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PLANT TISSUE CULTURE

In vitro effects of GA_3 on morphogenesis of CIP potato explants and acclimatization of plantlets in field

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Abstract Improvement of potato has been accomplished using conventional and non-conventional approaches coupled with numerous tissue culture procedures. The aim of the present study was to assess the efficacy of gibberellic acid (GA_3) on the morphogenesis of International Potato Center (CIP) potato explants and acclimatization of plantlets in the field. Nodal segments as an explant source (1–1.5 cm) were isolated from 31 CIP potato plantlets and were inoculated into Murashige and Skoog (MS) medium supplemented with 0.0 (control), 0.1, 0.5, or 1.0 mg $L^{-1}$of GA_3. The variation in growth parameters of the cultivars was then observed. The highest shoot induction occurred in MS medium containing 1.0 mg L^{-1} GA_3 with an increase in the inter-nodal distance between nodes as compared to other treatments. Higher concentration (1.0 mg L^{-1}) of GA_3 significantly increased plant height and root length in the treated germplasm however; this concentration was inhibitory to the number of nodes and roots per plant. The number of leaves was significantly increased in plants receiving GA_3 treatment at lower concentration (0.1 mg L^{-1}). The 31 CIP genotypes were transplanted to the field and checked for yield quality traits. It was concluded from the results that GA_3 had significant effects on morphogenesis and was effective in the acclimatization of CIP potato plantlets in field.

Keywords CIP · Gibberellic acid · Genotypes · MS medium

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Introduction

Potato (*Solanum tuberosum* L.) is considered one of the most important crops after wheat, rice, and maize (Food and Agriculture Organization of the United Nations 2008). As a food crop, potato has great potential and can supply high quality food within a relatively short period of time and is one of the cheapest sources of energy. According to Stevenson *et al.* (2001), potato produces 54% more protein per unit land area than wheat and 78% more than rice. Potato originated in the Andes Mountains of South America (Khoso 1988). Micro-propagation is used for the improvement of cultivated potato plants (Badoni and Chauhan 2009; Rahman *et al.* 2010). Propagation methods using meristems tips, nodal cuttings, and microtubers are also used in *in vitro* propagation and are more effective for maintaining the genetic integrity of the multiplied clones (Khanam *et al.* 2013).

Propagation in tissue culture is used to produce a large number of plantlets in a short time from single plantlets. Through micro-propagation, sufficient progeny can be produced for cultivars lacking seeds. Micro-propagation is a viable process that produces rooted plants ready for acclimatization in the field (Bhatia and Ashwath 2008).

Different crop modification techniques such as cross breeding, mutagenesis, polyploidy, protoplast fusion, transgenesis, and genome editing are used to improve desirable traits. A rapid rise in population requires an increase in food

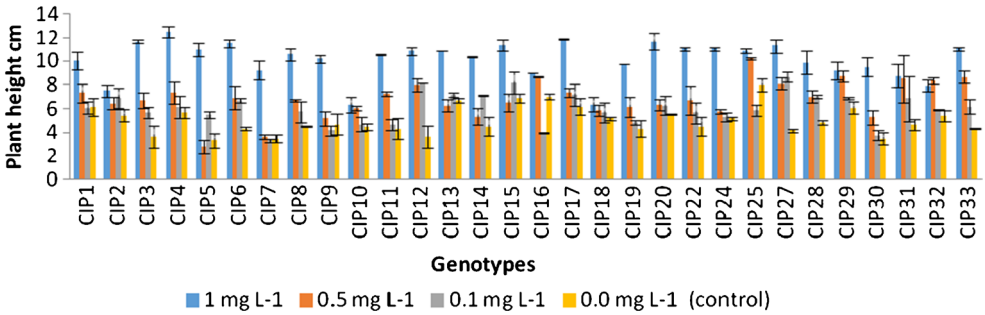

Figure 1. Effects of different concentration of gibberellic acid (GA_3) on plant height of potato germplasms.

productivity, feed, fuel, and fiber, which can be achieved through plant breeding and selection techniques (Fu 2015). However, plant breeding can produce a narrow genetic base in cultivars and hence, increase their susceptibility to diseases and devastation, due to adverse environmental factors and uniformity in response (Nejat *et al.* 2017).

