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Evaluation of groundnut (*Arachis hypogaea* L.) genotypes for cadmium tolerance

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ABSTRACT

Seedlings of 13 genotypes of groundnut [Arachis hypogaea L.] were raised in sand culture using modified Hoagland solution and were exposed to cadmium stress by supplementing 300 µM cadmium. Perusal of data indicated significant reduction in root length, fresh and dry weight of root as well as total dry weight of the plant under cadmium treatment. On the basis of reduction in seedling dry weight the tolerance indices (TI) were calculated for the genotypes under study and three most tolerant and susceptible genotypes were identified to study the physiological basis of tolerance. The results showed that the tolerant genotypes registered much higher increase in phenol content and guaiacol peroxidase activity in their leaves but less increase in lipid peroxidation and electrolyte leakage of solutes in roots as compared to the susceptible ones. The tolerant genotypes also recorded lower reduction in leaf chlorophyll under cadmium treatment. Three genotypes, ISK-2014-04, ISK-2014-12 and TG 51 were identified as the most tolerant in the present experiment.

Keywords **:** *Antioxidative enzyme, cadmium stress, groundnut, phenol*

Among the class of non-essential heavy metals, cadmium is considered as a major toxic pollutant for human, animals and plants. It is released into the environment mainly from power stations, rubber tyres, paint industries, metal-working industries and fertilizers produced from rock phosphates, sewage sludge and then enter the food chain (McLaughlin *et al.*, 1999). In plants, cadmium exerts strong toxicity even at relatively low concentrations due to its high absorption rate and rapid translocation to different parts. Increasing concentration of cadmium in soil has posed a serious threat to sustainable agriculture and human health worldwide. Accumulation of cadmium in plant tissues may cause a variety of toxicity symptoms ranging from chlorosis, wilting, and growth reduction, to cell death (Muneer *et al.,* 2011 and Tao *et al.,* 2015). It can adversely affect different physiological processes of plants. Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crop and Asia is the one of the biggest producer of the crop. In India, it accounts for approximately 50 per cent of oilseed production (Mulgir *et al.*, 2014). 70 per cent of area and 75 per cent of the production has been concentrated in the four stages of Gujrat, Andhra Pradesh, Tamil Nadu and Orissa (Ghosh *et al.,* 2015). It is a unique leguminous plant for its characteristic behaviour to bear the pods underground in direct contact with the ground. Recent evidences suggested that this oil seed crop shows considerable metal tolerance (Ching *et al.*, 2008 and Bianucci *et al*., 2012). Some research works have already been carried out on physiological response of groundnut to cadmium stress Dinakar *et al*., 2008; Shan *et al.*, 2012 and Nagaraju *et al*., 2015). However, the information on this aspect in groundnut is still meager. The present experiment has been designed to study the differential cadmium tolerance of some genotypes of groundnut and to study the effect of cadmium stress on some physiological parameters at seedling growth stage. Such a study will help in exploring the existence of genotypic variation which is very important for the identification of potential candidate genotype for the cadmium tolerance in this crop and also to elucidate the metal tolerance mechanism.

MATERIALS AND METHODS

Seeds of 13 genotypes of groundnut [*Arachis hypogaea* L.] were collected from AICRP on groundnut, Kalyani Centre. The seeds were germinated and seedlings were raised in sand culture using modified Hoagland solution in the laboratory at a temperature of 28±10 ^{f}C and relative humidity around 80 per cent. Cadmium stress was imposed by supplementing 300 µM cadmium in the form of $CdCl_2$, H_2O. A control set containing only Hoagland solution was also prepared for comparison. Observations were recorded on 21 days old seedlings for seedling growth parameters. Stress response index (SRI) for individual character was calculated as as per Chen *et al*. (2007) :

$$\text{SRI \%} = \frac{\text{Mean value of the genotype under stress}}{\text{Mean value of the genotype under non-stress condition}} \times 100$$

On the basis of stress response index (SRI) of all the growth parameters studied, the 13 genotypes were analyzed for genetic similarity based on Euclidean distance ausing NTSYS-PC version 2.0 software. A dendrogram was constructed by Sequential Agglomerative Hierarical Nested (SAHN) clustering

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using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm. Three most tolerant and susceptible genotypes were selected on the basis of tolerance index. Further physiological studies were conducted on these selected genotypes.

