

## Effect of 6-Benzylaminopurine and Abscisic Acid on Gas Exchange, Biochemical Traits, and Minituber Production of Two Potato Cultivars (*Solanum tuberosum* L.)

M. J. Ahmadi Lahijani<sup>1</sup>, M. Kafi<sup>1\*</sup>, A. Nezami<sup>1</sup>, J. Nabati<sup>2</sup>, and J. Erwin<sup>3</sup>

### ABSTRACT

Possibility of improving physiological traits and minituber yield of potato cultivars (*cvs.* Agria and Fontane) was investigated by application of plant growth regulators (BAP, ABA and BAP+ABA) at tuber initiation stage. Regardless of the cultivars, Net photosynthesis rate ( $N_p$ ), actual quantum yield ( $\Phi$ ), stomatal conductance ( $g_s$ ) and Transpiration rate ( $T_r$ ) of BAP-treated leaves were superior to those of the control. For Agria, the greatest Chlorophyll content (Chl) was observed in BAP-treated plants, while the highest Chl for Fontane was observed in ABA-treated plants. Increasing  $N_p$  and Chl content were associated with higher Soluble Carbohydrate content (SC). BAP+ABA application increased SC of leaflets in both cultivars compared with the control. Tuber Yield per Plant (Y/P), Mean Tuber Weight (MTW), and Tuber Number (TN) were stimulated by foliar treatment of plants with PGRs compared with the untreated ones, but there were significant interactions between cultivar and hormone type. Positive correlation between SC and Y/P ( $r=0.97^*$ ) and MTW ( $r=0.97^*$ ) were observed in Agria. Leaf area as well as dry and fresh weight of aerial parts of the BAP+ABA-treated plants were more than the untreated plants and other PGR treatments. These results indicate that either of BAP, ABA, or their combination could be effectively used to improve physiological traits and tuber yield of these cultivars, although, Agria responded more prominently to PGRs than Fontane.

**Keywords:** Net photosynthesis rate, Photosynthetic pigments, Plant growth regulators, Soluble carbohydrate, Tuberization.

### INTRODUCTION

Minitubers are small potato (*Solanum tuberosum* L.) tubers produced on *in vitro* plantlets after transplanting them into substrates in the greenhouse. The term minituber refers to their size and weight, which range from 5-25 mm in size and 1-10 g or more in weight (Struik, 2007a). Genotype, growth regulators, growth conditions and nutrients are among the main factors affecting minituber production (Sharma and Pandey,

2013). Genotypes are widely different in minituber production and some are more prolific and produce yield over 10-fold higher than the others (Sharma *et al.*, 2013). Tuber formation is a complex process and is affected by several external and internal factors such as photoperiod, temperature, levels of carbohydrates and plant hormones (Ramawat and Merillon, 2013).

Phytohormones are key factors for regulation of physiological and biochemical processes in plants (Borzenkova and

<sup>1</sup> Department of Agronomy and Plant Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Islamic Republic of Iran.

<sup>2</sup> Research Centre of Plant Sciences, Ferdowsi University of Mashhad, Islamic Republic of Iran.

<sup>3</sup> Department of Horticultural Science, University of Minnesota, 305 Alderman Hall, St. Paul, MN 55108, USA.

\* Corresponding author; email: m.kafi@um.ac.ir



Borovkova, 2003). Plant Growth Regulators (PGRs) are chemical compounds that regulate a wide range of processes in plants. These PGRs provide an opportunity to find out the potential of plant growth and development (Wang and Xiao, 2009). Numerous studies have revealed remarkable effects of phytohormones on all stages of potato tuberization (Ewing, 1995; Rodríguez-Falcón *et al.*, 2006). Among PGRs, Cytokinin (CK) and Abscisic Acid (ABA) are involved in regulating a wide range of processes in plants. There are evidences that ABA content increases under short day conditions, which is essential for tuber initiation (Ewing, 1995). It has been proved that ABA has an inhibitory effect on Gibberellin (GA) activities (Wang *et al.*, 2015). Xu *et al.* (1998) found that ABA/GA ratio has a determining role in the initiation of tuberization in potato. Foliar application of ABA provoked tuber formation in potato and deactivated inhibitory effect of GA (Xu *et al.*, 1998). However, it was suggested that ABA does not directly play a role in tuberization, and the positive effects of ABA might be due to its inhibitory effect on GA action and signalling (Ramawat and Merillon, 2013).

The role of CK in potato tuberization has long been suggested by Palmer and Smith (1970), but less attention has been paid to CK compared to GA and ABA. CK stimulates plant cell division (Romanov, 2009), and since tuber initiation is firmly related to cell division, CK is considered as a necessary part of this process. Furthermore, CK stimulates starch biosynthesis by activating enzymes, which results in accumulation of starch and strengthens sink capacity of the developing tubers (Ramawat and Merillon, 2013). These effects correlate with antagonism of CK to GA signalling (Romanov, 2009). For *in vitro* tuberization, exogenous cytokinins, especially BA, are used extensively to induce microtubers on a variety of explants including stolons, shoot cuttings and intact microplantlets (Donnelly *et al.*, 2003). Roosta *et al.* (2015) reported that plant dry weight, diameter of tubers and tuber yield of potato plants were increased by application of BAP *in*

*vitro*. Liu and Xie (2001) declared that various concentrations of cytokinin increased the size and weight of minitubers, and a linear relationship was observed between the size and weight.

