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Regeneration of Potato Plantlets Through Shoot Tip Culture Comparison Between GA3 and BAP

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Abstract

The research was conducted to investigate on the regeneration of potato plantlets through shoot tip culture, were carried out in the Virology Laboratory of Plant Pathology Section. Agriculture Research Institute, Tandojam during 2012. The regeneration of potato plantlets was compared between media based on various BAP and GA3 concentrations. The results revealed significant ($P<0.05$) effect of Murshigue & Skoog (M S) media and their concentrations on root/shoot number, root/shoot length, leaves plant^{-1} and callus formation. It was also observed that GA3 based media at 2.00mg liter^{-1} concentration to produce roots plant^{-1} 34.65, shoots plant^{-1} 11.48, cm root length 4.13, cm shoot length 5.56, leaves plant^{-1} 6.37 and percent callus formation 83.12; while the values for these characters under rest of the GA3 concentrations (2.50, 1.50 and 1.00 mg liter^{-1} were significantly lower than this optimum GA3 concentration. In BAP based media, 4.00 mg liter^{-1} concentration showed better results with 24.38 roots plant^{-1}, 7.07 shoots plant^{-1}, 3.44 cm root length, 4.29 cm shoot length, 5.29 leaves plant^{-1} and 66.63 percent callus formation; and values for these traits for other BAP concentrations were significantly lower. On an overall average, the BAP and GA3 based media resulted in 21.20 and 28.70 roots plant^{-1}, 5.60 and 8.72 shoots plant^{-1}, 2.58 and 2.92 cm root length, 2.93 and 3.86 cm shoot length, 3.94 and 4.83 leaves plant^{-1} and 54.87 and 67.60 percent callus formation. In conclusion, results clearly indicate that GA3 was more effective to produce higher values for all the traits examined for regeneration of plantlets through shoot tip culture.

Keywords

Regeneration, Potato Plantlets, Nitrogen, Shoot Tip Culture, BAP, and GA3

1. Introduction

Potato (*Solanum tuberosum* L.) is the member of Solanaceae family. Potato is classified to tuber crop which has an important impact on human nourishing, this crop is usually high yielding and vitality contented and produced protein are superior to wheat and rice. The Spanish Royal Academy declares in Spanish word is multipart of the Taíno batata and Quechua papa 'potato' [1]. Overall 99% of the current potatoes cultivated worldwide inclined from varieties that invented in the lowlands of south-central Chile, which

have colonial previously popular varieties from the Andes [2]. The word potato can refer to the plant itself as well as the comestible tuber [3]. *Tuberosum* is cultivated in the Northern Hemisphere [4]. While there are close to 4000 different varieties of potato, it has been farmed into many standard or well-known varieties, each of varieties is separate agricultural or culinary characteristics [5]. In general, varieties are categorized into a few main individual groups, such as whites, russets, yellows (also called Yukons), reds and purples—based on conjoint individualities. are frequently described in terms of their waxiness [6]. For example, the adoption of virus free planting material increased average yield by 30 percent in Ghana (FAO 2013) and more than five times in Uganda (CIP 2014). Naturally, the potato is low in cholesterol, a basic source of fiber and high in vitamins and minerals. To preserve the purple color best, heating is the best way for cook it, however, it can be also steamed and be baked. The purple or blue potato tends to have a screwier flavor than its relatives of other colors. Single and complex infection of potato virus diseases can contribute to following yield decline [7]. Potatoes can be cooked and prepared by boiling, frying, roasting or eating raw. In the world potato production during the year 2010 remained 324.4 million tons. China is the foremost fabricator with annual production of 74.8mt, followed by India (36.6mt), Russia (21.1mt), Ukraine (18.7mt), United States (18.3mt) and Germany (10.2mt). However, as compared to the European countries, the vegetative growth periods are shorter, especially during the fall and spring seasons. The production area potato under cultivation in Pakistan during the year 2011-12, the area under potato cultivation in the country increased to 185.1 thousand hectares with a production of 4104.4 thousand tons showing 17.5% increase in production over the preceding year [8]. In Pakistan, the basic problem for potato growth is the unavailability of good quality seed. Imported potato seed is used to reduce the threat of potato viral diseases and seed tolerated. The plant's breeders are also have been trying to increase the sustainable yield of the potato genotypes tolerant to abiotic and biotic stresses throughout the world. The potential value of the tissue culture in potato production has been recognized to produce the disease-free seed production widely either plantlets regeneration or micro-tuber production which is very convenient to transport and easily stored for a long time. Now, it is necessary to launch a protocol for the in-vitro production of micro tubes in rapid multiplication. A number of the substances are known that have relatively broad spectrum effects such as a Gibberellic acid (GA_3), primary morphological effects are associated with cell enlargement and cell division. GA_3 stimulates the development of nodal cutting on Murshigue & Skoog (M S) media but at high concentrations, it produces narrow and elongated shoots depends on genotypes. Keeping in the view of previous results of investigations, present study has been aimed to evaluate the effect of Gibberellic acid (GA_3) and 6-benzylaminopurine (BAP) on regeneration of potato seedling in-vitro condition from potato variety "Desiree", which ultimately leads to mass multiplication of healthy the seed and successfully in-vitro seed tuber production. A tissue culture method for the rapid propagation of potatoes will be studied using single variety named Desiree. Plantlets will be regenerated in-vitro, the progressive procedure from a single isolated shoot tips culture using Murshigue & Skoog (M S) media supplemented with GA_3 and BAP. However, the *in-vitro* regeneration of potato is usually prepared from different the explants on Murshigue & Skoog (M S) medium augmented with different auxins and cytokine for free diseases good quality seeds and the pathogen is free planting materials [9]. A suitable concentration of plant growth regulators in the culture media are required to cultivate rapid plants propagation from the explants [10].