The contents of chlorophyll and total phenol in the leaves of 21 days old seedlings were estimated as per Arnon (1949) and McDonald *et al.* (2001), respectively. The level of lipid peroxidation was measured in terms of the content of thiobarbituric acid reactive substances (TBARS) following the method of Heath and Packer (1968). Activity of guaiacol peroxidase enzyme in the leaf was measured by the method of Siegel and Galston (1967), while the electrolyte leakage of the root sample was estimated as per Guo *et al*. (2006). The mean values were subjected to statistical analysis following two-factor factorial design.

RESULTS AND DISCUSSION

The results indicated that the genotypes showed significant reduction in root length, fresh and dry weight of root as well as total dry weight of the plant when subjected to cadmium treatment. Taylor and Foy (1985) and Tao *et al.* (2015) concluded that the inhibition of root elongation was the first evident effect of metal toxicity in plants. In the present experiment, the genotypes, ISK-2014-04, TG 51 and ISK-2014-12 with 1.56, 4.11 and 4.50 per cent decrease in root length under cadmium treatment, respectively, exhibited the minimum detrimental effect of cadmium, while ISK-2014-14 and ISK-2014-02 with 21.51 and 22.25 per cent decrease over control registered the most severe effect of cadmium on root length. Lerda (1992) reported that reduction in root length under heavy metal stress could be due to reduction in mitotic cell division in meristematic zone of roots. The three genotypes, ISK-2014-04, ISK-2014-12 and TG 51 also recorded minimum reduction in fresh and dry weight of root under cadmium stress as compared to ISK-2014-02, ISK-2014-14 and ISK-2014-15 which showed most drastic reduction of root growth under stress among all the genotypes. Sensitivity of root growth to cadmium accumulation was reported earlier by several workers (Al-Yemeni, 2001; Cheng *et al.,* 2008 and Subin and Steffy 2013 and Nagaraju *et al*., 2015). Cadmium treatment also caused a significant reduction in total dry weight of the seedlings in the present experiment The results corroborated the early findings of Muneer*et al.* (2011), Siddhu and Khan (2012) and Mukhaelyan *et al.* (2015). The reduction in seedling growth might be attributed to decrease in chlorophyll content (Somashekaraiah *et al.,* 1992) as well as disturbances in photochemistry and photosynthetic electron transport chain (Pagliano *et al*., 2006). In the present experiment, the reduction in dry weight of groundnut seedlings under cadmium stress ranged from 6.19 to 34.28 per cent. On the basis of reduction in seedling dry weight the tolerance indices (TI) were calculated for the genotypes under study. The genotype ISK-2014-12 (TI= 92.86%) was found to be the most tolerant among all the genotypes closely followed by ISK-2014-04 (TI = 92.02%) and TG 51 (TI = 88.97%). On the contrary, ISK-2014-02 (TI= 66.46%), ISK-2014-14 (TI= 66.85%) and ISK-2014-15 (TI = 67.86%) were among the most susceptible genotypes.

On the basis of stress response index (SRI) of all the growth parameters studied, the 13 genotypes were analyzed for genetic similarity based on Euclidean distance. The dendrogram showed that three most tolerant genotypes showed close similarity and formed a cluster in which TG 51 separated out as a single genotype (Fig. 1). The most susceptible ones also formed a cluster together showing high level of similarity. The genotypes with intermediate tolerance belonged to separate cluster which could be discriminated from the most tolerant cluster at the coefficient of 3.36 showing substantial dissimilarity.

Based on the initial screening of genotypes, further physiological studies were conducted on three most tolerant (ISK-2014-12, ISK-2014-04 and TG 51) and susceptible genotypes (ISK-2014-02, ISK-2014-14 and ISK-2014-15). The data indicated that all the genotypes registered increase in lipid peroxidation of leaf and electrolyte leakage (EL) of root under cadmium stress indicating membrane damage of both leaf and root under stress (Table 2). The genotypes recorded 4.94 to 118.70 per cent increase in lipid peroxidation and 12.46 to 63.59 per cent increase in root EL under stress over that of unstressed control. Cadmium-induced lipid peroxidation was reported earlier by different research workers (Bora *et al.*, 2003; Zhao *et al.*, 2011; Shan *et al*., 2012; Mukhaelyan *et al.*, 2015; Gowayed and Kadasa, 2016). But the susceptible genotypes recorded much higher extent of such membrane damage than the tolerant ones. The three tolerant genotypes also recorded lower reduction in leaf chlorophyll content as compared to the susceptible genotypes (Table 2). The tolerant genotypes registered decrease in chlorophyll content that ranged from 8.82 to 12.67 per cent over unstressed control, whereas the corresponding values in the susceptible genotypes varied from 18.24 to 24.83 per cent over control. The decrease in chlorophyll content by cadmium treatment might be attributed to degradation of chlorophyll or inhibition of its biosynthesis and has been proposed to be responsible for observed reduction in photosynthesis and growth (Somashekaraiah *et al.*, 1992). There were significant differences among the genotypes in respect of content of phenol and activity of guaiacol peroxidase (GPOX) enzyme in the leaf. Out of three susceptible genotypes, ISK-2014-14 and ISK-

