EFFECT OF GROWTH REGULATORS IN MERISTEM CULTURE OF POTATO (Solanum *Tuberosmum* L.)

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Abstract

Two growth regulators, viz., Gibberellic acid (GA_3) and Naphthalene acetic acid (NAA) and a combination of both (GA_3 and NAA) were evaluated at three different levels (200, 300 and 400µl) to check the growth rate of root, shoot, number of leaves and nodes of potato plantlets in two potato varieties, namely cardinal and SH-5, grown by meristem culture. Analysis of variance revealed highly significant differences between varieties, hormones and their interaction for all the studied traits. Mean values revealed that among two varieties Cardinal produced root length (0.70 cm), shoot length (2.50 cm), number of leaves (6.96) and number of nodes (8) are better than the SH-5 which produced root length (0.54 cm), shoot length (1.38 cm), number of leaves (4.29) and number of nodes (5.3). The variety cardinal and GA_3 at 300 µl followed by 200 µl produced most suitable results. By using the concentration of 300 µl maximum root length of (1.40 cm), shoot length of (3.43 cm), number of leaves (10) and no of nodes (11) was obtained. At 200 µl, though root and shoot length obtained was very similar at 300 µl but produced less number of leaves and nodes.

Keywords: Potato, Tissue culture, Hormones, Meristems

Introduction

Potato is the most important non-cereal crop cultivated in the temperate and subtropical zones of the world. Its importance is evident from the fact that it ranks fourth after three major cereals, i.e., wheat, rice and maize (Jones et al., 1994). Among food crops, it possesses the largest quantity of carbohydrates per day per unit area (Zaag and Horton, 1983). It plays an excellent role in human diet by serving as a supplement to wheat and rice. It is composed of 80% water, 2-3% protein and 18% carbohydrates (Thompson and Kelly, 1957). In seed production system, potato is propagated through seed tubers but this method has disadvantages like poor seed health and low multiplication rate (Beukema and van der Zaag, 1990). For rapid seed production on large scale, tissue culture techniques may be employed. Such method of seed production allows rapid disease-free multiplication as potato production is severely affected by potato viruses, like, PLRV, PVX and PVY (Khalid et al. 2000). These viruses are tuber borne transmitted by aphids *Myzus persicae* that results in reduction of plant vigor and yield potential. For virus elimination from infected plants meristem culture, thermotherapy has been proven as a powerful tool to eradicate this problem (Paet and Zamora, 1990, Khan et al., 2013). Commercial production of disease free seeds for the enhancement of potato yield through in vitro techniques alongwith traditional procedures provided excellent results (Faccioli and Colombarini, 1996). Potato is also the first major crop where biotechnological procedures have been successfully employed to eliminate virus (Bajaj and Sopory, 1986). Meristem culture on MS-zero medium (Murashige and Skoog, 1962) with concentration of 2mg/liter NAA has been previously used to develop virus free potato (Yousuf et al, 1997). Plant Growth regulators sway potato tuberisation (Villafranca et al., 1998; Silva et al., 2001). Various studies have been made on plant hormones, but the interactions between them are still being discovered (Ross and O'Neill, 2001). Potato tuberisation is mainly controlled by hormones (Vreugdenhil and Struik, 1989), and the effect of exogenous plant growth hormones is commercially important for the induction of potato tuberisation (Zhang et al., 2005). Gibberellic acid plays a broad spectrum role in the form of cell enlargement and division. On MS medium, it stimulates nodal cutting but at high concentrations, it produces narrow and elongated shoots (Novak et al., 1980), depending upon the varieties/genotypes. Present research was designed to obtain virus free potatoes, using meristem culture as a technique and various

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hormone concentrations to check the response of two varieties of potato which may be helpful in developing virus free seed of potato.

Materials and Methods

The present experiment was carried out at the tissue culture lab of Plant virology section of Ayub Agricultural Research Institute, Faisalabad, Pakistan, in the year 2009-10. Two varieties namely Cardinal and SH-5 were selected for tissue culture studies. The hormones naming NAA, GA_3 and Combination of both were used at three levels, i.e., 200 µl, 300 µl and 400 µl, respectively. The meristems of above mentioned varieties were used as explants. The meristems were disinfected with 10% Clorox for 5 minutes and placed in test tubes containing MS-zero medium (Murashige and Skoog, 1962). The test tubes were then placed in growth chamber at 25ºC in continuous light of 100 Lux intensity. After six weeks plantlets produced were multiplied through stem cuttings on MS-zero medium having NAA, GA, and GA+NAA at three levels (200, 300 and 400 µl) and incubated at 25 ºC at 2500 Lux. After 2-3 weeks plantlets were studied for root length, shoot length, number of leaves and nodes.

