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EXISTENCE OF ALTERNATE DEFENSE MECHANISMS FOR COMBATING MOISTURE STRESS IN HORSE GRAM [*MACROTYLOMA UNIFLORUM* (LAM.) VERDC.]

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ABSTRACT

Screening of horse gram (*Macrotyloma uniflorum*) core collection was carried out under moisture stress conditions in glass house and in poly ethylene glycol 8000 solutions with an osmotic potential of -0.15 MPa to -1.76 MPa after germination. However, selected 50 accessions from the above study were raised under controlled moisture stress conditions to analyze variations in the magnitude of different enzyme activities among the surviving accessions. RWC remained unaltered during moisture stress condition but alteration in the plant structure was observed. Catalase activity and superoxide dismutase increased during moisture stress condition, which varied among the contrasting accessions. However, reduction in polyphenol oxidase and ascorbic acid oxidase activity was observed. The observed difference in anatomy confirms the presence of mechanisms other than antioxidant enzymes. Based on the performance in varying moisture stress conditions, the contrasting D9 and D14 were selected for breeding programme while D13 is recommended as a suitable cultivar under both irrigated and rainfed conditions. Differential enzyme activity, reduction in total sugar production and structural compaction were observed as mechanisms of energy conservation in horse gram to resist moisture stress conditions.

Key words: Drought stress, Enzyme activity, Oxidative stress, Relative water content, Tolerance.

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. ABBREVIATIONS

AAO- Ascorbic acid oxidase
APX- Ascorbic peroxidase
CAT- Catalase
CRD- Completely Randomised Design
DW-Dry weight
EC- Electrical conductivity
FW- Fresh Weight
GPX- Glutathione peroxidase
GR- Glutathione reductase
GST- Glutathione S- transferase
MPa- Mega Pascal
NBPGR- National Bureau of Plant Genetic Resources
PEG- Poly ethylene glycol
POD- Peroxidase
PPO- Polyphenol oxidase
ROS- Reactive oxygen species
RWC- Relative water content
SD- Standard deviation
SE-Standard error
SOD- Superoxide dismutase
TW- Total weight

INTRODUCTION

Horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] known as a poor man's pulse crop is used for human consumption in Africa and India. Its centre of origin is south west India (Arora and Chandel, 1972). Three species namely *Macrotyloma axillare*, *M. geocarpam* and *M. uniflorum* are currently under cultivation. Of these, *M. axillare* and *M. uniflorum* are used as forage plants in tropics and sub-tropics (Blumenthal and Staples, 1993).

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Macrotyloma is among potential dry season livestock feed of Australia and dry regions of Africa, indicating the ability of *Macrotyloma* in withstanding drought and being a suitable candidate for studying moisture stress tolerance and potential source of genes/QTLs for the same.

In India, horse gram is cultivated as a pulse crop contributing about 0.33% of the total food grain production (http://www.apdes.ap.gov.in/). Reports on nutritive value of horse gram indicate it as an excellent source of protein (up to 25 %), carbohydrates (60%), essential amino acids, energy, and low content of lipid (0.58%), iron and molybdenum (Bravo *et al.*, 1999). Anticalcifying inhibitors of crystallization present in seed extract of horse gram are water soluble, heat stable, polar, non-tannin and non-protein in nature (Peshin and Singla, 1994) and hence, it is being used in treatment of kidney stones.

Horse gram is a hardy and a potential crop of future for dryland areas as well as a fodder crop of economic importance. It grows and thrives in a wide range of geographical locations varying in water availability, thus, providing the basis to search for genetic variability and the mechanism of stress tolerance in horse gram. Hence, the present investigation was formulated to analyse the enzymatic activity in horse gram with special reference to available soil moisture levels.

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MATERIALS AND METHODS

Horse gram germplasm used in this study was collected from the National Gene Bank (NGB) of NBPGR, New Delhi, India and collected from farmers' holdings in south and western parts of India. Phenotypic diversity of the 345 accessions (core collection developed and evaluated by Yasin *et al.*, 2012) was assessed on the basis of information in their passport data and field evaluation. *In vitro* screening and pot culture experiments were carried out with 50 diverse accessions selected from preliminary screening using PEG (8000), which showed contrasting response to the artificially created moisture stress. Accessions in the present investigation were raised in the field for screening based upon phenotypic traits at NBPGR Regional Station, Akola under rainfed conditions. Phenotypic traits like growth habit, plant height and root length were recorded under irrigated and unirrigated condition from phytotron grown plants.

