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Comparison of Kinetin and 6- Banzyl Amino Purine Effect on *in vitro* Microtuberization of Two Cultivars of Potato (*Solanum tuberosum* L.)

E. Aryakia and Y. Hamidoghli

Department of Horticultural Sciences,
Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

Abstract: In the present study, *in vitro* microtuberization of two potato cultivars, "Arinda" and "Diamant" was investigated. The basal medium consisted of the Murashige and Skoog medium supplemented with 80 gl^{-1} sucrose and 8 gl^{-1} agar. The effects of 4 concentrations (0.25, 0.50, 0.75 and 1 $mg.l^{-1}$) of kinetin and 6- banzyl amino purine (BAP) on physical characteristics such as number, size and weight of potato microtuber were investigated. Sixty days after culture, microtubers were harvested and data analyzed. Results showed that the highest effect on microtuber number especially in cv. Diamant with an average number 1.49 per single node explants, was obtained due to kinetin role in tuber initiation. BAP especially in high concentrations (0.75 and 1 $mg.l^{-1}$) have an incremental effect on weight and size of microtuber. Since a large size of microtuber is suitable for commercial production, high concentration the BAP (0.75 and 1 $mg.l^{-1}$) for increasing of microtuber weight is recommended.

Key words: Microtuber · Potato · Kinetin · BAP

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important agricultural plants all over the world. Its annual worldwide output is just after rice (*Oryza sativa*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) [1]. Among the six main food crops in the world, potato produces both the most protein and calories [2, 3]. In some countries, potato constitutes the main daily food due to their low price and highly nutritive value [1]. Tubers are the most common source of planting material in potato reproduction. Approximately 15% of the total area under potato cultivation around the world is used for the production of tuber seeds that can be considered [2]. However, tubers formed through these conventional methods are susceptible to pathogen infections, thereby resulting in poor quality and yield and are difficult to transport and store due to their large size [3].

Plant tissue culture is the only technique that can eliminate viruses in tuber seed production programs and microtuber is one of the strategies in this perspective [4]. Microtubers can now be produced and stored in the laboratory year-round in order to be directly transported to market without transferring to fresh media [2, 3]. Cytokinin has been predominantly used for microtuber production [4]. Since the effect of Cytokinins in potato microtuberization has been established, in this experiment, influence of four concentrations (0.25, 0.50, 0.75 and 1 $mg.l^{-1}$) of two Cytokinin hormones, kinetin (Kin) and 6-benzyl amino purine (BAP) on physical characteristics such as number, size and weight of potato microtuber was investigated. The objective of this study is to determine the best concentration of two cytokinin contents, BAP and Kin, on increasing potato microtuberization of cv. "Arinda" and "Diamant".

MATERIALS AND METHODS

Shoot cultures of cv. "Arinda" and "Diamant" those were already disinfected for PVY and PLRV viruses and kindly provided by Ghaedsharaf [5]. For plantlet proliferation, single nodes were used as explants and cultured in test tubes (25×150 mm) containing 15 ml of solidified (0.8% agar) Murashige and Skooge (MS) medium supplemented with 3% sucrose and filter-sterilized 1 $mg.l^{-1}$ gibberellic acid (GA_3). The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were incubated at 25±1°C under 16 h photoperiod (fluorescent, 100 $\mu mole/m^2/s$). After a 4-week incubation, single-node cuttings were subcultured.

Corresponding Author: Y. Hamidoghli, Department of Horticultural Sciences, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran, Tel: (+98)9113315897, E-mail: hamidogli@guilan.ac.ir.

Microtuberization: The experiment was carried out on sub-cultured single-node explants. The basal media consisted of MS medium supplemented with 8% sucrose and different concentrations of Kin and BAP (0.25, 0.50, 0.75 and 1 mg/l). Five nodes were transferred into each jar containing 40 ml of the above media with 3 replicates for each cytokinin concentration treatment. The cultures were then incubated in complete darkness at 20±1°C. After 60 days, the number, fresh weight and size of harvested microtubers were measured.

Statistical Analysis: This experiment was arranged in factorial completely randomized design tree replications with fifteen samples per replicate. SAS software was used for data analyses, Excel software was used for designing graphs and Tukey's test was used for comparison of mean values.

