



Investigation of GA₃ effect on *in-vitro* micropropagation of potato varieties

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Abstract

In this investigation MS media supplemented with different concentrations of GA₃ viz. 0.00, 0.12, 0.25, 0.50, 0.75mg/L were used for in vitro micro propagation of potato varieties Cardinal and Desiree. The parameters included were percent sprout, plantlet height, number of nodes per plantlet, number of days to root initiation, and number of days to transferable height of the plantlets. Statistical analysis showed that the varieties, GA₃ concentration and varieties vs GA₃ concentration were highly significant for plant height, number of nodes per plantlet and number of days to transferable height of the plantlets. Plantlets developed in M.S media supplemented with 0.25 mg/L of GA₃ for Desiree and 0.12mg/L of GA₃ for Cardinal produce maximum plant height, more number of nodes, reduced no. of days to root initiation and took less number of days to transferable height of the plant. Plantlets developed in these treatments were healthy and vigorous than the plantlets developed in other treatments. The results have shown that sprout percentage was high in variety Cardinal and Desiree proved efficient in response of no. of days to transferable height of the plant.

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Introduction

Potato (*Solanum tuberosum* L.) is tuberous crop, belongs to family Solanaceae. Potato exceeds only by wheat, rice and maize in the world production for human consumption (Ross, 1986; Ben Dowling, 1995). In term of dry matter production per hectare, potato is among the most productive crops (Malik, 1995). Potato gives an remarkably high yield and produces more edible energy and protein per unit area

and time than many other crops (Kaur *et al.*, 2015). Nutritionally potato is best source of carbohydrate. It is major crop of Pakistan and three crops are grown. Because of importance of this crop, the UNO named 2008 as international year of the potato (IYP). During the year 2006-7 the area under potato cultivation was 0.131 million hectare with total production of 2.6 million tons (MINFAL 2006-7). Although there was considerable increase over the time in area and production of crop in the country, the average yield is still lower than the potential. One reason for low yield is the infection of potato plant with large number of fungal, viral and bacterial diseases. Some virus can alone decrease yield by 40%, in combination with other virus the loss is 90% (Siddiqui *et al.*, 1996). The potato is usually propagated by mean of tuber. However with conventional methods of vegetative propagation, potatoes are prone to pathogen i.e fungi, bacteria and virus, thereby resulting poor quality and yield. *In vitro* micro propagation can yield virus free plantlets. By improving tissue culture technique we can increase the yield of potato and other crops. Tissue culture technique provides the material free from virus and other pathogens (Blom Barnhoorn and Aartrijk, 1985). In addition, *in vitro* micro propagation can be used for conservation, storage and easy distribution of potato germplasm (Chaudhary and Mitta, 2014). Efficient plant regeneration depends upon several factors including the composition of culture media, concentrations of growth hormones and especially the genotype of the explants (Madhu *et al.*, 2014). In tissue culture different media are used. MS media is widely used in micropropagation as it shows best results for all vegetable, fruits and ornamental plants.

The different cultivars of same genus may show different response to media. Plant regulators are the important component of the media. The role of balanced plant hormone is also important for maintaining the growth and physiology of potato microplants. These are used in micromolar concentration, but it is very much effective in regulating physiology of plants including height, leaf expansion and stem elongation (Venkatasalam *et al.*, 2015). GA₃ is one of the important plant hormone used in micro propagation. It stimulates growth and elongation of cell. GA promotes cell division by stimulating cells in G1 phase to enter the S phase and by shortening the duration of 'S' phase (Liu & Loy, 1976). Kiapour *et al.*, 2015 reported that the application of gibberellin and titanium nanoparticles improve the negative effects of the stress in basil (*Ocimum basilicum*). Keeping in view of economic value of potato crop and important role of GA₃ on *in vitro* micro propagation of potato the present study was made. Desiree and Cardinal widely popular varieties were chosen for the present study. These varieties are suitable to agro climatic condition of our country. The main focus was to formulate cost effective protocol for rapid multiplication of virus free plantlets in shortest possible time and study the variations in requirement of phytohormone for two varieties.