2. Materials and Methods

Study design

The present study was carried out at the Tissue Culture/Virology Laboratory of Plant Pathology Section, Agriculture Research Institute Tandojam. The samples of Desiree potato variety were provided by NARC Islamabad. The sterilized plant material was, again and again, washed three times in the sterile laminar hood using distilled autoclaved water to remove all traces of bleach and detergent. The excised pieces explants (5-10 mm) were in-vitro cultured in seal bottles. The temperature of the growth room was maintained at 25±3°C. Marinating of the light intensity was 2000 lux during the 16-hours period for growth and development of tissue culture. The effect of different concentrations of BAP and GA_3 on the callus formation and root/shoot development was investigated. The details regarding the BAP and GA_3 concentrations were as under:

The experiment was conducted by growing three potato varieties viz. Patroness, diamond, and Desiree. The samples of Desiree potato variety were provided by NARC Islamabad. The potato tubers were grown in the field and earthen pots after chilling to break the dormancy. Parallel to this potato tuber of above varies was also incubated in the refrigerator at a temperature of 9-11^{o}C to break the dormancy. The tubers planted in the field, earthen pots and refrigerator started to show an emergence of shoot buds within 3 days. Then the shoot tips measuring about 5 mm were excised from 3 varieties. These excised shoot tips were washed under the running tap water for about 30 minutes to remove dirt etc. The shoot tips were surface sterilized by a quick dip in 70% ethanol followed by emersion in 10% sodium hypochlorite (NaOH) solution plus 2-3 drops of tween-20. The tween-20 was added to eliminate the surface tightness of the seed. Laminar airflow cabinet was also washed within the spirit and prepared for working before culturing. After washing, all the required maturity i.e. media, rubber bands, plastic papers or aluminum foil, spirit lamp, sprit, forceps, and scissors were kept inside the hood. UV light was switched for 30 minutes to remove any traces of contamination. The explants material was surface sterilized as described in figures. After sterilization, the shoot tips were

rinsed three times with distilled autoclaved water (dH_2O) to remove all the traces of effect-able disease under aseptic conditions in laminar airflow cabinet. Shoot tips were placed in the sterile under the distilled water. The excised portion of explants measuring about the 5mm was inoculated in the cultural bottles containing 5ml nutrient media with the help of forceps and scissors. Then the inoculated bottles were covered with plastic papers or aluminum foil (Depending on availability). After covering these bottles were transferred to the growth room under the temperature of 25±3°C in a white fluorescent light with intensity about 3000 lux during 16 hours for the photo period time. Murashigue and Skoog (M S) medium was used as a nutrient medium for the tissue culturing with the different concentration of BAP (benzyl aminopurine or Benzyl adenine) for initiation and multiplication of excised shoot tips. For rooting purpose IBA (Indole butyric acid) was used. After regeneration of complete plantlets transferred was in the field

3. Results and Discussion

The present study was carried out during the year 2012 on the regeneration of potato plantlets through shoot tip culture in the Virology Laboratory of Plant Pathology Section, Agriculture Research Institute, Tandojam. The regeneration of potato plantlets was compared between media based on BAP and GA_3. The cumulative effect of various concentrations of BAP (3.0, 3.5, 4.0 and 4.mg liter^{-1}) and GA_3 (1.0, 1.5, 2.0 and 2.5 mg liter^{-1}) on number of roots plant^{-1}, number of shoots plant^{-1}, root length (cm), shoot length (cm), number of leaves plant^{-1} and callus formation (%) as investigated and the results are presented in (Figures 1-6) along with their analysis of variance showing significance of the effect of BAP and GA_3 concentrations on the shoot/root development and callus formation.

