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IN VITRO DEVELOPMENT AND IMPROVEMENT OF CHROMIUM (VI)-AFFECTED ADVENTITIOUS ROOTS OF *SOLANUM TUBEROSUM* L. WITH GA_3 AND IAA APPLICATION

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Abstract

This study was undertaken *In vitro* to investigate the inhibitory effects of Chromium (Cr) VI by using $K_2Cr_2O_7$ on the growth of adventitious roots of potato (*Solanum tuberosum* L. cv Desiree). Another objective was to determine possible reversal of the detrimental growth effects with suitable combinations of GA_3 and IAA. It was observed that the applied Cr VI (100 ppm) alone in MS media inhibited root growth. However, addition of selected combinations of GA_3 and IAA in the MS media along with Cr VI improved the root growth. The plants grown on MS medium + 100 ppm $K_2Cr_2O_7$ resulted in reduction in number of adventitious roots up to 36.26%, number of rootlets (80.64%), diameter (37.27%), fresh and dry weights (72.96 and 84.74%, respectively) in comparison with control (MS medium). MS media containing 100 ppm $K_2Cr_2O_7$ + 20 ppm GA_3 + 16 ppm IAA enhanced the average number of roots from 4.78 to 62.6, number of rootlets from 2.6 to 28.2, diameter from 0.13 mm to 0.68 mm, fresh weight from 34.4 mg to 1234.75 mg and dry weight from 1.8 mg to 80.40 mg compared with cultures grown on MS + 100 ppm Cr (VI). The results thus highlight the fact that the deleterious effects of Cr VI on potato growth *In vitro* can be reversed with suitable hormonal treatments. Furthermore, this research has possible implications on large scale potato cultivation under heavy metal stress in a broader sense.

Introduction

Solanum tuberosum L., a dicotyledonous herbaceous plant species is grown in over 130 countries of the world and used as a staple food (Salem *et al*., 2010; Sajid & Aftab, 2012). Demand for potato is rising gradually because of increasing population and low production of potato and other food crops *viz*., wheat, rice, maize etc. In comparison with many developed countries, potato yield in Pakistan is not satisfactory (Farhatullah *et al*., 2007). Root system plays an important role in increasing the yield of plants (Ahmad *et al*., 2004). Potato plants developed from nodal explants or tubers form fine fibrous adventitious roots. Adventitious roots develop from organs such as leaves and stems under unusual circumstances and are considered to be an important phenomenon for the production of large number of plants through tissue culture means (Ford *et al*., 2001). Roots are concerned mainly with the absorption of water and minerals. A well developed root system is therefore important for increasing the crop yield. Problems in root formation result usually in decreased yield (de Klerk *et al*., 1999).

Soil contamination by heavy metals causes inhibition of root growth (Maksymiec, 2007; Khan *et al*., 2013). Out of these heavy metals, chromium (Cr) is considered as a severe environmental contaminant. Cr is present in the environment in two oxidation states i.e., trivalent and hexavalent (Chidambaram *et al*., 2009). Excessive amount of both chromium forms (Cr VI or Cr III) are usually toxic and detrimental to the growth and development of plants (Shahzadi *et al*., 2013). Hexavalent (Cr VI) form of chromium, however, is more toxic than the trivalent (Cr III) form (Jamal *et al*., 2006) as the later is considered to be less mobile (Becquer *et al*., 2003). Anthropogenic activities have increased bioavailability and bio-mobility of Cr in the soil (Shanker *et al*., 2005). Cr compounds are used in many industries such as manufacturing of inks, industrial dyes, paint, in chrome tanning, mining industries and other metal cleaning, electroplating operations, leather tanning, waste-water exchange, filtration, and electrochemical treatments etc., (Aksu *et al*., 2002; Davis *et al*., 2003; Rai *et al*., 2004; Dube *et al*., 2009; Wani *et al*., 2009). Effluents from these industries contain unwanted amounts of chromium ions.