Minituber production techniques require more studies and research on pre and post treatments of plantlets to increase size and number of minitubers for more successful multiplication in the open field (Sharma and Pandey, 2013). In evaluations of hormonal regulation of tuberization in potato crops, the most attention has been paid to the role of GA; to our knowledge, less attention has been paid to the simultaneous application of BAP and ABA on micro propagated potato plantlets *in vivo*. Hence, our study aimed to examine the response of two varieties of potato minituber plants to exogenous application of BAP, ABA and their combination in terms of tuber yield, biochemical and physiological parameters.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

The experiment was conducted in the research greenhouse of the Faculty of Agriculture, Ferdowsi University of Mashhad, in 2014. Plantlets of two potato cultivars (*Solanum tuberosum* L. cvs. Agria and Fontane) were grown in 27-liter plastic boxes (30×30×30 cm) in a greenhouse at 12-hours photoperiod, natural irradiance of  $400\pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, average day/night temperature of 25/18°C, and relative humidity of 50±5%. Plantlets were produced on *in vitro* micropropagated tissue cuttings grown in medium culture at Yekta Seed Technology Company, Mashhad, Iran. Four uniform plantlets were transplanted into each box (three boxes each cultivar) containing sterilized perlite-coco peat in 1:1 (v/v) ratio as substrate. Thirty and fifty days after transplanting, additional five cm substrate was added to the boxes to cover the lower plant nodes. Standard Hoagland solution (Hoagland and Arnon, 1950) was used weekly to supply nutrient demands.

### Hormonal Treatments

Fifty  $\mu\text{M}$  ABA ( $\pm$ -Abscisic Acid, Sigma-Aldrich<sup>®</sup>) and BAP (6-BenzylAminoPurine, Sigma-Aldrich<sup>®</sup>) concentrations were used to evaluate the effect of ABA and BAP on physiological and biochemical parameters of potato plantlets. At the time of tuber initiation (42 Days After Transplanting-DAT), foliar application was made of: (a) BAP, 50  $\mu\text{M}$ ; (b) ABA, 50  $\mu\text{M}$ ; (c) BAP+ABA, 50  $\mu\text{M}$ , and (d) Control, with three replications in a factorial arrangement based on Completely Randomized Design (CRD) (two cultivars and four chemical treatments). PGRs were applied in the end of day-time to prevent degradation or oxidation of hormones. Also, 10 mL of solution was applied on each plant by means of a handheld sprinkler. Control plantlets were sprayed with distilled water.

### Collection of Experimental Samples

One week after foliar application of PGRs, the third youngest fully expanded leaves were used for determination of leaflet photosynthetic pigments, soluble carbohydrate content and photosynthetic parameters (Cao and Tibbitts, 1991). Measurements were taken on three replicate plants of each cultivar.

### Chlorophyll and Soluble Carbohydrate Assay

Leaf samples were collected and immediately frozen in liquid nitrogen and kept at  $-80^{\circ}\text{C}$  for further analysis. To evaluate the chlorophyll content, the method of Knudson *et al.* (1977) was used. A sample of 100 mg leaf fresh weight was homogenized in 98% ethanol using a mortar and pestle. Absorbance of the Chl extract at 665 and 649 nm were measured spectrophotometrically (Unico 2100, USA). To convert these readings to Chl content of the leaf, the following equations were used:

$$\text{Chl } a = (13.70)(A_{665}) - (5.76)(A_{649}) \quad (1)$$

$$\text{Chl } b = (25.80)(A_{649}) - (7.60)(A_{665}) \quad (2)$$

Here, *Chl a* is chlorophyll a, *Chl b* is chlorophyll b, and A is absorption at the given wave length.

To assay the soluble carbohydrate content of leaves, the methods of Dubois *et al.* (1956) were used with some modifications. Samples of 100 mg leaf fresh weight were homogenized in 70% methanol using a mortar and pestle. Soluble carbohydrate content of leaflets was measured using a glucose standard curve.

### Gas Exchange Variables

Net photosynthesis rate, stomatal conductance, and transpiration rate were measured using a portable LCA4 photosynthesis system (ADC Bio Scientific Ltd, UK) in a relative humidity of  $50 \pm 5\%$ , approximately  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, leaf temperature of  $25^{\circ}\text{C}$  and ambient  $\text{CO}_2$  concentration. Measurements were taken on three replications of each cultivar-treatment combination, between 11:00-13:00 hours at midday. At the same time, Chlorophyll a fluorescence characteristics of the adaxial surface of attached leaves were measured after a 15-minute dark period using a handheld PEA Chlorophyll Fluorimeter (*Hansatech*, UK) at room temperature and ambient  $\text{CO}_2$  concentration. The actual quantum yield of photosystem II photochemistry ( $\Phi$ ), was calculated as  $(Fm' - Fs)/Fm'$ , where  $Fm'$  and  $Fs$  were defined as maximum fluorescence elicited by a saturating light pulse and steady state chlorophyll fluorescence, respectively (Genty *et al.*, 1989).