Materials and methods

Study Site

The research work was conducted at tissue culture lab. Hazara Agricultural research Station Abbottabad, Khyber Pakhtunkhwa, Pakistan.

Study Design, Plant material and treatments

A completely randomized design was used with variety and treatment as two factors. It comprised of five treatments consisting of MS media supplemented with different concentrations of GA₃ viz. T1 (0.00mg/L, T2 (0.12mg/L), T3 (0.25mg/L), T4 (0.50mg/L) and T5 (0.75mg/L) with four replications for each.

The certified seeds of varieties Cardinal and Desiree were obtained from HARS Abbottabad. The plants were grown in sterile conditions and placed in thermotherapy. The meristem tips with 0.3mm length were excised with sterile needle and razor under dissecting microscope and placed in test tubes containing liquid M.S media. The meristem derived plantlets were multiplied through stem cutting containing single node. Nodal culture results in production of true to type and nodal explants had maximum regeneration. For nodal culture the media was solidified with 8g/L agar and 30 gram sugar and pH was adjusted at 5.8 and autoclaved at 121°C for 15 minutes. After autoclaving different concentration of filtered GA₃ was added to five different flasks. GA₃ was added after autoclaving because autoclaving and microwaving appeared to reduce GA₃ activity (Tisserat *et al.*, 1992). Under aseptic conditions in culture room single node cutting were excised from *In vitro* plantlets (Fig.2).

The nodal cuttings were then inoculated on fresh medium (Fig.3). There were five treatments, (T₁, T₂, T₃, 4 & T₅) which were replicated four times. Ten test tubes were cultured for each replication. Thus total 200 test tubes were cultured for each variety. After culturing the test tubes were shifted to growth chamber.

The growth chamber was maintained at 25°C providing 16 hrs photoperiod of 200 lux light intensity. The growth of inoculated explants was monitored regularly.

Measured parameters

The parameters were Percent sprouting, plant height (cm), number of nodes per plant, number of days to root initiation & number of days to transferable height of the plant. The evaluations were performed on weekly basis for four consecutive weeks except for number of days to root initiation. At the end of four week the results were compiled averaged and expressed as a mean for each treatment of GA₃.

Data analysis

Data was analysed statistically by a computer program MSTATC and various treatment means were compared by applying Least Significant Differences (LSD) test (Steel and Torrie, 1980).

Results and discussions

The effect of different concentrations of GA₃ on *in-vitro* different parameters is shown in the table 1. The effect of different concentrations of GA₃ as well as the interaction between cultivar and GA₃ concentrations were significant (p<0.05) for most of the studied parameters.

Table 1. Percent sprout, plant height, & No. of nodes/plant observed at various levels of GA₃ on potato cultivar Cardinal and Desiree.

Variety	Treatments	Percent sprout	plant height	No. of nodes/plant	Days to root initiation	No. of days to transferable height
Desiree	T1	78.03 E	5.00 D	5.00 D	7.22 AB	32.20 A
	T2	79.00 DE	7.75 BC	5.90 BC	6.37 CD	28.53 B
	T3	80.08 D	10.30 A	7.35 A	6.12 CD	23.03 F
	T4	79.50 DE	9.73 A	5.37 BCD	6.00 CD	24.48 E
	T5	79.00 DE	8.53 B	4.25 E	6.25 CD	26.43 D
Cardinal	T1	82.00 C	7.00C	6.00 B	7.50 A	32.13 A
	T2	84.00 AB	10.00 A	7.40 A	6.27 CD	27.00 CD
	T3	85.00 A	9.85 A	6.90 A	5.90 D	28.18 BC
	T4	83.00 BC	8.63 B	5.2 CD	6.00 CD	28.8-0 BC
	T5	82.00 C	7.53 C	4.22 E	6.00 CD	29.25 B
LSD 0.05		1.547	0.9137	0.7832	0.7513	1.249

Means followed by different letters within columns are statistically different at .05 level of significant by using LSD test.

Percent sprouting

The results have shown that both treatment and variety were statistically significant at 5% level of probability. The highest percent sprouting (85.00 %) was obtained from T₃ (0.25 mg L⁻¹ of GA₃) treatment for Cardinal, followed by T₂ and T₄ of the same cultivar. while lowest percent sprouting (78.03%) was observed in the control treatment for Desiree cultivar.