Adventitious root formation is a complex phenomenon that involves many endogenous factors such as phyto-hormones and a suitable environment (Sorin *et al*., 2005). Plant growth regulators are involved in root growth regulation as well (Konishi *et al*., 2005) and suitable levels can also reverse some of the limiting effects of biotic and abiotic stress in plants (Ogawa *et al.*, 2003). According to Shanker *et al*., (2005), root growth is usually well thought-out as a valuable feature for tolerance of heavy metals in plants. Plant hormones are concerned with growth and development of plants (Spartz, & Gray, 2008), biochemical processes (Gul *et al.*, 2006), in yield of the crops (Farhatullah *et al*., 2007), provide directional and positional information (Bai & DeMason, 2008), signal genetic program for regulation of growth and increasing cell division and cell expansion (Wang *et al*., 2007). Gibberellins and auxins are involved in growth and development of roots, and crosstalk with various hormonal signaling pathways (Nemhauser *et al*., 2006; Yaxley *et al*., 2001).

Gibberellic acid (GA_3) is involved in many plant responses to the external environment (Akbari *et al*., 2008). The role of GAs, in general, is poorly studied in root development (Fukaki & Tasaka, 2009) and in particular with reference to lateral root formation (Gou *et al*., 2010). Auxins are involved in cell elongation, tissue enlargement, cell division, root induction, cell wall loosening (Cosgrove, 2005), defense responses (Bhandari *et al*., 2009) and gene expression etc., (Santner *et al*., 2009). Higher doses of auxins stimulate root induction and differentiation of vascular tissues (Tanimoto, 2005; Davies, 2004). For lateral root initiation, local tissue auxin accumulation is required (Heisler *et al*., 2005). Amongst the various auxins, Indolacetic acid (IAA) is fairly predominant and plant root tissues are known to be sensitive to it (Teale *et al*., 2006). Exogenous auxins can stimulate root induction, affect further development and root patterning (Kepinski & Leyser 2005). A balanced hormonal approach is therefore of utmost importance to yield desirable cellular response. This approach is further supported by the fact that usually a well-defined correlation exists between levels of various hormones and a certain developmental stage. The inhibitory effects of heavy metals such as Cr in our case can theoretically be controlled and potentially be reversed to greater or lesser extent by exposing the plants to suitable hormonal treatments. Since root development of potato in our preliminary *In vitro* studies was significantly hampered with higher Cr concentration, it was considered worthwhile to see the effects of some suitable levels of both GA_3 as well as IAA on release of its detrimental effects. From the view point of contemporary literature on potato also, it is not known whether a certain hormonal treatment can reverse the inhibitory effects of Cr or not? Hypothetically if yes, then such suitable level/s needs to be determined in potato. The objective of this study therefore was to investigate the inhibitory effect of chromium ($K_2Cr_2O_7$) on adventitious root growth of *Solanum tuberosum* L. (cv. Desiree) and its possible reversal with suitable combination of GA_3 and IAA under *In vitro* conditions.

Materials and Methods

Experimental plan: Nodal explants (1.0 cm long) of potato cv. Desiree from 30-day-old *In vitro*-grown plants were inoculated on Murashige & Skoog (1962) medium containing 100 ppm of Cr IV ($K_2Cr_2O_7$), 12 or 20 ppm GA_3, and 4 or 16 ppm of IAA (Best concentrations of IAA and GA_3 selected during preliminary experiments). Sucrose (30 g/l) was used as a carbohydrate source and agar (7g/l) was used for MS medium solidification. Culture tubes (25 × 150 mm) were used to pour 10 ml medium per tube. All media variants were autoclaved after pH (5.7) adjustment for 15 minutes at 121°C. The culture conditions were 25 ± 2°C and 16 h photoperiod from cool white fluorescent tube lights (35 m mole/m/sec). Fifteen replicate culture vessels were inoculated for each treatment. After 30 days of inoculation, plants were harvested, washed gently and dried on filter papers for the removal of excess water. Following treatments were included in the present work (Table 1).

Table 1. Various treatments involving medium, chromium VI, GA_3, and IAA.

Treatment	MS medium	Chromium VI (ppm)	GA_3 (ppm)	IAA (ppm)
Control*	+	-	-	-
1	+	100	-	-
2	+	-	12	-
4	+	-	20	-
5	+	-	-	4
6	+	-	-	16
7	+	100	12	4
9	+	100	20	16

*MS medium without GA_3, IAA and $K_2Cr_2O_7$ was used as control

Growth measurements: Variables measured in the present study included number, diameter, length, fresh/dry weights of roots. Longest roots and rootlets were measured with the help of a standard scale and their diameter was measured using Vernier Caliper. Fresh and dry weight of roots was measured with digital electric balance. For fresh weights, regenerated plants were harvested and weighed directly. Plants were then placed in paper bags and placed at 60 °C for five days before weighing. Increase or decrease in growth parameters was calculated with the help of the following expression.

$$\text{Relative growth} = \frac{\text{Treatment} - \text{control}}{\text{control}} \times 100$$

Data collected were subjected to ANOVA and presented as average ± standard error of the means.

