

## Different Plant Growth Regulators on Improvement of Potato (*Solanum tuberosum* L.) Micropropagation

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**ABSTRACT:** The study compared the effects of MS medium containing 0.1 mg L<sup>-1</sup> gibberellic acid (GA<sub>3</sub>) and  $\alpha$ -naphthaleneacetic acid (NAA) in combination with 2.0 mg L<sup>-1</sup> of kinetin (KIN), 2.0 mg L<sup>-1</sup> of benzyl aminopurine (BAP), 1.0 mg L<sup>-1</sup> of zeatine riboside (ZR), and 0.5 mg L<sup>-1</sup> of jasmonic acid (JA) for the micropropagation development of three potato (*Solanum tuberosum* L.) cultivars namely Caspar, Granola and Pasinler-92 using binodal stem explants. The results of this research clearly indicated that inclusion of JA among the other plant growth regulators significantly increased shoot regeneration and other characteristics of all potato cultivars used in the study. The minimum days to shoot proliferation on three cultivars ranged 4.0–5.5 d (best result on cv. Pasinler-92) on MS medium containing 0.5 mg L<sup>-1</sup> JA. The minimum time to root initiation (11.0 d) was observed on cv. Pasinler-92 on the same medium. The maximum number of axillary shoots (15.25) and nodes (19.0), maximum shoot length (17.25 cm), leaves (19.0) and roots (25.25) were noted on cv. Caspar. The longest roots (18.45 cm) on cv. Pasinler-92, and the maximum fresh weight (404.87 mg) and dry weight (61.85 mg) of plantlets from cv. Granola were also recorded on MS medium fortified with 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA.

**Keywords:** Potato, *Solanum tuberosum* L., micropropagation, *in vitro*, plant growth regulators

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Bu çalışma 9-12 Mayıs 2018 tarihleri arasında Van Yüzüncüyıl Üniversitesi tarafından yapılan "I. International Agricultural Science Congress'de" özet bildiri olarak sunulmuştur.

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the significant industrial crops all over the world used for multipurposes. Researchers are always interested in improvement of tissue culture techniques of potato to increase high-quality pathogen-free plantlets production with consistent genetic and physiological traits that are highly efficient in terms of photosynthesis (Pruski, 2007; Mohapatra and Batra, 2017; de Morais et al., 2018). *In vitro* techniques could also be employed to stimulate the strength and improvement of potato breeding programs and to overcome negative effects related to conventional breeding methods (Tazeb, 2017). Previous reports suggest that the regeneration of potato is highly explant, PGR combinations and cultivar dependent (Hussain et al., 2005; Kumlay, 2014; Nuwagira et al., 2015; Naqvi et al., 2019). Under appropriate growth conditions, potato shoots, roots and stem explants with nodes have ability to regenerate, even without any plant growth regulators (PGRs) (Kumlay et al., 2014a; Quiroz et al., 2017; Kolachevskaya et al., 2019). However, regeneration of plantlets without PGRs is time extensive inducing low number of shoots, leaves and roots per explants (Kumlay, 2014). Therefore, adding exogenous PGRs is an effective way to accelerate plantlet regeneration and to reduce growth cycle during potato *in vitro* micropropagation (Kumlay et al., 2014b; Mendel et al., 2020). Most of the researchers prefer use of cytokinins with auxins to enhance root and shoot production (Ghaffoor et al., 2003; Kumlay and Eryiğit, 2011; Wani et al., 2018). Even though gibberellic acid (GA<sub>3</sub>), cytokinins, auxins and jasmonic acid (JA) are thought to play a significant effect in arrangement of root and shoot development in potato *in vitro* (Kumlay and Eryiğit, 2011; Kumlay, 2016), there is no study reporting effects of these in combinations on improvement of potato micropropagation.

