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Evaluation of BAP Effects on Plantlets Micro Tuberization of Five Potato Cultivars

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Author's contributions

This work was carried out by Author MSP, who designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript, managed the analyses of the study and the literature searches. The author read and approved the final manuscript.

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ABSTRACT

The effects of BAP on the micro tuberization were evaluated using five commercial cultivars of potato. Potato tubers were planted in the greenhouse and young shoots were cut and transferred to Tissue Culture Laboratory of Agricultural Research Institute. Apical and axillary bud explants were cultured on liquid MS medium containing 2 mgl^{-1} GA_3. The biological samples were subculture every four weeks on the same medium for plantlet production and propagation. Then, for plantlets micro tuberization, plantlets were cultured on liquid MS medium with different concentrations of BAP (0, 5, 10, 15 and 20 mgl^{-1}). After eight weeks characteristics, such as the number of micro tubers, micro tuber fresh weight, micro tuber dry weight, the mean of micro tuber diameter, the mean of eyes number, the mean of small micro tuber diameter, the mean of large micro tuber diameter, the mean of eyes number on the small micro tubers and the mean of eyes number on the large micro tubers were recorded. Morphological parameters of micro tubers varied significantly among cultivars and BAP concentrations at $P < 0.05$ or $P < 0.01$. Results indicated that cultivars produced significantly higher number of micro tubers in the presence of BAP. For the tested cultivars, micro tuberization was maximum in liquid MS medium containing 5 mgl^{-1} BAP and it was lowest in 0 mgl^{-1} BAP.

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Keywords: BAP (6-benzylaminopurine); in vitro; plant growth regulator; potato; micro tuberization.

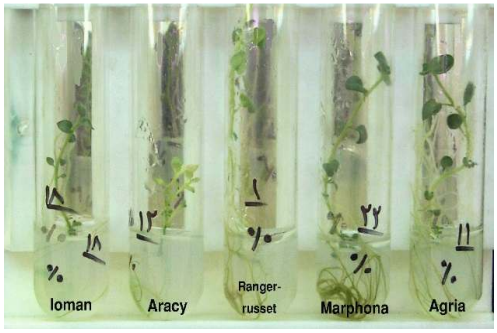
1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world and is ranked fourth after rice, wheat and maize in terms of total production of fresh weight [1]. Micro propagation is a potential biotechnological tool that has become a commercially viable method of in vitro (clonal) propagation of a wide range of herbaceous (mostly) and woody plant species [2]. It has been proved that micro propagation is a very efficient technique to speed-up the production of high quality pathogen-free plantlets, in terms of genetic and physiological uniformities at competitive costs [3-5]. In potato, various tissues can be used as explants for direct shoot formation [6,7]. The most important step before the inoculation of explants is surface disinfection [8]. Segments of potato sprouts were used as the experimental plant material and were surface disinfected with 10% commercial bleach (Tween-20) for 10 minutes [9, 10]. Disease-free and genetically uniform plantlets may be produced by meristem culture through tissue culture techniques [11,12]. Researchers reported that some viruses can decrease the yield by 40% solely and in combination with other viruses the loss is 90%. Therefore, production of virus-free plantlets and micro tubers is important. Meristem culture provides a reproducible and economically viable method for producing pathogen-free plants. As meristem tips are free from viruses, production of virus-free plants are possible through meristem culture [13]. There were many reported media formula, which were used for in vitro propagation of potato [9,14-22]. It has been reported the effects of different concentrations of GA_3 on micro propagation and suggested that the dosage of 0.248 mgl^{-1} of GA_3 boosted all the morphological characteristics over control and other treatments. It was suggested that this level (0.248 mgl^{-1}) could be used as standard dose for micro propagation of potato [11]. it was indicated that the best medium for primary establishment of meristem for 'Agria' was MS medium containing 0.5 mgl^{-1}, the best media for subculture of plantlets of 'Agria' was liquid MS medium containing 1 mgl^{-1} GA_3 [23]. Also MS medium was used with different concentration of KIN and IAA for root proliferation [9,24]. Different concentration of NAA and BAP were used for root induction and studied that application of BAP and NAA decreased shooting and rooting of single nodes [25]. Micro tubers are an ideal propagating material for producing high-quality seed potatoes [26]. Micro tubers have several merits over in vitro plantlets due to their little size, reduced weight and vigorous nature. Micro tubers offer advantages of small space accommodation, ease of transport and storage in addition to solving the problems of transplanting of plantlets [27]. Micro tubers are also useful in other applications, including germplasm storage and exchange, or as experimental research tools in the areas of plant metabolism, germplasm selection and evaluation, transformation, somatic hybridization or molecular farming, and for in vitro selection of agronomical important characteristics, such as maturity and abiotic stress tolerance [28]. It was indicated that micro tuber induction of potato was highly dependent on sucrose and genotype interaction [29]. The use of micro tubers instead of in vitro plantlets is advantageous, as they are easier to store and handle and do not require any acclimatization treatment [30]. In general, the use of micro tuber technology appears to have enormous potential in seed tuber production, breeding programs, germplasm conservation and research. The technology helps to reduce the time necessary to supply seed tuber of greatly improved quality in large scale with low cost [31]. In this experiment, the effect of different concentrations of BAP in the MS medium on micro tuberization in five cultivars of potato was studied.