Gibberellic acid (GA_3) has been shown to modulate developmental processes in the plant life cycle (Xu *et al.* 1998; Khan and Bano 2016a) and to stimulate mitotic division in stems, roots, and leaves, but mainly it induces growth and cell elongation (Hedden and Thomas 2016). In germinating seeds, the gibberellins regulate the formation of various types of mRNA that code for different enzymes, and hence increase the percent germination (Richards *et al.* 2001). Various types of gibberellins have been isolated, and they are synthesized primarily in plant leaves (Vivanco and Flores 2000).

Gibberellins are used to artificially break seed dormancy and to improve crop growth and development. However, plants produce GA_3 in very minute quantities. Therefore, to use this phytohormone, it needs to be produced by microorganisms through expensive fermentation and applied to plants exogenously (Khan and Bano 2016b).

Potato varieties are produced by hybridization and selection of a few lines of *S. tuberosum* sub-species. Genetic diversity evaluation is important to know the sources of genes, which are responsible for quality traits within the available germplasm (Haydar *et al.* 2007). The genetic diversity in potato research programs globally is important for the delivery of varieties bred to adjust to the changing climate and for humanitarian needs. The farmer uses the optimal potato varieties for production, according to the demands of the modern world. Different potato varieties show different responses to disease and climatic change. In fact, it is now acknowledged that the genetic diversity of crop loss is a complex process and depends on the local variations in the environment, culture, and economy (Brush 2004).

The long-range goal of the present work was to develop an economically practical, disease-free planting material system

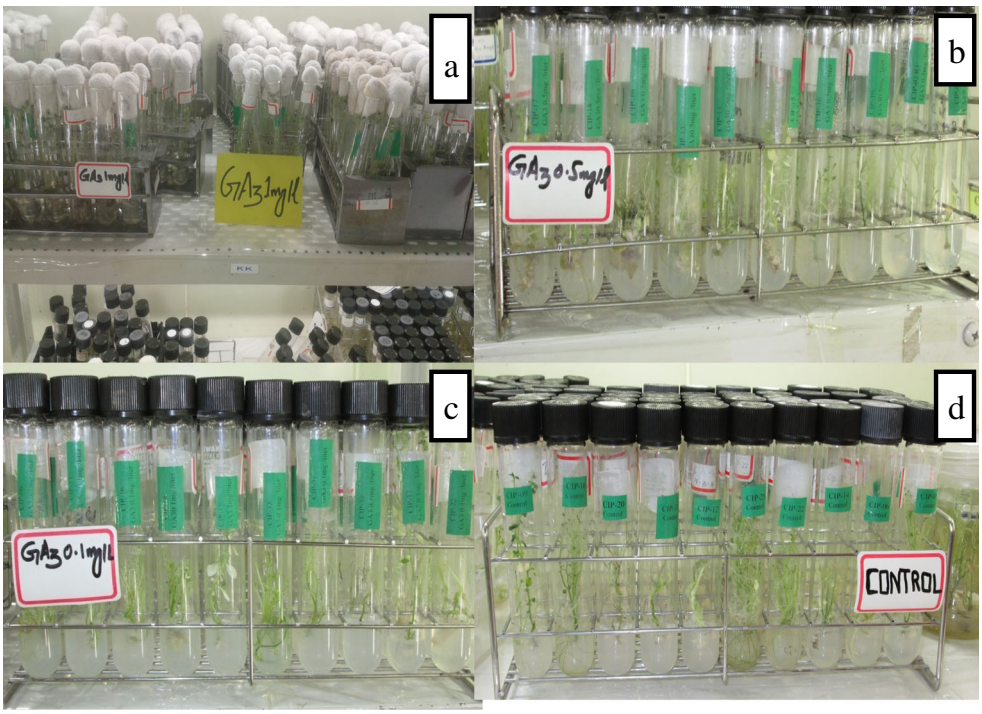

Figure 2. *a–d* Effect of different concentration of gibberellic acid (GA_3) on plant height of potato germplasms.