Results and Discussion

The recent development in tissue culture and the flexibility of organ development in potato allows for alternative methods of propagation through *in vitro* techniques (Farhatullah et al. 2007). According to Badoni and Chauhan (2009), *in vitro* propagation methods using meristem tips or nodal cuttings are more reliable methods for maintaining genetic integrity of the multiplied clones. Although, there are many reports on potato micropropagation (Yousef et al., 2001; Badoni and Chauhan, 2009; Rahman et al., 2010), It is a well known fact that the regeneration potential of micropropagated plants is genotype dependent (Abe and Futsuhara, 1986). As meristem tips are free from viruses, elimination and generation of virus free plants are possible through meristem culture (Jha and Ghosh, 2005). Similar observations was found in the studies of Bhuiyan (2013), who described a regeneration protocol by *in vitro* meristem culture to get disease free potato plantlets. In the current experiment the hormones NAA and GA, were used both separately and in combination to study the role of these hormones on root length, shoot length, number of leaves and node development on potato meristems. The Gibberelic acid GA_3 has relatively broad spectrum effects as it plays a vital role in cell division and enlargement (Farhatullah et al. 2007). The results of analysis of variance revealed highly significant variation between varieties, treatments levels and all interactions presented in Table 1. Two varieties namely Cardinal and SH-5 were used in current studies for tissue culture study. The plantlet development of two varieties is given in Figs. 1 and 2. Among the two varieties studied, Cardinal responded well to different hormones at different concentrations. Mean values presented in Table 2 revealed that Cardinal produced root length (0.70 cm), shoot length (2.50 cm), number of leaves (6.96) and number of nodes (8) better than the SH-5 which produced root length (0.54 cm), shoot length (1.38 cm), number of leaves (4.29) and number of nodes (5.3). Table 3 reveals that variety Cardinal and GA_3 at 300 µl followed by 200 µl produced most suitable results. Khan et al. (2013) also used Gibberellic acid in their studies and found desirable results for number of nodes and number of leaves. By using the concentration of 300 µl maximum root length of 1.40 cm, shoot length of 3.43 cm, number of leaves (10) and no of nodes (11) was obtained. At 200 µl though root and shoot length obtained was very similar at 300 µl but produced less number of leaves and nodes. Farhatullah et al. (2007) used different concentration of GA_3 hormone and found it suitable for the growth of microtubers, however, (Badoni and Chauhan, 2009) applied this hormone on meristems and obtained the desired results. The GA_3 at a concentration of 400 µl did not respond well and produced lesser mean values than 200 and 300 µl concentrations. The combination of Cardinal+NAA at concentration of 300 µl produced the best results producing root length of (0.50 cm), shoot length (1.50 cm), number of leaves (6.33) and number of nodes (7.67). The same combination at 200 µl produced better results compared to 400 µl concentration. The variety cardinal produced root length of (0.97 cm), shoot length (4.03 cm), number of leaves (10) and number of nodes (10.95) when treated with combinations of both hormones at concentrations of 200 µl. But at higher concentrations desirable results were not obtained. While considering the variety SH-5, GA_3 hormone at a concentration of 300 µl gave better results. At this concentration the root length remained at (1.20cm), shoot length (2.53 cm), number of leaves (7) and number of nodes (8.33). The concentration of 400 µl though produced at par the number of leaves and nodes but did not show good results while considering root and shoot length. The concentration of 200 µl did not give better results for variety SH-5. The combination of NAA with variety SH-5 at all concentrations did not give promising results. A combination of both hormones for variety SH-5 proved good when cultured at 300 µl but did not perform well as it produced very low mean values of the trait studied. The combination of GA_3 and NAA in the studies of Badoni and Chauhan (2009) with different variety proved very successful. It may be concluded that if we use both these hormones with some other varieties the results may be the same as obtained by the above mentioned scientists.

Table 1. Analysis of variance for different traits studied.

Source of variation	*DF*	*Root length (cm)*	*Shoot length (cm)*	*No. of leaves*	*No. of nodes*
Reps	**2**	**0.05**	**0.07**	**2.35**	**2.74**
Varieties	**1**	**0.34****	**16.84****	**96.0****	**98.68****
Treatments	**2**	**2.99****	**6.12****	**44.12****	**45.24****
Levels	**2**	**0.23****	**1.86****	**20.07****	**21.12****
Varieties × Treatment	**2**	**0.04****	**1.00****	**5.05****	**6.35****
Varieties × Levels	**2**	**0.31****	**3.10****	**8.22****	**7.90****
Treatments × levels	**4**	**0.07****	**0.82****	**7.21****	**8.10****
Varieties × Treatments × levels	**4**	**0.11****	**0.33****	**8.86****	**8.90****
Error	**34**	**0.01****	**0.04**	**1.42**	**1.43**
Total	**53**				

Table 2. Performance of two potato varieties in terms of different traits.