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Fig. 1. Preparation of media.



Fig. 2. Excision of nodes.

The treatment T3 with 0.25 mg L^{-1} of GA_3 was proved as a best treatment for both the cultivars.

The interaction between variety and treatments revealed non-significant difference. The maximum percent sprouting (85%) was recorded in Cardinal in T3, while maximum percent sprouting recorded in the variety Desiree was 80% in T3.

The minimum percent sprouting was found in control treatment of Desiree cultivar. Most of the treatments of the Cardinal showed higher percent sprouting than the Desiree (Fig.9). The difference in the percentage

sprout may be due to genetic diversity. Similar results have been reported by Akhtar *et al.*, (2006) that variety Cardinal shows higher regeneration than Desiree and other cultivars. High regeneration capacity of cultivar Cardinal has been reported by Hussain *et al.*, (2005).

Plantlets height (cm)

The data regarding the plantlet height at different level of GA_3 reflect that plantlets attain more height with increase in the concentration of GA_3 as compared to control treatment but too high concentration of GA_3 resulted to weak and tender plantlets.



Fig. 3. Inoculation of ex plant.



Fig. 4. Desiree plantlets cultured in different concentration of GA₃.

The tallest plantlets (10.3 cm) were produced when (T3) 0.25 mg L⁻¹ of GA₃ was applied to the medium in the cultivar Desiree followed by (T2) 0.12 mg L⁻¹ and (T3) 0.25mg L⁻¹ of GA₃ treatment of Cardinal (Fig.4). T1 (control) of the Desiree produced shortest plant and showed significant difference from rest of the treatments of Desiree and Cardinal.

Interaction between varieties and treatments showed significant difference. The cultivar Desiree produced tallest plants (10.30cm and 9.725cm) when medium was supplemented with 0.25 (T3) and (T4) 0.50 mg L⁻¹ of GA₃ and

Cardinal produced tallest (10cm and 9.85cm) plantlets in the medium with (T2) 0.12 and (T3) 0.25 mg L⁻¹ of GA₃. The plantlets produced in these growth regulator treatments were taller than the control and other treatments (Fig.7).

It was reported by Rabbani *et al.*, (2001) that maximum shoot length was obtained in Desiree plant when 4mg L⁻¹ was applied to the MS medium. In our study it was observed that higher concentration of GA₃ resulted to a weak and narrow stem (Fig.5) which results in the low growth in the green house. It is required that plantlet must be healthy.

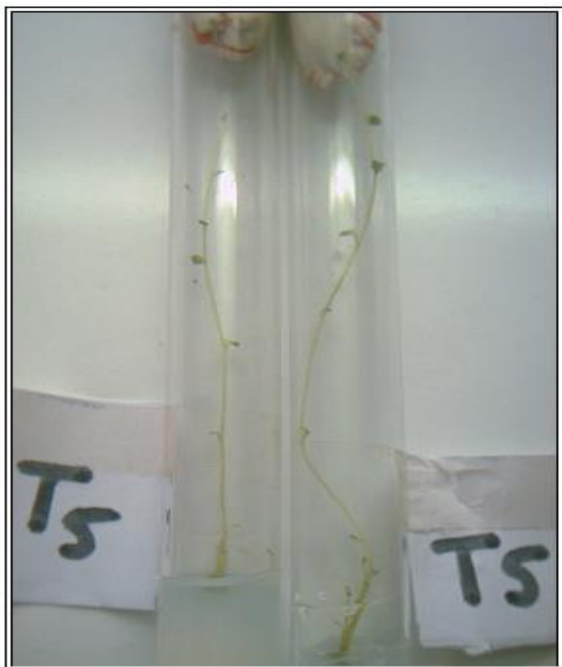


Fig. 5. Cardinal plantlet cultured in T5 produced with long internodes and weak stem.

