all the plantlet characteristics studied was significantly different ($p < 0.01$). The effect of cultivars on all of the plantlet characteristics except regeneration percentage also differed significantly ($p < 0.01$ and $p < 0.05$). The interaction between hormonal concentration (treatments) and cultivar potentiality in terms of the length of the shoots and roots; the number of shoots, nodes, leaves, and roots; and FW of plantlets also showed significant variations ($p < 0.01$); however, the interaction was insignificant in terms of regeneration percentage, shoot and root induction days, and DW of plantlets ($p > 0.05$). The results of each treatment and cultivar interactions are presented under different sub-headings.

Regeneration Percentage

The regeneration percentage of explants was considerably influenced by the different concentrations of JA ($p < 0.01$). However, the differences among potato cultivars and JA \times cultivars interaction was insignificant ($p > 0.01$) (Table 1). Application of JA increased regeneration potential of all potato cultivars grown *in vitro*. The three cultivars showed 100% regeneration on agar solidified MS medium containing 0.5, 1.0 and 2.0 μM JA. Although previous reports have emphasized positive effect of JA on potato micropropagation (Martin-Closas and Pelacho, 1997; Martin-Closas et al. 2000; Ravnikar et al. 1992), no report has described the effect of JA on the regeneration percentage of potato plantlets grown *in vitro*.

Days to Shoot Induction

Number of days required for shoot appearance was significantly influenced by different concentrations of JA and potato cultivars *in vitro* ($p < 0.01$). However, interaction between JA \times cultivars was insignificant ($p > 0.01$) (Table 1). The minimum days to shoot induction was observed on cv. Pasinler (7.25 days), followed by cv. Granola (9.00 days) and cv. Caspar (11.25 days), on MS medium containing 2.0 μM JA and cv. Caspar (11.00 days) on MS medium containing 1.0 μM JA (Table 2). However, the control treatment lacking JA showed a delay in shoot initiation. Table 2 showed that the use of JA supplemented MS medium for the *in vitro* culture of potato explants induced the appearance of shoots 11.75 days earlier compared to the controls. Similar results were observed by Zhang and Cheng (1996) who noted that *in vitro*-grown nodal cultures of potato cultivars showed different sensitivities to JA in terms of shoot induction.

Table 2. Effects of 1.0 \times MS medium containing various JA concentrations on days to shoot and root induction in potato cultivars Pasinler, Granola, and Caspar.

JA (μM)	Days to shoot induction			Days to root induction		
	Pasinler	Granola	Caspar	Pasinler	Granola	Caspar
0	16.50 \pm 0.65 ^C	19.00 \pm 0.58 ^C	17.50 \pm 2.02 ^C	28.00 \pm 0.82 ^C	29.50 \pm 0.96 ^C	31.75 \pm 1.18 ^D
0.5	10.75 \pm 0.48 ^B	11.50 \pm 0.50 ^B	13.50 \pm 0.50 ^B	18.25 \pm 0.63 ^B	21.75 \pm 0.63 ^B	25.25 \pm 1.49 ^C
1.0	7.75 \pm 0.48 ^A	9.50 \pm 0.65 ^A	11.00 \pm 0.41 ^A	14.00 \pm 0.71 ^A	15.75 \pm 0.75 ^A	19.75 \pm 0.63 ^A
2.0	7.25 \pm 0.25 ^A	9.00 \pm 0.41 ^A	11.25 \pm 0.48 ^A	14.25 \pm 0.75 ^A	20.00 \pm 0.71 ^B	21.50 \pm 0.9 ^B

Note: Means of different values shown by different letters in the same column are statistically different using Duncan's Multiple Range Test at 0.01 level of significance. Standard error (\pm SE) was calculated from 4 replications (n=4).