Table 1: Effect of cadmium stress on seedling growth of 13 genotypes of groundnut

Genotypes	Root length (cm)		Root fresh wt (g)		Root dry wt (g)		Total dry wt (g)	
	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM
ISK-2014-01	14.47	13.54 (-6.43)	1.31	1.28 (-2.28)	0.15	0.12 (-20.00)	0.51	0.40 (-22.07)
ISK-2014-02	13.53	10.52 (-22.25)	1.14	0.90 (-21.34)	0.17	0.10 (-41.18)	0.48	0.31 (-34.28)
ISK-2014-04	14.77	14.54 (-1.56)	1.20	1.19 (-0.56)	0.13	0.12 (-7.69)	0.32	0.30 (-6.19)
ISK-2014-05	14.70	13.16 (-10.50)	1.24	1.02 (-17.96)	0.12	0.10 (-16.67)	0.36	0.29 (-19.44)
ISK-2014-06	13.80	12.89 (-6.59)	1.10	0.90 (-18.48)	0.12	0.09 (-27.75)	0.38	0.30 (-20.18)
ISK-2014-09	16.26	14.26 (-12.30)	1.37	1.14 (-16.35)	0.11	0.09 (-18.18)	0.37	0.29 (-21.43)
ISK-2014-10	15.86	14.10 (-11.10)	1.37	1.27 (-7.06)	0.13	0.09 (-30.77)	0.40	0.31 (-21.86)
ISK-2014-11	15.90	14.07 (-11.51)	1.17	1.06 (-9.66)	0.19	0.13 (-31.58)	0.49	0.36 (-25.86)
ISK-2014-12	14.01	13.38 (-6.59)	1.42	1.40 (-1.41)	0.12	0.11 (-8.33)	0.42	0.39 (-7.14)
ISK-2014-14	12.41	9.74 (-21.51)	1.87	1.33 (-28.70)	0.18	0.11 (-39.99)	0.45	0.30 (-33.09)
ISK-2014-15	14.73	12.47 (-15.34)	1.54	1.11 (-28.08)	0.15	0.10 (-33.33)	0.42	0.28 (-32.55)
ISK-2014-34	15.20	14.31 (-15.34)	1.71	1.49 (-13.06)	0.14	0.10 (-28.57)	0.37	0.29 (-21.43)
TG-51	15.98	15.32 (-4.11)	1.03	1.01 (-1.94)	0.14	0.12 (-9.80)	0.41	0.37 (-10.56)
LSD (P-0.05)								
Genotype (G)	**0.366**		**0.034**		**0.003**		**0.011**	
Treatment (T)	**0.144**		**0.013**		**0.001**		**0.004**	
G x T	**0.518**		**0.048**		**0.004**		**0.015**	

Table 2: Changes in some biochemical parameters in tolerant and susceptible genotypes under cadmium stress

Genotypes	Lipid peroxidation (µM of TBARS content g^{-1} fresh weight)		EL (%)		Total chlorophyll (mg g^{-1} fresh weight)		Phenol (mM of gallic acid g^{-1} fresh weight)		GPOX (ÄA470 min^{-1} g^{-1} fresh weight)	
	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM	Cd 0 µM	Cd 300 µM
ISK-2014-02	22.96	50.22(118.70)	45.63	74.65 (63.59)	2.50	1.88 (-24.83)	4.13	4.30 (4.12)	22.27	16.29 (-26.88)
ISK-2014-04	20.95	27.51 (31.32)	56.08	63.06 (12.46)	2.92	2.55 (-12.67)	2.94	4.60 (56.46)	14.01	14.30 (2.07)
ISK-2014-12	32.30	38.86 (20.31)	49.44	65.03(31.52)	2.66	2.35(-11.42)	3.95	4.71 (19.32)	19.04	27.55(44.74)
ISK-2014-14	30.03	41.13 (36.96)	52.10	79.59 (55.77)	2.92	2.30(-21.03)	4.46	4.03 (-9.72)	19.33	17.15 (-11.28)
ISK-2014-15	19.68	27.25 (38.46)	41.64	60.30(44.79)	2.96	2.42 (-18.24)	4.96	3.43 (-30.83)	19.07	16.67 (-12.60)
TG-51	20.44	21.45 (4.94)	57.01	65.79 (15.41)	2.65	2.41 (-8.82)	3.94	4.78 (21.32)	14.40	26.43 (83.54)
LSD(0.05)										
Genotype (G)	**0.845**		**1.613**		**0.068**		**0.111**		**0.512**	
Treatment (T)	**0.488**		**0.931**		**0.040**		**0.064**		**0.296**	
G x T	**1.195**		**2.281**		**0.100**		**0.157**		**0.724**	