Therefore 0.25 mg L^{-1} is the best treatment for Desiree and 0.12 mg L^{-1} of GA_3 for Cardinal variety. The plantlets in the control treatments were 5 and 7 cm in the cultivars Desiree and Cardinal respectively. These results are in line with finding of Novak *et al.*, (1983) who studied that medium supplemented with low concentration of growth regulators may be beneficial to shoot growth and Farhatullah *et al.*, (2007) studied that Desiree plantlets produce tallest shoot when medium is supplemented with 0.248 mg L^{-1} of GA_3 .

Number of nodes per plantlets

The results indicated that the cultivars, GA_3 concentration and their interaction were statistically significant at 5% level of probability. Maximum number of nodes per plants (7.40) were produced by GA_3 at 0.12 mg L^{-1} (T2) for Cardinal cultivar, followed by T3 (7.35) of Desiree and T3 (6.9) for Cardinal variety. It was indicated that the moderate dose of GA_3 is effective for increase in number of nodes. Lowest number of nodes was obtained in T5 of Cardinal and T5 & T1 of Desiree variety.

Increase in the number of nodes increases the rate of *in vitro* micro propagation of the plant. It was studied by Rabbani *et al.*, (2001) that number of nodes was not significantly affected by GA_3 concentrations.

In our study it was observed that GA_3 affected number of nodes in moderate concentration while higher concentration of GA_3 did not increase the number of nodes. Our results are in agreement with previous study conducted by Bostan and Demirci (2004). They observed that highest number of nodes was obtained from $0.00/0.50 \text{ mg L}^{-1}$ BA/ GA_3 Granola, Pasinler 92 and $0.00/0.25 \text{ mg L}^{-1}$ BA/ GA_3 treatments for Caspar cultivar of potato. Adhikari *et al.*, (2005) reported that potato cultivar Multa and Petronese developed in a medium with 0.1 ppm gibberellic acid had more number of nodes than the plantlets developed in other media. It can be concluded that treatment (T3) with 0.25 mg L^{-1} of GA_3 is best treatment for cultivar Desiree and (T2) 0.12 mg L^{-1} is the best treatment for Cardinal variety of potato to increase the number of nodes per plantlet. Miller *et al.*, (1985) studied that combination of 1 mg/L of GA_3 + 0.1 mg/L NAA was more effective in increasing the number of node.



Fig. 6. Cardinal plantlets cultured in T2 (Vigorous & healthy plantlets).

Days to root initiation

Data on number of days to root initiation reveals that numbers of days to root initiation were significantly different for different concentration of GA_3 . Interaction among treatments and different concentration of GA_3 showed non-significant difference.

Minimum number of days (5.90 and 6.275) to root initiation was observed in the treatments T3 and T2 of Cardinal variety where as

minimum number of days (6 and 6.12) to root initiation in the cultivar Desiree was observed in the treatments T4 and T3 respectively.