Table 1. Analysis of variance for the effects of cultivars and various JA concentrations on studied plantlet characteristics

Source of Variation	DF	Mean squares										
		Regeneration percentage (%)	Days to shoot induction	Days to root induction	Shoot length (cm)	Number of shoots	Number of nodes	Number of leaves	Number of roots	Root length (cm)	FW of plantlets (mg)	DW of plantlets (mg)
Replications	3	47.74 ^{ns}	0.97 ^{ns}	1.19 ^{ns}	2.82 ^{**}	0.06 ^{ns}	0.91 ^{ns}	1.19 ^{ns}	1.22 ^{ns}	0.15 ^{ns}	794.02 ^{ns}	0.53 ^{ns}
Cultivars (C)	2	39.66 ^{ns}	30.77 ^{**}	141.15 ^{**}	11.69 ^{**}	1.90 [*]	15.15 ^{**}	10.02 ^{**}	10.02 ^{**}	14.16 ^{**}	15247.58 ^{**}	2.25 [*]
Hormones (H)	3	8589.41 ^{**}	187.25 ^{**}	406.19 ^{**}	256.10 ^{**}	45.72 ^{**}	311.80 ^{**}	411.85 ^{**}	311.67 ^{**}	166.16 ^{**}	377014.04 ^{**}	93.46 ^{**}
C x H	6	21.70 ^{ns}	3.44 ^{ns}	5.15 ^{ns}	1.84 ^{**}	1.87 ^{**}	14.42 ^{**}	5.27 ^{**}	3.85 ^{**}	6.71 ^{**}	5826.55 ^{**}	1.19 ^{ns}
Error	33	38.27	2.40	3.26	0.451	0.465	1.08	1.38	0.72	0.37	337.63	0.56
Corrected total	47	582.61	15.45	34.96	17.58	3.57	23.20	28.44	21.40	12.33	25745.12	6.64

Significant at 0.01 level (**); significant at 0.05 level (*); not significant (ns).

Days to Root Induction

The number of days required for root appearance and differences among potato cultivars was significantly influenced by the different concentrations of JA ($p < 0.01$). However, the interaction between JA \times cultivars was insignificant ($p > 0.01$) (Table 1). The minimum days to root induction was observed in cv. Pasinler on MS medium containing 1.0 μM JA (14.00 days), followed by cv. Pasinler (14.25 days) on 2.0 μM JA and cv. Granola (15.75 days) on 1.0 μM JA (Table 2). Root induction was retarded and delayed in the absence of JA in late-maturing cv. Caspar. Table 2 shows that the use of JA-supplemented MS medium for the *in vitro* culture of potato explants induced the development of roots 17.75 days earlier compared to the controls. No previous report suggests any information pertaining to effects of JA on root induction; however, Kovac and Ravnikar (1994) noted that 1 μM JA enhanced stem elongation, leaf expansion, and development of root systems in potatoes under *in vitro* conditions.

Shoot Length

The application of JA resulted in a significant increase in explant development and shoot elongation. Significant variations were observed among JA concentrations, potato cultivars, and the interaction of JA \times cultivars on shoot length ($p < 0.01$) (Table 1). After 6 weeks of cultivation, the longest shoots were noted on cv. Granola (15.13 cm), followed by cv. Pasinler (14.93 cm) and cv. Caspar (13.50 cm), using 1.0 μM JA in 1.0 \times MS medium (Table 3). Addition of JA resulted in a 5.67-fold increase in shoot length compared to the control. The results of the present experiment are in agreement with those of Ravnikar et al. (1992), Martin-Closas and Pelacho (1997), Martin-Closas et al. (2000), and Pruski et al. (2002), who suggested that JA could play an important role by inducing a general increase in the vegetative development of the *in vitro*-cultured potato explants. The results of this study show similarities with those of Ravnikar et al. (1990), Dermastia et al. (1994), and Dermastia et al. (1996). Ravnikar et al. (1990) reported that when JA concentrations were increased from 0.1 μM to 1 μM , they considerably improved shoot length. JA-supplemented MS medium produced taller and thicker shoots, and taller plantlets using 1.0 \times MS medium containing 1 μM JA (Dermastia et al. 1994; Dermastia et al. 1996).

