



**ASSESSMENT OF GENOTYPIC VARIATION IN GROUNDNUT (*Arachis hypogaea L.*) GENOTYPES FOR CHROMIUM TOLERANCE USING PHYSIOLOGICAL AND BIOCHEMICAL TRAITS**

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**ABSTRACT**

Chromium (Cr) is a well known toxic metal that affect the growth and development of plants. Assessment of genotypic variation for Cr tolerance is crucial to understand the insights of tolerance mechanism. A pot experiment was conducted in a green house to evaluate the genotypic variability in groundnut for Cr tolerance, with different concentrations of Cr. Thirteen groundnut genotypes were exposed to different levels of Cr and studied their relative responses in terms of growth and biochemical traits underlying across the genotypes. After one month of stress imposition, clearly differentiates the genotypes - in their growth parameters, viz., chlorophyll content, free proline, electrolyte leakage and antioxidative enzymes. The effect of Cr was severe with increasing concentration of Cr (100, 200, and 300 mg kg<sup>-1</sup> soil) on all genotypes studied. The shoot and root growth, biomass and chlorophyll content was severely affected at - higher concentration of Cr (300 kg<sup>-1</sup> soil). Free proline content and antioxidative enzymes like catalase (CAT) and Ascorbate peroxidase (APX) activity was significantly increased at (100 mg kg<sup>-1</sup> soil) Cr contamination, however the enzyme activities were gradually decreased at higher concentration of Cr (200, and 300 mg kg<sup>-1</sup> soil). The electrolyte leakage was also found to be increased with the increase in concentration of Cr. Based on -the data - genotypes studied can be categorized as - genotypes - highly tolerant (Abhaya, Anantha and Dharani) - as moderately tolerant (ICGV-91114, K-6, K-1375, TG-47 and Rohini) and sensitive (TPT-4, JL-24 and Narayahi) to Cr stress.

**KEY WORDS:** Heavy metal stress, Chromium (Cr), Groundnut, Proline, Antioxidative Enzymes



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## INTRODUCTION

Chromium (Cr) is the seventh most abundant transition element, which is available in two stable forms in nature, i.e., trivalent Cr (III) and hexavalent Cr (VI) <sup>64,44</sup>. Cr (VI) the forms of chromate ( $\text{CrO}_4^{2-}$ ), dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) and  $\text{CrO}_3$  is considered as highly toxic to biological systems as these forms are highly soluble and having high oxidization potential, whereas Cr (III) in the forms of oxides, hydroxides and sulphates is relatively insoluble in water, thus less mobile and less toxic to the biological systems <sup>40</sup>. In recent years contamination of the environment by chromium has become a major concern. Due to the use of the hexavalent Cr in several industries and municipal sewage, led to the high influx of Cr to the biosphere increased the bioavailability and permeability of the metal <sup>74, 38,71,24</sup>. In general, the concentration of chromium in the soil depends on the natural composition and anthropogenic deposition. Both the forms Cr (III) and Cr (VI) undergoes a variety of transformations such as oxidation, reduction, sorption, precipitation, and dissolution <sup>33</sup>. However, Cr (VI) might persist in the soil or sediments for years together when the concentration levels increase more than reducing capacity of the soil <sup>40</sup>. Chromium enters in the biological systems through the food chain all the way mediated mainly through the plants. Since, Cr is a nonessential element of the plant, uptake mechanism depends on the available form of a metal in the surrounding soils. The pathway of Cr (III) transport was a passive process and Cr (VI) can be uptaken by plant in an energy dependent process mediated by transporters <sup>64</sup>. Chromium compounds are highly toxic to plants and they severely affect the development of the plants. Several plant species exhibit genotypic differences in chromium toxicity tolerance and variations in the accumulation of Cr among plant tissues <sup>62,76,36</sup>. The response of plants to Cr contamination include the decrease in germination rate <sup>49,57,59</sup>, root elongation and biomass <sup>60,77</sup> height and growth of the stem <sup>16,57</sup> reduction in leaf area <sup>39</sup> and yield <sup>68</sup>. Cr can also alter the production of chlorophyll pigments <sup>56</sup> and reduces the activity of Nitrate reductase <sup>46</sup>. In general, elevated concentrations of heavy metals damage the plant tissues, stimulate the output of free radicals by imposing oxidative stress <sup>21</sup>. Similarly, in several studies it was identified that, an elevated Cr content increases the reactive oxygen species (ROS), which can peroxidize the membrane lipids and affects the function and morphological unity of biological membranes, resulting in increased plasma membrane permeability leading to leakage of potassium ions and other solutes <sup>44</sup>. To cope up such damages, plants induces a number of anti-oxidative enzymes, such as superoxide dismutase and ascorbate peroxidase that protect them against oxidative damage <sup>69</sup>. Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crop and Asia is the one of the biggest producer of the crop. Groundnut is a unique leguminous plant for its characteristic behavior to get the pods underground in direct contact with the ground. Most of the plants which were considered so far as metal

tolerances are oil seed crops belonging to the family Brassicaceae 14,72 (Clemens, 2001; Verbruggen et al., 2009). Recently accumulating evidences suggested that another important oil seed crop groundnut - is also distinguished as the metal tolerant plant 13,8 (Ching et al., 2008; Bianucci et al., 2012). However, most of the recent studies revealed the existence of genotypic variation within the genotypes of the same species, which is very important for the identification of potential candidate genotypes for the metal tolerance and also to elucidate the metal tolerance mechanism <sup>7,17,15</sup>. Thus, understanding the enzymes whose expression is altered by Cr stress can possibly led in the development of genotypes with improved levels of Cr tolerance. Previous study on Indian Mustard revealed the role of detoxification mechanism in Cr tolerance in genotypic variation for Cr accumulation and tolerance <sup>17</sup>. Hence, the present study was focused to evaluate the genotypic variability of groundnut under different Cr concentrations to find out the most sensitive and tolerant genotype. Any difference in Cr tolerance mechanisms between the genotypes can contribute to better understand the mechanism, why a particular genotype can tolerate to Cr toxicity than the other genotypes. The effects of elevated Cr across contrasting genotypes provide the potential mechanisms that involved in genotypic variation for Cr tolerance. Further, the promising genotypes could be exploited for cultivating in contaminated soils or as parental lines in breeding programs for development of Cr tolerant genotypes.