Previous studies suggest that higher concentrations of benzyl aminopurine (BAP) together with lower concentrations of  $\alpha$ -naphthaleneacetic acid (NAA), and GA<sub>3</sub> induce shoot formation and organogenesis in potato (Hussain et al., 2005; Kaur et al., 2014). Mehmood et al. (2017) noted that potato plantlets from cv. Cardinal on MS + 0.12 mg L<sup>-1</sup> of GA<sub>3</sub> and cv. Desiree on MS + 0.25 mg L<sup>-1</sup> of GA<sub>3</sub> illustrated reduced days to root initiation, developed maximum plantlet height and more node numbers, and took minimum days to transferable height of the plantlets. Although the employment of BAP in the culture media increased the average rate of multiplication to 3–6 shoots per plantlet (Quiroz et al., 2017), the combination of GA<sub>3</sub>+NAA gave better results since this integration improved the rate of multiplication in shoot tip culture by developing the plantlet height, the number of nodes on the plantlets (Nuwagira et al., 2015). However, Xhulaj and Gixhari (2018) suggested effective combination of GA<sub>3</sub>+BAP in the development of better explant at organogenesis phase. In another research, Xhulaj (2019) recorded better plantlet development in shoot height (8.7 cm), root length (11.2 cm) and node number (5.1) on 0.2 mg L<sup>-1</sup> GA<sub>3</sub>+0.02 mg L<sup>-1</sup> NAA hormonal combinations.

It has been suggested that supplementation of MS medium by zeatin riboside (ZR) and zeatin showed better plantlet growth compared to the other cytokinin-containing media (Molla et al., 2011; Chaudhary and Mittal, 2014). Similarly, Dhital et al. (2010) suggested that integration of zeatin,  $\alpha$ -naphthaleneacetic acid (NAA), and GA<sub>3</sub>, in addition to BAP and GA<sub>3</sub>, might be used for direct regeneration of potato shoots under *in vitro* conditions. Minimum days to shoot induction (17 days) with maximum shoot number (12.93) and leaf number (3.22) were recorded on 5.0 mg L<sup>-1</sup> ZR supplemented MS medium (Huda et al. 2013). In another study, Campos et al. (2016) used 3 mg L<sup>-1</sup> concentration of ZR supplemented MS medium with different concentrations of NAA+GA<sub>3</sub>, and the best for shoot regeneration from potato internode explants were obtained from 0.05 mg L<sup>-1</sup> NAA+0.10 mg L<sup>-1</sup> GA<sub>3</sub>+3 mg L<sup>-1</sup> ZR including MS medium.

JA is generally well-known because of its tuber-stimulating property *in vitro* (Koda et al., 1991; van den Berg and Ewing, 1991) and has been associated with the promotion of senescence (Pelacho et al., 1997) in the micropropagated potatoes. However, it has been recently shown that JA has outstanding and potentially beneficial features that affect plant growth and improvement when applied exogenously to potato plantlets (Ravnikar et al., 1992; Dermastia et al., 1994). Kumlay et al. (2014) and Kumlay (2016) suggested that root formation as well as vegetative development could be stimulated on *in vitro* cultured potato explants by application of JA.

The purpose of this research was to determine the influence of integration of adenine type cytokinins, naphthalene type auxins, jasmonic acid and GA<sub>3</sub> on improvement of *in vitro* grown potato cultivars Caspar, Granola and Pasinler-92 using meristem originated binodal stem segments as explants.

## MATERIALS AND METHODS

### Preparation, Concentrations and Combinations of Plant Growth Regulators Used in the Research

MS medium including vitamins, sucrose [3%, (w/v)] and agar [0.8% (w/v)] was used in the study. The pH was modulated to 5.7±0.1 by using 1.0 N HCl or 1.0 N NaOH after addition of all medium constituents except agar and Ca-pantothenate. Since the combination of GA<sub>3</sub>+NAA gave better results by developing the plantlet height, the number of stems and nodes on the plantlets (Nuwagira et al., 2015), the integration of these PGRs was used as control. The stem node segments of meristem originated potato explants were regenerated on MS medium including 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA (control) with the combination of 2.0 mg L<sup>-1</sup> KIN, 2.0 mg L<sup>-1</sup> BAP, 1.0 mg L<sup>-1</sup> ZR, and 0.5 mg L<sup>-1</sup> JA. Because of thermolabile properties of GA<sub>3</sub>, ZR and JA hormones, they were not autoclaved but they were filter-sterilized by passing through 0.22-µm Millipore filters (Schleicher & Schuell, FP 30/0.2 CA-S; 0.2 µm; 7 bar max) inside a laminar flow cabin, and added to the autoclaved medium after cooling to 40–45 °C. The other PGRs were sterilized along with culture medium by autoclaving at 120 °C, 104 kPa for 15 min.