Table 3. Effects of 1.0 \times MS medium containing various JA concentrations on shoot length and the number of shoots in potato cultivars Pasinler, Granola, and Caspar.

JA (μM)	Shoot length (cm)			Number of shoots		
	Pasinler	Granola	Caspar	Pasinler	Granola	Caspar
0	2.95 \pm 0.22 ^D	4.23 \pm 0.62 ^D	2.63 \pm 0.26 ^C	2.25 \pm 0.25 ^C	2.25 \pm 0.25 ^C	1.75 \pm 0.25 ^C
0.5	9.18 \pm 0.57 ^C	8.33 \pm 0.48 ^C	7.83 \pm 0.51 ^B	3.25 \pm 0.48 ^B	3.00 \pm 0.4b ^B	3.50 \pm 0.29 ^B
1.0	14.93 \pm 0.39 ^A	15.13 \pm 0.3 ^A	13.5 \pm 0.36 ^A	5.50 \pm 0.28 ^A	6.50 \pm 0.29 ^A	8.00 \pm 0.41 ^A
2.0	10.98 \pm 0.37 ^B	9.98 \pm 0.37 ^B	7.98 \pm 0.14 ^B	3.75 \pm 0.25 ^B	3.25 \pm 0.25 ^B	4.00 \pm 0.41 ^B

Note: Means of different values shown by different letters in the same column are statistically different using Duncan's Multiple Range Test at 0.01 level of significance. Standard error (\pm SE) was calculated from 4 replications (n=4).

Number of Shoots

The effect of different concentrations of JA treatments and the interaction of JA \times cultivars on the number of shoots showed significant variations ($p < 0.01$). Differences among potato cultivars showed significant variations at the ($p < 0.05$) (Table 1). A maximum number of 8.00, 6.50, and 5.50 shoots for cvs. Caspar, Granola, and Pasinler, respectively, were obtained using 1.0 \times MS medium containing 1.0 μM JA. Addition of JA also resulted in 4.57-fold increase in the number of shoots compared to the control (Table 3). Present results are in agreement with those of Ravnikar et al. (1992) and Pruski et al. (2002), who showed that application of 0.1–1.0 μM JA at lower concentrations resulted in extensive lateral branching on *in vitro*-grown potato plantlets. Zhang and Cheng (1996) also showed that number of axillary shoots increased significantly using media supplemented with low concentrations of JA (0.1–5 μM).

Table 4. Effects of $1.0 \times$ MS medium containing various JA concentrations on the number of nodes and leaves in potato cultivars Pasinler, Granola, and Caspar.

JA (μ M)	Number of nodes			Number of leaves		
	Pasinler	Granola	Caspar	Pasinler	Granola	Caspar
0	3.25 \pm 0.25 ^D	4.25 \pm 0.63 ^C	2.25 \pm 0.25 ^C	3.75 \pm 0.48 ^D	3.50 \pm 0.57 ^D	2.50 \pm 0.29 ^D
0.5	6.00 \pm 0.41 ^C	6.50 \pm 0.65 ^B	6.75 \pm 0.75 ^B	11.5 \pm 0.64 ^B	11.0 \pm 0.41 ^B	13.5 \pm 0.65 ^B
1.0	11.8 \pm 0.63 ^A	14.75 \pm 0.48 ^A	19.0 \pm 0.58 ^A	16.3 \pm 0.75 ^A	16.25 \pm 0.48 ^A	19.0 \pm 1.22 ^A
2.0	6.50 \pm 0.64 ^B	6.50 \pm 0.29 ^B	7.25 \pm 0.25 ^B	7.00 \pm 0.40 ^C	9.0 \pm 0.41 ^C	9.5 \pm 0.29 ^C

Note: Means of different values shown by different letters in the same column are statistically different using Duncan's Multiple Range Test at 0.01 level of significance. Standard error (\pm SE) was calculated from 4 replications (n=4).

