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EFFECT OF VARIOUS CONCENTRATIONS OF PLANT REGULATORS ON MERISTEM CULTURE OF POTATO CULTIVARS (*SOLANUM TUBEROSUM* L.)

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ABSTRACT

Large quantity of potato seed is produced by *in vitro* virus free mini tubers worldwide. Therefore, evaluation of commercial varieties for production of virus free potato mini tuber is critical. In the current study, the effects of various combinations of Abscisic acid (ABA) and Gibberellic acid (GA) each at three levels i.e. 0.5 , 1.0 and 2.0 mg liter^{-1} were evaluated on meristem culture of two potato cultivars namely "Agria" and "Savalan". Meristems were excised from plantlets grown in wooden boxes. The meristems were then transferred into tubes containing 2 mL liquid MS medium (Prematilake and Mendis, 1999) containing above mentioned growth hormones. Meristems were placed on the M bridges. Then the tubes were placed in germinator with 25 ± 2°C and 18:6 hours Day : Night photoperiod. After 3 weeks, the plantlets were sub-cultured into jars with 30 mL semi-solid medium with same treatments. In this study, the plantlets were sub-cultured twice again each three weeks. At the end, data on morphological traits such as stem length, bud and shoot number were recorded. The results showed that there existed significant differences among treatments. For stem length, there was a significant difference between varieties, various concentrations of ABA and GA and also double and triple interactions between factors. For bud number, the varieties showed significant differences. GA had a significant interaction with variety and ABA. Shoot number was affected by different varieties and different ABA concentrations.

Keywords: Abscisic acid (ABA), Gibberellic acid (GA), Meristem culture, Potato.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the important vegetable crops and a staple food in many countries. After wheat, rice and maize it is the fourth major cultivated food crop (Moeinil *et al.*, 2011). Potato as an annual herbaceous plant is vegetatively propagated. Tuberization in potato is a highly complex developmental process, which may be modified in various ways. However, during vegetative propagation, tubers are contaminated with different diseases resulting in poor quality and yields. Alternatively, micropropagation methods are ideal for easy multiplication of disease free plant in masses. Since there is more than 20 viruses that infect potatoes and hence meristem culture and tissue culture techniques have been adopted for virus elimination and maintenance and propagation of nuclear seed stocks, respectively (Bryan, 1988; Jones, 1988).

Because meristems are virus free so meristem tip culture is an effective method for the production of virus free plants (Badoni and Chauhan, 2009). We can induce tuberization under *in vitro* condition. In addition, *in vitro* methods can be used for conservation, storage and easy distribution of potato germplasm in the form of breeding lines, new varieties and microtubers. Many researchers used different growth regulators for *in vitro* induction of meristem culture in potato (Hossain and Sultana, 1998). The Gibbberellic acid (GA) and Abscisic acid (ABA) have strong effects on *in vitro* culture (Dodds *et al*., 1988; Tovar *et al*., 1985). Although, there are many reports on potato micropropagation (Badoni and Chauhan, 2009; Rahman *et al.*, 2010; Yousef *et al.*, 2001). Yet it is a well known fact that the response of micropropagated explants is genotype dependent (Abe and Futsuhara, 1986). In potato production the potential value of tissue culture has been widely recognized. In the current study, two cultivars named "Agria" and "Savalan" were used. Extensive physiological research has revealed that several factors, such as hormonal combination, ratio of photo period, nutrient compositions etc. affected tuberization (Coleman *et al*., 2001; Tugrul and Samanci, 2001; Vreugdenhil and Struik, 1989).