### Plant Harvest

Plants were harvested 115 DAT when they had signs of physiological maturity, and separated into shoots and tubers. Fresh weight of shoots and tubers were measured separately. Mean tuber number per plant was also measured. Leaf area of the plant was



measured using Li-3100 area meter (LI-COR, Lincoln, NE). Shoots were oven dried at 75°C for 48 hours to a constant weight and dry weight was determined.

### Statistical analysis

Data were analyzed by ANOVA using SAS software. Statistical significance, where indicated, is at least at the 5% level as determined by the analysis of variance and the Fisher's least significant difference test. Correlation among parameters were computed when applicable.

## RESULTS

Based on a preliminary experiment, 0, 25, 50 and 100 µM ABA and BAP were used to determine the optimum concentration of PGRs. Among the treatments, 50 µM ABA and BAP advanced gas exchange variables including net photosynthesis rate. Higher concentration of PGRs slightly decreased growth and gas exchange variables (data not shown).

### Chlorophyll and Soluble Carbohydrates Content

Leaflet Chlorophyll content (Chl) tended to increase with foliar application of PGR treatments (Table 1). Agria leaves had greater Chl on fresh weight basis than Fontane by an average of 9%. Application of BAP in tuber initiation stage increased Chl of Agria by an average of 45% over the control, whereas, foliar application of ABA in Fontane increased Chl by 14% compared with the control. There were no significant differences between hormonal treatments in Chlorophyll a content (Chla) in Fontane, but in Agria, BAP was more effective and significantly increased Chl a of Agria by 42% over the control. In Agria, BAP-treated plants had also significantly greater concentration of Chlorophyll b (Chl b) by

Table 1. Growth regulators application induced changes in chlorophyll and soluble carbohydrates content of potato plants.<sup>a</sup>

Treatment	Chl a <sup>b</sup> (µg g FW <sup>-1 h</sup> )		Chl b <sup>c</sup> (µg g FW <sup>-1</sup> )		Chl a+b <sup>d</sup> (µg g FW <sup>-1</sup> )		SC <sup>e</sup> (µg g FW <sup>-1</sup> )
	Agria	Fontane	Agria	Fontane	Agria	Fontane	
Control	0.67±0.06	0.67±0.02	0.71±0.09	0.86±0.07	1.38±0.12	1.54±0.06	17.7±1.79
BAP <sup>f</sup>	1.16±0.01	0.70±0.07	1.36±0.13	0.92±0.01	2.52±0.14	1.62±0.06	21.9±2.22
ABA <sup>g</sup>	0.98±0.14	0.68±0.06	0.74±0.07	1.12±0.04	1.72±0.21	1.81±0.05	21.9±1.93
BAP+ABA	0.80±0.06	0.71±0.02	0.85±0.05	0.87±0.05	1.66±0.05	1.58±0.04	27.2±2.00
Analysis of variance	**		ns		**		**
Cultivar (C)	**		**		**		**
Treatment (T)	**		**		**		**
C×T							ns

<sup>a</sup> Values are mean±SD of three replicates (n=3), <sup>b</sup> Chlorophyll a; <sup>c</sup> Chlorophyll b; <sup>d</sup> Total Chlorophyll content, <sup>e</sup> Leaf Soluble Carbohydrate, <sup>f</sup> Benzyl Amino Purine; <sup>g</sup> Abscisic Acid; <sup>h</sup> Leaf Fresh Weight. \* Significant at P<0.05; \*\* Significant at P<0.01, ns: Not significant at P> 0.05, LSD test.

47% compared with the control, while, in Fontane, ABA-treated plants had 23% greater Chlb compared with the control. Hormonal treatments significantly increased Soluble Carbohydrate content (SC) of leaflets compared with the untreated control plants. Fontane had significantly higher SC than Agria. Irrespective of the cultivar, ABA+BAP application significantly increased SC in leaflets by 35 and 37% in Agria and Fontane, respectively.

### Gas Exchange Variables

Net photosynthetic rate ( $N_p$ ) was affected by either cultivars or PGRs (Table 2). Net photosynthetic rate of Agria was always higher than Fontane. The highest  $N_p$  was recorded when BAP was applied at tuber initiation, which was 44 and 38% higher than the control in Agria and Fontane, respectively. No significant effects of foliar application of PGRs were observed on  $g_s$ , however, irrespective of the cultivar, BAP- and ABA-treated plants recorded the highest and the lowest values of  $g_s$ . Cultivar and PGR interacted to affect  $T_r$ . BAP-treated plants had significantly higher  $T_r$  than the control in Agria, but not in Fontane, in contrary, ABA-treated plant recorded lower  $T_r$  than the control in Fontane, but no significant effect of which was observed in Agria. Fontane recorded higher  $\Phi$  than Agria. Although there was significant difference between cultivars in terms of  $\Phi$ , no significant differences between PGR treatments were observed.