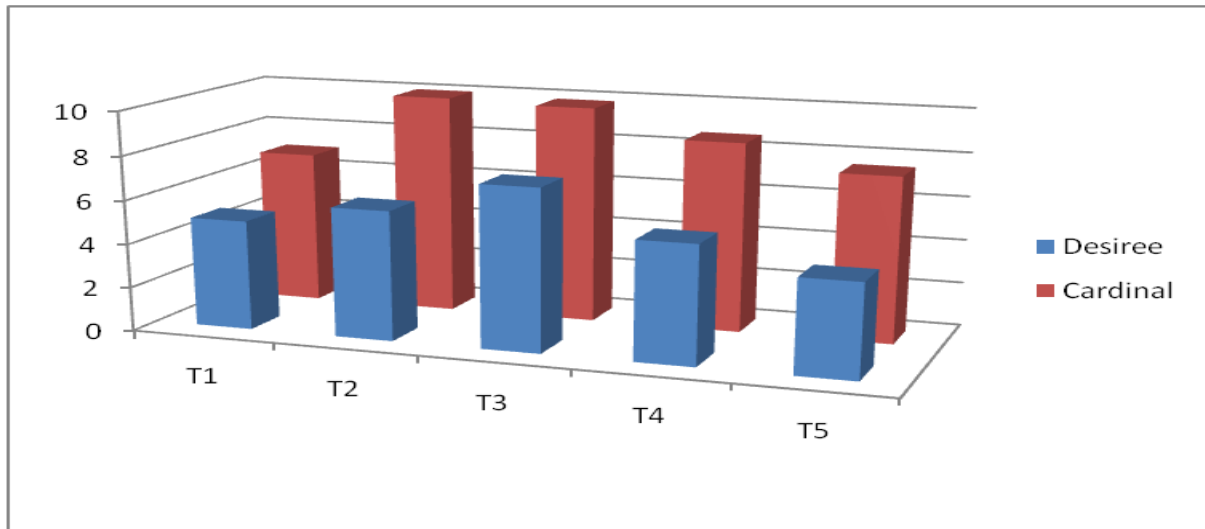


Fig. 7. Effect of GA₃ on Plantlet height of two varieties of potato.

Maximum number of days (7.50) and (7.225) to root initiation was observed in the treatment T1 (control) of cultivars Cardinal and Desiree respectively. These results showed that GA₃ application decrease the number of days to root initiation. The treatments T1 (control) of both cultivars Desiree and Cardinal showed significant difference from most of the treatments of both cultivars. The treatments T3 and T4 for Desiree and T2 and T3 of Cardinal are recommended as the best treatments.

Adhikari *et al.*, (2005) noted that plantlets developed in the MS media supplemented with 0.1 ppm of GA₃ in the cultivar Multa and Petronese have better rooting than the plantlets developed in other media. Faratullah *et al.*, (2007) has studied that the addition of GA₃ to MS medium enhanced root initiation in the cultivar Desiree of potato. Early initiation of root affects the growth of the plantlets. Because the micro propagated plants are transfer to soil, it is required that plantlet must be better rooted.

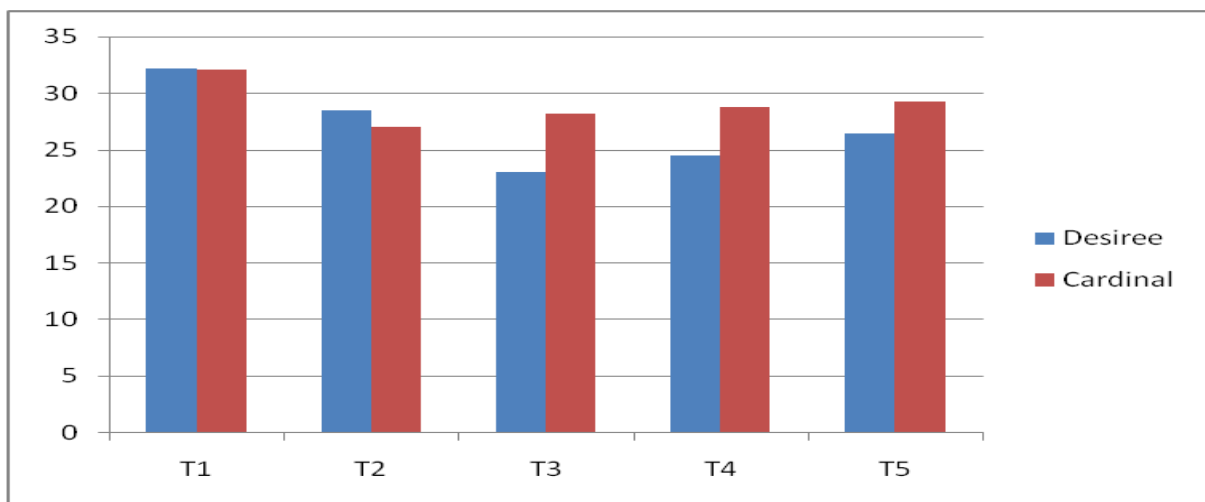


Fig. 8. Effect of GA₃ on number of days to transferable height of the plantlets.

Number of days to transferable height of the plantlets

The data pertaining to number of days to transferable height of the plantlets showed that both the varieties and various concentrations of GA₃ and their interaction were statistically significant at 5% level of probability. Minimum number of days (23.03) to transferable height of the plantlets was obtained at concentration level of 0.25 mg l⁻¹ (T₃)

followed by 0.50 mg L⁻¹ (T₄) of Desiree variety. Maximum number of days (32.20 and 32.13) to transferable height of the plantlets was observed in control treatments (T₁) of Desiree and Cardinal respectively. The control treatments were different significantly from other treatments (Fig.8). The treatments T₃ of Desiree differ significantly from rest of the treatments.