## MATERIALS AND METHODS

### Plant materials

Thirteen groundnut genotypes i.e., Abhaya, Anantha, Dharani, Greeshma (TCGS-888), ICGV-91114, JL-24, K-6, K-1375, Narayani, Prasuna (TCGS-341), Rohini (TCGS-913), TG-47 and TPT-4 were procured from the Regional Agricultural Research Station, Tirupati 517502, Andhra Pradesh, India.

### Pot experiment

Pot experiment was conducted under greenhouse conditions at the Yogi Vemana University, Kadapa, India (Latitude 14°47'N, Longitude 78°71'E). Each pot was filled with 3kg of air dried soil and mixed with varied concentrations, 0.0 (Control), 100, 200, and 300 mg kg<sup>-1</sup> soil of chromium ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ). The seeds of each and every genotype were surface-sterilized with 0.1% mercuric chloridethoroughly washed with distilled water and seeds were raised in earthen pots in three replications. The treatment was continued for 30 Days - after -sowing (DAS) and measurements were taken on the 30<sup>th</sup> day across all genotypes and replications.

### Measurement of morphological characters

On the 30<sup>th</sup> day, all the plants were carefully harvested, gently washed to remove sand and other debris and spread on filter papers to remove surface moisture if any. All the phenotypic characters viz., root length (cm), shoot

length (cm), total plant weight (g), total fresh root weight (g) and total fresh shoot weight (g) were measured immediately across all genotypes and treatments. Total root and shoot dry weight was recorded after exposing the samples to 60 °C for 72 hrs.

#### **SPAD (Soil Plant Analysis Data) Assay**

SPAD Chlorophyll Meter Reading (SCMR) was recorded at 30 days after sowing (DAS) by a Minolta handheld portable SCMR meter (SPAD-502, Konica Minolta, Japan) using the third leaf for a sample at 9.00-10.00 A.M across all groundnut genotypes and different Cr treatments.

#### **Proline assay**<sup>6</sup>

Fresh leaves (100mg) were collected after 30 days and homogenised in 3ml of 3% Sulfo-Salicylic acid and centrifuged at 12,000g at 4°C for 15 min. Take 2 ml of supernatant and add 2 ml of acid Ninhydrine reagent (1.24 gm of Ninhydrin in a mixture of glacial acetic acid and 6M ortho-phosphoric acid in 3:2 ratios) and glacial Acetic acid in 1:1:1ratio were added, the tubes were heated in a water bath at 100°C for 1 hr and cooled on ice for 10 min. To the resulting mixture, 4 ml of toluene was added and incubated at room temperature for 30 min. The tubes were shaken for 15Sec and allowed to stand for 10 min to separate the phases. The upper organic phase was separated and absorbance was measured at 520 nm using toluene as a blank.

#### **Antioxidant enzymes extraction and assays**<sup>19</sup>

A 200mg of fresh leaves tissue was collected from heavy metal treated and control plants, ground to a fine powder in liquid nitrogen using a pre cooled mortar and pestle. The exact weight of each powdered sample was determined before it was thoroughly homogenized in 1.2 ml of 0.2M potassium phosphate buffer (pH 7.8 with 0.1mM EDTA) and samples were centrifuged at 15,000Xg for 20 min at 4°C and the supernatant was removed and pellet was resuspended in 0.8mL of the same buffer, and the suspension centrifuged for another 15 min at 15,000Xg. The combined supernatant was stored on ice and used to determine following antioxidative enzymes.

#### **Catalase Assay (CAT)**<sup>1</sup>

The decomposition of H<sub>2</sub>O<sub>2</sub> was followed as a decrease in absorbance at 240nm in a UV/Vis spectrophotometer. The 3 mL assay mixture contained 2 mL leaf extract (Diluted 200 times with 50 mM potassium phosphate buffer, pH 7.0) and 1 ml of 10 mM H<sub>2</sub>O<sub>2</sub>. The extinction coefficient of H<sub>2</sub>O<sub>2</sub> (40 mM<sup>-1</sup>cm<sup>-1</sup> at 240nm) was used to calculate the enzyme activity that was expressed in terms of milli moles of H<sub>2</sub>O<sub>2</sub> per minute per gram fresh weight.

#### **Ascorbate peroxidase Assay (APX)**<sup>37</sup>

APX activity was determined from the decrease in absorbance at 290nm due to oxidation of Ascorbate in the reaction. The 1 mL assay mixture contained 50mM potassium phosphate buffer (pH 7.0), 0.5mM Ascorbate,

0.5 mM H<sub>2</sub>O<sub>2</sub> and 10 µL of crude leaf extract. H<sub>2</sub>O<sub>2</sub> was added last to initiate the reaction, and the decrease in absorbance was recorded every 30 seconds for 3 min. The extinction coefficient of 2.8 mM<sup>-1</sup> cm<sup>-1</sup> for reduced Ascorbate was used in calculating the enzyme activity that was expressed in terms of milli mole of Ascorbate per minute gram fresh weight.

#### **Measurement of electrolyte leakage**<sup>23</sup>

Cell damage was assayed by measured electrolyte leakage. Twenty five leaf discs of 0.3 cm<sup>2</sup> were excised from the third / fourth leaf using cork borer, rinsed briefly with water and floated on 5 ml of double distilled water for 6 hours at room temperature. The conductivity of the water was measured using a Crison conductivity meter. This represented the electrolyte leakage from the leaf discs (reading 1). Then, the samples were boiled for 20 min at 90°C. After the liquid cooled down, the conductivity of the water was measured again. This represented the total ions present in the leaf discs (reading 2). Electrolyte leakage was represented as the percentage of the total ions released [(reading 1/reading 2) × 100].

#### **Statistical analysis**

The data were processed by analysis of variance (ANOVA) and Pearson correlation using the Software IBM SPSS Statistics v. 2.0

#### **Phenotypic distance analysis**<sup>65</sup>

Mean data for phenotype traits was taken in the present study and used to develop clustering among the groundnut genotypes studied. STATISTICA Cluster analysis software was employed to develop Hierarchical clustering (joining) of groundnut varieties based on morpho-physiological traits using Ward's minimum variance method with the Euclidean distances to measure the distances among the accessions.

