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Physiological basis of cadmium tolerance in groundnut (*Arachis hypogaea* L.)

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Abstract
Seedlings of thirteen groundnut [*Arachis hypogaea* L.] genotypes 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 dry weight, shoot dry weight and total dry weight of the seedlings under cadmium treatment. The stress tolerance indices (STI) of thirteen genotypes ranged from 65.72% to 93.81% under 300 µM cadmium stress. Among all the genotypes, ISK-2014-04 93.81%), ISK -2014-12and TG-51 were found to be the most tolerant, while the genotypes, ISK-2014-02, ISK-2014-14 and ISK-2014-15 were found to have much lower STI and were considered to be the most susceptible to cadmium stress. Further physiological studies showed that the tolerant genotypes registered much higher increase in proline content, super oxide dismutase (SOD) and guaiacol peroxidase (GPOX) activity in their leaves under cadmium treatment compared to the susceptible ones. But the genotypes exhibited varied response to cadmium stress in respect of catalase (CAT) activity in leaf. The tolerant genotypes also recorded lower reduction in leaf protein content and nitrate reductase (NR) enzyme activity under cadmium treatment.

Keywords: Cadmium stress, groundnut, proline, superoxide dismutase, guaiacol peroxidase, catalase, nitrate reductase

Introduction
Groundnut (*Arachis hypogaea* L.) is one of the important oil seed crops of Asia as well as India. It is a unique leguminous plant for its characteristic behaviour to bear the pods underground in direct contact with the ground. It is grown as oil seed, food and feed crop. Groundnut kernels contain 45-50% oil and 25-30% digestable protein (Nath and Alam, 2002) [32]. Recent evidences suggested that groundnut shows considerable metal tolerance (Ching *et al*., 2008 and Bianucci *et al*. 2012) [5, 1]. Cadmium is considered as a major toxic trace pollutant for humans, animals and plants. It is released into the environment mainly from power stations, rubber tyres, paint industries, metal-working industries, sewage sludge and waste materials. In addition, some phosphate fertilizers applied to crops have been found to contain high levels of cadmium (He and Singh, 1994) [12]. Accumulation of cadmium in plant tissues may cause chlorosis, wilting, and growth reduction and ultimately cell death (Sreedevi *et al.*, 2008; Shaukat *et al.* 2010; Siddhu and Khan, 2012 and Tao *et al.*, 2015) [30, 26, 27, 31]. Cadmium-induced cellular toxicity may result in interferences with many processes, such as nitrate absorption and reduction (Hernandez *et al.*, 1996) [13], enzyme catalysis (van Assche and Clijsters, 1990) [33], osmotic regulation (Costa and Morel, 1994 and Perfus-Barbeoch *et al.* 2002) [6, 22] reactive oxygen species production (Dixit *et al.*, 2001 and Khan *et al.*, 2007) [8, 27] etc. Some research works have been conducted on the effect of cadmium toxicity in groundnut (Dinakar *et al*. 2009 and Nagaraju *et al*., 2015) [7, 21]. But the information is still a meager. The present experiment has been designed to evaluate a few genotypes of groundnut for their tolerance against cadmium toxicity and to understand the physiological basis of tolerance at seedling growth stage.

Materials and Methods
Seeds of 13 genotypes of groundnut [*Arachis hypogaea* L.] were collected from AICRP on groundnut, Kalyani Centre. The experiment was conducted in sand culture using modified Hoagland solution (Epstein. 1972) under laboratory condition with 80 ±1% relative humidity (R.H.) and at a temperature of 28±1 °C. 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. Stress tolerance index (STI) for each genotype was calculated as per Chen *et*

al. (2007) [3]. Three most tolerant and susceptible genotypes were selected on the basis of tolerance index. Further physiological studies were conducted on these selected genotypes. Proline and soluble protein content in the leaf was determined as per the methods of Mohanty and Sridhar (1982) [18] and Lowry *et al.* (1951) [17], respectively. Estimation of the activities of nitrate reductase and superoxide dismutase (SOD) enzymes were done following the methods of Jaworski (1971) [15] and Giannopolitis and Ries (1977) [10], respectively. While the activities of Catalase (CAT) and Guaiacol peroxidase (GPOX) in the leaf were estimated as per the methods of Goth (1991) [11] and Siegel and Galston (1967) [28], respectively. The mean values were subjected to statistical analysis following two-factor factorial design with three replications.