### Growth Parameters

Leaf Area (LA), Shoot Dry (DW) and Fresh Weight (FW) of plants increased with foliar application of PGRs (Table 3). Agria had greater DW, FW and LA than Fontane either in PGR-treated or untreated control plants. Among the PGR treatments, the highest value of shoot DW was recorded when BAP+ABA was applied, which was 34

**Table 2.** Growth regulators application induced changes in gas exchange variables of potato plantlets.<sup>a</sup>

Treatment	$N_p^b$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )		$g_s^c$ ( $\text{mol m}^{-2} \text{s}^{-1}$ )		$T_r^d$ ( $\text{mmol}^{-2} \text{s}^{-1}$ )		$\Phi^e$
	Agria	Fontane	Agria	Fontane	Agria	Fontane	
Control	1.30±0.42	1.18±0.07	27.8±2.19	25.3±0.01	0.87±0.02	0.93±0.01	0.816±0.60
BAP <sup>f</sup>	2.34±0.37	1.93±0.04	28.9±1.11	29.0±2.47	0.93±0.03	0.96±0.03	0.818±0.60
ABA <sup>g</sup>	2.28±0.20	1.88±0.24	24.6±5.23	23.8±4.65	0.86±0.02	0.83±0.04	0.814±0.37
BAP+ABA	1.95±0.35	1.91±0.28	25.8±0.60	25.5±1.65	0.91±0.01	0.94±0.03	0.818±0.53
Analysis of variance							
Cultivar (C)	*		ns		ns		*
Treatment (T)	**		ns		**		ns
C×T	ns		ns		*		ns

<sup>a</sup> Values are mean±SD of three replicates (n=3). <sup>b</sup> Net photosynthesis rate; <sup>c</sup> Stomatal conductance; <sup>d</sup> Transpiration rate; <sup>e</sup> Actual quantum yield. <sup>f</sup> Abscisic Acid; <sup>g</sup> Benzyl Amino Purine; \* Significant at  $P < 0.05$ ; \*\* Significant at  $P < 0.01$ , ns: Not significant at  $P > 0.05$ , LSD test.



**Table 3.** Growth regulators induced changes in growth parameters of potato plants.<sup>a</sup>

Treatment	FW <sup>b</sup> (g plant <sup>-1</sup> )		DW <sup>c</sup> (g plant <sup>-1</sup> )		LA <sup>d</sup> (cm <sup>2</sup> plant <sup>-1</sup> )	
	Agria	Fontane	Agria	Fontane	Agria	Fontane
Control	3.65±0.29	2.83±1.15	0.269±0.10	0.244±0.05	107±19.3	76±16.12
BAP <sup>e</sup>	4.53±0.80	3.47±0.30	0.316±0.03	0.267±0.05	140±23.1	89±20.5
ABA <sup>f</sup>	4.53±1.30	2.86±0.55	0.295±0.01	0.247±0.04	136±23.8	78±19.0
BAP+ABA	6.14±0.52	4.18±0.34	0.411±0.01	0.338±0.06	190±45.5	103±1.55
Analysis of variance						
Cultivar (C)	**		*		**	
Treatment (T)	**		*		**	
C×T	ns		ns		ns	

<sup>a</sup> Values are mean±SD of three replicates (n= 12). <sup>b</sup> Shoot Fresh Weight per plant; <sup>c</sup> Shoot Dry Weight per plant, <sup>d</sup> Leaf Area per plant. <sup>e</sup> Benzyl Amino Purine; <sup>f</sup> Abscisic Acid; \*Significant at  $P < 0.05$ ; \*\* Significant at  $P < 0.01$ , ns: Not significant at  $P > 0.05$ , LSD test.

and 21% greater than the control in Agria and Fontane, respectively. Regardless of the cultivars, BAP+ABA-treated plants had also greater FW and LA compared with the controls. The BAP+ABA treatment significantly increased LA by 43 and 26% relative to the control in Agria and Fontane, respectively.

### Minituber Yield

PGR treatments significantly affected tuber yield per plant (Table 4). Agria produced greater tuber yield per plant than Fontane either in PGR-treated or untreated control plants. Compared with the control, application of BAP+ABA increased tuber yield of Agria by 20%, while, application of ABA increased tuber yield of Fontane by 21%. Similarly, for Agria, the BAP+ABA treatment significantly increased mean tuber weight by 28% compared to the controls. In