## **RESULTS AND DISCUSSION**

#### **Physiological traits to identify genotypic differentiation**

In the presence of various concentrations of Cr in the soil i.e., 0 (Control), 100, 200, and 300 mg kg<sup>-1</sup> soil, groundnut genotypes exhibited significant variability in terms of both physiological and biochemical traits. Irrespective of the genotype, the incidence of Cr in the soil, reduced the growth (Fig:1) fresh weights of the shoot and roots as presented in the (Fig: 3 ). Groundnut genotypes showed a linear decrease in their shoot and root lengths up on exposure to various concentrations of Cr as compared to their respective control plants (Fig: 2). Maximum shoot and root growth for all the genotypes was observed under control conditions. The reduction in the shoot, root growth was increased with the increased concentration of Cr in the soil and maximum reduction in shoot, root growth and biomass was observed under 300 mg kg<sup>-1</sup> Cr concentration in the genotypes Narayani, TPT-4 and JL-24 (Fig: 4). The genotypes Abhaya,

Greeshma, Anantha and Dharani maintained better shoot rates when compared with the other genotypes. However, the genotypes Greeshma, ICGV-9114 and K-1375 exhibited superior root length compared with other genotypes. Similarly, genotypes Abhaya, Anantha and Dharani maintained higher biomass compared to the other genotypes. The data clearly indicates that, Cr toxicity inhibited growth and biomass production of roots to a greater extent than of shoots. The results presented in this study on growth and biomass of groundnut genotypes connects with previous studies. Decrease in growth and biomass due to Cr toxicity have been observed in mung bean<sup>58</sup>, *Phaseolus vulgaris*<sup>50</sup>, wheat<sup>65,12</sup>, *V. radiata* 61. The increased concentration of Cr significantly reduced the root, stem and leaf dry masses in the sensitive cultivar "Zheda 622" of oilseed rape<sup>25</sup>. The increased concentration of Cr might deteriorate water and nutrient uptake, as resulted there could be inhibition of cell division and cell elongation caused reduced growth rates<sup>59,4</sup>. In data on groundnut indicates that, possibly Narayani, TPT-4 and JL-24 are sensitive genotypes, thus recorded reduced growth rates, compared with the other genotypes. In a previous study our group successfully used the SPAD chlorophyll meter to measure the groundnut genotypic variation for Nickel induced stress<sup>29</sup>. The Chlorophyll meter is a simple, non-destructive tool for measuring the -relative Chlorophyll concentration of leaves<sup>32</sup>. The SPAD data obtained in the present study clearly indicates that, there a linear decrease in the SAPD values of all genotypes with increasing concentrations of Cr content in the soils (Fig: 5). SAPD values indicates the amount of chlorophyll present in the leaves. Greater SPAD values under various concentrations of Cr were observed for the genotypes Abhaya (39.7±1.27(Control), 30.2±0.34 (100mg), 21±1(200mg) and 13.3±1.52 (300mg)); Anantha (40.6±1.19, 28.6±1.52, 20±1 and 13.6±1.52) and Dharani (42.5±1.7, 27.7±1.1, 19.3±0.5 and 13.2±0.2) and lesser values were recorded for the genotypes TPT-4 (37.4±0.34,20.1±0.6, 15.33±0.5 and 10±0), Narayani (36.7±0.8, 21.06±1.1, 15±2 and 10.6±0.5); JL-24 (40±0.45, 22.6±1.7, 16.3±1.5 and 10.6±1.1). The data presented in here is in correlation with SPAD data of tomato plants exposed to Cadmium stress<sup>18</sup> and groundnut genotypes under Ni stress<sup>29</sup>. The positive correlation between the plant growth and chlorophyll retention under Ni stress was observed in groundnut genotypes<sup>29</sup>. The effect of Cr stress on chlorophyll content was well studied in several plant species<sup>70,53,63</sup>. The increased levels of Cr in the plant possibly reduces the activity of δ-aminolevulinic acid dehydratase (ALAD), which affects the synthesis of δ-aminolevulinic acid (ALA) a key enzyme involved in the first step in tetrapyrrole biosynthesis, leading to the formation of heme, chlorophyll, billins, vitamin-B and specialized products<sup>67,51,41,70</sup>. Proline is the only one amino acid that accumulates during stress conditions and play an important role in osmoregulation, protecting the enzymes and protein synthesis machinery from environmental stress<sup>3,42,30</sup>. In the present study, drastic increase in the