### Cultivars, Origin of Plant Material and Micropropagation of Explants in the Research

Since *in vitro* grown plantlet is more reliable explants source than field grown plant material (de Morais, 2018), binodal stem explants taken from four weeks old meristem originated seedlings regenerated on micropropagated sprouts of Caspar, Granola and Pasinler-92 potato genotypes were used in the research. Binodal stem explants were aseptically incubated on the MS medium including 10 explants per replication (n=6), totally 60 binodal cuttings per treatment. Explants were cultured under 2,000 lux light intensity with 16-h of long day photoperiod at a temperature of 24±2 °C for 6 weeks.

### Observations and Statistical Method for Evaluation the Results

The results of experiment were recorded as days to shoot and root initiation, shoot and root length (cm), the number of leaves, nodes, axillary shoots and roots per plantlet, fresh weight (FW) and dry weight (DW) of plantlet (mg). FW of harvested plantlets was determined as in mg after washing completely to remove the attached agar, sugar and salt residues from roots, and drying on soft sterile tissue paper. Plantlets were dehydrated to a stable weight at 60°C for 2 d, and then DW of plantlets was determined as in mg. A completely randomized design (CRD) was employed to reveal the performance of three potato cultivars, using five PGR combinations with six replications. Results of the study were subjected to the analysis of variance, the means were evaluated by using Duncan's multiple comparison test and data presented by various letters in the same column are different statistically. Results on all parameters were expressed as means from six replications (n=6) with standard error (± SE).

## RESULTS AND DISCUSSION

The influence of PGRs on the stimulation of plantlet characteristics is presented in Tables 1 through 10. The results showed significantly ( $P < 0.01$ ) different effects of PGRs on the length of shoots and roots, number of nodes, leaves, axillary shoots, roots per shoots along with FW and DW of the plantlets. The results indicated a significant interaction (at the 1% probability level) between PGR combinations (treatments) and cultivars potentiality for the number of leaves and nodes per explant and the length of root. Similarly, an interaction ( $P < 0.05$ ) was also recorded between variety capability and PGRs for the mean shoot length, the mean branch number, FW and DW content of regenerating plantlets.

The days to induce shoot and root initiation ranged 4.00 – 15.75 d and 11.00 – 28.00 d, respectively (Table 1). The average shoot length varied 2.70–17.25 cm, the number of axillary shoots ranged 2.50–15.25 (Table 2), the number of nodes differed 2.75–19.00, the number of leaves ranged 2.75–19.00 (Table 3), the number of roots varied 3.75–25.25, root length changed 2.45–18.45 cm (Table 4). Furthermore, the results had a clear effect on FW of plantlets with range of 186.48–809.73 mg, and the DW of the plantlets differed in range of 19.32–123.73 mg on all PGR combinations treatments. The combined use of KIN, BAP, ZR, and JA with GA<sub>3</sub> plus NAA in 1.0×MS medium resulted in 5.0–11.5 d to induce shoots and 7.5–17.0 d to induce roots. The treatments of hormones ended up with 1.2–5.4 fold increase in shoot length, 1.7–5.8 fold increment in the number of axillary shoots, 1.5–6.9 fold higher node number, 1.3–6.3 fold rise in the number of leaves, 1.5–5.7 fold higher growth in the number of roots, 1.4–6.5 fold rise in root length, 1.3–4.2 fold increase in FW of plantlets, and 1.6–6.4 fold increase in DW of plantlets compared to single application of GA<sub>3</sub> with NAA. The data of this research also illustrated that among the PGRs, the effect of JA was more pronounced in all the studied seedling properties compared to the PGRs. Because the single application of GA<sub>3</sub> and NAA at a 0.1 mg L<sup>-1</sup> concentration (control) showed weaker plantlet growth parameters and their number was much lower than the other PGR treatments, the lowest results from each character were not be discussed in detail in text. The data from of each application and their interactions with cultivar are presented below under different subheadings.

### Days to Shoot Initiation

The influence of all hormone treatments on days to shoot initiation varied significantly ( $P < 0.01$ ). The minimum days to shoot initiation were recorded on Pasinler-92 (4.00 d), followed by Granola (4.75 d) and Caspar (5.50 d) on MS medium including 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA after 6 weeks of cultivation. However, late-maturing Caspar had the most slowed shoot initiation period of 15.75 d, followed by 14.25 on Granola, and 13.75 d on Pasinler-92 on control treatment (Table 1). The results displayed that the PGR applications were efficient to shorten days to shoot initiation compared to regeneration on control medium. Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly reduced days to shoot initiation.