Figure 3. Effect of gibberellic acid (GA_3) at 1.0 mg L^{-1} on potato plant.

using nodal stem culture of the CIP potato cultivars grown in Pakistan, and to identify the influence of different plant hormones on multiplication and different concentrations of growth regulators on CIP potato germplasm. The present study was carried out to assess the *in vitro* effects of GA_3 on morphogenesis and on acclimatization of CIP potato explants in field conditions and to study the morphological characteristics of different CIP potato cultivars.

Materials and methods

The present study was carried out with 1.5 to 2.0 cm aseptic nodal segments from plant materials acquired from the International Potato Center (CIP), Lima, Peru and *in vitro* cultured at the Plant Genetic Resource Institute (PGRI), National Agriculture Research Center (NARC) Islamabad, Pakistan. For tissue culture, MS (Murashige and Skoog 1962) medium (Bioworld, Visalia, CA) was used and supplemented with 0.0 (control), 0.1, 0.5, or 1.0 mg L^{-1} gibberellic acid (TradeFord.com). After completion of the growth period, the plantlets were transferred to a greenhouse located at Murree, 49.5 km away from NARC, Islamabad, for additional examination of the qualitative and quantitative traits of the selected cultivars.

Culture media Stock solutions were prepared, and then dilutions were made from these stock solutions to prepare MS medium. Full strength MS medium with all macronutrients, micronutrients, and vitamins were used in the experiments, which was best for the growth of different parts of the potato cultivars. Sucrose at 30 g L^{-1} was added to the medium as a source of energy and carbon for plant development. After the addition of 2.3 g L^{-1} agar (Plant TC agar, Merck, Kenilworth, NJ), the media were boiled in a microwave (Argos Technologies, Vernon Hills, IL) to equally distribute components in the medium before pouring into tubes (size 25 × 190 mm; Globe Scientific Supply, Wilmington, NC), 10 mL per tube. The pH of all culture media was adjusted to 5.8 with 1 M HCl and 1 M NaOH (Ittehad Chemicals Limited, Kala Shah Kaku, Pakistan) before boiling the media. All the equipment (forceps, scalpels, Petri plates, and test tubes) were surface sterilized in an autoclave (LABRepCo, Horsham, PA) at 121°C and 103.42 kPa pressure for 1 h. Autoclaving was increased for media with a volume more than 10 mL in a test tube.

***In vitro* propagation** The routinely cultivated CIP cultivars were cultured in MS media supplemented with different concentrations of gibberellic acid under aseptic conditions. The cultures were incubated at 25 ± 1°C with a 16 h photoperiod (fluorescent white tube light; Philips, Lahore, Pakistan) under a light intensity of 17.98 μmol m^{-2} s^{-1} for 4 wk. Additional subculturing was carried out for a month for germplasm multiplication. Data from different parameters including plant height, number of nodes, shoots, leaves and roots, and length of roots were recorded after 1 mo of culturing.

Acclimatization of plants The fully developed *in vitro* plants were transferred to peat moss bags (PREMIER peat moss,

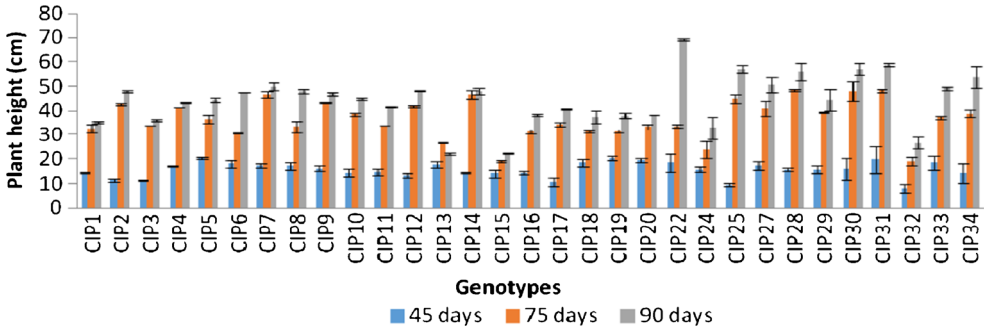

Figure 4. Plant height at different times after acclimatization.