free proline content was observed under 100 mg kg<sup>-1</sup> Cr concentration, further increasing concentration of Cr reduced proline content in all genotypes (Fig: 6). This trend was contradictory to the previous report, where an increase in Cr content gradually increased the proline accumulation in soybean<sup>22</sup>. However, in a recent study, increasing concentration of aluminium decreases the proline content of *Sorghum* genotypes<sup>54</sup>, suggesting that, it is difficult to pinpoint the exact role of proline under the heavy metal toxicity. This trend could be due the inhibition of proline synthesis at higher concentration of metal. Higher concentrations of heavy metals damage the cellular structures by degrading the cellular components through the generation of free radicals and reactive oxygen species (ROS), which ultimately affect the plant growth and development<sup>47,52</sup>. In protecting the plants from the metal induced oxidative damage, the antioxidative enzymes play an important role<sup>60</sup>. Thus antioxidative mechanism is one of the key plant trait that determine the hypertolerance. In the present investigation we have measured the activity of the of the two antioxidative enzymes i.e. catalase (CAT) and ascorbate peroxidase (APX) to access the tolerance of groundnut genotypes for Cr. Catalase is an important enzyme which decomposes the H<sub>2</sub>O<sub>2</sub> by breaking down into water and oxygen<sup>11,27</sup>. The activity of CAT, significantly increased in groundnut genotypes upon of 100 mg kg<sup>-1</sup> Cr treatment compared to their respective control plants, however, with increasing concentration of Cr further to 200 mg kg<sup>-1</sup> and 300 mg kg<sup>-1</sup> the activity was decreased ( Fig: 7A). The higher CAT enzyme activity was observed in the genotype Abhaya (0.15±0.02, 0.87±0.09, 0.45±0.02, and 0.16±0.01) Followed by Dharani (0.13±0.01,0.85±0.05,0.39±0.01 and 0.13±0.0) and Anantha (0.13±0.01,0.82±0.02,0.39±0.07 and 0.14±0.01 ). However, the increase in the activity was less in the genotypes Narayani ( 0.16±0.01 ,0.49±0.05 ,0.25±0.01 and 0.05±0.02 ); Rohini ( 0.15±0.02 ,0.49±0.01 ,0.23±0.01 and 0.05±0.02 ) and JL-24 (0.2±0.01 ,0.51±0.03,0.25±0.03 and 0.05±0.01 ). The activity of CAT was different from plant to plant. In rice, *Brassica* spp.<sup>45,73,31</sup> the Cr either induced or suppressed the CAT activity. The increase levels of O<sub>2</sub><sup>-</sup> radicals might be inactivated the CAT activity as it is sensitive to O<sub>2</sub><sup>-</sup> radicals. However, in several plants species gradual increase in CAT activity by Cr was reported<sup>43,46,47,15</sup>. Ascorbate peroxidase (APX) is a heme-containing enzyme that catalyzes the ascorbate-dependent reduction of hydrogen peroxide). APX along with the other enzymes glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) constitute the ascorbate-glutathione cycle which is one of the main antioxidant system in plants to keep H<sub>2</sub>O<sub>2</sub> under control under many environmental stresses, including heavy metals<sup>2,20,75</sup>. In the present study, the activity of APX in the groundnut genotypes upon Cr stress was recorded. As depicted in the Fig: 7B the activity of APX, increased in groundnut genotypes upon of 100 mg kg<sup>-1</sup> Cr

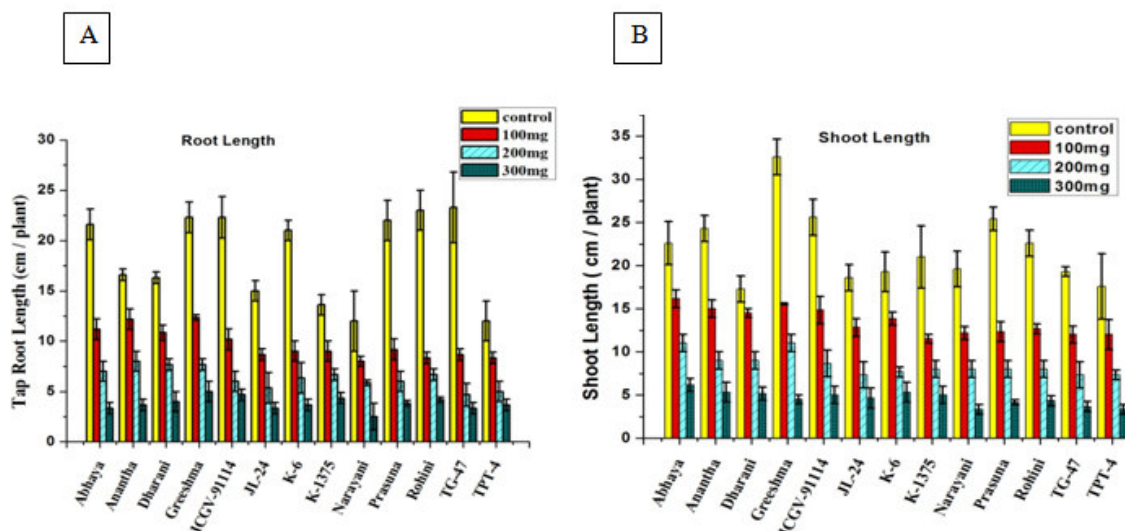
treatment compared to their respective control plants, however, the activity was gradually decreased with increasing concentration of Cr further to 200 mg kg<sup>-1</sup> and 300 mg kg<sup>-1</sup> of Cr in the soil. The greater APX enzyme activity was observed in the genotype Abhaya ( 4.4±0.8, 17.38±1.09, 9.52±0.54 and 3.69±0.54 ) Followed by Dharani ( 3.6±0.5, 17.7±1.25, 7.9±0.7 and 3.21±5.4 ) and Anantha ( 4.1±0.5, 16.3±1.76, 7.9±0.2 and 3.45±0.2). However, the APX activity was less in the genotypes TPT-4 (4.1±0.2, 9.04±0.2, 4.28±0 and 1.42±0.35); Rohini ( 3.4±0.5, 9.4±1.25, 4.4±0.2 and 2.26±0.1) and Narayani (3.5±0.7, 9.64±0.3, 4.5±0.2 and 2.1±0.02). The gradual decrease in the APX activity was observed in *Brassica* spp<sup>73,26</sup>; cotton genotypes<sup>15</sup>. The enzyme activity might be inhibited by the higher concentration of Cr. Electrolyte leakage is one of the standard trait measured to determine the stress induced injury of plant cells and plant stress tolerance<sup>35,9,5,34</sup>. Membrane damage increases the leakage of potassium ions and other solutes from plant cell<sup>10</sup>. Electrolyte leakage was measured for the groundnut genotypes under Cr stress. The data depicted in the Fig: 8 clearly indicates that, the electrolyte leakage results were correlated with the antioxidative enzymes results. Electrolyte leakage gradually increased with an increase in the concentration of Cr irrespective of the genotypes. However, the electrolyte leakage was recorded lesser in the genotypes Abhaya ( 65.3% (control), 75% (100mg), 88% (200mg)

and 92% (300mg)) Anantha (64.1% ,77%, 85% and 92.6% ) and Dharani (67.5%, 79.1%, 86% and 93% ) genotypes and higher for the genotypes Narayani ( 66.4%,78.7%,91.5% and 97.5% ), Prasuna ( 65.8% ,78.5% ,92% and 97%) and JL-24(70.1%, 78.1%, 92% and 96%). The increase in the activities of antioxidative enzymes protect the membranes from the free radicals, as resulted reduced levels of electrolytes have been observed in several studies<sup>28</sup>. Moreover, the dendrogram generated by Hierarchical clustering (joining) of groundnut varieties based on morpho-physiological traits using Ward's minimum variance method also clearly discriminate the groundnut genotypes in agreement to the above findings. Based on the tolerance and susceptibility of the genotypes for Cr contamination, the varieties can be divided into two main clusters, resistance cluster linked to the genotype Abhaya and susceptible cluster linked to the genotype JL-24. The varieties that form a different grouping pattern at control conditions, gradually reoriented along with the increased concentration of the Cr (Figures: 9, 10, 11 and 12). At the highest Cr concentration in the present study, genotypes Anantha, Dharani, Greeshma, ICGV-91114 and K-6 form a cluster with the Abhaya where as the varieties Rohini, Prasuna, K-1375, Narayani, TPT-4 and TG-47 cluster with JL-24, which is in agreement with the present findings studying individual growth and biochemical parameter in present study.