During 2010 and 2011, preliminary screening of 345 accessions (treatment numbers AC1 to AC345) in PEG 8000 solutions (after germination) with an osmotic potential of -0.15 MPa to -1.76 MPa (Michel and Kaufmann, 1973) and pot cultures of selected 50 accessions under controlled conditions were done in replicated CRD trial with five replications to select the extremely susceptible and tolerant lines.

Further, twenty most diverse accessions were selected and raised in two sets of pots as irrigated and unirrigated. The seeds were surface sterilized with 70% ethanol for 10 min; rinsed thrice with distilled water and sown in pots containing sand: soil: composted coir pith: peat mixture (1:1:1:1) under controlled conditions in three replications at the National Phytotron Facility (NPF), Indian Agricultural Research Institute, New Delhi. The plants were maintained in a glass house under a natural photoperiod of 10/14h within a temperature range of 28-35°C. Irrigated pots were maintained at 100% field capacity by watering daily. Unirrigated pots were maintained at 10% of field capacity by recharging the evapo-transpiration loss. In the present investigation, the available water content in the soil was maintained throughout the experiment to get uniform result as suggested by Monteiro *et al.* (2011). The accessions which did not survive under unirrigated condition were not considered for biochemical analysis. The selected accessions were provided with treatment code numbers from D1 to D20.

Substrate solution for AAO (EC 1.10.3.3) enzyme activity was prepared by dissolving 8.8mg of ascorbic acid in 300ml of 0.1M phosphate buffer (pH-5.6) and enzyme activity was measured as explained by Vines and Oberbacher (1963). CAT (EC 1.11.1.6) was quantified as detailed by Aebi (1984). PPO (EC 1.14.18.1) was measured as explained by Sarvesh and Reddy (1988). POD (EC 1.11.1) activity was assayed as increase in optical density due to the oxidation of guaiacol to tetra-guaiacol (Castillo *et al.*, 1984). SOD (EC 1.15.1.1) estimation was done by recording the decrease in optical density of formazone made by superoxide radical and nitro-blue tetrazolium dye due to enzyme activity (Dhindsa *et al.*, 1981), while total sugar content was estimated by anthrone method (Yemm and Willis, 1954) and chlorophyll was extracted as

per the protocol of Tait and Hik (2003). Chlorophyll extract was transferred to cuvette and spectrophotometer readings were recorded at 649 and 665nm. Relative water content was estimated as described by Barrs and Weatherley (1962) and calculated as RWC = (FW-DW) / (TW-DW) X100.

Sections of root, stem and leaf were prepared using standard microtome procedure explained by Steven (1999) for observation under light microscope.

Hotelling test was done to identify significant treatment of the moisture stress. The comparisons of means were executed separately for each trait. Differences were determined by Tukey's multiple range tests at $p \leq 0.05$, correlations were determined and tested using Pearson test at $p \leq 0.05$ and Principal Component Analysis (PCA) was done to find out the contribution of individual components. All statistical analyses were carried out using SAS 9.2. (SAS institute, 2009).

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RESULTS AND DISCUSSION

The results of *in vitro* screening experiment showed significant differences in the survival rate of all accessions (core collection of 345 accessions developed by Yasin *et al.*, 2012) screened for moisture stress tolerance at different concentrations of at 10, 20, 30 and 40% PEG 8000. . Moisture stress sensitivity was used as criteria for selection of accessions. At 10% of PEG 8000, all the accessions except AC20 survived; hence it was identified as the most susceptible accessions. At 20% of PEG 8000, more than 50% survival was recorded of which, five accessions were selected as moderately tolerant lines. While at 30%, only five accessions survived and were selected as tolerant lines. No survival was observed at 40% PEG 8000. Thus, 30% of PEG 8000 was selected as a suitable concentration for preliminary screening of horse gram accessions for moisture stress tolerance.