Table 1 Phenotypic diversity among different CIP potato germplasms

Genotypes	Plant height (cm)	Number of branches	Number of leaves	Number of tubers
CIP-01	35.333^{fghi}	4.667^{ij}	18.667^{lm}	5.667^{bcdefg}
CIP-02	48.000^{bcdefg}	8.667^{bcdefgh}	22.333^{klm}	5.333^{bcdefg}
CIP-03	36.667^{fghi}	10.000^{abcde}	22.333^{klm}	6.667^{bcd}
CIP-04	43.333^{bcdefg}	10.000^{abcde}	27.000^{ghijklm}	5.333^{bcdefg}
CIP-05	44.333^{bcdefg}	9.333^{abcdef}	38.333^{abcdef}	13.000^{a}
CIP-06	47.667^{bcdefg}	6.333^{fghij}	16.667^{m}	3.667^{cdefg}
CIP-07	50.000^{bcdef}	7.000^{efghij}	23.333^{jklm}	7.000^{bc}
CIP-08	48.000^{bcdefg}	8.000^{cdefgh}	44.667^{ab}	5.333^{bcdefg}
CIP-09	47.000^{bcdefg}	5.667^{hij}	24.000^{ijklm}	4.667^{bcdefg}
CIP-10	45.000^{bcdefg}	4.333^{j}	21.667^{klm}	5.000^{bcdefg}
CIP-11	41.667^{cdefgh}	6.333^{fghij}	25.000^{ijklm}	6.000^{bcdef}
CIP-12	48.333^{bcdefg}	10.667^{abcd}	30.000^{efghijk}	7.333^{b}
CIP-13	22.000^{i}	8.000^{cdefgh}	26.333^{ghijklm}	6.333^{bcde}
CIP-14	48.000^{bcdefg}	10.667^{abcd}	44.333^{abc}	4.667^{bcdefg}
CIP-15	22.333^{i}	7.667^{defghi}	41.333^{abcd}	6.333^{bcde}
CIP-16	38.333^{efgh}	10.000^{abcde}	36.667^{abcdefg}	4.333^{bcdefg}
CIP-17	40.667^{defgh}	11.333^{ab}	28.667^{fghijkl}	5.667^{bcdefg}
CIP-18	37.333^{fghi}	9.667^{abcde}	41.000^{abcd}	7.333^{b}
CIP-19	38.000^{fgh}	11.333^{ab}	29.667^{efghijk}	11.000^{a}
CIP-20	38.333^{efgh}	10.000^{abcde}	39.333^{abcde}	3.667^{cdefg}
CIP-22	69.000^{a}	11.000^{abc}	33.667^{defghij}	3.333^{defg}
CIP-24	33.000^{ghi}	12.333^{a}	25.333^{hijklm}	6.000^{bcdef}
CIP-25	57.000^{abc}	5.667^{hij}	35.667^{bcdefgh}	3.000^{efg}
CIP-27	50.667^{bcdef}	7.667^{defghi}	39.333^{abcde}	2.667^{fg}
CIP-28	56.000^{abcd}	7.667^{defghi}	34.000^{cdefghi}	5.667^{bcdefg}
CIP-29	44.667^{bcdefg}	5.667^{HIJ}	47.000^{a}	6.000^{bcdef}
CIP-30	57.000^{abc}	10.000^{abcde}	39.667^{abcde}	2.667^{fg}
CIP-31	58.667^{ab}	7.333^{efghij}	44.333^{abc}	2.667^{fg}
CIP-32	26.667^{hi}	9.000^{bcdefg}	23.333^{jklm}	2.333^{g}
CIP-33	49.333^{bcdef}	10.000^{abcde}	24.000^{ijklm}	4.000^{bcdefg}
CIP-34	53.667^{abcde}	6.000^{ghij}	33.333^{defghij}	4.667^{bcdefg}

Values followed by different letters in a column were significantly different ($P < 0.005$)

Brighton, Ontario, Canada) and were kept in the greenhouse for additional studies. After 2 mo of growth in greenhouse, data for different parameters viz. plant height, number of nodes, roots, shoots, and leaves per plant was recorded.

Data analysis The data was analyzed using Statistica™ 8.1 (Tibco®, Palo Alto, CA) statistical software. Analysis of Variance (ANOVA) was used for comparison among different potato species.