The results are in partial agreement with Zhang and Cheng (1996) who recorded significant consequences of JA on *in vitro*-grown potato nodal explants in terms of shoot induction. In another study, it has been illustrated that days to shoot appearance started 11.75 days earlier on MS medium including 2µM JA compared to the controls (Kumlay, 2016).

### Days to Root Initiation

Significant differences were noted among PGR treatments on the number of days to root initiation ( $P < 0.01$ ). The treatment with KIN, BAP, ZR and JA had a meaningful influence in lowering the days to root initiation compared to regeneration on control medium. Single application of GA<sub>3</sub> and NAA or

in combination with any of the adenine type cytokinin did not significantly reduced days to root induction.

**Table 1.** Effects of various PGR combinations on the days to shoot initiation

PGR Combinations (mg L <sup>-1</sup> )						Days to Shoot Initiation		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					13.75±0.65e	14.25±1.75d	15.75±1.75d
0.1	0.1	2.0				10.75±1.11d	9.50±0.48c	9.25±0.48c
0.1	0.1		2.0			8.50±0.87c	8.00±0.41bc	8.75±0.48bc
0.1	0.1			1.0		6.25±0.25b	7.50±0.64b	8.00±0.58b
0.1	0.1				0.5	4.00±0.25a	4.75±0.25a	5.50±0.41a

Average values presented by various letters in the same column are statistically different

**Table 2.** Effects of various PGR combinations on the days to root initiation

PGR Combinations (mg L <sup>-1</sup> )						Days to Root Initiation		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					22.75±0.48e	24.50±0.82e	28.00±0.82e
0.1	0.1	2.0				17.25±1.11d	18.75±0.25d	20.50±0.29d
0.1	0.1		2.0			15.00±0.57c	16.50±1.00c	18.00±0.82c
0.1	0.1			1.0		13.00±0.50b	14.25±0.25b	15.50±0.96b
0.1	0.1				0.5	11.00±0.63a	12.00±0.50a	12.50±0.48a

Average values presented by various letters in the same column are statistically different

It can be seen on Table 2 that the minimum days required for root initiation were recorded on MS medium supplemented with 0.1 mg L<sup>-1</sup> GA<sub>3</sub> + 0.1 mg L<sup>-1</sup> NAA + 0.5 mg L<sup>-1</sup> JA (11.00 d for Pasinler-92, 12.00 for Granola, and 12.50 for Caspar). Although there are no many studies of JA supplemented medium regarding days to root induction; in a previous study, involvement of 2µM JA to MS medium of micropropagated potato plantlets stimulated the improvement of roots 17.75 days earlier compared to the control (Kumlay, 2016).

#### Average Shoot Length per Plantlet (cm)

Elongation of regenerated shoots plays important roles in the initial growth stage: It facilitates easy distinction between fast- and slow-regenerating cultivars. It also helps researcher to easily distinguish cultivars with elongating shoots and fast regenerating nodes per explant (Kovac and Ravnkar, 1994).

Combinations of GA<sub>3</sub>+NAA together with KIN, BAP, ZR, and JA affected the length of shoots, demonstrating significant differences among them ( $P<0.01$ ). Reduced shoot length was noted on MS medium including only GA<sub>3</sub>+NAA. Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly improved shoot length (Table 3). The interaction between cultivars and PGR treatments also illustrated noteworthy differences ( $P<0.01$ ). Among the five PGRs treatments, a considerable increase was determined using GA<sub>3</sub>+NAA+JA; the longest shoots were observed in the Caspar (17.25 cm), proceeded by Granola (15.88 cm) and Pasinler-92 (15.18 cm) using MS medium including 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA after 6 weeks of cultivation (Table 3).

Irrespective of PGR combinations, MS medium containing JA showed significantly improved shoot length. The outcomes of the present experiment are in consensus with the findings of Dermastia et al. (1994), who revealed that plantlets grown in a medium supplemented with JA were taller when compared to the other PGR treatments. Zhang and Cheng (1996) noted potato cultivars exhibited various

responses to JA in shoot development when subjected to different JA concentrations in the MS medium. Pelacho et al. (1997) revealed that the application of 0.1 mg L<sup>-1</sup> JA increased the length shoot to 15.65 cm, however shoot elongation decreased to 10.56 cm on 1 mg L<sup>-1</sup> JA including MS medium. Zhang et al. (2006) obtained 8.83 cm-long shoots from cv. Helanwuhua on 2.0 mg L<sup>-1</sup> JA comprising culture medium. Kumlay (2016) obtained 5.67-times longer shoots on 1.0 µM JA including medium in comparison with control treatment.