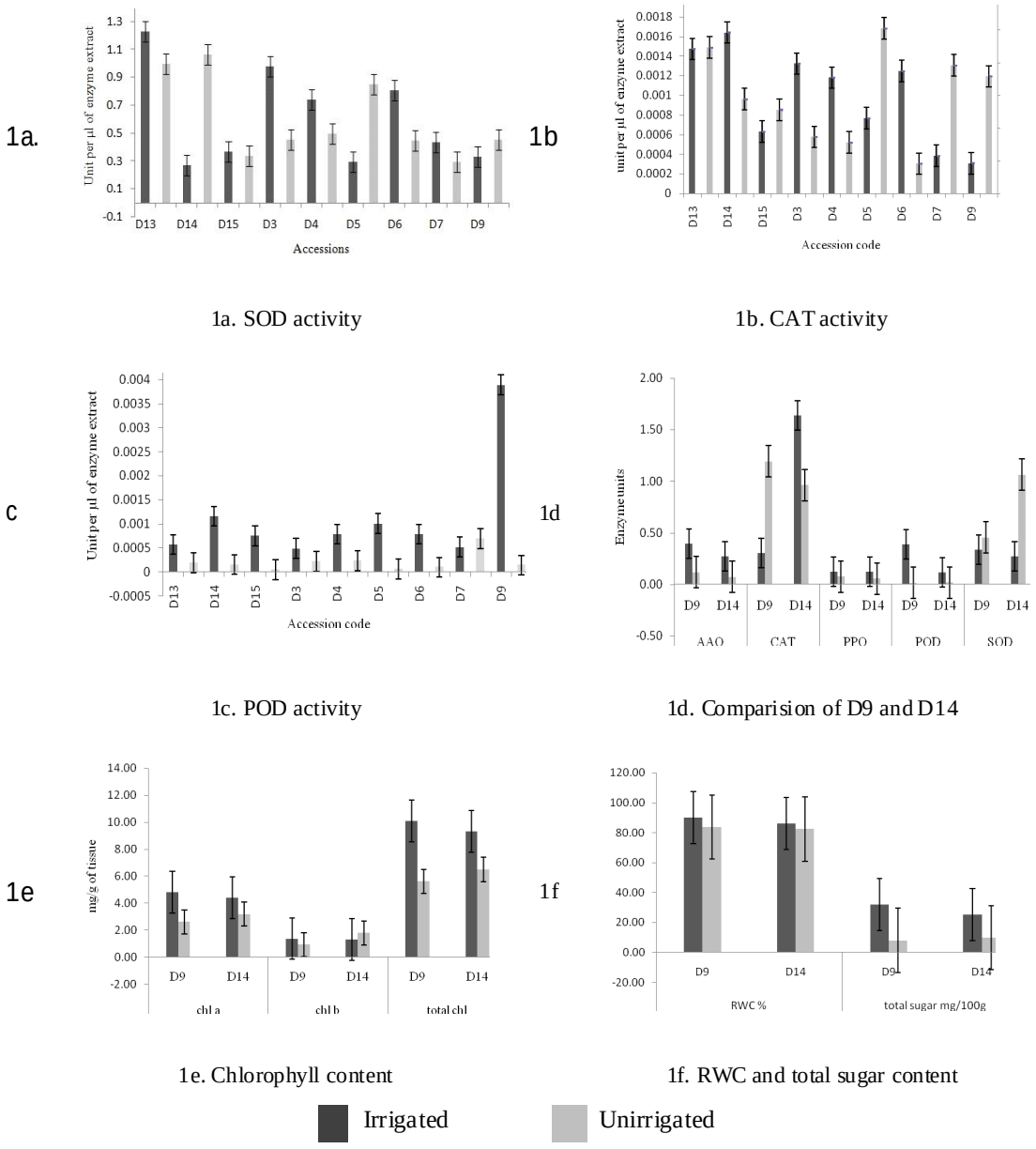
From pot culture experiments, twenty accessions were selected based on soil moisture stress tolerance for screening under glass house condition at NPF, of which, ten were susceptible and ten were tolerant. Accessions which were unable to survive for 80 days after germination (3/4th of the maximum recorded crop duration) under both irrigated and unirrigated conditions were eliminated from statistical analysis resulting in reduction in number of accessions to nine for final enzymatic study. Observations on various parameters were recorded on remaining nine accessions (D3, D4, D5, D6, D7, D9, D13, D14, and D15).

SOD, POD, CAT and PPO activity expressed in unit per µl of leaf extract varied among accessions and treatments (irrigated and unirrigated conditions). Highest enzyme activity was exhibited by D13 irrigated and D14 unirrigated for SOD (Fig. 1a); D14 unirrigated and D5 irrigated for CAT (Fig. 1b) and D9, D8 followed by D14 irrigated for POD (Fig. 1c).