The United Nations by calling the crop a “hidden treasure” officially declared the year 2008 as the International Year of the Potato, to raise its profile in developing countries. However, the average yield of potato in Iran is several times lower than that of many European countries. Although potato is being considered as one of the main food crop in Iran, its productivity is disturbed due to infection of fungus, virus and bacterial diseases. The total loss caused by these diseases during cultivation and storage is 30-100%. To overcome these impediments, both conventional and biotechnological breeding programs need to be applied. We can obtain disease free and genetically uniform plantlets by meristem culture through tissue culture techniques (Hoque *et al*., 2007). Over the last 40 years to eliminate

viruses from important cultivars meristem culture technique has been applied to many crops, especially vegetative propagated crops such as potato, (Bhojwani and Razdan, 1983; Hartmann *et al*., 1990). Plant tissue culture techniques have been employed in many important potato varieties in agriculture (Hashem *et al*., 1990). Huda and Sikdar (2006) developed a protocol for *in vitro* plant production through apical meristem culture of bitter gourd (*Momordica charantia* L.). Aasim *et al*. (2008) established a protocol for *in vitro* micropropagation from shoot meristems of Turkish cowpea (*Vigna unguiculata* L.).

The application of tissue culture and rapid propagation method for potato production continues to become more widely used in both developed and developing countries. To increase propagation rates and also modify the germplasm itself while conserving the present resources we can use tissue culture techniques. For several years tissue culture has been applied to improve potato production by means of micropropagation, disease free propagule development, and germplasm conservation (Roca *et al*., 1978; 1979).

The present investigation was carried out to optimize the best combination of growth regulators for the multiplication of local potato varieties "Agria" and "Savalan" using meristem tips as explants; the most regenerative variety could then be efficiently micropropagated for commercial purposes and also molecular studies.

Materials and methods

Plant materials

Two potato varieties "Agria" and "Savalan" were used in this study. Tubers were collected from Vegetable and Integrated Pulses Research Department of Seed and Plant Improvement Institute of Karaj, Iran. Collected potato tubers were sown in the wooden pots filled with soil at 18°C. The distinct sign of sprouting was visible within 15 to 20 days as shown in Fig. 1. A single tuber could be used several times to collect sprouts.

Surface sterilization

Potato sprouting measuring 1.0 to 2.0 cm in length were cut from the tubers and washed with distilled water followed by dipping in 70% ethanol for 30 seconds and immediately thereafter washed with distilled water and subsequently sterilized in the laminar air flow cabinet with 0.1% aqueous solution of $HgCl_2$ for 4 to 7 minutes. Sterilized sprouts were washed 4 to 5 times with sterilized distilled water.

Fig. 1. Growth of sprouts in the wooden pots.

The surface sterilized sprouts were transferred into a sterilized petri dish. The lateral meristems were obtained with the help of a loop, sterilized scalpel and forceps under a laminar air flow. The cuttings based on different treatments were inoculated into liquid MS medium (Basal) with 30 g lit^{-1} sucrose at the top of a paper M bridge. A single meristem was placed in each test tube. The test tubes were incubated under florescent light (2500-3000 lux) at 25±2°C temperature and 18:6 hours (day:night) photoperiod in a germinator. After three weeks, for shoot elongation meristems were transferred to MS semi-liquid medium supplemented with different concentration of ABA and GA, 30g lit^{-1} sucrose and 7g lit^{-1} agarose. The meristems give rise to shoot buds within 2 to 3 weeks as shown in Fig.2. In order to get large scale of shoot apex and nodal segments the shoot buds were sub-cultured regularly after every 3 weeks. For sub-culturing glass jars containing 30 ml semi-liquid MS medium and same treatments were used.