Results and discussion

Effect of GA_3 concentration on plant height Micro-propagated plants treated with GA_3 had significantly increased plant height compared to the control (Figs. 1, 2, 3). The maximum increase (10 cm) was recorded in genotypes CIP 3, 4, 6, 12, 15, 17, 20, 22, 24, 27, and 33 treated with 1.0 mg L^{-1} GA_3. Different genotypes responded differently to micro-propagation, as previously reported by Danci and Danci (2007). According to Bhuiyan (2013), GA_3 was most effective in increasing shoot length in different potato varieties. Plants treated with 0.5 mg L^{-1} GA_3 had increased plant height up to 6.8 cm (Fig. 1). However, an increase of 6 cm in plant height was recorded in response to 0.1 mg L^{-1} of GA_3. The GA_3 supplements reduced the regeneration time of shoots on nodal explants of CIP germplasms. The GA_3 stimulated plant height by increasing inter-nodal distance by cell elongation or cell division as reported earlier (Mahajan *et al.* 2016).

In the greenhouse, the plant height of acclimatized micro-propagated plants was recorded at regular intervals of time, and the plant height varied from 69 to 22 cm. The maximum plant height was observed in the CIP 22 cultivar followed by CIP 31 (58.6 cm) and CIP 30 (57 cm). As indicated by Ranjbar and Mirzakhan (2012), the difference in the plant height in CIP cultivars showed the genetic diversity in these varieties (Fig. 4; Table 1).

Number of nodes The numbers of nodes per plant were visually counted which showed that the control had the highest number of nodes (average 31 nodes per plant). These results demonstrated that plants supplemented with GA_3 at the rate of 1 mg L^{-1} had significantly lower number of nodes (average 12

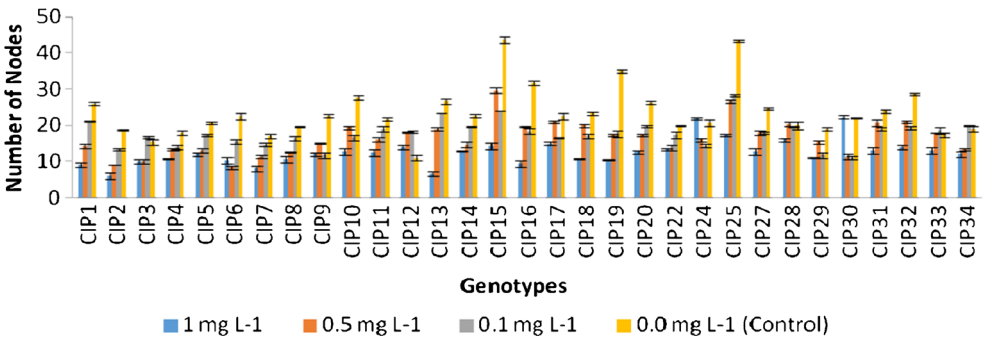

Figure 5. Effects of different concentration of gibberellic acid (GA_3) on number of nodes.

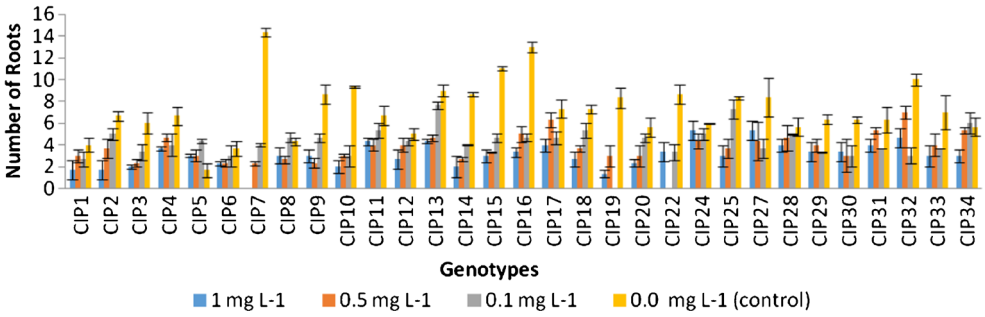

Figure 6. Effects of different concentration of gibberellic acid (GA_3) on number of roots of potato germplasm.