Results

Mean number of various growth parameters is given in Table 2. The number of roots (7.5) and rootlets (12.4), diameter, (0.22 mm), length (8.18 cm), fresh and dry weights of roots (127 and 11.8 mg) were observed in the control (MS medium) after 30 days of inoculation.

Table 2. Effect of various levels of Cr VI, GA_3 and IAA on number of roots/ rootlets, diameter, length, fresh and dry weight of *Solanum tuberosum* L. roots.

Treatments	Number of roots	Number of rootlets	Diameter of roots (mm)	Roots length (cm)	Fresh weight of roots (mg)	Dry weight of roots (mg)
Control	7.5 ± 0.98	12.4 ± 1.93	0.22 ± 0.01	8.18 ± 0.47	127 ± 33	11.8 ± 2.85
100 ppm Cr VI	4.78 ± 0.62 (- 36.26%)	2.4 ± 1.07 (- 80.64%)	0.13 ± 0.01 (- 37.27)	5.6 ± 0.42 (- 31.54%)	34.4± 7.10 (- 72.95%)	1.8 ± 0.48 (- 84.74%)
12 ppm GA_3	10.5 ± 2.12 (+ 40.00%)	13.55 ± 2.77 (+ 9.27%)	0.29 ± 0.03 (+ 32.27%)	8.66 ± 0.44 (+ 5.86%)	292 ± 36.47 (+ 129.92%)	21. 66 ± 1.15 (+ 83.61%)
20 ppm GA_3	5.2 ± 1.58 (- 30.66%)	6.0 ± 1.36 (- 51.61%)	0.12 ± 0.02 (- 45.45%)	6.23 ± 0.63 (- 23.83%)	67.4 ± 9.84 (- 46.29%)	2.8 ± 1.11 (- 76.27%)
4 ppm IAA	76.7 ± 7.16 (+ 922.66%)	16.4 ± 1.62 (32.25%)	0.50 ± 0.03 (127.27%)	5.48 ± 0.25 - 33.98%	1056.33 ±55.16 (+ 731.73%)	66.50 ± 7.82 (+ 463.55%)
16 ppm IAA	34.2 ± 5.90 (+ 356.00%)	20.7 ± 3.63 (+ 66.93%)	0.70 ± 0.13 (+ 218.18%)	3.79 ± 0.24 (-53.66%)	827.8 ± 24.66 (+ 551.81%)	37± 6.34 (+ 213.55%)
100 ppm Cr VI+ 12 ppm GA_3 + 4 ppm IAA	63. 4 ± 8.81 (+745.33%)	13.0 ± 2.48 (+ 4.83%)	0.20 ± 0.01 (- 9.09%)	7.57 ± 0.44 -7.45%	390.6 ± 25.39 (+ 207.55%)	27.6 ± 6.83 (+133.89%)
100 ppm Cr VI+ 20 ppm GA_3 +16 ppm IAA	62.6 ± 5.21 (+734.66%)	28.5 ± 5.52 (129.83%)	0.68 ± 0.05 (+ 209.09%)	5.33 ± 0.66 (- 34.84%)	1234.75 ± 37.39 (872.24%)	143.2 ± 5.27 1113.55%

All values are means of 15 replicates (± SEM) after 30 days of inoculation

Addition of 100 ppm $K_2Cr_2O_7$ to MS medium proved inhibitory as a decrease in the number of roots as well as rootlets was up to 36.26% and 80.64%. Likewise, reduction of 37.27, 31.54, 72.95 and 84.74% was observed in diameter, length, fresh and dry weight of roots in comparison with the control after 30 days of explant inoculation. MS medium fortified with 12 ppm of GA_3 enhanced the number of roots as well as rootlets by 40% (Fig. 1A) and 9.27% (Fig. 1B) respectively. However, average diameter, length, fresh and dry weight of roots increased up to 32.27, 5.86, 129.92 and 83.61% (Fig. 1C, D, E, F).