**Table 4.** Growth regulators application induced changes in yield parameters of potato plantlets.<sup>a</sup>

Treatment	Y/P <sup>b</sup> (g plant <sup>-1</sup> )		MTW <sup>c</sup> (g)		TN	
	Agria	Fontane	Agria	Fontane	Agria	Fontane
Control	25.0±2.23	20.1±1.12	8.3±1.00	6.4±1.30	2.3±0.58	3.0±0.66
BAP <sup>e</sup>	26.5±2.22	22.2±1.39	9.1±1.45	9.0±0.66	3.9±0.80	2.5±0.50
ABA <sup>f</sup>	27.0±2.18	26.5±1.32	9.2±0.93	7.9±1.56	3.0±0.50	3.4±0.24
BAP+ABA	31.3±2.79	23.1±1.28	11.5±0.46	8.0±1.13	3.3±0.80	3.6±0.38
Analysis of variance						
Cultivar (C)	**		××		ns	
Treatment (T)	**		*		ns	
C×T	*		ns		*	

<sup>a</sup> Values are mean±SD of three replicates (n= 12). <sup>b</sup> Yield per Plant; <sup>c</sup> Mean Tuber Weight, <sup>d</sup> Tuber Number per plant; <sup>e</sup> Benzyl Amino Purine; <sup>f</sup> Abscisic Acid;

contrast, application of BAP increased mean tuber weight of Fontane by 28% compared with the control. Cultivar and PGR treatments interacted to affect tuber number per plant. In Agria, application of BAP increased the tubers number by 40% compared with the control, but no significant effect of PGRs on the tuber number was observed in Fontane.

## DISCUSSION

It is generally accepted that hormonal regulation of different processes in potato provide a basis for distribution of assimilates and productivity. It was shown that this regulation depends on either content of or ratio between phytohormones (Wang, H. and Xiao, 2009). BAP application at tuber initiation stage significantly increased Np in both cultivars. However, the magnitude of the effect was different between cultivars, and Agria responded more strongly. Synková *et al.* (1997) claimed that cytokinins directly affect photosynthesis parameters such as chlorophyll synthesis and degradation, chloroplast composition, electron transport, and enzymes activities. In agreement with our study, Caldiz *et al.* (1998) found that BAP increased photosynthetic rate of potato and BAP-treated plants maintained photosynthetic activity longer than that of untreated control plants.

We observed that BAP-treated plants of Agria had higher transpiration rates compared with the controls. It has been reported that high concentrations of BenzylAdenine (BA) inhibit stomatal conductance and transpiration rate, but low concentrations enhance them (Pospíšilová, 2003), although this effect could be species specific and dependent on type of cytokinin, concentration, and method of application. Irrespective of the cultivar, application of ABA slightly, but not significantly, decreased  $g_s$  compared with the controls. It has been proven that exogenous application of ABA stimulates stomatal closure during

post-treatment (Davies *et al.*, 2005). Our result is in agreement with Reinoso *et al.* (2011) who found reduction in  $g_s$  of ABA-treated soybean within 24 hours after the hormone was applied. The partial stomatal closure may result in a higher photosynthetic rate due to maintenance of a higher intercellular  $CO_2$  concentration and lower water losses (Kang *et al.*, 1998). In agreement with this, we also found that ABA-treated plants have significantly higher NP than controls. We observed that the subtractive effects of ABA treatment on  $g_s$  was amended when ABA was applied in combination with BAP. Tichá (2004) also reported that the adverse effect of ABA on stomatal closure was ameliorated when ABA was applied in combination with BA.

Application of BAP+ABA at tuber initiation stage increased soluble carbohydrate content of both cultivars. Yadav *et al.* (1997) also reported that application of BAP stimulated accumulation of soluble sugar, proline and amino acids in *Cicer* plants. Carbohydrates play a critical role for tuber growth and starch biosynthesis, and are an efficient inducer of tuberization (Ramawat and Merillon, 2013). Greater content of SC activates a great number of genes involved in starch synthesis and, as a consequence, tubers with higher assimilates capacity are formed (Kloosterman *et al.*, 2005). Greater SC of leaflets could be a result of the higher photosynthetic rate of PGR-treated plants. Work on transgenic potato plants revealed that intensified sucrose biosynthesis in leaves has efficient effects on tuberization (Fischer *et al.*, 2008).

The greatest tuber yield and mean tuber weight in Agria was recorded in BAP+ABA-treated plants. Grappin *et al.* (2000) suggested that application of fluridone, a carotene and ABA synthesis inhibitor, reduces the activity of enzymes and contributes to starch synthesis. Hence, it seems that application of BAP+ABA promoted photosynthetic activities to produce higher photoassimilates. ABA leads to mobilization of these assimilates toward



tubers and stimulates starch synthesis enzymes to produce heavier tubers. It is likely that higher maintained rate and duration of photosynthesis during tuberization might be the cause of greater shoot carbohydrate content and tuber weights of PGR-treated plants.