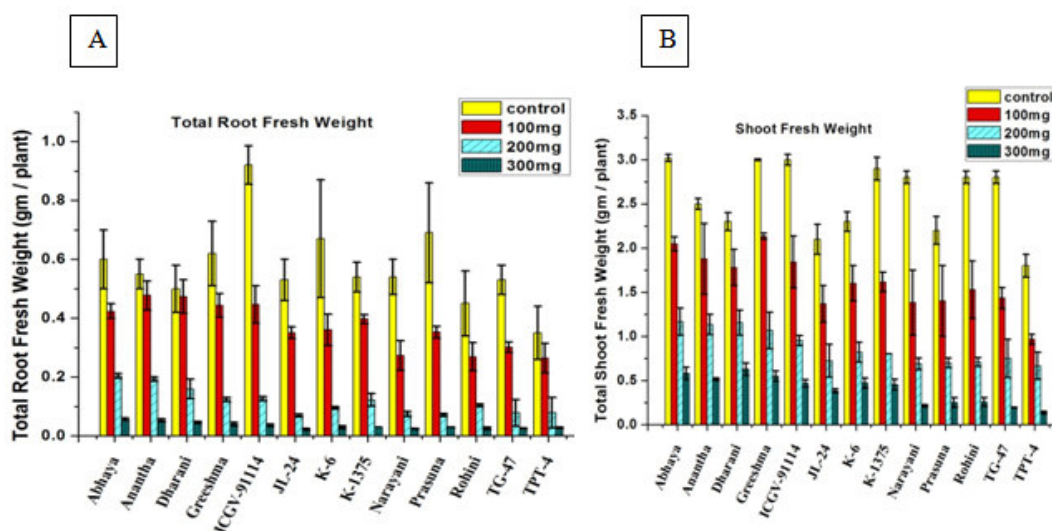


Figure 1

**Effect of Chromium (100 mg kg<sup>-1</sup>) on groundnut genotypes 1) Abhaya, 2) Anantha 3) Dharani, 4) Greeshma (TCGS-888), 5) ICGV-91114, 6) JL-24, 7) K-6, 8) K-1375, 9) Narayani, 10) Prasuna (TCGS-341), 11) Rohini (TCGS-913), 12) TG-47 and 13) TPT-4**



**Figure 2**  
**Effect of Cr on root and Shoot length of different groundnut genotypes grown in different concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl<sub>3</sub>.6H<sub>2</sub>O) (A) Root Length (B) Shoot Length. Error bars indicate ±SD.**



**Figure 3**  
**Effect of Cr on root and Shoot length of different groundnut genotypes grown in different concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl<sub>3</sub>.6H<sub>2</sub>O) (A) Total Root Fresh Weight (B) Total Shoot Fresh Weight. Error bars indicate ±SD**

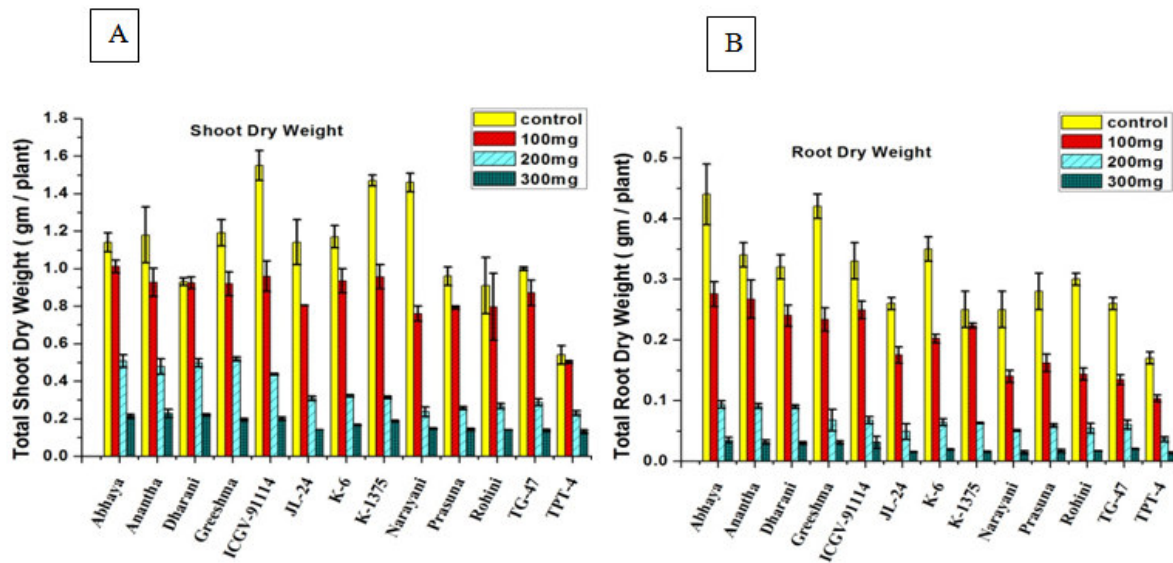


Figure 4

Effect of Cr on root and Shoot length of different groundnut genotypes grown in different concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl<sub>3</sub>.6H<sub>2</sub>O) (A) Total Root Dry Weight (B) Total Shoot Dry Weight. Error bars indicate ±SD