Number of Nodes

The number of nodes per plantlet showed significant variations ($p < 0.01$) after treatment with $1.0 \times$ MS medium containing various JA concentrations (Table 1). The highest number of nodes was observed in cv. Caspar (19.00), followed by cv. Granola (14.75) and cv. Pasinler (11.75), using MS medium supplemented with 1.0 μ M JA. Addition of JA also resulted in a 8.44-fold increase in the number of nodes compared to the control (Table 4). These results are consistent with JA stimulated shoot elongation and the increased number of nodes reported in potato stem nodes when JA was present at concentrations of 0.01 to 1 μ M (Ravnikar et al. 1992). Zhang and Cheng (1996) reported that the number of nodes increased significantly using media with JA compared to medium without JA; in agreement with Pruski et al. (2002), who obtained maximum number of nodes using potato plantlets grown on medium with or without 0.5 and 1.0 μ M JA.

Number of Leaves

The application of various JA concentrations resulted in significant changes in the number of leaves on potato plantlets ($p < 0.01$) (Table 1). The highest number of leaves was observed on cv. Caspar (19.00), followed by cv. Pasinler and cv. Granola (both 16.25) using $1.0 \times$ MS medium supplemented with 1.0 μ M JA (Table 4). Addition of JA also resulted in a 7.60-fold increase in the number of leaves compared to the control. Present results are both in agreement with those of Dermastia *et al.* (1994) but have edge over their results by demonstrating that plantlets grown on the medium supplemented with JA promoted number of leaves.

Number of Roots

All JA concentrations resulted in significant ($p < 0.01$) differences in number of roots (Table 1). The maximum number of roots, 15.75 and 15.25, were observed in cv. Caspar using $1.0 \times$ MS medium supplemented with 2.0 and 1.0 μ M JA, respectively. Addition of JA also resulted in a 4.69-fold increase in the number of roots compared to the control (Table 5). Potato roots readily in regenerating medium or MS medium, and nodal explants of potato do not require exogenous hormone for rooting (Kumlay, 2014; Kumlay and Ercisli, 2015; Vinterhalter et al. 1997). The present results show similarities to those of Ravnikar et al. (1990), Martin-Closas et al. (2000), and Zhang et al. (2006). Ravnikar et al. (1990) reported that the number of roots increased with increasing JA concentration; however, the roots were retarded with increased diameter. Martin-Closas et al. (2000) suggested that JA could stimulate root formation on *in vitro*-cultured potato regenerated shoots. Pruski et al. (2002) also showed that JA supplementation retarded roots length with extensively branched root systems. Although Zhang et al. (2006) recorded a significant increase in the number of roots, Zhang and Cheng (1996) pointed out that number of lateral roots increased significantly without any effect on the number of adventitious roots under the influence of JA in the culture medium.

Table 5. Effects of $1.0 \times$ MS medium containing various JA concentrations on the number of roots and root length in potato cultivars Pasinler, Granola, and Caspar.

JA (μ M)	Number of roots			Root length (cm)		
	Pasinler	Granola	Caspar	Pasinler	Granola	Caspar
0	3.25 \pm 0.25 ^C	4.50 \pm 0.58 ^D	3.25 \pm 0.25 ^C	2.45 \pm 0.14 ^D	3.85 \pm 0.19 ^C	3.63 \pm 0.1 ^D
0.5	8.50 \pm 0.65 ^B	9.50 \pm 0.29 ^C	11.5 \pm 0.65 ^B	7.00 \pm 0.20 ^C	6.00 \pm 0.38 ^B	5.00 \pm 0.2 ^C
1.0	14.3 \pm 0.48 ^A	13.0 \pm 0.41 ^B	15.25 \pm 0.25 ^A	14.95 \pm 0.22 ^A	11.3 \pm 0.39 ^A	10.3 \pm 0.5 ^A
2.0	13.5 \pm 0.65 ^A	14.8 \pm 0.48 ^A	15.75 \pm 0.25 ^A	8.10 \pm 0.27 ^B	6.6 \pm 0.38 ^B	6.1 \pm 0.3 ^B

Note: Means of different values shown by different letters in the same column are statistically different using Duncan's Multiple Range Test at 0.01 level of significance. Standard error (\pm SE) was calculated from 4 replications (n=4).