Cytokinins have been proven to have a considerable regulatory effect on the source-sink relationship during tuberization *in vitro* (Sarkar *et al.*, 2006). Increases in total tuber yield by application of BAP were previously reported by other researchers in potato (Caldiz *et al.*, 1998). We observed a positive correlation between soluble carbohydrate content and tuber yield ( $r= 0.97$ ,  $P\leq 0.05$ ) and mean tuber weight ( $r= 0.97$ ,  $P\leq 0.05$ ) in Agria (Table 5). During tuber growth stage, starch is synthesized and deposited actively in amyloplasts of tuber cells. The intense assimilate synthesis and inflow toward tubers plays an important role in tuber growth (Ramawat and Merillon, 2013). Aksenova *et al.* (2009) reported that kinetin markedly altered the proportion of total plant biomass toward tubers at the expense of a reduction in shoots.

Application of BAP in Agria increased the number of tubers at the expense of reduced mean tuber weight. There was an antagonism between tuber number and tuber weight in Agria. During tuber formation stage, there are numerous potential tuber initiates on potato plants, but some of them do not develop due to limited assimilates transfer toward tubers (Struik, 2007b). It is possible that BAP enhances net photosynthetic rate and chlorophyll content,

and production of greater carbohydrates alleviate source limitation and cause more potential tubers to be translated to minitubers. Aksenova *et al.* (2012) showed that CK inflow from the roots located near the stolons regulates sink capacity of the tubers. Addition of kinetin to the medium markedly increased the proportion of tubers as a part of total biomass in potato *cv.* Desiree (Aksenova *et al.*, 2009).

BAP+ABA-treated plants showed significantly greater FW, DW and LA compared with the controls. ABA is an important regulator of cell and whole plant water relations, and possibly due to increasing turgor pressure and optimal cellular expansion, higher leaf area and shoot biomass are obtained (Acevedo *et al.*, 1971). Our results indicated that BAP+ABA stimulated increases of total biomass of both aerial and underground parts of the plants. This hormonal treatment advanced Np of both cultivars compared with the controls as well. Positive correlation between the carbohydrate content and DW, FW and LA of both cultivars were observed (Table 5). Greater tuber yield could be a result of greater LA and higher Np per leaf area basis. Plants with greater leaf area can also benefit from the greater light interception, particularly in greenhouse grown plants, where light is more limited than in field conditions.

## CONCLUSIONS

Overall, the results of this experiment

**Table 5.** Correlation coefficient between soluble carbohydrate content and yield and growth parameters of two potato minituber cultivars.

	Y/P <sup>a</sup>	MTW <sup>b</sup>	TN/P <sup>c</sup>	DW <sup>d</sup>	FW <sup>e</sup>	LA <sup>f</sup>
SC <sup>g</sup>						
Agria	0.974*	0.971*	0.573ns	0.959*	0.992**	0.993**
Fontane	0.143ns	0.511ns	0.812ns	0.957*	0.959*	0.970*

<sup>a</sup> Yield per plant; <sup>b</sup> Mean Tuber Weight; <sup>c</sup> Mean Tuber Number per Plant; <sup>d</sup> Shoot Dry Weight; <sup>e</sup> Shoot Fresh Weight; <sup>f</sup> Leaf Area; <sup>g</sup> Leaf Soluble Carbohydrate, \* Significant at  $P < 0.05$ ; \*\* Significant at  $P < 0.01$ , ns: Not significant at  $P > 0.05$ .

show that plant hormones are closely involved in regulation of morpho-physiological and tuberization processes in micropropagated potato plants. Generally, PGR applications had more significant effects on the cultivar Agria than on Fontane. Although the effects of different hormonal treatments were not consistent between cultivars, it was shown that these treatments can improve physiological parameters and minituber yield attributes in these two potato cultivars. It can be concluded that effectiveness of application of either BAP, ABA, or their combination could be highly cultivar specific, hence, it seems that both of these PGRs, or their combination can usefully be applied to improve physiological traits and yield of these two potato minituber cultivars.