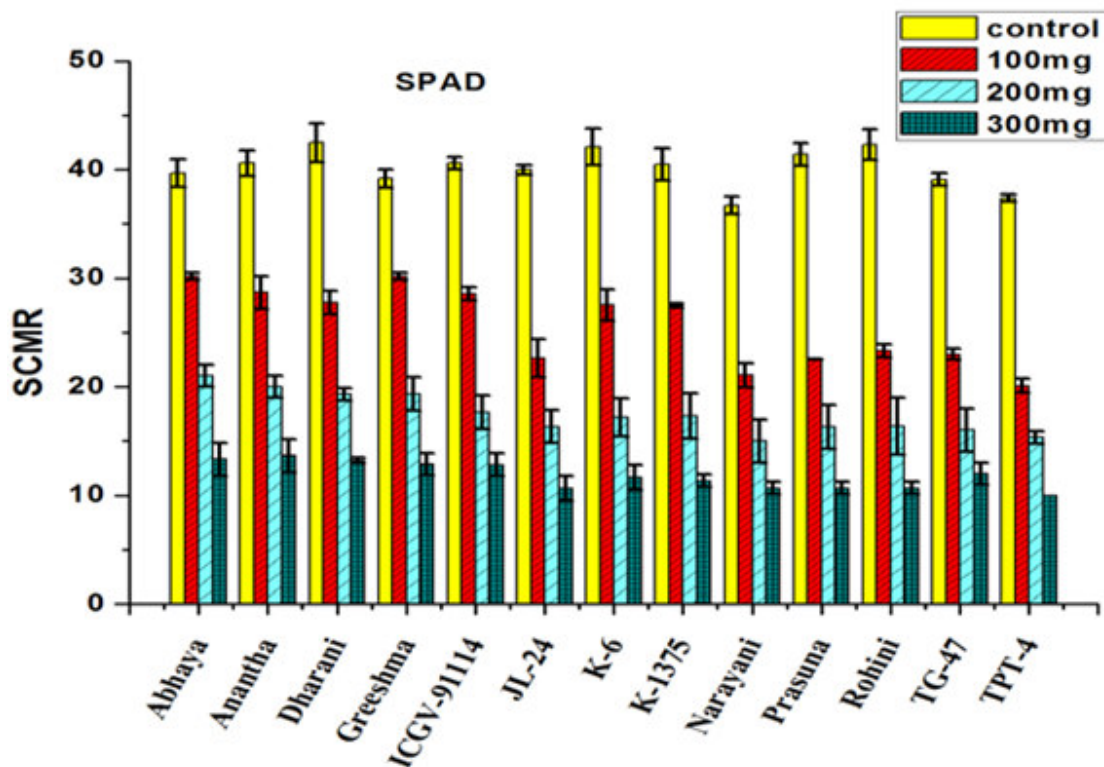


Figure 5

Effect of Cr on SPAD values (Chlorophyll content) of different groundnut genotypes grown in different concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl<sub>3</sub>.6H<sub>2</sub>O). Error bars indicate ±SD.

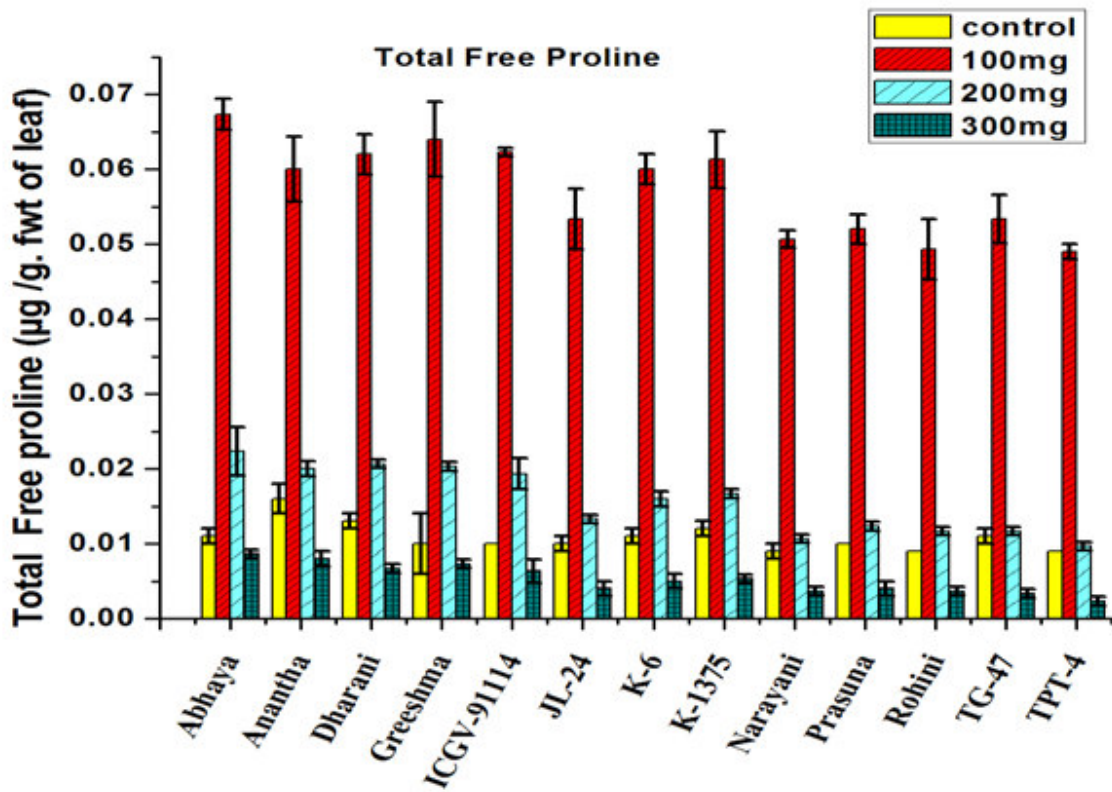


Figure 6

Free Proline content in different groundnut genotypes grown in various concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl3.6H<sub>2</sub>O). Error bars indicate ±SD.