Results and Discussion

In the present experiment, the extent of inhibition for root and shoot dry weight under cadmium toxicity ranged from 7.69-41.18% and 6.67-35.71% over control, respectively. In general, the root dry weight was found to be more sensitive to cadmium-inhibition than shoot growth. Greater sensitivity of root growth than shoot growth to cadmium stress was reported earlier by several workers (Cheng *et al.*, 2008; Shan *et al*. 2012 and Tao *et al*., 2015) [4, 24, 31]. The three genotypes, ISK-2014-04, ISK-2014-12 and TG-51 registered the minimum reduction in total dry weight of whole seedling, while ISK-2014-02, ISK-2014-14 and ISK-2014-15 showed the most drastic effect of cadmium stress. On the basis of dry weight of whole seedling under cadmium stress and in unstressed control condition, the stress tolerance index (STI) was calculated separately for each genotype. The STI of thirteen genotypes ranged from 65.72% to 93.81% under 300 µM cadmium treatment in the present study (Fig. 1). Among all the genotypes, the highest STI was registered by ISK-2014-04 (93.81%). It was followed by ISK-2014-12 (92.86%) and TG-51 (89.44%). These genotypes were considered to be the most tolerant to cadmium stress. On the contrary, the genotypes, ISK-2014-02, ISK-2014-14 and ISK-2014-15 with 65.72%, 66.91% and 67.45% STI, respectively, were found to have much lower STI and were considered to be the most susceptible to cadmium treatment in the present study. Further biochemical studies were done on these six genotypes showing the higher tolerance and higher susceptibility than others.

The data on leaf proline content revealed that all the six genotypes, except ISK-2014-02 and ISK-2014-15 registered significant increase in proline content in their leaves under treatment as compared to the untreated control (Table 2). The range of increase varied from 13.90% in the susceptible genotype ISK-2014-14 to 38.28% over control in ISK-2014-12. The other two tolerant genotypes, TG-51 and ISK-2014-04, registered 19.02 and 32.20% increase in leaf proline, respectively in the stressed seedlings. While the two susceptible genotypes, ISK-2014-15 and ISK-2014-02 recorded 2.91 and 16.26% decrease in leaf proline over the control plants, respectively. Increase in proline content under cadmium stress has been reported earlier in different crops by Zhang *et al.* (2000) [35], Muneer *et al.* (2011) [20] and Mondal *et al.* (2013) [19]. It might be mentioned that proline is not only a compatible osmolyte, but also it acts as osmoprotectant as well as free radical scavenger.

The data on soluble protein content in the leaves exhibited that the leaf protein content significantly decreased under cadmium stress in all the six genotypes. The range of decrease varied from 2.38 to 38.08% over control. The results corroborated some early works of Dinakar *et al.* (2008), Sharma *et al.* (2010) [25] and Muneer *et al.*, (2011) [20] and might be attributed to degradation of protein under cadmium treatment. In the present experiment, the three tolerant genotypes ISK-2014-12, TG-51 and ISK-ISK-2014-04 indicated 2.38, 10.84 and 15.04% decrease in protein content, respectively, under cadmium stress as compared to control. On the contrary, the three susceptible genotypes, ISK-2014-15, ISK-2014-02 and ISK-2014-14 with 17.82, 38.08 and 40.60% decrease over control, recorded very high decrease in leaf protein content as a result of cadmium treatment.

All the six genotypes revealed significant decrease in the activity of nitrate reductase (NR) enzyme under cadmium stress as compared to that in the unstressed control seedlings. The tolerant genotype ISK-2014-12 showed the lowest decrease (9.52%) in NR under cadmium treatment as compared to control and the other two tolerant genotypes TG-51 (9.62% over control) and ISK-2014-04 (9.83% over control) also recorded comparatively lower reduction, whereas, the susceptible genotypes, ISK-2014-02, ISK-2014-15 and ISK-2014-14 revealed 16.81, 34.07 and 34.18% decrease over control, respectively, in NR activity. The first step in the process of conversion of nitrate to organic nitrogen is the reduction of nitrate to nitrite and it is catalyzed by the enzyme nitrate reductase (NR). This step is often considered to be the rate limiting step. Thus, NR is considered to be one of the most important enzyme in nitrogen assimilation. The reduced activity of NR under cadmium stress was reported earlier by several authors (Dinakar *et al.*, 2008; Muneer *et al.*, 2011; Siddhu and Khan, 2012 and Irfan *et al.*, 2014) [20, 27, 14].