Inoculation of explants in MS media containing 20 ppm GA_3 resulted in callus induction first and rooting started only later thus resulting in decreased number of roots and rootlets (up to 30.66, 51.61%) and thereby yielding further poor growth parameters i.e., diameter and length of roots were reduced up to 45.45 and 23.83%, respectively than the control. Likewise 46.29 and 76.2% reduction in fresh and dry weight of roots was observed respectively. When 4 ppm IAA was added in the MS medium, interesting results were recorded i.e., increase in average number of roots, rootlets and diameter of roots was 76.7 (922.66%), 16.4 (32.25%) and 0.5 mm (127.27%) respectively. In contrast, using MS medium containing 4 ppm IAA, a decrease in length of roots was registered that was to the tune of 33.98%. Fresh and dry weight of roots though increased significantly (731.73% and 463.55%, respectively). Higher concentration of IAA (16 ppm) in MS medium enhanced the number of roots up to 34.2 (356%) whereas increase in the number of rootlets was 66.93%. Similarly, the recorded increase in root diameter was 218.18%. However, decrease (53.66%) in length of roots was registered while fresh and dry weight increased sharply (551.81% and 213.55%, respectively). $K_2Cr_2O_7$ (100 ppm) in different combinations with GA_3 and IAA also yielded interesting results. MS medium containing 100 ppm $K_2Cr_2O_7$, 12 ppm GA_3 and 4 ppm IAA enhanced the average number of roots up to 63.4 (745.33%) in comparison with the control. A small increase (4.83%) in the number of rootlets was also noted. However, both diameter and length of roots were somehow reduced (up to 9.07 and 7.45%, respectively). Increase in fresh and dry weight was relatively large (207.55 and 133.89%, respectively). A big positive change was recorded in MS medium containing 100 ppm $K_2Cr_2O_7$, 20 ppm GA_3 and 16 ppm IAA for number of roots and rootlets, and diameter. The recorded increase was up to 62.6 (734.66%), 28.5 (129.83%) and 0.68 (209.09%), respectively (Table 2, Fig. 1A, B, C). Contrarily, decrease the root length was recorded as being 34.84% (Table 2, Fig. 1, A, B, C). Fresh and dry weight showed increase up to 872.24%, and 1113.55% (Fig. 1 E, F) in comparison with the control.

Discussion

In the present investigation, the root growth parameters of *Solanum tuberosum* cv. Desiree were recorded to be affected by the use of chromium compound ($K_2Cr_2O_7$) in MS medium. Reduction in corresponding values was therefore recoded in the mean number of root/rootlets, diameter, length, fresh and dry weights of roots. As the roots are in direct contact with the medium (Shanker *et al*., 2005) plant tissues cannot detoxify higher concentrations of heavy metals. The reduction may be due to deleterious effects of exogenous chromium on mitotic activities, inhibition in enlargement of cells, changes in physiological processes (Chaudhry & Khan, 2006) such as disturbance in carbohydrate and nitrogen metabolism, protein synthesis, photosynthesis and enzymatic activities (Yu & Gu 2007; Sinha *et al*., 2005) and low uptake of mineral nutrients because of elevated level of chromium in the roots (Kumar & Joshi, 2008). Iqbal *et al.*, (2001) and Chen *et al*., (2001) reported similar findings in Poinciana (*Caesalpinia pulcherrima*) and Wheat respectively.