#### REFERENCES

1. Acevedo, E., Hsiao, T. C. and Henderson, D. 1971. Immediate and Subsequent Growth Responses of Maize Leaves to Changes in Water Status. *Plant Physiol.*, **48(5)**: 631-636.
2. Aksenova, N., Konstantinova, T., Golyanovskaya, S., Sergeeva, L. and Romanov, G. 2012. Hormonal Regulation of Tuber Formation in Potato Plants. *Russ. J. Plant Physiol.*, **59(4)**: 451-466.
3. Aksenova, N., Konstantinova, T., Lozhnikova, V., Golyanovskaya, S. and Sergeeva, L. 2009. Interaction between Day Length and Phytohormones in the Control of Potato Tuberization in the *in Vitro* Culture. *Russ. J. Plant Physiol.*, **56(4)**: 454-461.
4. Borzenkova, R. and Borovkova, M. 2003. Developmental Patterns of Phytohormone Content in the Cortex and Pith of Potato Tubers as Related to Their Growth and Starch Content. *Russ. J. Plant Physiol.*, **50(1)**: 119-124.
5. Caldiz, D., Clua, A., Beltrano, J. and Tenenbaum, S. 1998. Ground Cover, Photosynthetic Rate and Tuber Yield of Potato (*Solanum tuberosum* L.) Crops from Seed Tubers with Different Physiological Age Modified by Foliar Applications of Plant Growth Regulators. *Potato Res.*, **41(2)**: 175-185.
6. Cao, W. and Tibbitts, T. W. 1991. Physiological Responses in Potato Plants under Continuous Irradiation. *J. Amer. Soc. Hort. Sci.*, **116(3)**: 525-527.
7. Davies, W. J., Kudoyarova, G. and Hartung, W. 2005. Long-Distance ABA Signaling and Its Relation to other Signaling Pathways in the Detection of Soil Drying and the Mediation of the Plant's Response to Drought. *J. Plant Growth Regul.*, **24(4)**: 285-295.
8. Donnelly, D. J., Coleman, W. K. and Coleman, S. E. 2003. Potato Microtuber Production and Performance: A Review. *Am. J. Potato Res.*, **80(2)**: 103-115.
9. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. and Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.*, **28(3)**: 350-356.
10. Ewing, E. E. 1995. The Role of Hormones in Potato (*Solanum tuberosum* L.) Tuberization. In: "*Plant Hormones Physiology, Biochemistry and Molecular Biology*", (Ed.): Davies, P. J. Springer, The Netherlands, PP. 698-724.
11. Fischer, L., Lipavska, H., Hausman, J. F. and Opatrny, Z. 2008. Morphological and Molecular Characterization of a Spontaneously Tuberizing Potato Mutant: An Insight into the Regulatory Mechanisms of Tuber Induction. *BMC Plant Biol.*, **8(1)**: 117.
12. Genty, B., Briantais, J. -M. and Baker, N. R. 1989. The Relationship between the Quantum Yield of Photosynthetic Electron Transport and Quenching of Chlorophyll Fluorescence. *Biochim. Biophys. Acta.*, **990(1)**: 87-92.
13. Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. and Jullien, M. 2000. Control of Seed Dormancy in *Nicotiana plumbaginifolia*: Post-Imbibition Abscisic Acid Synthesis Imposes Dormancy Maintenance. *Planta*, **210(2)**: 279-285.
14. Hoagland, D. R. and Arnon, D. I. 1950. *The Water-Culture Method for Growing Plants without Soil*. 34 PP.
15. Kang, S., Shi, W., Hu, X. and Liang, Y. 1998. Effects of Regulated Deficit Irrigation on Physiological Indices and Water Use Efficiency of Maize. *Trans. Chin. Soc. Agri. Eng.*, **14(4)**: 82-87.
16. Kloosterman, B., Vorst, O., Hall, R. D., Visser, R. G. and Bachem, C. W. 2005.