Root Length

The addition of JA significantly stimulated root development in all cultivars, resulting in approximately 3–4 times longer roots using 0.5 – 2.0 μ M JA compared to the control medium. The effect of JA, differences among potato cultivars, and the interaction between JA and cultivars on root length showed significant variations ($p < 0.01$) (Table 1). The longest roots were observed on cv. Pasinler (14.95 cm), followed by cv. Granola (11.28 cm) and cv. Caspar (10.35 cm), using $1.0 \times$ MS medium supplemented 1.0 μ M JA. Addition of JA also resulted in a 6.10-fold increase in the root length compared to the control (Table 5). The present results are similar or have edge over the previous finding by Ravnkar et al. (1992), Dermastia et al. (1994), and Zhang et al. (2006). It has been shown that MS medium supplemented with JA resulted in a highly developed and differentiated root system with several lateral branches (Ravnkar et al. 1992; Dermastia et al. 1994). Pruski et al. (2002) and Zhang et al. (2006) also witness a significant increase in root length after JA supplementation.

Fresh Weight of Plantlets

Significant variations in the FW of plantlets were observed ($p < 0.01$) (Table 1). The maximum FW was observed on cv. Pasinler (649.38 mg), followed by cv. Caspar (630.18 mg) and cv. Granola (534.73 mg), using MS medium supplemented with 1.0 μ M JA (Table 6). The present results have edge over the finding of Martin-Closas and Pelacho (1997) and Pruski et al. (2002), who concluded that JA supplementation resulted in a general increase in total FW and significantly enhanced plantlet biomass compared to the controls. Zhang et al. (2006) also reported that the application of JA significantly increased the shoot fresh mass of potato plantlets; however, high concentrations of JA inhibited the growth of potato explants.

Table 6. Effects of $1.0 \times$ MS medium containing various JA concentrations on fresh weight and dry matter content of plantlets in potato cultivars Pasinler, Granola, and Caspar.

JA (μ M)	Fresh weight of plantlets (mg)			Dry weight of plantlets (mg)		
	Pasinler	Granola	Caspar	Pasinler	Granola	Caspar
0	193.5 \pm 7.7 ^C	169.6 \pm 6.7 ^C	151.48 \pm 6.48 ^C	20.56 \pm 0.5 ^D	18.71 \pm 0.74 ^D	14.99 \pm 0.36 ^D
0.5	397.3 \pm 6.3 ^B	376.2 \pm 6.1 ^B	314.83 \pm 4.13 ^B	49.97 \pm 1.1 ^C	43.18 \pm 0.87 ^C	33.31 \pm 0.45 ^C
1.0	649.4 \pm 13.4 ^A	534.7 \pm 11.6 ^A	630.18 \pm 15.6 ^A	98.58 \pm 0.6 ^A	79.03 \pm 0.75 ^A	93.89 \pm 0.92 ^A
2.0	418.1 \pm 13.7 ^B	390.6 \pm 6.6 ^B	326.38 \pm 11.8 ^B	69.41 \pm 1.0 ^B	62.89 \pm 1.25 ^B	54.18 \pm 0.45 ^B

Note: Means of different values shown by different letters in the same column are statistically different using Duncan's Multiple Range Test at 0.01 level of significance. Standard error (\pm SE) was calculated from 4 replications (n=4).