Perusal of data indicated that cadmium treatment increased the activity of superoxide dismutase (SOD) enzyme over control in all the genotypes studied. The level of increase was much higher in the three tolerant genotypes. Among the tolerant genotypes, ISK-2014-14 recorded the maximum increase in SOD activity (44.54% over control) when the seedlings were exposed to cadmium stress. The susceptible genotypes showed an increase of SOD activity that varied from 32.57% in ISK-2014-02 to 44.54% in ISK-2014-14 over that of control plants. The tolerant genotypes also showed increase in the guaiacol peroxidase (GPOX) enzyme activity under cadmium treatment over control, while the enzyme activity decreased in three susceptible genotypes under stress. Out of three tolerant genotypes, TG-51 registered the maximum increase (83.54% over control) in GPOX activity in leaf, while the other two, viz, ISK-2014-04 and ISK-2014-12, exhibited 2.07 and 44.74% increase, respectively. The three susceptible genotypes, ISK-2014-14, ISK-2014-15 and ISK-2014-02 showed 11.28, 12.60 and 26.88% decrease in leaf GPOX activity, respectively, under cadmium treatment. Increased activity of peroxidase and SOD under cadmium stress were also reported earlier by Rout *et al.* (2000) [23], Bora *et al.* (2003) [2], Dinakar *et al.* (2009) [7] and Sharma *et al.* (2010) [25]. Such increased activities of SOD and GPOX might result in better scavenging of free radicals in the tolerant genotypes under cadmium stress. The genotypes exhibited varied response to cadmium stress in respect of catalase (CAT) activity in leaf. Out of all the six genotypes, only ISK-2014-12 registered an increase (9.71% over control) in CAT activity, while the remaining five genotypes showed inhibition of the enzyme activity when exposed to cadmium stress. The susceptible genotype, ISK-2014-15 showed the highest reduction in CAT activity under cadmium treatment as compared to the unstressed control seedlings. Earlier,

Vitoria *et al.* (2001) [1], Singh *et al.* (2008) [29] and Zhao (2011) [36] reported increased activity of catalase enzyme.

Summarizing the data it might be concluded that higher increase in leaf proline together with much higher range of activities of SOD and GPOX might help three genotypes ISK-2014-12, TG-51 and ISK-ISK-2014-04 to register comparatively greater tolerance to cadmium stress in the present experiment.

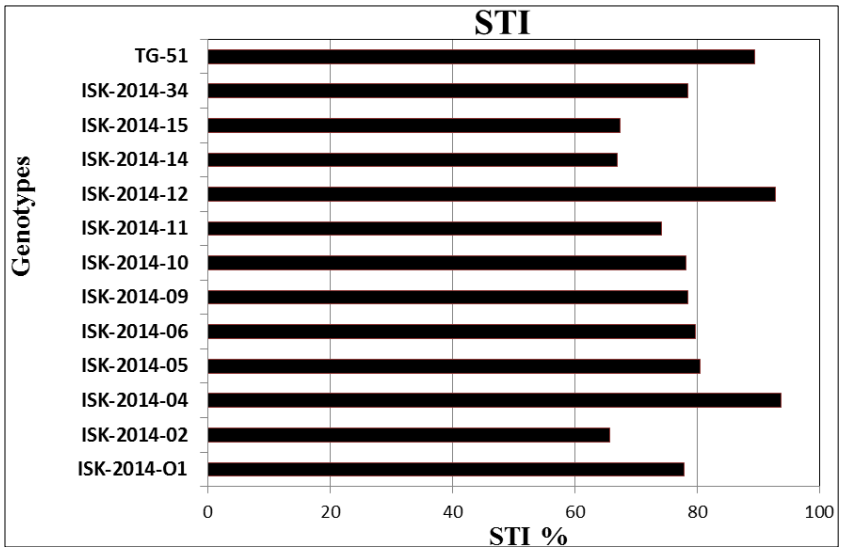

Fig 1: Stress tolerance index (STI) of 13 genotypes of groundnut under cadmium stress

Table 1: Effect of cadmium stress on dry weight of root, shoot and whole seedling in 13 genotypes ofgroundnut

Genotypes	Root dry wt (g)			Shoot dry wt (g)			Total dry wt (g)		
	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean
ISK-2014-O1	0.15	0.12 (-20.00)	0.14	0.21	0.15 (-29.68)	0.18	0.51	0.40 (-22.07)	0.46
ISK-2014-02	0.17	0.10 (-41.18)	0.14	0.18	0.12 (-33.33)	0.15	0.48	0.31(-34.28)	0.40
ISK-2014-04	0.13	0.12(-7.69)	0.13	0.09	0.08 (-11.11)	0.09	0.32	0.30 (-6.19)	0.31
ISK-2014-05	0.12	0.10 (-16.67)	0.11	0.12	0.09 (-25.00)	0.11	0.36	0.29 (-19.44)	0.33
ISK-2014-06	0.12	0.09 (-27.75)	0.10	0.11	0.10 (-11.74)	0.11	0.38	0.30 (-20.18)	0.34
ISK-2014-09	0.11	0.09 (-18.18)	0.10	0.10	0.08 (-20.00)	0.09	0.37	0.29 (-21.43)	0.33
ISK-2014-10	0.13	0.09 (-30.77)	0.11	0.13	0.12 (-9.98)	0.13	0.40	0.31 (-21.86)	0.35
ISK-2014-11	0.19	0.13 (-31.58)	0.16	0.12	0.09 (-22.25)	0.11	0.49	0.36 (-25.86)	0.43
ISK-2014-12	0.12	0.11(-8.33)	0.12	0.15	0.14 (-6.67)	0.15	0.42	0.39 (-7.14)	0.40
ISK-2014-14	0.18	0.11(-39.99)	0.15	0.13	0.09 (-32.48)	0.11	0.45	0.30 (-33.09)	0.38
ISK-2014-15	0.15	0.10(-33.33)	0.13	0.14	0.09 (-35.71)	0.12	0.42	0.28 (-32.55)	0.35
ISK-2014-34	0.14	0.10 (-28.57)	0.12	0.10	0.08 (-20.00)	0.09	0.37	0.29 (-21.43)	0.33
TG-51	0.14	0.12(-9.80)	0.13	0.11	0.10 (-8.83)	0.11	0.41	0.37 (-10.56)	0.38
Mean	0.14	0.11		0.12	0.10		0.41	0.32	
CD (P=0.05)									
Genotype (G)	0.003			0.003			0.011		
Treatment (T)	0.001			0.001			0.004		
G x T	0.004			0.005			0.015		