per plant), which was 91% less than that of control (Fig. 5). On the other hand, plants treated with 0.5 mg L^{-1} of GA_3 showed a decrease in the number of nodes by 43% as compared to the control followed by plants supplemented with 0.1 mg L^{-1} of gibberellic acid (26%). The numbers of nodes were decreased with an increase in GA_3 concentrations, and the primary effect of GA_3 was to increase plant height by increasing the inter-nodal length and by reducing the number of nodes per plant. Ndagijimana *et al.* (2014) reported that GA_3 increased plant height by increasing the inter-nodal length, and these finding are in agreement with the current study.

Number of roots In present study, maximum numbers of roots (7 per plant) were recorded in the control treatment, rather than in the gibberellic acid-treated plants. Higher concentration of GA_3 (1.0 mg L^{-1}) significantly reduced (threefold) the number of roots per plant. Highly significant reduction (97%) in number of roots per plant also occurred in plants treated with 0.5 mg L^{-1} of gibberellic acid followed by (72%) at a concentration of 0.1 mg L^{-1}, with respect to the control. The highest number of roots were observed in CIP 7 and CIP 16, but there was no significant difference between 0.5 and 0.1 mg L^{-1} of GA_3 (Fig. 6).

Plants treated with 1 mg L^{-1} GA_3 significantly increased (1.9 cm) root length compared to the other treatments (Fig. 7). Whereas, the increase in root length was not significant (0.89 cm) in plants treated with 0.5 mg L^{-1} GA_3. Plants treated with 0.1 mg L^{-1} of GA_3 did not show any significant increase in root length (Fig. 7). Similar to the present results, Doaigey *et al.* (2013) reported for date palm (*Phoenix dactylifera* L.) and Lulai *et al.* (2016) for potato, that GA_3-treated plants had a lower number of roots, but had increased root extension.

Number of shoots GA_3 levels of 0.1 and 0.5 mg L^{-1} did not cause any significant effect on the number of shoots (Fig. 8). However, data showed that 1.0 mg L^{-1} of GA_3 significantly reduced (twofold) the number of shoots as compared to lower concentrations of GA_3. There was contradiction between 0.5 and 0.1 mg L^{-1} concentrations of GA_3, because in some genotypes 0.5 mg L^{-1} GA_3 increased the number of shoots,

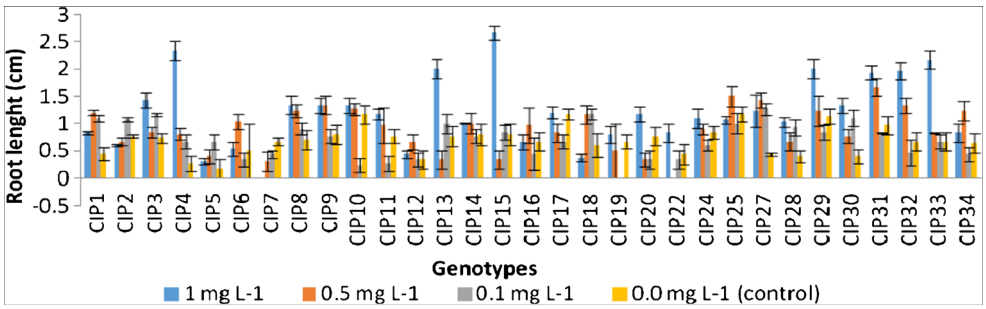

Figure 7. Effects of different concentration of gibberellic acid (GA_3) on root length of potato germplasm.