**Table 3.** Effects of various PGR combinations on average shoot length per plantlet

PGR Combinations (mg L <sup>-1</sup> )						Shoot Length (cm)		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					2.70±0.45d	4.23±0.62d	3.38±0.41d
0.1	0.1	2.0				7.15±0.98c	5.98±0.60d	6.50±0.47c
0.1	0.1		2.0			10.10±0.77b	8.60±0.67c	7.85±0.52c
0.1	0.1			1.0		10.63±0.33b	11.80±0.25b	11.58±0.54b
0.1	0.1				0.5	15.18±0.78a	15.88±0.84a	17.25±0.35a

Average values presented by various letters in the same column are statistically different

#### Average Number of Axillary Shoots per Plantlet

Since the increased number of axillary shoots could be multiplied easily for further shoot multiplication, the number of axillary shoots per plantlet is a significant parameter when studying the growth rate of explants. Significant differences were determined among PGRs ( $P < 0.01$ ), since variants of KIN, BAP, ZR, and JA affected the number of axillary shoots. The interaction between cultivars and PGR treatments demonstrated meaningful differences statistically ( $P < 0.05$ ). The highest quantity of axillary shoots was obtained in Caspar (15.25) pursued by Pasinler-92 (13.00) and Granola (12.00) using MS medium supplemented with 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA combination (Table 4). Reduced number of axillary shoots was noticed on control application. Single application of GA<sub>3</sub>+NAA or in combination with any of the adenine type cytokinin did not significantly improve the quantity of axillary stems per plantlet.

**Table 4.** Effects of various PGR combinations on average number of axillary shoots per plantlet

PGR Combinations (mg L <sup>-1</sup> )						Number of Axillary Shoots		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					2.50±0.29c	3.25±0.25d	2.75±0.25d
0.1	0.1	2.0				7.75±0.32b	7.50±0.29c	6.75±0.48c
0.1	0.1		2.0			8.25±0.48b	6.50±0.29cd	6.50±0.29c
0.1	0.1			1.0		8.75±0.48b	8.75±0.25b	9.00±0.41b
0.1	0.1				0.5	13.00±0.41a	12.00±0.41a	15.25±0.41a

Average values presented by various letters in the same column are statistically different

The data of the current research are in partial harmony with Ravnkar et al. (1992), Zhang and Cheng (1996) and Kumlay (2016). Ravnkar et al. (1992) expressed that the inclusion of JA at lower concentrations (0.1–1.0 µM) on MS medium resulted in the emergence of lateral branches. Zhang and Cheng (1996) suggested that the quantity of lateral branches enhanced significantly depending on low JA concentrations (0.1–5 µM). Kumlay (2016) obtained 4.57 times higher lateral shoots totally and a maximum number of 8.00 branches from Caspar by addition of 1.0 µM JA to MS medium compared to the control.

The results were not similar to the findings of Rabbani et al. (2001), Hussain et al. (2005), and Uddin (2006). Rabbani et al. (2001) determined that 2.0 mg L<sup>-1</sup> BAP+GA<sub>3</sub> containing MS medium could bring significant development on the *in vitro* multiple shoot induction by increasing the number axillary shoots. Hussain et al. (2005) reported the highest number of 9.0-shoot in cv. Altamash, and 14.3 shoots in cv. Diamond using nodal cuttings on 2.0 mg L<sup>-1</sup> of BAP including MS medium. Uddin (2006) regenerated the better results with the combination of 3.0 mg L<sup>-1</sup> BAP+2.0 mg L<sup>-1</sup> KIN comprised medium, and emphasized this application was superior to using BAP or KIN alone for multiple shoot induction (2.0 shoots).

### Average Number of Nodes per Plantlet

The number of nodes per binodal explants was affected by PGR applications significantly ( $P<0.01$ ). Variations among potato varieties were also significant statistically ( $P<0.05$ ). The presented results clearly indicate role of GA<sub>3</sub>, NAA, and JA in inducing multiple nodes. Maximum number of nodes was determined in Caspar (19.00), proceeded by Granola (16.75) and Pasinler-92 (12.00) on 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA including MS medium (Table 5). Reduced number of nodes was noted on control medium. Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly improve number of nodes per explant.