2. MATERIALS AND METHODS

Potato tubers of five cultivars, i.e. 'Loman', 'Aracy', 'Ranger-russet', 'Agria' and 'Marphona' were planted in the greenhouse and young shoots were cut and transferred to the Tissue Culture Laboratory of Agricultural Research Institute. Meristems isolated from tuber sprouts were sterilized by immersion for several seconds in 70% ethanol followed by immersion in mercuric chloride 0.1% for 2 minutes and rinsed 5 times with sterile, distilled water. The tip and sub-tending leaf primordia were removed with scalpel and needle. Afterwards apical and axillary bud explants were cultured on liquid MS medium (the medium pH was 5.8±0.1before autoclaving at 121℃ and 1-5 kg/cm for 25 min) containing 2.5 mgl^{-1} GA_3 and were maintained in the growth chamber with 24±2℃ temperature and a light period of 16 hours (irradiance of 100 µmol m^{-2} s^{-1}) and 8 hours darkness period [32]. The samples were sub cultured every four weeks on the same medium for plantlet production and

propagation. To study the effects of BAP on the plantlets micro tuberization, plantlets were cultured on liquid MS medium with different concentrations of BAP containing 0, 5, 10, 15 and 20 mgl^{-1} BAP and 80 gl^{-1} sucrose. Cultures were grown in complete darkness at 22±1℃ in a diurnal growth incubator for two months (Fig. 1). The experimental design was a completely randomized design (CRD) with four replications, three plantlets per one replication. The treatment design was a 5 x 5 factorial with five cultivars and five BAP levels for a total of 25 treatments. After eight weeks, characteristics, such as the number of micro tubers, micro tuber fresh weight, micro tuber dry weight, the mean of micro tuber diameter, the mean of eyes number, the mean of small micro tuber diameter, the mean of large micro tuber diameter, the mean of eyes number on the small micro tubers and the mean of eyes number on the large micro tubers were recorded. Data were subjected to analysis of variance (ANOVA) using statistical analysis system and followed by Duncan's multiple range tests and terms were considered significant at $P < 0.05$ and $P < 0.01$ by SPSS version 22 and MSTATC software.



(a)

(b)

Fig. 1 (a) Test tubes containing plantlets; (b) Micro tuber formation on MS medium supplemented with 5 mgl^{-1} BAP

3. RESULTS AND DISCUSSION

3.1 Variance Analysis

In the present study, micro tuberization of cultivars was studied by five levels of BAP concentration. After eight weeks of culture, morphological parameters of micro tubers varied significantly among cultivars and BAP levels at $P < 0.05$ or $P < 0.01$, according to the analysis of variance.