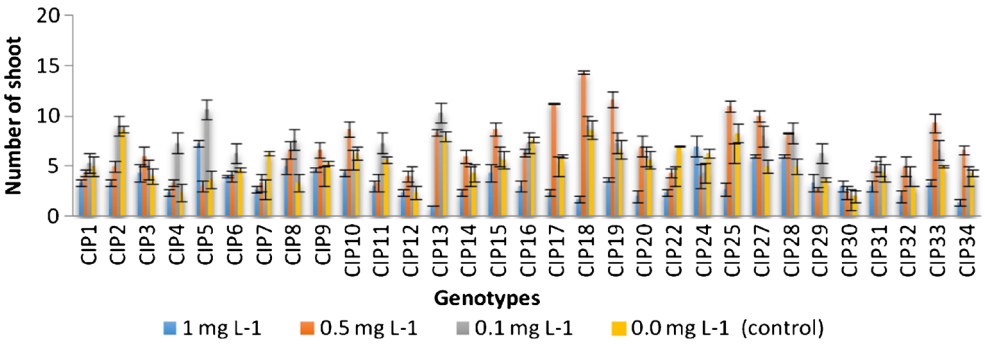

Figure 8. Effects of different concentration of gibberellic acid (GA_3) on number of shoots of potato germplasm.

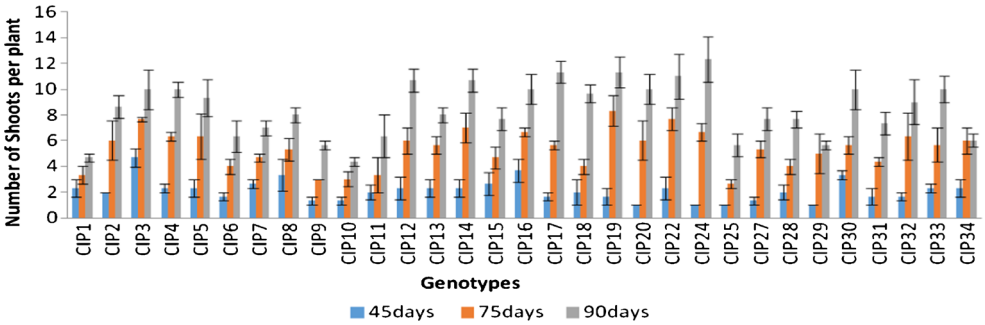

Figure 9. Shoot number of different genotypes at different times after acclimatization.

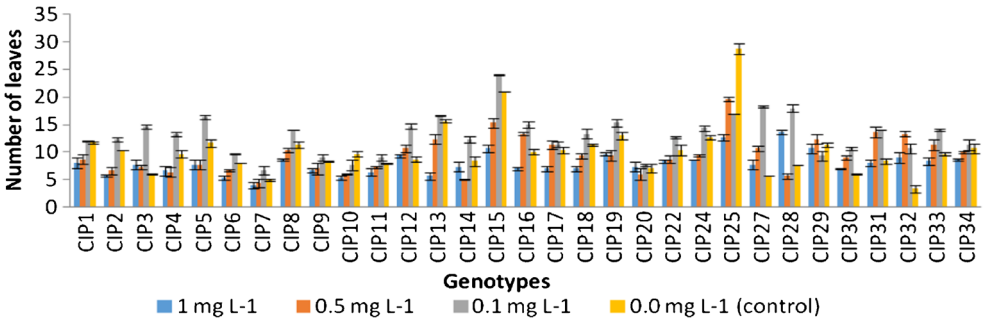

Figure 10. Effect of different concentration of gibberellic acid (GA_3) on number of leaves of potato germplasm.

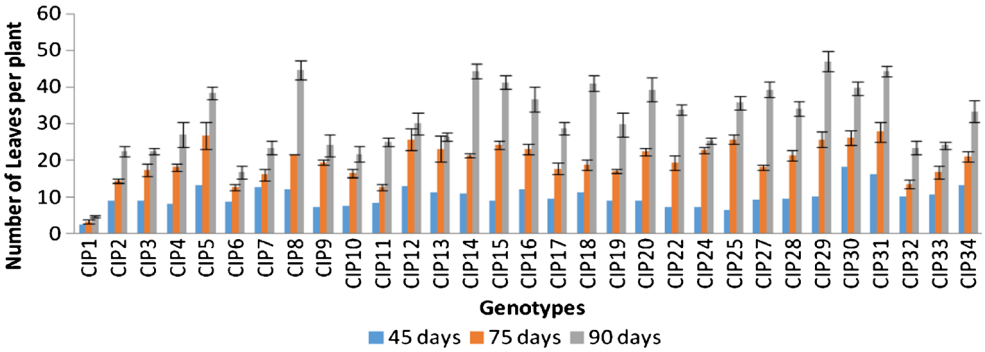

Figure 11. Number of leaves in different genotypes at different times after acclimatization.