The introduced findings are development over previous studies by Rabbani et al. (2001), and Uddin (2006). Rabbani et al. (2001) obtained the highest number of nodes (6.2) using 4.0 mg L<sup>-1</sup> GA<sub>3</sub> singly. Contrary to the results shown in this study, Uddin (2006) observed a better regeneration response using BAP compared to KIN. The results are in harmony with the findings of Ravnkar et al. (1990), Zhang and Cheng (1996) and Kumlay (2016) in which they recorded that JA concentration used variably showed a rise on node number per plantlet.

**Table 5.** Effects of various PGR combinations on average node number per plantlet

PGR Combinations (mg L <sup>-1</sup> )						Number of Nodes		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1		Control			3.00±0.41c	4.25±0.63c	2.75±0.25c
0.1	0.1	2.0				7.25±0.51b	6.50±0.29b	5.25±0.75b
0.1	0.1		2.0			6.00±0.71b	6.75±0.48b	6.75±0.25b
0.1	0.1			1.0		7.50±0.64b	6.25±0.25b	6.50±0.50b
0.1	0.1				0.5	12.00±0.41a	16.75±0.63a	19.00±0.58a

Average values presented by various letters in the same column are statistically different

### Average Number of Leaves per Plantlet

The number of leaves per plantlet demonstrated noteworthy differences ( $P<0.01$ ) after treatment with PGRs used in this research. The maximum number of leaves was detected in Caspar (19.00), succeeded by Pasinler-92 (15.50) and Granola (15.25) using MS medium supplemented with 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA (Table 6). Reduced number of leaves was noted on control medium. Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly improved number of leaves per explant.

The findings of the research have edge over all former studies with more number of leaves per explant emphasizing role of NAA, GA<sub>3</sub> and JA used in combination to induce leaves on regenerating plantlets. Dermastia et al. (1997) demonstrated that explants cultured on JA supplemented medium resulted in expanded leaves. Kumlay (2016) observed an increase on leaf number from Caspar (19.00), on 1.0 μM JA including medium, and emphasized that the addition of JA concluded in a 7.60-times rise in the leaf number compared to no PGRs including control medium.

**Table 6.** Effects of various PGR combinations on the average number of leaves per plantlet

PGR Combinations (mg L <sup>-1</sup> )						Number of Leaves		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					2.75±0.48c	3.50±0.29d	3.00±0.00c
0.1	0.1	2.0				7.25±0.68b	6.25±0.25b	4.50±0.29c
0.1	0.1		2.0			3.75±0.25c	5.00±0.41c	3.75±0.48c
0.1	0.1			1.0		7.50±1.26b	3.25±0.25d	10.50±0.65b
0.1	0.1				0.5	15.50±0.65a	15.25±0.63a	19.00±1.22a

Average values presented by various letters in the same column are statistically different

### Average Number of Roots per Plantlet

Data obtained from this research showed significant influence of all PGR treatments on root number ( $P < 0.01$ ). Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly improve number of roots per explant. The roots of the plantlets treated with the application of GA<sub>3</sub> and NAA at a 0.1 mg L<sup>-1</sup> or any concentration of adenine type cytokinin were much weaker and their number was much lower than compared to JA containing treatments. The maximum number of roots were recorded in Caspar (25.25), succeeded by Granola (24.00) and Pasinler-92 (23.75) using MS medium containing 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+0.1 mg L<sup>-1</sup> NAA+0.5 mg L<sup>-1</sup> JA (Table 7). The results of this study showed edge of NAA, GA<sub>3</sub>, JA regenerated shoots to induce higher number of roots and enhanced rooting efficiency indicate that the NAA, GA<sub>3</sub>, JA-supplemented medium prepares plantlets for easy acclimatization to *ex vitro* conditions and increases their chance to survival under greenhouse conditions.

**Table 7.** Effects of various PGR combinations on the average number of roots

PGR Combinations (mg L <sup>-1</sup> )						Number of Roots		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1					3.75±0.25e	5.50±0.65e	4.00±0.87e
0.1	0.1	2.0				9.75±1.00d	10.25±0.48d	9.50±0.65d
0.1	0.1		2.0			15.50±0.29c	14.75±0.25c	15.25±0.48c
0.1	0.1			1.0		18.00±0.82b	18.50±0.87b	18.00±0.41b
0.1	0.1				0.5	23.75±0.48a	24.00±0.41a	25.25±0.48a

Average values presented by various letters in the same column are statistically different