In-vitro regeneration of plantlets in potato has become a most important and interesting way of development of disease-free crop strains. Therefore, the present study was carried out on the regeneration of potato plantlets through shoot tip culture. In BAP based media, the highest number of roots (34.38) plant^{-1} was determined at 4.00 mg liter^{-1} BAP concentration and minimum (16.29) at lowest BAP concentration (3.00 mg liter^{-1}); while in GA3 based media, the highest number of roots (34.65) plant^{-1} was determined at 2.00 mg liter^{-1} GA3 concentration and lowest (19.75) at 1.00 mg liter^{-1} GA3 concentration. On average GA3 based media produced significantly higher number of roots (28.70) plant^{-1} in potato during the regeneration process as compared to 21.20 roots plant^{-1} in BAP based Murshigue & Skoog (M S) media. This research indicates that the GA3 based media was more effective to develop rooting potential of potato as compared to BAP based media. However, either GA3 or BAP based media on their concentration higher than 2.0 and 4.00 mg liter^{-1} showed severe adverse effects on rooting in potato regeneration process. The average results showed that the highest number of roots plant^{-1} (29.01) was observed in media containing 2.5 mg liter^{-1} GA3 or BAP, and increasing concentration resulted in a decrease in the number of roots plant^{-1}. The results reported by [11]. The further confirmed the findings of the present research who observed that GA3 based media resulted in highest number and length of shoots and number and length of roots in potato regeneration studies. The reported significant differences on the number of roots and shoots produced by different types of media [12]. The developed low-cost medium can be used the production of affordable disease free sweet potato seedlings. The significant differences in the root and shoot development of sweet potato under Murshigue & Skoog (M S) media based on different growth regulators [13]. In case of number of shoots plant^{-1}, 4.00 mg liter^{-1} BAP concentration resulted maximum shoots (7.07) plant^{-1} and minimum (3.84) in 3.00 mg liter^{-1}, while in GA3 based media the maximum shots (11.48) plant^{-1} were noted at 2.00 mg liter^{-1} concentration and minimum (5.25) at 1.00 mg liter^{-1} GA3 concentration. Shoot development under BAP and GA3 based Murshigue & Skoog (M S) media showed significant difference while and on average by GA3 based media resulted in a significantly greater number of shoots (8.72) plant^{-1} than BAP based Murshigue & Skoog (M S) media with 5.60 shoots plant^{-1}. It is obvious that GA3 based Murshigue & Skoog (M S) media was more effective for development of in-vitro shooting potential in potato as compared to BAP based media. These results are further supported by [15] (Rind et al. 2007) who tried Murshigue & Skoog (M S) medium with different BAP, GA3 and sucrose concentrations to determine the optimum level of BAP, GA3, and sucrose for optimum regeneration, and found that GA3 contained media resulted in higher number of shoots plant^{-1} as compared to BAP based media in potato regeneration. reported were that the maximum shoot length (8.96 cm) was obtained with 4 mg GA3 litre^{-1} was applied. The maximum number of shoots (1.4) was obtained when 2 mg BAP litre^{-1} was applied. The reported that Murshigue & Skoog (M S) medium are accompanied with different plants growth regulators (PGRS) (0.5 mg L^{-1} benzyl adenine (BA), 0.5 mg L^{-1} gibberellic acid (GA3) and medium without PGRS.) and results revealed significant effect of media based on different growth regulators and without growth regulators on number of roots and development of virus-free plantlets in potato [14]. The concluded that meristem tip culture technique is one of the most used for potato in-vitro culture beginning and most of used in order to obtain free virus plantlets [15]. They reported that growth regulators based Murshigue & Skoog (M S) medium was more effective to develop virus-free plantlets with sufficient shoot length which was desirable. The root length in BAP based media was highest (3.44 cm) at 4.00 mg liter^{-1} concentration and lowest (1.33 cm) at 3.00 mg liter^{-1}, while in GA3 based media root length was highest (4.13 cm) at 2.00 mg liter^{-1} concentration and lowest (1.32 cm) at 1.00 mg liter^{-1} GA3 concentration. The average effect of media type indicated that in GA3 based media, the average root length was significantly ($P<0.05$) higher (2.92 cm) as compared to the root length (2.58 cm) in BAP based media. This indicated that GA3 was more effective media for root development of