Varieties	*Root length*	*Shoot length*	*Number of leaves*	*Number of nodes*
Cardinal	0.70a	2.50a	6.96a	8.00a
SH-5	0.54b	1.38b	4.29b	5.30b
LSD (0.05)	0.075	0.13	0.66	0.67

Table 3. Performance of varieties at different concentrations of two hormones alone and in combination for various seedling traits.

Variety	*Hormone*	*Concentrations (µl)*	*Root length (cm)*	*Shoot length (cm)*	*No. of leaves*	*No. of nodes*
Cardinal	GA_3	200	**1.20**	**3.30**	**7.33**	**8.33**
Cardinal	GA_3	300	**1.40**	**3.43**	**10.00**	**11.00**
Cardinal	GA_3	400	1.06	2.11	7.00	8.00
Cardinal	NAA	200	0.43	2.06	6.00	7.00
Cardinal	NAA	300	0.50	1.50	6.33	7.67
Cardinal	NAA	400	0.40	1.23	5.66	6.65
Cardinal	GA_3+NAA	200	**0.97**	**4.03**	**10.00**	**10.95**
Cardinal	GA_3+NAA	300	0.17	2.80	6.67	7.66
Cardinal	GA_3+NAA	400	0.20	2.06	3.67	4.67
SH-5	GA_3	200	0.90	1.87	6.00	7.00
SH-5	GA_3	300	**1.20**	**2.53**	**7.00**	**8.33**
SH-5	GA_3	400	**0.80**	**1.02**	**7.00**	**7.95**
SH-5	NAA	200	0.30	0.95	2.33	3.33
SH-5	NAA	300	0.40	1.14	3.66	4.66
SH-5	NAA	400	0.17	0.80	2.33	3.33
SH-5	GA_3+NAA	200	0.33	1.30	2.35	3.33
SH-5	GA_3+NAA	300	**0.50**	**1.76**	**6.33**	**7.33**
SH-5	GA_3+NAA	400	0.30	1.10	1.67	2.33

Note: Values in bold showing superior combinations

Fig. 1. Plantlet growth of variety SH-5.

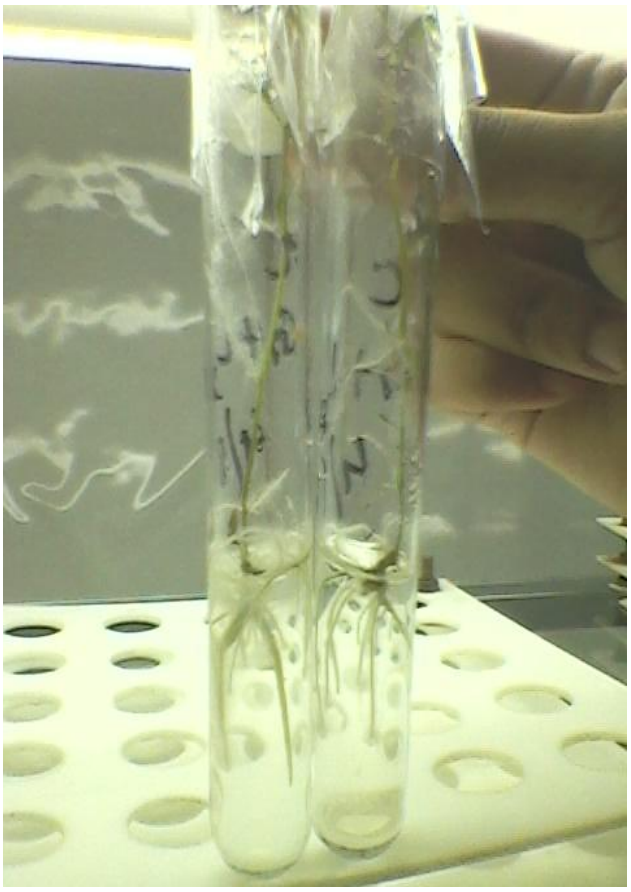

Fig. 2. Plantlet growth of variety Cardinal.

Present study indicated that application of GA_3 alone is better suited to variety Cardinal at 300 µl. Though the same hormone provided better results with SH-5 at 300 µl but it was far less than the Cardinal variety.

Conclusions

Present research using tissue culture on meristems may be helpful in developing virus free seeds of potato.

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