RESULTS AND DISCUSSION

Number of Microtuber: Data analyses of variance showed that the main effects of cultivars, hormone concentrations and hormone content were significant on microtuber number (p= 0.01). Cultivar × hormone content interaction was also significant (p= 0.01) but cultivar × hormone concentration, hormone content × hormone concentration and finally cultivar × hormone content × hormone concentration interactions were not significant on microtuber number.

As indicated in Table 1, Comparison of means showed that cv. "Diamant" with number of 1.495 induced more microtuber number than cv. "Arinda" with 1.247 microtuber (p= 0.01). Many of researchers have shown that cultivars have a different potential in production of microtuber [6, 7]. It seems that in the same condition, genotypic potential of cultivars has the greatest effect and will result in different yields because of innate capacity of the genotypes in production of endogenous levels of growth regulators. The main factor of microtuber initiation and induction are different, since increase in BAP concentration, the number of genotypes responding to microtuberization decreases [7] whether some cultivars of the potato genus have not potential for microtuber production [8].

Comparison of means showed that hormonal content had a main significant effect on the microtuber number since the Kin with mean number of 1.459 produced more microtuber than BAP with the mean number of 1.283. Effect of Kin on microtuberization is due to its relationship with the ethylene biosynthesis [9]. Because Kin mainly influenced the microtuber initiation, therefore microtuber number increased; besides, effect of phytohormones on microtuberization parameters depends on plant genotype and the amount of sucrose in media [8, 10]. Hormone concentration of 0.25 $mg.l^{-1}$ with the mean number of 1.641 induced the highest microtuber.

Because cytokinins interfere with cell division, induction and production of potato were increased [10] and among the various concentrations, with an increase in concentration, size and weight of microtuber increased since there is linear relation between them [11] but the mean number of microtuber were decreased because the external using of hormone disturbs the balance of endogenous levels of growth regulators [7].

Eventhough it proved "Diamant" genetically produced microtuber more than "Arinda" but in the present study there were no significant differences between BAP and Kin on microtuber production of "Diamant" (Fig. 1).

In the case of "Arinda", Kin because of its effect on microtuber initiation induced more increase in microtuber number [8]. BAP albeit mainly increased microtuber weight [2].

Weight of microtuber: Data analysis of variance showed that the effect of hormone content and hormone concentration was significant (p=0.01).

Table 1: Comparison mean of the main effects of cultivar, hormone concentration and content on number, weight and size of produced mirotubers

		Characters		
Treatment		Microtuber number	Microtuber weight (mg)	Microtuber size (mm)
Cultivar	Diamant	1.495[a]	118.057[a]	5.136[a]
	Arinda	1.247[b]	113.698[a]	5.111[a]
Hormone content	BAP	1.283[a]	134.345[a]	5.326[a]
	Kin	1.459[a]	97.411[b]	4.920[b]
Hormone concentration	0.25	1.641[a]	91.797[b]	4.876[b]
	0.50	1.375[ab]	105.206[b]	4.98[b]
	0.75	1.235[b]	135.461[a]	5.360[a]
	1	1.233[b]	131.048[a]	5.277[a]