potato tubers, while the root length under BAP based media was considerably shorter than GA3. The above results are further supported by (Rind et al. 2007) who reported that GA3 based media was more effective to produce higher root length in regeneration studies. Similarly, examined the effect of GA3 are use on in-vitro propagation through different concentration on Potato variety "Desiree" [16]. For example, these three treatments of GA3 use, 0.1, 0.25, 0.5 mg L^{-1} in Murshigue & Skoog (M S) medium were under observation. 0.25 mg L-1 dosage of GA3 in the Murshigue & Skoog (M S) medium also gave the best results are compared to other dosages when data was recorded from Shoot length, Root length and a number of leaves per plantlet. 0.25 mg L^{-1} dosage of GA3 in the Murshigue & Skoog (M S) medium can be used for in-vitro culture of potato plantlet for best results as compared to other dosages. The supplemented Murshigue & Skoog (M S) media for potato regeneration in 0.1 mg L^{-1} gibberellic acid (GA3) and this regeneration protocol was found useful for micropropagation and genetic transformation study [17]. The based media in shoot length BAP was highest (4.29 cm) at 4.00 mg $liter^{-1}$ concentration and lowest (1.02 cm); while in GA3 based media maximum shoot length (5.56 cm) was noted at 2.00 mg $liter^{-1}$ concentration and lowest shoot length (1.44 cm) at 1.00 mg $liter^{-1}$ GA3 concentration. On an overall average basis, in GA3 based media the shoot length was significantly ($P<0.05$) higher (3.86 cm) as compared to the shoot length (2.93 cm) in BAP based media. This argued that shoot length responded more positively to GA3 based media as compared to BAP. Similar results have also been reported by (Rind et al. 2007) who found that under GA3 based media, the shoot length was higher than the BAP concentration. The numbers of roots were significantly affected by several of the GA3 concentrations used in this study. The maximum number of shoots (1.4) was obtained when 2 mg BAP $litre^{-1}$, while concluded that significant reduction in stem and internode size was observed by increasing BAP and kinetin concentrations [18]. In another study in Pakistan observed that the highest number of nodes per plantlet was obtained with IBA at 0.35 mg $litre^{-1}$ [19]. The significant differences in the root and shoot development of sweet potato under the Murshigue & Skoog (M S) media based on different growth regulators. Similarly, development of leaves under BAP and GA3 based Murshigue & Skoog (M S) media showed significantly different trends. Hence on average GA3 based media produced significantly more leaves (4.82) $plant^{-1}$ as compared to BAP based Murshigue & Skoog (M S) media with 3.94 leaves $plant^{-1}$ on an overall average basis

Number of roots plant-1

In tissue culture studies on potato, a number of roots length development in the seed tuber is primary importance and the media results in the emergence of more roots in a seed tuber are generally recommended for further propagation. This could obviously be assumed on the basis of experimental results that GA3 based Murshigue & Skoog (M S) media was more effective for growth and development of in-vitro regeneration in potato as compared to BAP based media. These results are further supported by who also reported that type of media and growth regulator results variation in regeneration outcomes in the laboratory [20]. The callus formation in BAP based Murshigue & Skoog (M S) media was maximum (66.63%) at 4.00 mg $liter^{-1}$ BAP concentration and minimum (38.98%) at 3.00 mg $liter^{-1}$ BAP concentrate; while in GA3 based media, the maximum callus formation (83.12%) was noted at 2.00 mg $liter^{-1}$ GA3 concentration and lowest (44.49%) in media containing 1.00 mg $liter^{-1}$ GA3 concentration. The effect of type of Murshigue & Skoog (M S) media indicated that on an overall average, in GA3 based media the callus formation was significantly ($P<0.05$) maximum (67.60%) as compared to 54.87% callus formation in BAP based media. This suggested that callus formation responded more positively to GA3 based media as compared to BAP based media in tissue culture studies on potato regeneration. These results are further in accordance with those of [21]. Who are reported that GA3 and BAP based media are effective in potato regeneration and reported that the callus formation is better under GA3 based media as compared to another BAP (Figure 1).