GA_3 application in the present study increased the number of root and rootlets, diameter, fresh and dry weight of *In vitro*-grown potato plants. Exogenous GA_3 improved root growth at lower concentration (12 ppm). This increase may be due to several factors such as increased cell division (Silverstone & Sun, 2000), size of root meristem (Ubeda-Tomás *et al.,* 2009) and degradation of DELLA protein- an inhibitor of root growth (Nemhauser *et al*., 2006). Tanimoto (2005) reported that artificial depletion of GAs cause abnormal expansion and inhibition of root elongation. Low concentration of GA_3 in our studies improved root growth (increased roots and rootlets, diameter, fresh and dry weight of roots). Farhatullah *et al*., (2007) have also reported similar effects. Higher concentration of exogenous GA_3 proved to be insignificant in the present study. Higher concentration of GAs resulted in decreased growth as also reported by Yasmin *et al*., (2003). This may be due to the fact that higher concentration of GAs generally results in differentiation (Shani *et al*., 2006) whereas dedifferentiation is required in the meristems of lateral roots (Gou *et al*., 2010). In the present investigation, while 12 ppm GA_3 yielded better growth parameters, 20 ppm on the other hand, results in seemingly lower values. Higher level of GA_3 (20 ppm) in fact resulted in the formation of callus initially and root formation took place only later. This has resulted in apparently erroneous data but if the above point is taken into consideration, the anomaly for the reduced values of such growth parameters may be explained. However, Bakrim *et al*., (2007) reported that GA_3 treatment had no effect on root elongation. In *Populus tremula*, applied GAs suppressed development of adventitious as well as lateral roots (Gou *et al*., 2010) that may be due to the suppression of initiation of lateral root primordia (Casimiro *et al*., 2003).

Ricci *et al*., (2004) reported that the rooting of explants without pre-existing meristems depends on the supply of exogenous auxins. In the present work, the decrease in the length was accompanied by a corresponding increase in the number of adventitious roots. This may be due to the promotion of cell division (Perrot-Rechenmann, 2010). Auxins are involved in lateral root formation (Mishra *et al*., 2009) which again seems due to the enhancement of cell division. There are reports of adventitious root formation in the IAA-treated plants (Bhalerao *et al*., 2002). McDonald & Visser, (2003) working on tobacco observed that NPA (an auxin inhibitor) reduced the number of adventitious roots. Increase in the number of roots and rootlets in the present work can also be due to the effect of auxins on pericycle of plants as reported by Aloni, *et al.,* (2006) which in turn promotes root number thus increasing fresh and dry weight of roots. The width of roots increased with applied IAA in our studies. This fact may be attributed to the effects of auxins on cell wall's mechanical extensibility (Cosgrove, 1993). IAA softens the cell wall and increases the activity of enzymes acting on cell wall resulting in increased plasticity. The length of roots was inhibited as compared to the control in this present investigation. The root elongation inhibited with increasing concentration of IAA.