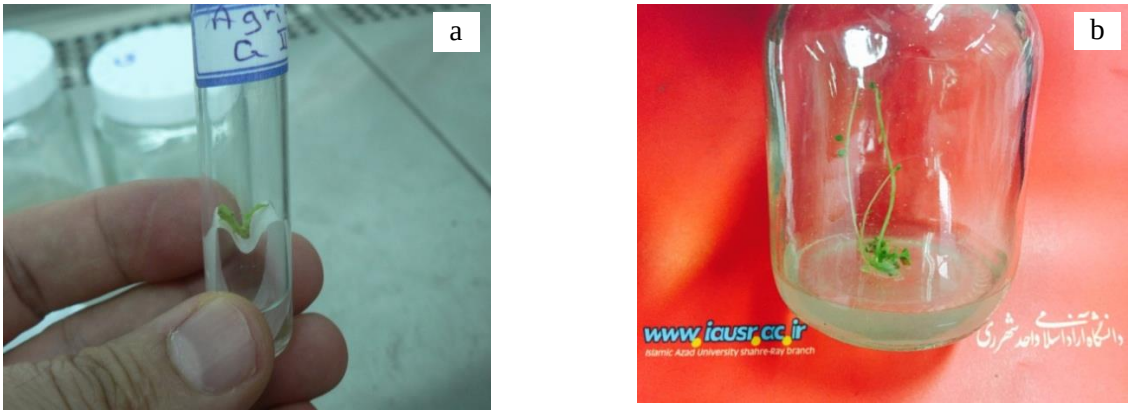

Fig. 2. Growth of meristems on a M bridge (a) and the growth of sub-cultured plantlets in the semi-solid medium (b)

After three sub-culturing, the data on intended traits such as stem length, bud number and number of shoots were recorded. The results were analyzed with SPSS (20) software. The experimental design was factorial based on completely randomized design with three replications. The factors were varieties (two levels), ABA (three levels i.e. 0.5, 1.0 and 2.0 mg lit^{-1}.) and GA (three levels i.e. 0.5, 1.0 and 2.0 mg lit^{-1}.). Thus the number of treatments was 18.

RESULTS AND DISCUSSION

The results obtained in the present study are elaborated as under:

Stem Length

There existed a significant difference between two varieties for this trait (α=0.01). The main effect of Abscisic acid (ABA) and Gibberellic acid (GA) is significant too. All of the double and triple interactions between the factors are significant as well. For ABA the highest and the lowest stem length are 13.78 cm and 7.03 cm with 2.0 and 0.5mg lit^{-1}, respectively. For GA this trait showed 12.19 cm and 9.37 cm by 2.0 and 0.5mg lit^{-1}, respectively (Fig.3 a,b).

Number of buds $plantlet^{-1}$

One of the most important traits in tuberization in potato is the number of buds. In the current study, the bud number showed significant difference between varieties (Table-1). The effect of ABA and GA and also the interactions between variety × ABA and variety × ABA × GA were not significant on the number of buds $plantlet^{-1}$. The results showed that there was a significant difference between variety × GA and also ABA × GA interactions (α=0.01) (Table-1). Mean grouping is mentioned in Table 2. For ABA the highest and the lowest number of buds $plantlet^{-1}$ are 5.28 and 4.89 with 1.0 and 2.0 mg lit^{-1}, respectively. For GA this trait showed 5.44 and 4.72 by 1.0 and 2.0 mg lit^{-1}, respectively (Fig.3 a,b).

Table 1. The results of analysis of variance for morphological traits.

S.O.V	df	Mean Squares		
		Stem Length	Number of Buds	Number of Shoots
Varieties	1	35.155^{**}	24.000^{**}	7.407^{**}
Abscisic Acid (ABA)	2	214.887^{**}	0.722^{ns}	3.852^{**}
Gibberellic Acid (GA)	2	35.796^{**}	2.389^{ns}	1.130^{ns}
Varieties × ABA	2	17.812^{**}	2.167^{ns}	1.407^{ns}
Varieties × GA	2	5.473^{**}	10.500^{**}	2.463^{*}
ABA × GA	4	25.643^{**}	4.194^{**}	1.213^{ns}
Variety × ABA × GA	4	9.380^{**}	1.583^{ns}	2.046^{*}
Error	36	0.659	24.00	0.741

** and * significant in α = 0.01 and α = 0.05, respectively; n.s. non significant

Table 2. The results of mean grouping for different traits

Hormone	Concentration Mg lit^{-1}	N	Stem Length			Number of buds		Number of shoots	
			subset						
			1	2	3	1	2	1	2
Abscisic Acid	0.5	18	7.03			5.17		2.33	
	1.0	18		11.65		5.28			3.22
	2.0	18			13.78	4.89			3.0
Gibberellic Acid	0.5	18	9.37			5.17	5.17	2.83	
	1.0	18		10.90			5.44	3.11	
	2.0	18			12.19	4.72		2.61	