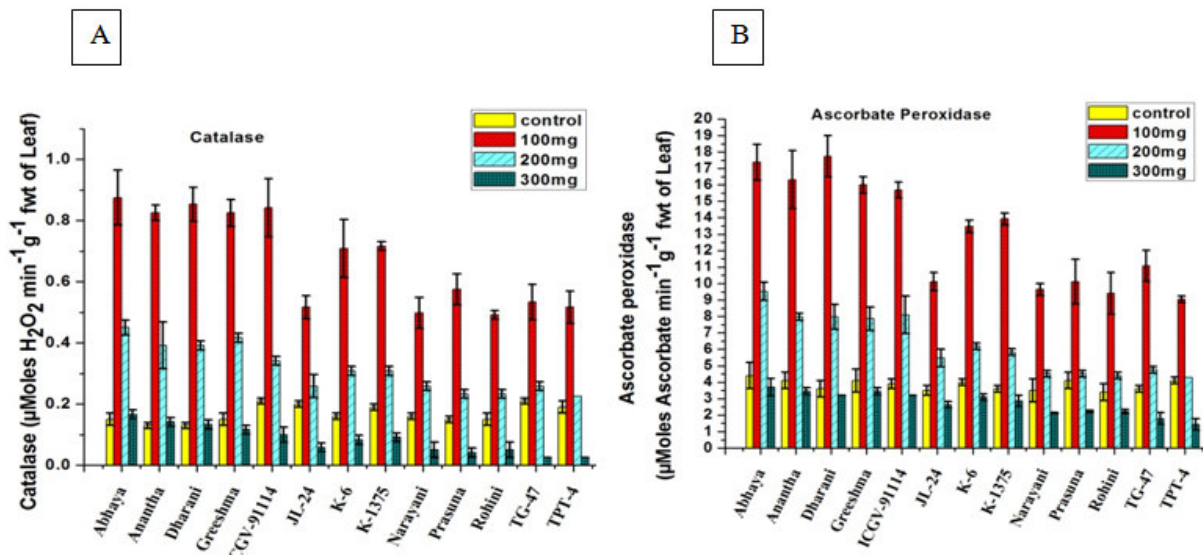


Figure 7

Activities of antioxidative enzymes in different groundnut genotypes grown in various concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl3.6H<sub>2</sub>O). (A) Catalase (CAT) (µMoles H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> g<sup>-1</sup> f.wt of leaf) and (B) Ascorbate Peroxidase (APX) (µMoles Ascorbate min<sup>-1</sup> g<sup>-1</sup> f.wt of leaf). Error bars indicate ±SD.



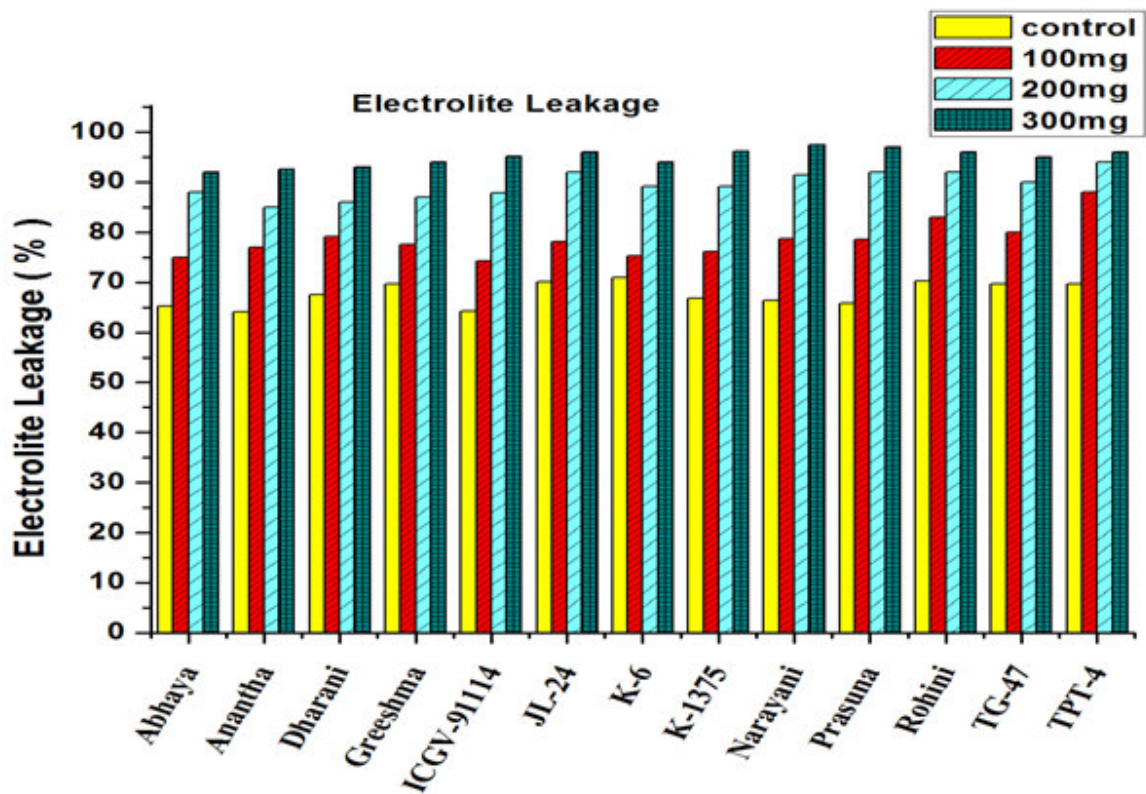


Figure 8

Electrolyte Leakage (%) which indicates the cell membrane damage in groundnut genotypes grown in various concentrations (0.0 (control), 100, 200 and 300 mg kg<sup>-1</sup> soil) of Cr (CrCl<sub>3</sub>.6H<sub>2</sub>O). Error bars indicate ±SD.