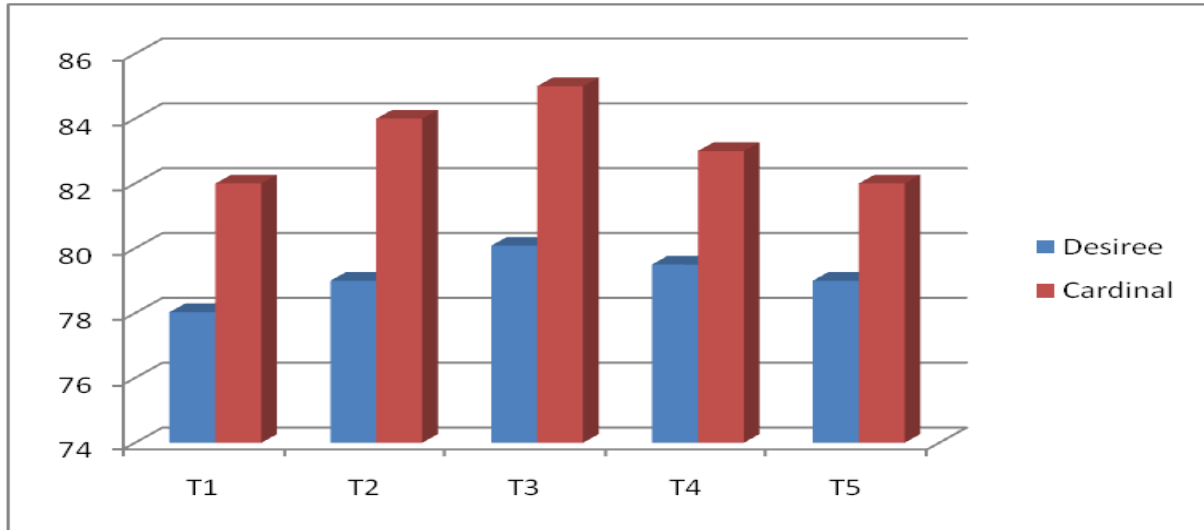


Fig. 9. Effect of different concentration of GA₃ on Percent sprout in potato plant.

The interaction between varieties and treatments were highly significant and revealed that both the variety behaved differently. The results indicated that Desiree variety took less number of days to transferable height of the plantlet than the Cardinal. The minimum numbers of days (23.03) to transferable height of the plantlet in Desiree variety were observed in T₃. Whereas minimum numbers of days (27.00) to transferable height of the plantlet were found in T₂ of the Cardinal variety.

The variable response of two varieties is due to genetic diversity. The treatments T₃ and T₂ are recommended as best treatments for Desiree and Cardinal varieties to decrease the number of days to transferable height of the plantlets. The above data helped us to conclude that the use of GA₃ in the media has reduced the number of days to transferable height of the plantlets. Webb *et al.*, (1983) reported that the addition of GA to the MS medium enhanced shoots.

Conclusion

The response of different concentrations (0.00, 0.12, 0.25, 0.50 and 0.75 mg L⁻¹) of GA₃ on *in vitro* micro propagation of two potato varieties, Desiree and Cardinal was evaluated in present study. Results have shown that there were significant differences among treatments and cultivars for most of the growth parameters (p<0.05) under stud.

y. From the above discussion, it is revealed that a genotype is dependent on *in vitro* protocol for the micro propagation. We assume that the findings from the study presented here provide efficient protocol for micro propagation of healthy and disease free potato plants. On the basis of results obtained from above experiments, it is recommended that T₃ (0.25 mg L⁻¹ of GA₃) is the best treatment for Desiree and T₂ (0.12 mg L⁻¹ of GA₃) for Cardinal variety. These treatments have shown best performance for most of the growth parameters.

The variable response of two varieties was due to genetic diversity which leads to *in vitro* regeneration response. The use of this protocol on commercial scale can lead to production and distribution of virus free seeds of potato.

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