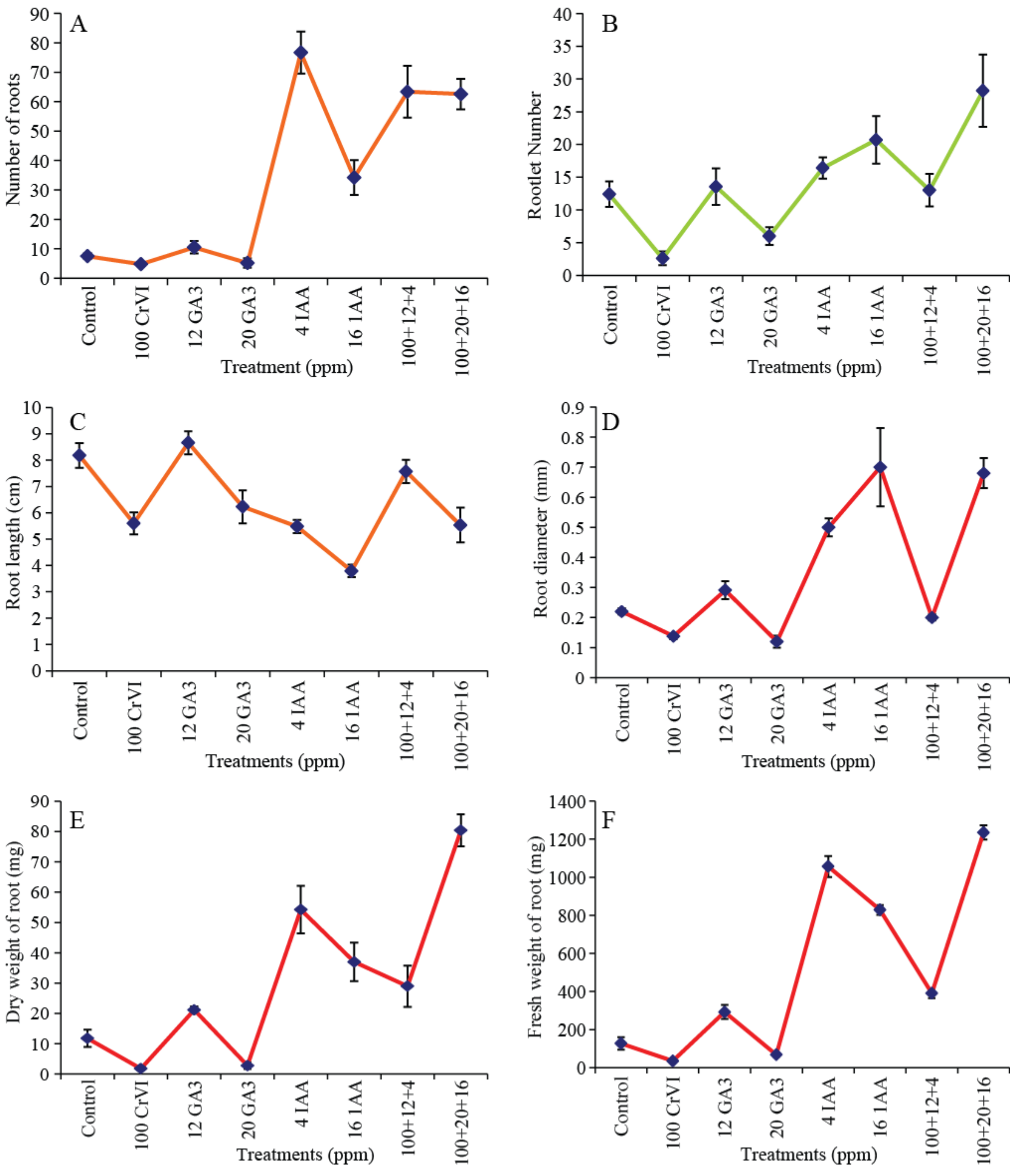

Fig. 1. Effect of various levels of IAA (4,16 ppm) and GA_3 (12, 20 ppm) individually or in combinations with 100 ppm $K_2Cr_2O_7$, when supplemented to full strength MS medium on (a) number of roots (b) number of rootlets (c) root length (d) root diameter (e) fresh weight and (f) dry weight of roots.

A concentration balance of GA_3 and IAA plays an important role in plant growth. Duca (2006) reported that there is an unequal distribution of GA_3 and IAA in the stem and root apices. The chromium-treated plants grown in MS medium containing mixed doses of both GA_3 + IAA in this study increased the number of roots as well as rootlets. The combination of 100 ppm Cr VI + 12 ppm GA_3 + 4 ppm IAA supplemented to MS medium improved the number of roots and rootlets in the current work (Table 2) that resulted in increased fresh and dry weight of roots. However, 16 ppm GA_3 and 4 ppm IAA decreased this number compared with the control. This may be due to complex interaction of hormones as a balanced hormonal combination is required for normal plant growth (Farhatullah *et al*., 2007). Auxins are involved in the synthesis of GA_3 which causes complex interaction (Ross & O'Neill, 2001, Ross *et al*., 2002, 2003) affecting several physiological processes (Chaudhry & Khan, 2006). Similarly, combination of 100 ppm Cr VI + 20 ppm GA_3 + 16 ppm IAA also enhanced this number and in fact proved to be the best combination. GA_3 in higher concentration inhibited root diameter while IAA application increased it. Inhibition in root growth parameters observed due to 100 ppm $K_2Cr_2O_7$ were therefore partly reversed by the application of both 12 ppm of GA_3 + 4 ppm IAA as well as 20 ppm of GA_3 + 16 ppm IAA in the MS medium.

Conclusion

The results from this investigation suggest that application of GA_3 and IAA in combination may mitigate adverse effects of exogenous chromium. Rooting response (number of roots as well as rootlets, diameter, fresh and dry weight) from nodal explants of *S. tuberosum* was adversely affected when applied with 100 ppm Cr VI in MS medium. The use of selected growth hormones was successful in the present investigation and inhibitory growth effects were reversed partially. Though two specific combinations of exogenous GA_3 and IAA improved the root growth of Cr-treated plants compared with the control, 20 ppm GA_3 and 16 ppm IAA proved to be the best combination as it enhanced the growth parameters better than the other applied combinations. These results may find application in further studies aiming at enhanced root formation and crop productivity in potato.

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