Results of Duncan's test shown in Tables 1 and 2 indicated that all measured characteristics were positively affected by BAP levels. At the MS medium containing 5, 10, 15 and 20 mgl^{-1}BAP, micro tuberization of cultivars was increased in comparison to the control (0 mgl^{-1} BAP). The range of the micro tuberization was positively correlated to the BAP levels in all samples. Therefore, micro tuberization characteristics, such as the number of micro tubers, micro tuber fresh weight, micro tuber dry weight, the mean of micro tuber diameter, the mean of eyes number, the mean of small micro tuber diameter, the mean of large micro tuber diameter, the mean of eyes number on the small micro tubers and the mean of eyes number on the large micro tubers were also increased in all samples subjected to the presence of BAP. Usage of plant growth regulators was essential in potato tissue culture and micro tuberization [33-37]. BAP had a major effect on micro tuberization in all samples. The most intensive micro tuberization was achieved at 5 mgl^{-1} BAP level. The study also showed that when BAP was not used, micro tuberization did not increase. Similarly, other researchers also presented the necessity of adding BAP in 5-10 mgl^{-1} BAP concentration into the culture medium [4,10,15,26,29,30,33].

Results presented that mean of fresh and dry weight of micro tubers were significantly higher in 'Agria'. Micro tuberization of studied potato cultivars was affected by BAP concentrations and it indicated that micro tuberization was limited in control treatment (0 mgl^{-1} BAP). Data indicated that 'Agria' and 'Marphona' produced significantly higher number of micro tubers for almost all BAP concentrations. For these cultivars, micro tuberization were maximum in liquid MS media containing 5 mgl^{-1} BAP and was lowest in 0 mgl^{-1} BAP.

Phenotypic correlations among characteristics indicated that there were highly significant negative correlations at $P<0.01$ between the

number of micro tubers with the mean of micro tuber diameter. There were highly significant positive correlations at P<0.01 between the micro tuber fresh weight and the micro tuber dry weight, and also between the mean of eyes number and the mean of eyes number on both large and small micro tubers, and between the mean of micro tuber diameter with micro tuber fresh weight and the micro tuber dry weight, and between the mean of micro tuber diameter and the mean of large micro tuber diameter. Significant positive correlations were also observed at p<0.05 between the micro tuber fresh weight and the mean of large micro tuber diameter, and between the micro tuber dry weight and the mean of large micro tuber diameter, and between the mean of micro tuber diameter and the mean of eyes number on large micro tubers, and between the mean of large micro tuber diameter and the mean of eyes number on large micro tubers, and between the mean of small micro tuber diameter and the mean of eyes number on small micro tubers (Table 3). Phenotypic correlations indicated that some associated genetic factors correlate with each other and contribute in the occurring of these characteristics.

Table 1. Means comparison of BAP levels for micro tuberization characteristics using Duncan's test at P < 0.01

BAP levels (mgl^{-1})	NM	MMD	MEN	MFW	MDW	MLMD	MSMD	MENLM	MENSM
5	8.4a	45a	4.7a	1738a	334.8a	69.35a	39.23a	5.403a	4a
10	6.4b	45a	4.9a	1590a	302.4a	67.78a	37.75a	5.583a	4.014a
15	5.9bc	43a	4.9a	1196b	214b	63.98a	33.54a	5.611a	4.028a
20	5.1c	40ab	4.9a	993.7bc	168.1bc	57.47ab	27.46ab	5.722a	4.097a
0	3.6d	31.5b	4.1b	734.4c	124.5c	45.82b	15.73b	4.722b	3.306b