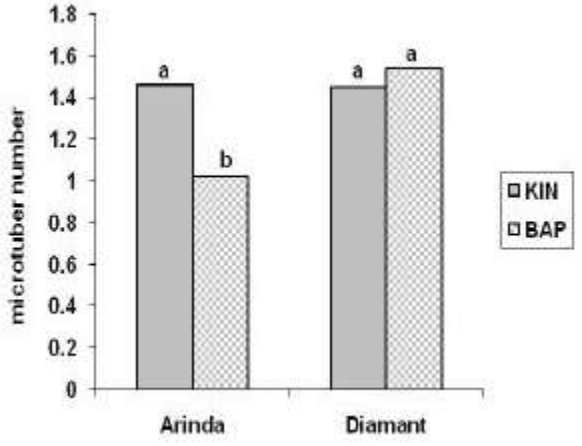

Fig. 1: Cultivar × hormone content interaction effect on microtuber number

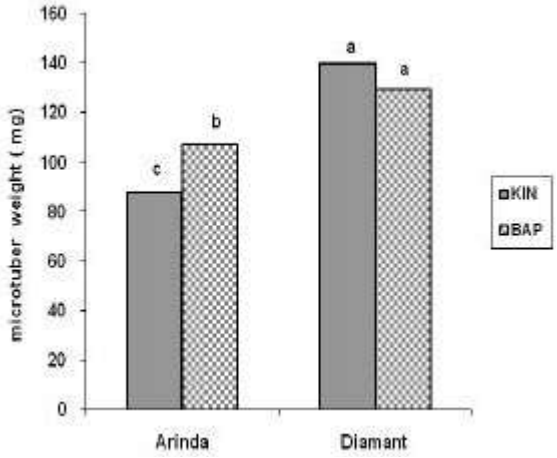

Fig. 2: Cultivar × hormone content interaction effect on microtuber weight

Effect of cultivar on microtuber weight was not significant. Data showed that cultivar × hormone content interaction on microtuber weight was significant (p=0.01). Cultivar × concentration, hormone content × concentration and cultivar × hormone content × concentration interaction effects on microtuber weight were not significant.

Comparison of means showed that there is significant difference in hormone contents (p=0.01) since BAP with mean weight of 135.345 mg resulted in a more microtuber weight than Kin with mean weight of 97.411 mg (Table 1). This is in accordance with the previous results [2]. They found that 0.75 mg.l^{-1} BAP produced highest microtuber weight while 0.25 mg.l^{-1}Kin resulted the least one . They also reported that with addition of BAP in medium, microtuber weight was increased [12]. In most cases, Kin produced no significant change in tuber size and mainly increased microtuber number [8]. Kin had no significant effect on growth, diameter ant weight of microtuber [10]. So it can be deducted that in comparison to Kin, BAP has the highest effect on increase of microtuber weight.

Comparison of means showed that there is a significant difference for microtubers weight between concentrations of 1 and 0.75 mg.l^{-1} with concentrations of 0.25 and 0.50 mg.l^{-1} (p=0.01).

Although there is no significant difference between concentrations of 1 and 0.75 mg.l^{-1} but concentration of 0.75 mg.l^{-1} with mean weight of 135.641 mg induced maximum yield (Table 1) that is conformed with Amina and Shoaib (2006). They found that 0.75 mg.l^{-1} BAP produced highest microtuber weight. Jasmonic acid increased microtuberization because of antagonistic effect on GA_3 activity while in advance it is noted that its effect is due to increase level of cytokinin activity [13]. It emphasies the special role of cytokinin. Endogenous levels of cytokinin in final phases of microtuber formation are high [14]. Cytokinins increase starch aggregation in tubers (contain of 70 percent of total solid substance) [15]. So with cytokinin increase in media, we can increase microtuber weight.

Stolon respond to high concentration of cytokinin depends on its interaction with other hormones specially GA_3 [16]. Cytokinins cause to increase microtuber formation but GA inhibits the growth of tuber [17]. So we can conclude, increase in cytokinin concentration is parallel with decrease of endogenous GA_3 as high concentration of cytokinin (1 and 0.75 mg.l^{-1}) than low concentration (0.25 and 0.50 mg.l^{-1}) cause increse in cell division and with increase in starch aggregation in tuber (containing of 70 percent of total solid substance), parameters of microtuberization such as fresh weight of microtuber were increased.

Both Kin and BAP in "Diamant" had the highest effect on increasing microtuber weight than "Arinda (p=0.01) and BAP effect on increasing microtuber fresh weight was more than its effect on "Arinda"(Fig. 2). In "Arinda", BAP produced more microtuber fresh weight and Kin had the least effect which conformed with Amina and Shoaib (2006) because in comparison to BAP, Kin mainly increased microtuber number [8].

Microtuber Size: Data analysis of variance showed that main effect of hormone content and hormone concentration were significant (p=0.01). Main effect of cultivar on microtuber size was not significant. Data showed that cultivar × hormone interaction on microtuber

size was significant (p=0.01). Hormone content × concentration, cultivar × hormone concentration and cultivar × content × concentration interaction effects on microtuber size were not significant.

Comparison of means showed that hormone content influenced the microtuber size (p=0.01) as BAP with mean size of 5.326 mm is first and Kin with mean size of 4.92 mm is the next that is conformed with Amina and Shoaib (2006) (Table 1).