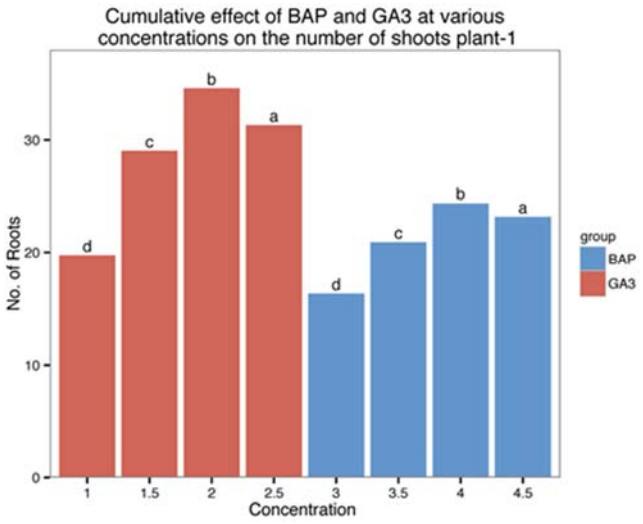

Figure 1. *The cumulative effect of BAP and GA_3 at various concentrations on the number of roots $plant^{-1}$.*

In a column means followed by same letters are not significantly different at P=0.05 as recommended by LSD test.

Number of shoots $plant^{-1}$

In tissue culture studies on potato, the shoot development in the seed tube is of primary importance and the media results in the emergence of more shoots in a seed tuber are generally recommended for further propagation. The data in regards to a number of shoots $plant^{-1}$ of potato under the effect of BAP and GA_3 concentrations are presented in (Figure 2). Whereas the analysis of variance (Appendix-II) suggested significant ($P<0.05$) effect of Murshigue & Skoog (M S) media, their concentrations (C) as well as the interaction between Murshigue & Skoog (M S) media and concentrations (M x C) on the number of shoots $plant^{-1}$.

The development of shoots in potato under BAP based

media indicates that the maximum shoots (7.07) plant^{-1} were developed at 4.00 mg liter^{-1} BAP concentration, while the number of shoots plant^{-1} declined to 6.45 and 5.04 under 4.50 and 3.50 mg liter^{-1} BAP concentrations at water levels respectively (Figure 2). However, the minimum number of shoots (3.84) plant^{-1} was determined in media containing 3.00 mg liter^{-1} BAP. The number of shoots reached to maximum level under 4.00 mg liter^{-1} BAP concentration and beyond this concentration affected the shoot development adversely. In GA_3 based media, the maximum shoot (11.48) plant^{-1} were noted at 2.00 percent concentration and shoots plant^{-1} decreased to 9.80 and 8.33 in MS Murshigue & Skoog (M S) media containing 2.50 and 1.50 mg liter^{-1} GA_3 concentrations respectively (Figure 2). The minimum shoots plant^{-1} (5.25) was found in media containing lowest GA_3 concentration (1.00 mg liter^{-1}). This suggested that shoots plant^{-1} reached to maximum level under 2.00 mg liter^{-1} GA_3 concentration either higher GA_3 lower concentrations resulted in a reduction in potato shoot development. It is obvious that GA_3 based Murshigue & Skoog (M S) media was averagely more effective for root development of in potato as compared to BAP based media (Figure 2).

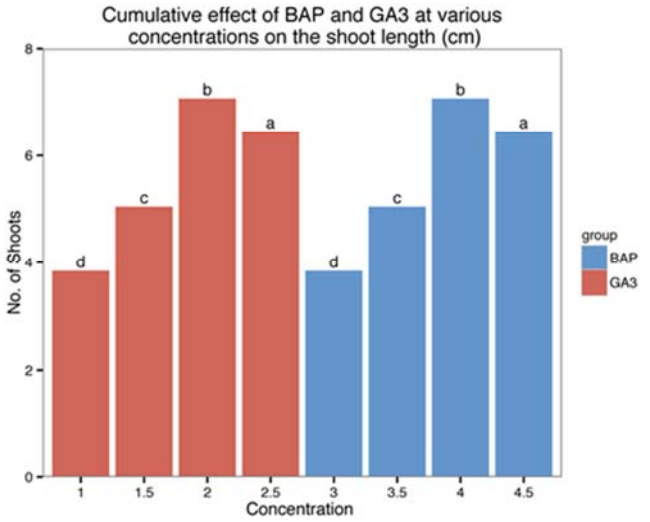

Figure 2. *The cumulative effect of BAP and GA_3 at various concentrations on the number of shoots length (cm).*

In the column mean followed by same letters are not significantly different at P=0.05 as recommended by LSD test.