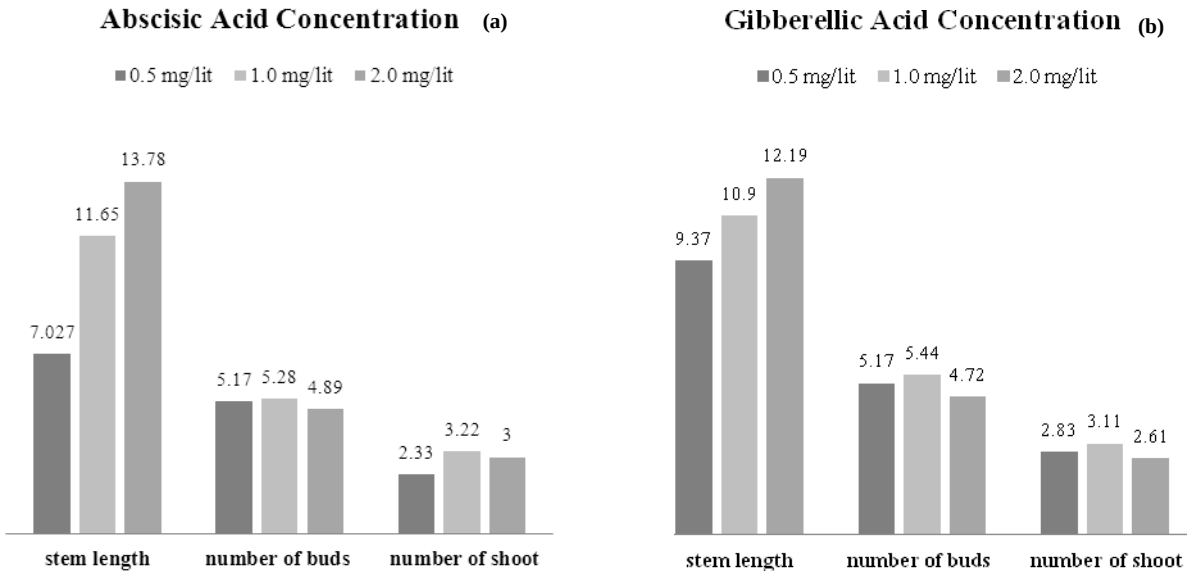

Fig. 3. Effect of ABA (a) and GA (b) on various morphological traits of potato

Number of Shoot

The results of analysis of variance showed that there was a significant difference between two varieties for shoot number (MS=7.407**) and different levels of ABA (MS=3.852**). But the different levels of GA and the interaction effects between variety × ABA and also ABA × GA were not significant (Table 1). Although the interactions between variety × GA and also variety × ABA × GA were significant (α=0.05). The highest number of shoots (3.22) was obtained with 1.0mg lit^{-1} and the lowest number (2.33) was for 0.5mg lit^{-1} ABA, respectively (Table 2). There was no difference between different levels of GA for this trait. For ABA the highest and the lowest number of shoots are 3.22 and 2.33 with 1.0 and 0.5 mg lit^{-1} respectively. For GA this trait showed 3.11 and 2.61 by 1.0 and 2.0 mg lit^{-1}, respectively (Fig.3. a,b).

Conclusion

The results showed that the ABA and GA have significant effects on meristem culture in potato. For purpose of shoot development and hence more stem growth the use of ABA in high concentration is effective. In the current study, the ABA had no significant effect on the number of buds plantlet^{-1}. GA had a significant effect only on stem length, but it had no significant effect either on number of buds or number of shoots. Since there is a significant relation between ABA and GA, it is suggested that complementary studies may be done. Of course, these results are dependent on varieties.

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