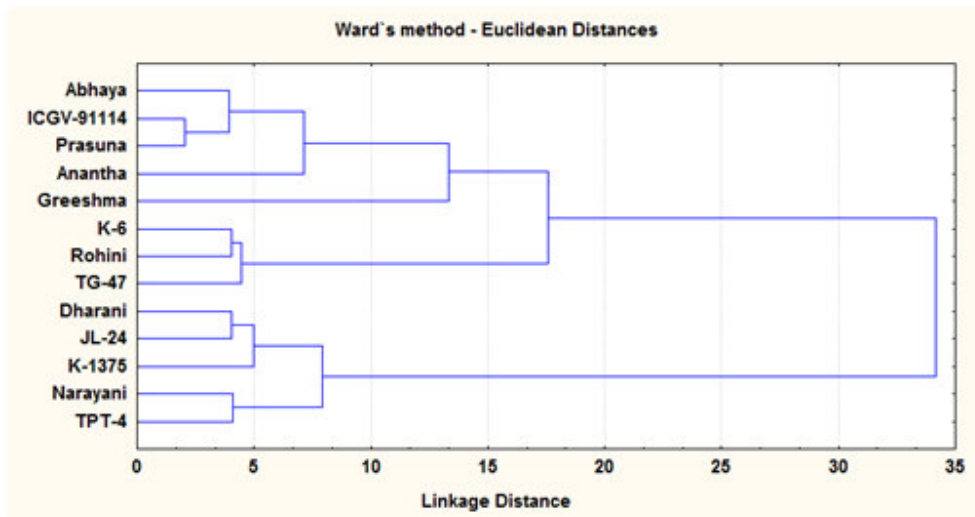
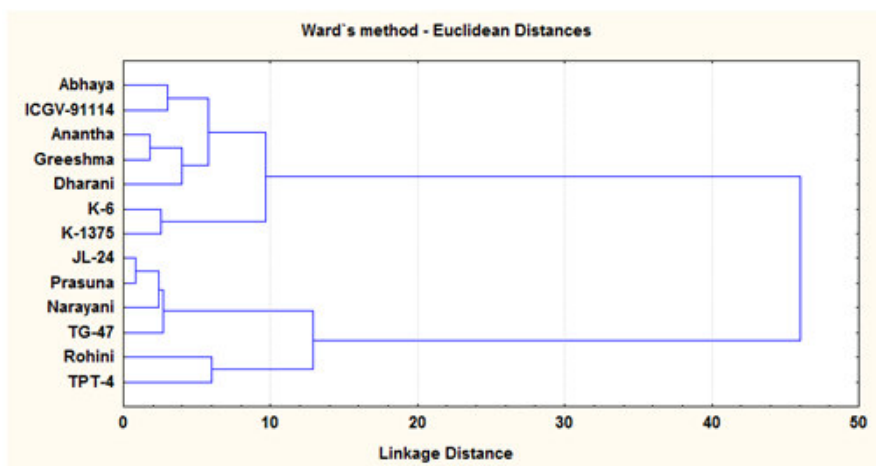
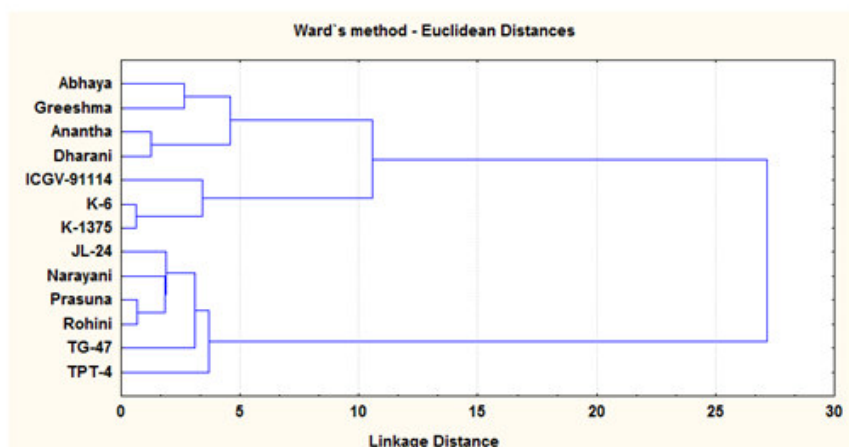


Figure 9

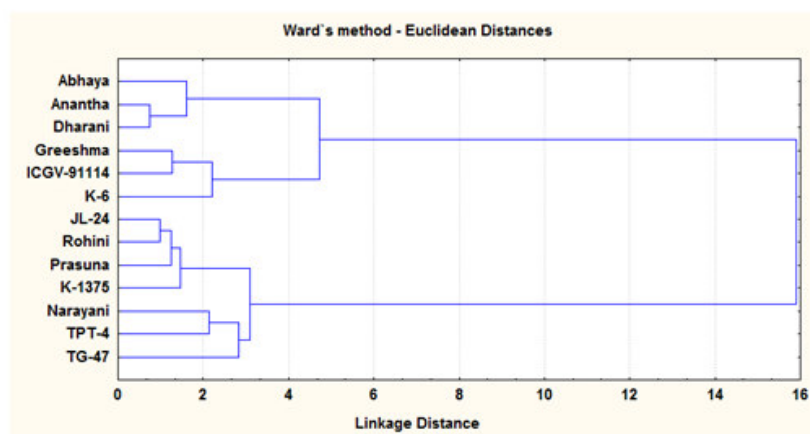
Ward's Minimum Variance Dendrogram for the Groundnut varieties studied under Control conditions



**Figure 10**  
*Ward's Minimum Variance Dendrogram for the Groundnut varieties studied under Chromium Stress (100 mg kg<sup>-1</sup>)*



**Figure 11**  
*Ward's Minimum Variance Dendrogram for the Groundnut varieties studied under Chromium Stress (200 mg kg<sup>-1</sup>)*



**Figure 12**  
*Ward's Minimum Variance Dendrogram for the Groundnut varieties studied under Chromium Stress (300 mg kg<sup>-1</sup>)*

## CONCLUSION

In conclusion, our data demonstrated that Cr in the soil has a deleterious effect on groundnut genotypes resulted in a decrease in biomass, growth and development especially with the increasing concentration of Cr. However, groundnut genotypes Abhaya, Anantha and Dharani genotypes are more tolerant to Cr stress, - demonstrated by their higher antioxidative capacity and better chlorophyll content. Based on our data all the thirteen genotypes studied can be categorized as followed. The genotypes Abhaya, Anantha and Dharani as highly tolerant for Cr; Greeshma, ICGV-91114, K-6, K-1375; TG-47; Rohini as moderately tolerant and TPT-4,

JL-24, Prasuna and Narayani are sensitive to Cr stress. The Cr tolerance genotypic variation could be directly applied as starting material towards the development of the metal tolerant genotypes and also the genotypic variation could also be used for further studies to understand the Cr tolerance mechanism.

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