Root length (cm)

The data pertaining to root length of potato under the effect of various concentrations of BAP and GA_3 are presented in (Figure 3). The analysis of variance showed the significant (P<0.05) effect of Murshigue & Skoog (M S) media, their concentrations (C) as well as the interaction between Murshigue & Skoog (M S) media and concentrations (M x C) on the root length of potato. The effect of concentrations showed that the maximum root length under BAP based media (3.44 cm) was observed at 4.00 mg liter^{-1} concentration, while the root length considerably decreased to 2.98 cm and 2.58 cm under 4.50 and 3.50 mg liter^{-1} BAP concentrations respectively. The minimum root length (1.33 cm) was found in media containing 3.00 mg liter^{-1} BAP. This showed that root length followed an adverse trend in media containing BAP beyond 4.00 percent concentration (Figure 3). The root length in GA_3 based media was maximum (4.13 cm) at 2.00 percent concentration which reduced significantly to 3.64 cm and 2.59 cm in Murshigue & Skoog (M S) media containing 2.50 and 1.50 mg liter^{-1} GA_3 concentrations respectively. The minimum root length (1.32 cm) was noted in media containing lowest GA_3 concentration (1.00 mg liter^{-1}). This indicated that increasing GA_3 concentration 2.00 percent showed a negative impact on root length (Figure 3). The average effect of media type GA_3 indicated the average root length was significantly (P<0.05) higher (2.92 cm) as compared to the root length (2.58 cm) in BAP based media (Figure 3). this is indicated that GA_3 was more effective media for root development of potato tubers. Then the root length under BAP based which media was considerably shorter than GA_3.

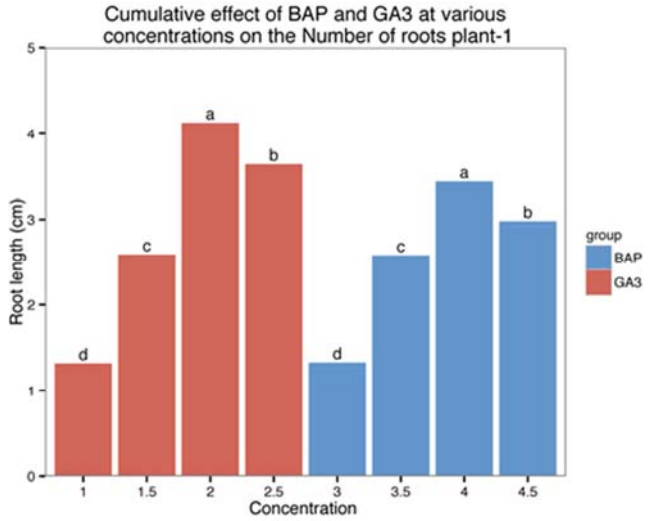

Figure 3. *The cumulative effect of BAP and GA_3 at various concentrations on the root length plant^{-1}.*

In column mean followed by the same letters are not significantly different at P=0.05 as suggested by LSD test.

Shoot length (cm)

In-vitro shoot length undershoots tip culture is generally influenced by the type of Murshigue & Skoog (M S) media and its concentration used for regeneration, but the influence of proper handling of the tissue culture process is of significance. The results pertaining to shoot length of potato under the effect of various concentrations of BAP and GA_3 are given in (Figure 4). The analysis of variance demonstrated significant (P<0.05) effect of Murshigue & Skoog (M S) media and their concentrations (C), while the effect of interaction between Murshigue & Skoog (M S) media and concentrations (M x C) on the shoot length of potato was non-significant (P>0.05). In BAP based Murshigue & Skoog (M S) media, the maximum shoot length (4.29 cm) was observed at 4.00 mg liter^{-1} concentration, while the shoot length markedly reduced to 3.64 cm and 2.75

cm under 4.50 and 3.50 mg liter^{-1} BAP concentrations, respectively. The minimum shoot length (1.02 cm) was noted in media containing 3.00 mg liter^{-1} BAP. This showed that for shoot length development in potato, the optimum BAP concentration would be 4.00 mg liter^{-1} and a further increase in BAP concentration will impact shoot development in the negative direction. The maximum shoot length in GA_3 based media (5.56 cm) was noted at 2.00 mg liter^{-1} concentration which declined markedly to 4.61 cm and 3.82 cm in Murshigue & Skoog (M S) media containing 2.50 and 1.50 mg liter^{-1} GA_3 concentrations, respectively. The minimum shoot length (1.44 cm) was noted in media containing lowest GA_3 concentration (1.00 mg liter^{-1}). This indicated that 2.00 mg liter^{-1} GA_3 was an optimum concentration to result in maximum shoot length, and a further increase in GA_3 affected the shoot length in a negative manner. The data showing the effect of type of Murshigue & Skoog (M S) media indicated that on an overall average, in GA_3 based media the shoot length was significantly ($P<0.05$) higher (3.86 cm) as compared to the shoot length (2.93 cm) in BAP based media. This argued that shoot length responded more positively to GA_3 based media as compared to BAP.