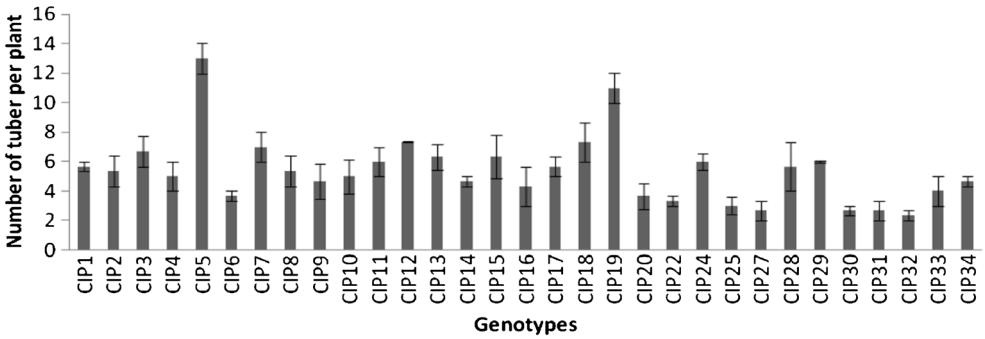

Figure 12. Number of tubers in different exotic potato germplasm.

while in others 0.1 mg L^{-1} was effective. Yasmin *et al.* (2011) reported that the combination of 1.0 mg L^{-1} pantothenic acid and 0.5 mg L^{-1} gibberellic acid, induced the highest number of shoots and roots in different varieties of potato.

Data related to number of shoots per plant showed highly significant differences among the analyzed cultivars (Fig. 9; Table 1). The number of shoots per plant was highest (12.3) in CIP 24, followed by CIP 19 (11.333). However, the lowest number of shoots was observed in CIP 10 (4.333).

Number of leaves The results showed that high concentrations of GA_3 were inhibitory to the number of leaves produced (Fig. 10; Table 1). The highest number of leaves (13 per plant) were recorded in plants treated with 0.1 mg L^{-1} of GA_3 as compared to other concentrations of GA_3 (0.5 and 1 mg L^{-1}). The data revealed that the minimum number of leaves (7.92 per plant) was recorded in 1.0 mg L^{-1} followed by 0.5 mg L^{-1} of GA_3 (9.5 per plant) compared to 0.1 mg $^{-1}$ of GA_3. According to Lolaei *et al.* (2013), gibberellins are widely involved in all stages of growth and development of plants and increase the number of leaf and the length of stolon when applied at low concentration as compared to control. Xu et al. (2016) also reported that GA_3-treated plants exhibited a change in leaf size and number. In the present study, the number of leaves was highest (47 per plant) in CIP 29 followed by CIP 8 (44 per plant). The lowest number of leaves was noted in CIP 06 (16.6 per plant) followed by CIP 01 (18.6 per plant) (Fig. 11; Table 1).

Number of tubers per plant Maximum number of tubers per plant was obtained from CIP 05 13) followed by CIP 19 (11 (Table 1). However, the minimum number of tubers (2.3 per plant) was recorded in CIP 32 (Fig. 12 and Table 1). These results are consistent with Luthra *et al.* (2005), who reported variations in number of tubers in different genotypes. The differences in tuber number could be due to varietal character, including which variety performs better (Kumar *et al.* 2007). The heredity effect was significant with regard to the number of tubers (Muthuraj *et al.* 2005). Ravikant and Chadha (2009) reported that excessive vegetative growth retarded tuber initiation, while restricted foliage growth help in the onset of tuberization. Similar findings were also reported by Knowles and Knowles (2006).

Conclusion

The increase in plant height and other growth attributes indicated that potato responded well to the different concentrations of GA_3. The results also confirmed that GA_3-treated plants were thin as compared to untreated plants. Furthermore, it is suggested that different physicochemical cultural conditions have a significant influence on the micropropagation studies and optimization protocols for potato could be valuable for future research studies.

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