The results reported here are improvement over previous studies. Ravnkar et al. (1990) noted the highest root number on higher JA including MS medium, although its roots shortened and its diameter increased. Kovac and Ravnkar (1994) reported that 1.0 μM JA concentration improved the root systems of potatoes grown *in vitro*. Martin-Closas et al. (2000) implied that JA induced root establishment in *in vitro* cultured potato explants. Although Pelacho et al. (1997) obtained the highest number of 14.8 roots on 1.0 mg L<sup>-1</sup> JA including medium, and Zhang et al. (2006) determined the highest number of 12.00 roots in cv. Helanwuhua on 2.0 mg L<sup>-1</sup> JA containing MS medium. Kumlay (2016) pointed out that the inclusion of JA showed a 4.69-times higher root number. It is clear from these results that when the concentrations increased the number of roots decreased or did not increase significantly.

### Average Root Length per Plantlet (cm)

The treatment of PGRs varied the length of roots significantly ( $P < 0.01$ ) among potato cultivars. The longest roots were determined from Pasinler-92 (18.45 cm), followed by Caspar (17.10 cm) and Granola (17.03 cm) using control medium + 0.5 mg L<sup>-1</sup> JA (Table 8) without having any adverse effect

on texture of roots. Irrespective of the cultivar, the developing roots were morphologically strong did not show any abnormality or inhibition, branched and had tendency to grow with the passage of time. The results clearly demonstrate that presence of GA<sub>3</sub>+NAA+JA in one medium significantly improve root length in potato cultivars. Single application of GA<sub>3</sub> and NAA or in combination with any of the adenine type cytokinin did not significantly improve root length.

There are contradictory results and no agreement about JA containing medium in previous studies. Ravnkar et al. (1992) illustrated that JA treatment using lower concentrations (0.1–1.0 μM) resulted in a very distinction root structure with many irregular branches. Dermastia et al. (1994) demonstrated that explants cultures in JA containing medium generated a well-developed root system. Although Pelacho et al. (1997) obtained the increased the length of root on 1.0 mg L<sup>-1</sup> JA including medium (14.8 cm), Zhang et al. (2006) illustrated that the application 2 mg L<sup>-1</sup> JA including medium significantly increased root length (16.37 cm in cv. Favorita), and Kumlay (2016) determined the longest roots from cv. Pasinler-92 (14.95 cm), including 1.0 μM JA containing medium. The findings of the current research are in disagreement with the results of Vilhar et al. (1997), which showed that JA changed the root structure and inhibited root proliferation, but caused thicker roots through cell division and not cell enlargement.

**Table 8.** Effects of various PGR combinations on the average root length

PGR Combinations (mg L <sup>-1</sup> )						Root Length (cm)				
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar		
0.1	0.1		Control			2.45±0.35d	4.85±0.55d	3.63±0.36e		
0.1	0.1	2.0				7.28±0.38c	5.95±0.48cd	6.20±0.45d		
0.1	0.1		2.0				8.78±0.53c	6.50±0.44c	7.68±0.59c	
0.1	0.1			1.0				11.03±0.65b	10.25±0.48b	10.35±0.27b
0.1	0.1				0.5	18.45±0.59a	17.03±0.68a	17.10±0.36a		

Average values presented by various letters in the same column are statistically different

#### Average Fresh Weight per Plantlet (mg)

The application of PGRs to the MS medium influenced the FW of cultured explants significantly ( $P<0.01$ ) among cultivars. The interaction of PGRs and potato cultivars on the FW of plantlets illustrated significant differences ( $P<0.05$ ). The heaviest FW of cultured explants was observed in Granola (404.87 mg), followed by Pasinler-92 (399.68 mg) and Caspar (390.08 mg) using MS medium containing control+0.5 mg L<sup>-1</sup> JA (Table 5). However, the minimum FW of plantlets was observed in cv. Caspar (93.24 mg) and cv. Pasinler-92 (94.23 mg) using MS medium containing control application (Table 9). These results clearly show that presence of GA<sub>3</sub>+NAA+JA in one medium significantly improve fresh weight in potato cultivars.