Data in parentheses indicate percentage increase (+) or decrease (-) over control

Table 2: Effect of cadmium stress on proline and soluble protein content and nitrate reductase (NR) activity in the leaves of tolerant and susceptible genotypes of groundnut

Genotypes	Proline (micromol/g)			Protein (mg/g fw)			NR (milimol/hr/g fw)		
	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean
ISK-2014-02	306.30	256.49(-16.26)	281.40	216.24	133.90(-38.08)	175.07	1.13	0.94(-16.81)	1.04
ISK-2014-04	218.17	288.42(32.20)	253.30	157.16	133.53(-15.04)	145.35	0.78	0.70(-9.83)	0.74
ISK-2014-12	216.89	299.92(38.28)	258.40	201.47	196.67(-2.38)	199.07	1.05	0.95(-9.52)	1.00
ISK-2014-14	229.66	261.60(13.90)	245.63	242.82	144.24(-40.60)	193.53	1.18	0.78(-34.18)	0.98
ISK-2014-15	307.58	298.64(-2.91)	303.11	161.59	132.79(-17.82)	147.19	1.36	0.90(-34.07)	1.13
TG-51	282.04	335.68(19.02)	308.86	206.29	183.93(-10.84)	195.11	1.14	1.03(-9.62)	1.09
Mean	260.11	290.13		197.60	154.18		1.11	0.88	
CD (P=0.05)									
Genotype (G)	7.320			4.790			0.026		
Treatment (T)	4.226			2.766			0.015		
G x T	10.351			6.775			0.037		

Data in parentheses indicate percentage increase (+) or decrease (-) over control

[ISK-2014-04, ISK-2014-12 and TG-51 are the tolerant and ISK-2014-02, ISK-2014-14 and ISK-2014-15 are the succeptible genotypes]

Table 3: Effect of cadmium stress on activities of superoxide dismutase (SOD), guaiacol peroxidase (GPOX) and catalase (CAT) enzymes in leaves of tolerant and susceptible genotypes of groundnut

Genotypes	SOD (Unit/min/g fw)			GPOX (ΔA470/min/g fw)			CAT (micromol H_2O_2/min/g fw)		
	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean	Cd 0 µM	Cd 300 µM	Mean
ISK-2014-02	9.18	12.17 (32.57)	10.68	22.27	16.29 (-26.88)	19.28	709.96	545.94 (-23.10)	627.95
ISK-2014-04	10.34	17.67 (70.86)	14.00	14.01	14.30 (2.07)	14.16	844.97	744.55 (-11.88)	794.76
ISK-2014-12	8.28	16.78 (102.66)	12.53	19.04	27.55 (44.74)	23.30	666.44	731.16 (9.71)	698.80
ISK-2014-14	6.78	9.80 (44.54)	8.29	19.33	17.15 (-11.28)	18.24	923.07	709.96 (-23.09)	816.51
ISK-2014-15	10.78	15.23 (41.28)	13.01	19.07	16.67 (-12.60)	17.87	933.11	687.64 (-26.31)	810.38
TG-51	7.34	11.23 (53.00)	9.29	14.40	26.43 (83.54)	20.42	981.09	738.97 (-24.68)	860.03
Mean	8.78	13.81		18.02	19.73		843.11	693.04	
CD (P=0.05)									
Genotype (G)	0.306			0.511			20.488		
Treatment (T)	0.177			0.296			11.829		
G x T	0.432			0.724			28.974		

Data in parentheses indicate percentage increase (+) or decrease (-) over control
[ISK-2014-04, ISK-2014-12 and TG-51 are the tolerant and ISK-2014-02, ISK-2014-14 and ISK-2014-15 are the succeptible genotypes]

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