They found that 0.75 mg.l^{-1}BAP induces micotuber size more while 0.25 mg.l^{-1} Kin induces the least effect. It is reported that big microtubers are suitable for commercial production, because we can plant these tubers in the field without acclimation and long storage which reduced microtuber weight [18].

There is a linear relation between size and weight of microtuber [11], means each factor that influence microtuber weight, directly influence microtuber size. In many cases, Kin usually induce no significant change in microtuber size while in presence of IAA, microtuber growth prominently increased [8]. Among cytokinins, benzyl adenine (BA) has more potential for microtuberization than Kin and have promoted effect on reduction of total sugar and subsequently have increased starch content [15]. Although cytokinins such as BA have antagonistic effect on jasmonates, microtuber yielded from Kin in comparison to microtuber yielded from jasmonic acid (JA), has smaller size [15]. on the other hand Kin and thidiazuron induce no significant difference on diameter, weight and growth of microtuber [10]. So we can conclude that BAP in comparison to Kin shows more potential for increasing microtuber size.

Hormone concentration influenced the size of microtuber as concentrations of 1 and 0.75 mg.l^{-1}were significant in comparison to concentrations 0.25 and 0.50 mg.l^{-1}(p=0.01). Concentration of 0.75 mg.l^{-1} with mean size of 5.365 mm was highest and concentrations of 1, 0.50 and 0.25 mg.l^{-1} respectably with mean size of 5.277, 4.98 and 4.876 mm were the next, the results which conform with Amina and Shoaib [2] that they related its cause to genotypic differences (Table 1). As comparison means of data showed that with increasing cytokinin concentration, microtuber size increased.

Increasing parameters of microtuberization on short days and low temperature, which induce cytokinin production, are simultaneous with reduce level of GA_3. GA_3 inhibits microtuberization process [19]. Stolon respond to high level of cytokinin in microtuberization process depended to its interaction with other hormones specially GA_3 [16]. So we can conclude with increasing cytokinin concentration and reduce activity of GA_3 microtuberization parameters such as microtuber size increases.In both cultivars, BAP had highest effect on increasing microtuber size and its effect in "Arinda" was more than "Diamant" (Fig. 3).

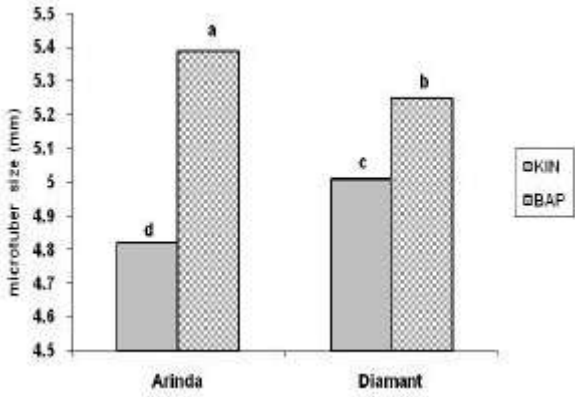

Fig. 3: Cultivar × hormone content interaction effect on microtuber size

Kin has the least effect in increasing microtuber size of both cultivars and in "Arinda" this effect was lesser since Kin with attention to its role on tuber initiation, mainly increase microtuber number. This result is in accordance to Amina and Shoaib [2]. They conclude that concentration of 0.75 mg.l^{-1} BAP produces more microtuber size while concentration of 0.25 mg.l^{-1} Kin has the least effect. As it was mentioned cultivar × hormone content interaction was significant which implys to importance of genotypic charecteristics to maximize microtuberization [7].

CONCLUSION

With attention to high consumption of potato in our country, it is necessary to work enough to optimize its production. Of those we can mention production of virus free potato plantlets which increase production of crop at 30%. In this process, microtuber production is important. Because increasing weight and size of microtuber make advantage in field performance, in comparison to microtuber number, effort for increasing microtuber weight would be in priority. Results of this study suggest that high concentration of BAP (in this experiment 0.75 and 1 mg.l^{-1}) can be used for *in vitro* microtuber produntion because it results in increasing microtuber weight and size more than others.

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