Dry Weight of Plantlets

The effect of JA supplementation on the DW of plantlets was significantly different ($p < 0.01$) (Table 1). Differences among potato cultivars also showed significant variations at the 5% probability level. The maximum dry weight of plantlets measuring 98.58 mg and 93.89 mg was recorded for cvs. Pasinler and Caspar respectively, followed by cv. Granola (79.03 mg), using $1.0 \times$ MS medium supplemented with 1.0 μ M JA (Table 6). The present results are in agreement with those of Martin-Closas et al. (2000) who reported that JA increased the average dry weight of micropropagated single-node explants (60%) and

plantlet root systems (300%) of *in vitro*-grown potato plantlets. Martin-Closas and Pelacho (1997) also showed that JA caused a general increase in the DM content of plantlets.

Previous reports revealed that the use of MS medium supplemented with 1 μM of JA resulted in expanded root systems, extended leaf areas, taller plantlets with well-developed root systems, expanded leaves, and thickened stems (Dermastia et al. 1994; Dermastia et al. 1996). Similar results were also reported by Pruski et al. (2002), who reported that the longest shoots were observed in plantlets grown on media either without JA or with low (0.5 and 1.0 μM) JA concentrations. A correlation between tuber initiation and appearance of some morphological changes, such as a decrease in shoot development, rooting & branching, and cessation of longitudinal growth has been previously established (Pelacho et al. 1997). Previous reports suggests that high concentrations of JA (1.0 $\text{mg}\cdot\text{L}^{-1}$) resulting in an increase in the dry matter (%) of plantlets, whereas shorter plantlets with less branched shoots & roots of plantlets showed decreased fresh weight using a higher concentration (Zhang et al. 2006). Takahashi et al. (1994) and Cenzano et al. (2003) suggested that the observed microtuber formation in the presence of JA could be due to increased cell expansion, a reduction in the length of leaf primordia, enlargement of meristems, and early vascular tissue differentiation. However, Vilhar et al. (1997) suggested that JA supplementation altered morphology of roots through cell division and inhibited root elongation.

Conclusions

The growth of potato plantlets cultured for 6 weeks on the propagation 1.0 \times MS medium stimulated development of the longest stems and roots and the highest number of shoots, roots, leaves, and nodes when 0.5, 1.0, and 2.0 μM of JA were used. However, control treatment lacking JA showed an inhibitory effect on all of the studied plantlet characteristics; furthermore, it hardly allowed shoot and root development. These results suggest that *in vitro*-grown potato explants can easily be micropropagated on JA-supplemented MS medium. The present study also showed that appropriate concentrations of JA is essential for direct and efficient regeneration of explants without callus formation, abnormal axillary shoot growth, and that moderate JA concentrations (0.5 and 1.0 μM) have improved effects on potato explants cultured *in vitro*. Since supplementation of MS medium with 1.0 μM JA showed significant increase in all characteristics of plantlet growth, this optimal concentration then could be used to efficiently micropropagate healthy plantlet stocks for *in vitro* seed tuber production for commercial purposes. The effect of PGRs on plantlet characteristics varied according to genotype. With the exception of explant regeneration percentage, all plant characteristics differed among three potato cultivars. Since potato cultivars showed different sensitivities and various maturity groups responded differently to various JA concentrations, it may be concluded that optimal concentration of JA may vary for each potato cultivar. It seems as if 1.0 μM JA concentration in culture medium is a threshold for development of shoots, leaves, and roots for explant growth in this study. On the basis of these findings, it can be concluded that since 2.0 μM JA concentration caused significant delay in explant development that resulted in deterioration in morphological characteristics of and fresh weight of morphological characteristics of developing plantlets with expectation of promotion of microtuber induction at higher concentrations. These results may serve as a foundation for the mass production of cultivars of interest using *in vitro* micropropagation techniques. Further research on the effect of 2.0 μM and higher concentrations of JA on the microtuberization of micropropagated potato plantlets is warranted.

Acknowledgement

The author is thankful to the Ministry of Food, Agriculture and Livestock, Eastern Anatolia Agricultural Research Institute for providing financial support to carry out this research.

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