Notes : Figures in parentheses indicate percentage increase (+) or decrease (-) over the control

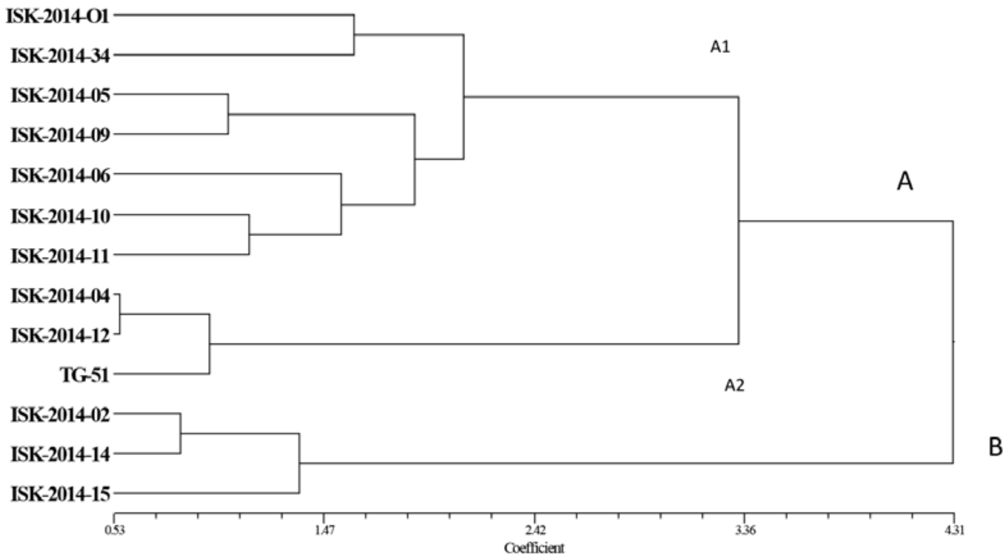

Fig. 1: Dendrogram showing clustering of groundnut genotypes on the basis of seedling growth parameters under cadmium stress

2014-15 showed decreased content of phenol in leaf under cadmium stress while the other susceptible genotype ISK-2014-02 showed slight increase over control. On the contrary, the three tolerant genotypes registered considerable increase in phenol content when treated with cadmium and the increase ranged from 19.32 to 56.46 per cent over control. Thus, the tolerant genotypes in the present experiment responded by synthesizing antioxidant metabolite. The increase in content of total phenols due to cadmium treatment was also reported earlier by Dudjak *et al.*(2004) and Garcia *et al.* (2012). The GPOX activity in the leaf of tolerant genotypes also increased under stress with TG 51 registering the maximum increase of 83.54 per cent and it was followed by ISK-2014-12 and ISK-2014-04 which recorded 44.74 and 2.07 per cent increase over unstressed control. Unlike the tolerant ones, the three susceptible genotypes showed decreased activity of GPOX in their leaves as a result of cadmium treatment. The high activities of GPOX in tolerant genotypes appear to be involved in effective scavenging ROS generated by Cd treatments, whereas its suppression in susceptible genotypes might make them more vulnerable to oxidative stress induced by reactive oxygen species (ROS). Increased activities of peroxidase in groundnut under cadmium stress were also reported earlier by Dinakar *et al.* (2008) and Shan *et al.* (2012).

Summarizing the results it might be concluded that cadmium stress caused significant growth reduction in groundnut genotypes. The differences among genotypes for chlorophyll and phenol content and GPOX activity in leaf and electrolyte leakage of solutes in the root might contribute to observed variation in respect of cadmium tolerance.

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