- Tuber on a Chip: Differential Gene Expression during Potato Tuber Development. *Plant Biotech. J.*, **3(5)**: 505-519.
17. Knudson, L. L., Tibbitts, T. W. and Edwards, G. E. 1977. Measurement of Ozone Injury by Determination of Leaf Chlorophyll Concentration. *Plant Physiol.*, **60(4)**: 606-608.
  18. Liu, J. and Xie, C. 2001. Correlation of Cell Division and Cell Expansion to Potato Microtuber Growth *In Vitro*. *Plant Cell Tissue Organ Cul.*, **67(2)**: 159-164.
  19. Palmer, C. and Smith, O. 1970. Effect of Kinetin on Tuber Formation on Isolated Stolons of *Solanum tuberosum* L. Cultured *In Vitro*. *Plant Cell Physiol.*, **11(2)**: 303-314.
  20. Pospíšilová, J. 2003. Participation of Phytohormones in the Stomatal Regulation of Gas Exchange during Water Stress. *Biol. Plantarum.*, **46(4)**: 491-506.
  21. Ramawat, K. G. and Merillon, J. M. 2013. *Bulbous Plants Biotechnology*. CRC Press, USA, 450 PP.
  22. Reinoso, H., Travaglia, C. and Bottini, R. 2011. ABA Increased Soybean Yield by Enhancing Production of Carbohydrates and Their Allocation in Seed. INTECH Open Access Publisher.
  23. Rodríguez-Falcón, M., Bou, J. and Prat, S. 2006. Seasonal Control of Tuberization in Potato: Conserved Elements with the Flowering Response. *Ann. Rev. Plant Biol.*, **57**: 151-180.
  24. Romanov, G. 2009. How Do Cytokinins Affect the Cell? *Russ. J. Plant Physiol.*, **56(2)**: 268-290.
  25. Roosta, H., Vazirinasab, S. and Raghmi, M. 2015. Effect of 6-Benzylaminopurine and Cycocel on Minituber Production in Two Potato Cultivars *In Vitro*. *Iran. J. Hotr. Sci.*, **46(1)**: 141-156.
  26. Sarkar, D., Pandey, S. K. and Sharma, S. 2006. Cytokinins Antagonize the Jasmonates Action on the Regulation of Potato (*Solanum tuberosum*) Tuber Formation *In Vitro*. *Plant Cell Tissue Organ Cul.*, **87(3)**: 285-295.
  27. Sharma, A. and Pandey, K. 2013. Potato Mini-Tuber Production through Direct Transplanting of *In Vitro* Plantlets in Green or Screen Houses: A Review. *Potato J.*, **40(2)**: 95-103.
  28. Sharma, A., Venkatasalam, E. and Kumar, V. 2013. Potato Mini-Tuber Production during Main and off Crop Seasons in High Hills of North-Western Himalaya. *Potato J.*, **40(1)**: 29-37.
  29. Struik, P. C. 2007a. The Canon of Potato sScience. 25. Minitubers. *Potato Res.*, **50(3-4)**: 305-308.
  30. Struik, P. C. 2007b. Above-Ground and Below-Ground Plant Development. In: "*Potato Biology and Biotechnology: Advances and Perspectives*", (Ed.): Vreugdenhil, D. Elsevier, Amsterdam, PP. 219-236.
  31. Synková, H., Wilhelmová, N., Šesták, Z. and Pospíšilová, J. 1997. Photosynthesis in Transgenic Plants with Elevated Cytokinin Contents. In: "*Handbook of Photosynthesis*", (Ed.): Pessaraki, M. Marcel Dekker, New York, PP. 541-552.
  32. Tichá, I. 2004. Photosynthesis and Stomatal Opening in *Vicia faba* L. as Affected by Abscisic Acid and Benzyladenine. *Acta Physiol Plantarum.*, **26(3)**: 104-104.
  33. Wang, X., Xu, C., Cang, J., Zeng, Y., Yu, J., Liu, L., Zhang, D. and Wang, J. 2015. Effects of Exogenous GA3 on Wheat Cold Tolerance. *J. Agr. Sci. Tech.*, **17(4)**: 921-934.
  34. Wang, H. and Xiao, L. 2009. Effects of Chlorocholine Chloride on Phytohormones and Photosynthetic Characteristics in Potato (*Solanum tuberosum* L.). *J. Plant Growth Regul.*, **28(1)**: 21-27.
  35. Xu, X., van Lammeren, A. A., Vermeer, E. and Vreugdenhil, D. 1998. The Role of Gibberellin, Abscisic Acid, and Sucrose in the Regulation of Potato Tuber Formation *In Vitro*. *Plant Physiol.*, **117(2)**: 575-584.
  36. Yadav, N., Gupta, V. and Yadav, V. 1997. Role of Benzyladenine and Gibberellic Acid in Alleviating Water-Stress Effect in Gram (*Cicer arietinum*). *Indian J. Agr. Sci.*, **67(9)**: 381-387.

محلول پاشی ۶-بنزیل آمینوپورین و آبسزیک اسید بر تبادل گازی، ویژگی های بیوشیمیایی و تولید دو رقم ریزغده سیب زمینی (*Solanum tuberosum* L.) موثر است

م. ج. احمدی لاهیجانی، م. کافی، ا. نظامی، ج. نباتی، و ج. اروین

### چکیده

در این پژوهش، امکان بهبود ویژگی های فیزیولوژیک و عملکرد ریزغده دو رقم سیب زمینی (رقم های آگria و فونتانه) توسط کاربرد دو تنظیم کننده رشد (بنزیل آمینوپورین، آبسزیک اسید و کاربرد توام آن-ها) در مرحله شروع غده دهی مورد ارزیابی قرار گرفت. کاربرد بنزیل آمینوپورین میزان فتوسنتز، عملکرد کوانتومی، هدایت روزنه ای و میزان تعرق هر دو رقم نسبت به گیاهان شاهد را افزایش داد. بیشترین محتوای کلروفیل برگ در رقم آگria در تیمار بنزیل آمینوپورین اندازه گیری شد، در حالی که، در مورد فونتانه بیشترین محتوای کلروفیل در گیاهان تیمار شده با آبسزیک اسید اندازه گیری شد. افزایش محتوای کلروفیل و میزان فتوسنتز برگ با افزایش محتوای کربوهیدرات محلول برگ ها همراه بود. کاربرد همزمان بنزیل آمینوپورین و آبسزیک اسید موجب افزایش محتوای کربوهیدرات محلول برگ هر دو رقم شد. عملکرد در بوته، میانگین وزن و تعداد غده نیز تحت تاثیر کاربرد تنظیم کننده های-رشد قرار گرفت. همبستگی مثبت و معنی داری بین محتوای کربوهیدرات محلول و عملکرد در بوته ( $r=0/97^*$ ) و میانگین وزن تک غده ( $r=0/97^*$ ) در رقم آگria مشاهده شد. سطح برگ، وزن تر و وزن خشک اندام های هوایی گیاهان تیمار شده با بنزیل آمینوپورین+آبسزیک اسید نسبت به گیاهان شاهد بیشتر بود. این نتایج نشان می دهد که کاربرد این تنظیم کننده های رشد می تواند به طور موثری در بهبود ویژگی های فیزیولوژیک و عملکرد این دو رقم مورد استفاده قرار گیرند، البته رقم آگria پاسخ بیشتری به این تیمارها از خود نشان داد.