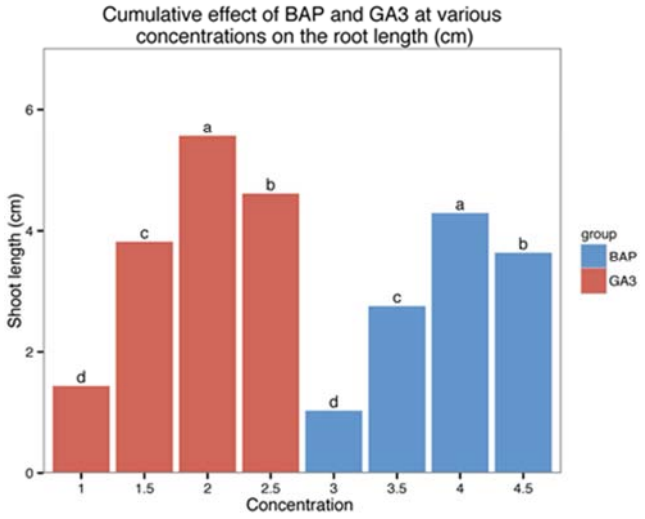

Figure 4. *The cumulative effect of BAP and GA_3 at various concentrations on the shoot length (cm).*

In a column means followed by same letters are not significantly different at P=0.05 as suggested by LSD test.

Number of leaves plant^{-1}

A number of leaves are a growth trait and under circumstances in research, leaves plant^{-1} provides relatively better growth measurement to compare the treatments. The results related to a number of leaves plant^{-1} of potato under the effect of BAP and GA_3 concentrations are given in (Figure 5) and the analysis of variance as Appendix-V. The analysis of variance demonstrated significantly ($P<0.05$) effect of Murshigue & Skoog (M S) media and their concentrations (C), while the effect of interaction between Murshigue & Skoog (M S) media and concentrations (M x C) was non-significant ($P>0.05$) on potato leaves plant^{-1}. In BAP based media, the maximum number of leaves (5.29) plant^{-1} was noted at 4.00 mg liter^{-1} BAP concentration and leaves plant^{-1} decreased to 4.38 and 4.09 under 4.50 and 3.50 mg liter^{-1} BAP concentrations, respectively. However, the minimum leaves (2.02) plant^{-1} were observed in media containing 3.00mg liter^{-1} BAP. The leaves plant^{-1} followed a negative direction when BAP application was higher than 4.00 mg liter^{-1}. In GA_3 based media, the maximum leaves (6.37) plant^{-1} were noted at 2.00 percent concentration and leaves plant^{-1} decreased to 5.15 and 4.65 in Murshigue & Skoog (M S) media containing 2.50 and 1.50 mg liter^{-1} GA_3 concentrations, respectively. The least number of leaves plant^{-1} (3.09) was found in media containing lowest GA_3 concentration (1.00 mg liter^{-1}). This indicates that 2.00 mg liter^{-1} GA_3 would be an optimum concentration for achieving maximum results in regards to leaves plant^{-1} in potato regeneration *in-vitro*.

Development of leaves under BAP and GA_3 based Murshigue & Skoog (M S) media showed significantly different trends, and on average GA_3 based media produced significantly more leaves (4.82) plant^{-1} as compared to BAP based Murshigue & Skoog (M S) media with 3.94 leaves plant^{-1} on an overall average basis. This could obviously be assumed on the basis of experimental results that GA_3 based Murshigue & Skoog (M S) media was more effective for growth and development of *in-vitro* regeneration in potato as compared to BAP based media.

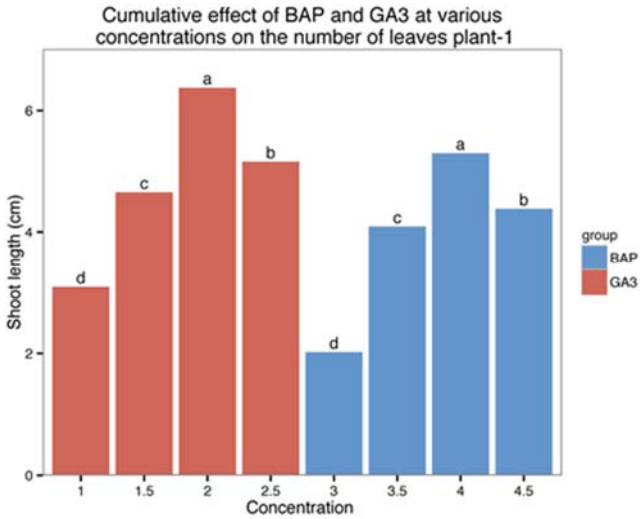

Figure 5. *The cumulative effect of BAP and GA_3 at various concentrations on the number of leaves plant^{-1}.*

In a column means followed by same letters are not significantly different at P=0.05 as suggested by LSD test.