NM = Number of micro tubers, MMD = The mean of micro tuber diameter, MEN = The mean of eyes number, MFW = Micro tuber fresh weight, MDW = Micro tuber dry weight, MLMD = The mean of large micro tuber diameter, MSMD = The mean of small micro tuber diameter, MENLM = The mean of eyes number on the large micro tubers, MENSM = The mean of eyes number on the small micro tubers

Table 2. Means comparison of potato cultivars using Duncan's test at P < 0.01

Cultivars	NM	MMD	MEN	MFW	MDW	MLMD	MSMD	MENLM	MENSM
Agria	10.25a	0.7064b	9.533a	79.79a	229a	3.044b	20.74abc	10.5a	97.06bc
Marphona	9.778a	1.003a	7.039b	15.39b	190.9ab	13.59a	15.28bc	1.611b	70.15c
Ranger-russet	8.444b	0.5509c	5.017c	12.46bc	156.8abc	2.675b	23.06ab	1.583b	112.8b
Aracy	7.778b	0.2336e	3.383d	8.172bc	117.2bc	0.4278c	28.28a	0.8333b	172.9a
Loman	4.944c	0.4099d	1.764e	1.889c	91.98c	2.6b	13.51c	0.4167b	30.83d

NM = Number of micro tubers, MMD = The mean of micro tuber diameter, MEN = The mean of eyes number, MFW = Micro tuber fresh weight, MDW = Micro tuber dry weight, MLMD = The mean of large micro tuber diameter, MSMD = The mean of small micro tuber diameter, MENLM = The mean of eyes number on the large micro tubers, MENSM = The mean of eyes number on the small micro tubers

Table 3. Phenotypic correlation of micro tuberization characteristics

Characteristics	NM	MMD	MEN	MFW	MDW	MLMD	MSMD	MENLM	MENSM
NM	1	-0.787^{**}	0.016^{ns}	0.811^{**}	0.751^{**}	-0.265^{ns}	0.351^{ns}	-0.226^{ns}	0.245^{ns}
MMD		1	0.281^{ns}	0.987^{**}	0.823^{**}	0.806^{**}	0.660^{*}	0.547^{*}	0.501^{ns}
MEN			1	0.228^{ns}	0.437^{ns}	0.505^{ns}	0.295^{ns}	0.891^{**}	0.859^{**}
MFW				1	0.839^{**}	0.559^{*}	0.602^{*}	0.003^{ns}	0.451^{ns}
MDW					1	0.510^{*}	0.937^{**}	0.209^{ns}	0.589^{*}
MLMD						1	0.178^{ns}	0.693^{*}	0.190^{ns}
MSMD							1	-0.042^{ns}	0.597^{*}
MENLM								1	0.561^{ns}
MENSM									1

*ns = no significant, * significant (P < 0.05), ** significant (P < 0.01)*

NM = Number of micro tubers, MMD = The mean of micro tuber diameter, MEN = The mean of eyes number, MFW = Micro tuber fresh weight, MDW = Micro tuber dry weight, MLMD = The mean of large micro tuber diameter, MSMD = The mean of small micro tuber diameter, MENLM = The mean of eyes number on the large micro tubers, MENSM = The mean of eyes number on the small micro tubers

4. CONCLUSION

Micro tuberization of cultivars was studied by five levels of BAP concentration. Morphological parameters of micro tubers varied significantly among cultivars and BAP levels. The range of the micro tuberization is positively correlated to the BAP levels. The most efficient micro tuberization was achieved in 5 mgl^{-1} BAP level. Micro tuberization of the studied potato cultivars is limited without the presence of BAP. Results indicated that 'Agria' and 'Marphona' produced a significantly higher number of micro tubers for almost all BAP concentrations. For these cultivars, micro tuberization was maximum in liquid MS media containing 5 mgl^{-1} BAP and was lowest in 0 mgl^{-1} BAP. Similar conclusion was reported by [15,29,30,33,35]. Phenotypic correlations indicated that some associated genetic factors correlate with each other and contribute in the occurring of these characteristics.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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