**Table 9.** Effects of various PGR combinations on the average fresh weight of plantlets

PGR Combinations (mg L <sup>-1</sup> )						Fresh Weight of Plantlet (mg)				
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar		
0.1	0.1		Control			94.23±6.9d	108.57±6.3d	93.24±4.3d		
0.1	0.1	2.0				205.30±9.7c	185.62±10.0c	195.73±12.0c		
0.1	0.1		2.0				208.02±6.5c	139.34±14.5d	153.84±16.9c	
0.1	0.1			1.0				317.07±13.8b	298.78±20.8b	331.95±17.2b
0.1	0.1				0.5	399.68±16.9a	404.87±16.8a	390.08±27.4a		

Average values presented by various letters in the same column are statistically different

The current findings are in partial harmony with previous studies, all of which used variable concentrations of JA on variable cultivars to induce maximum fresh weight. Pelacho et al. (1997) showed that increasing JA concentration decreased total FW of plantlet. Zhang et al. (2006) reported that the fresh mass of potato plantlets cultured *in vitro* significantly increased with application of JA, but higher amount of JA applications delayed the FW of cultured potato plantlets. Findings of Kumlay (2016) are in agreement with above researches that pointed out the maximum FW was obtained on cv. Pasinler of 649.38 mg on 1.0  $\mu\text{M}$  JA, but same cultivar gave 418.1 mg on 2.0  $\mu\text{M}$  JA containing MS medium.

### Average Dry Weight per Plantlet (mg)

The influence of all PGR treatments on the DW of cultured explants varied significantly ( $P < 0.01$ ). Variations among potato varieties, and the interaction of PGRs and varieties on the DW of plantlets also indicated significant differences at the 5% probability level. The highest DW were observed in Granola (61.85 mg), followed by Pasinler-92 (59.68 mg) and Caspar (57.15 mg) using MS medium including control+0.5 mg L<sup>-1</sup> JA. The results clearly indicate that presence of JA in MS medium significantly improved DW of cultured explants. However, the lowest DW content of plantlets was observed in Pasinler-92 (19.32 mg) using control medium, as well as in Caspar (10.17 mg) and Granola (11.98 mg) using 0.1 mg L<sup>-1</sup> GA<sub>3</sub>+2 mg L<sup>-1</sup> BAP containing MS medium (Table 10).

**Table 10.** Effects of various PGR combinations on the average dry weight of plantlets

PGR Combinations (mg L <sup>-1</sup> )						Dry Weight of Plantlet (mg)		
GA <sub>3</sub>	NAA	KIN	BAP	ZR	JA	Pasinler-92	Granola	Caspar
0.1	0.1		Control			9.66±0.39c	11.98±0.96b	10.17±0.50c
0.1	0.1	2.0				22.54±0.97bc	19.83±0.76b	24.76±0.73b
0.1	0.1		2.0			20.91±0.79c	15.05±0.50b	16.08±0.53c
0.1	0.1			1.0		38.37±1.08b	35.35±0.96b	47.14±1.16a
0.1	0.1				0.5	59.68±1.66a	61.85±1.74a	57.15±1.87a

Average values presented by various letters in the same column are statistically different

The present findings are in agreement with previous studies by Castro et al. (2000) reported that JA significantly increased DW when compared to control treatment in the presence of GA<sub>3</sub>. Martin-Closas et al. (2000) also suggested that inclusion of JA increased the mean DW of shoots (60%) and average DW of roots (300%) of cultured potato explants *in vitro*. It is also presented that, although inclusion of JA increased DW at 1.0  $\mu\text{M}$  concentration, the addition of 2.0  $\mu\text{M}$  JA decreased DW of micropropagated potato plantlets (Kumlay, 2016).

### CONCLUSION

All studied cultivars responded positively to PGRs supplemented to MS medium resulting in healthy *in vitro* grown plantlets with green leaves and normal morphological traits. The results of this study revealed that irrespective of the parameter studied, presence of JA in one medium had significantly positive impact on potato cultivars used in this study compared to the results reported in previous researches. Since the exogenous addition of JA in combination with GA<sub>3</sub>+NAA effectively reduces propagation interval and increases the number of cultured potato explants under *in vitro* conditions, it can be concluded that this PGR combination plays a significant role in governing shoot and root improvement on micropropagated plantlets.

Because this study has shown the influence of different hormones on cultured explants properties differed with the cultivar and the appropriate PGR concentration may be unique for each potato cultivar, further researches might be conducted to determine the optimal PGR combinations for new improved

promising potato clones or lines. The studies could also be further expanded by investigating the factors that affect acclimatization to *ex vitro* conditions and microtuberization in micropropagated potato plantlets.

### Conflict of Interest

The article authors declare that there is no conflict of interest between them.

### Author's Contributions

The authors declare that they have contributed equally to the article.

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