Callus formation (%)

Callus formation is the fundamental investigative micropropagation applied for the tissue culture procedures and callus can be multiplied and later used to clone numerous whole plants. Explants from several parts of large intact plants can be used to form callus. The most successful explants are often young tissues of one or a few cell types. The data show to callus formation of potato under the effect of various concentrations of BAP and GA_3 are given in (Figure 6). The analysis of variance indicated significant

(P<0.05) effect of Murshigue & Skoog (M S) media and their concentrations (C), while the effect of interaction between Murshigue & Skoog (M S) media and concentrations (M x C) on the callus formation of potato was non-significant (P>0.05). It is obvious from the data that in BAP based Murshigue & Skoog (M S) media, the maximum callus formation (66.63%) was observed at 4.00 mg liter^{-1} BAP concentration, while the callus formation declined to 61.66% and 52.24% under 4.50 and 3.50 mg liter^{-1} BAP concentrations, respectively. The minimum callus formation (38.98%) was found in media containing 3.00 mg liter^{-1} BAP. This indicates that 4.00 mg liter^{-1} BAP concentration showed optimistic results for callus development and application of higher BAP concentration resulted in reduced callus formation. In the case of GA_3 based media, the highest percentage of callus formation (83.12%) was noted at 2.00 mg liter^{-1} GA_3 concentration but followed a declining trend with 73.04% and 69.74% callus formation in Murshigue & Skoog (M S) media containing 2.50 and 1.50 mg liter^{-1} GA_3 concentrations, respectively. The callus formation was lowest (44.49%) in media containing lowest GA_3 concentration (1.00 mg liter^{-1}). This indicated that 2.00 mg liter^{-1} GA_3 was an optimum concentration to result in maximum callus formation. The effect of type of Murshigue & Skoog (M S) media indicated that on an overall average, in GA_3 based media the callus formation was significantly (P<0.05) higher (67.60%) as compared to 54.87% callus formation in BAP based media. This suggested that callus formation responded more positively to GA_3 based media as compared to BAP based media in tissue culture studies on potato regeneration.

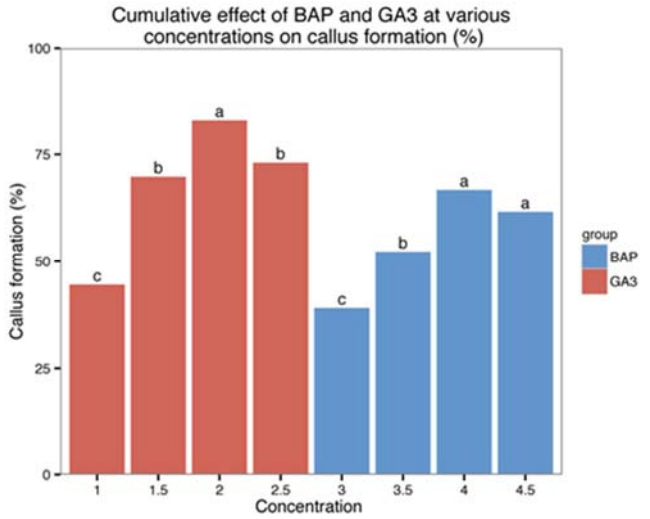

Figure 6. *The cumulative effect of BAP and GA_3 at various concentrations on callus formation (%).*

In a column means followed by same letters are not significantly different at P=0.05 as suggested by LSD test.

4. Conclusions

GA_3 based media proved to be more effective than the BAP based media for root/shoot development, leaves plant^{-1} and callus formation. GA_3 at 2.00 mg liter^{-1} concentration produced optimistic results for root/shoot development, leaves plant^{-1} and callus formation and increasing up to 2.50% resulted in adverse impact on these characters. BAP at 4.00 mg liter^{-1} concentration showed maximum values for root/shoot development, leaves plant^{-1} and callus formation characters and increasing up to 4.50 mg liter^{-1} showed a negative effect.

5. Suggestions

On the basis of findings from the present research, following suggestions are made:

- GA_3 may be used at 2.00 mg liter^{-1} concentration to achieve optimum regeneration of potato plantlets.
- In case BAP based media is used, 4.00 mg liter^{-1} concentration would be optimum for regeneration of potato plantlets.
- GA_3 based media may preferably be used for achieving remarkable results in regeneration of potato plantlets in the laboratory.

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