SOD and CAT activities were significantly different in the most contrasting accessions i.e. D9 and D14. SOD activity was high for D14 unirrigated and D9 irrigated condition. CAT activity was high in D14 irrigated and D9 unirrigated (Fig. 1d). However, in all the accessions as a group, results from Hotelling test for significance of unirrigated and irrigated conditions showed different AAO and PPO activities. PPO and AAO activities declined in unirrigated plants whereas CAT activity increased in the same. Although four genotypes exhibited more than double PPO and AAO enzyme activity as compared to others, PPO and AAO activities were not influenced by the genotypes under treatment conditions. As CAT was found to be influenced by genotypes, it was not significant as a group in Hotelling test (Table 1). AAO was negatively correlated with CAT and positively correlated with PPO, whereas CAT was found to be negatively correlated with PPO (Table 2). SOD and CAT indicate significant relationship for individual accessions. However, as a group of observations, the relationship is insignificant and tends to exhibit a weak relationship. This can be better explained by regression equation developed between CAT and SOD (SOD = 333.7 CAT + 0.272) with an R^2 value of 0.248 (Fig. 2). Accession D9 and D14 were found to be diverse among the accessions where the CAT and SOD difference is enhanced.

RWC pattern in the present investigation indicates that irrigated and unirrigated plants tend to maintain the RWC within a considerable limit. The RWC was unaffected to a greater extent by moisture stress (Fig.1f). As per Hotelling test, the accessions under irrigated condition, as a group

FIG. 1. Impact of available moisture level on Horse gram accessions under irrigated and unirrigated conditions (data are mean of three replicates ± SE)



were significantly different from those under unirrigated condition for RWC indicating the significance of treatments and not the accessions. However, the treatments as a group are significant for total sugar content. RWC was positively correlated with AAO, PPO and total sugar content but negatively correlated with CAT and chlorophyll content (Table 2).

Chlorophyll b content was significantly different between group of irrigated and unirrigated plants based on Hotelling test. Chlorophyll a and total chlorophyll were not significant as there was significant variation for irrigated and unirrigated accessions D9 and D14 (Fig. 1e). Chlorophyll content was negatively correlated with AAO, CAT, PPO, RWC and total sugar content (Table 2). PCA of POD, SOD and CAT (Fig. 3 and 4) indicates that the first two components contribute up to 83% of total variation (Table 3). The scatter plot of the first two components showed grouping of accessions differentiating irrigated and unirrigated condition (Fig. 5). Chlorophyll b showed high positive loadings

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TABLE 1: Group analysis for associated traits of Horse gram accessions under controlled conditions

Biochemical	Irrigated	Unirrigated
Parameter	Mean ± SD	Mean± SD
AAO	0.19± 0.12**	0.08± 0.04**
CAT	0.23± 0.08	0.48± 0.33
PPO	0.14± 0.03**	0.07± 0.01**
RWC	0.88± 0.03**	0.80± 0.06**
Total Sugar	20.34± 11.46	10.34± 4.13
Chlorophyll a	3.73± 0.84	4.45± 2.00
Chlorophyll b	1.15± 0.24**	1.85± 0.73**
Total Chlorophyll	7.90± 1.76	9.74± 4.27

**Treatments as group of Irrigated and Unirrigated were significantly different based on Hotelling test

in component 1; chlorophyll a in component 2 and negative for CAT (Table 4).

In irrigated condition the epidermis of stem and its single layer endodermis were clearly separated from thin layer of three cell thick cortex. Passage cells were merged with protoxylem whereas, the central core was covered with larger area of pith and fibre cells were not prominent. The parenchyma cells in cortex region were irregularly spaced with conspicuous air spaces. Trichomes were very less and scattered. Adaxial surface was pubescent in irrigated plants. Contrast to the irrigated condition, in plants under unirrigated condition, the epidermal layer was thicker than the control. Cortex layer was expanded with two to three more layers of cells, pith region was reduced and relatively endodermis was not clearly separable from cortex. Fibre cells and tracheids were found to be prominent, however, no difference was observed in the presence of trichomes. Palisade parenchyma was compact whereas spongy parenchyma was sparse with more air space. Numerous trichomes were present at the abaxial surface of leaf; while the adaxial surface was waxy and shiny. Number of stomata was also less in plants growing in unirrigated conditions compared to the irrigated ones.

The presence of structural compaction (Yasin *et al.*, 2013), retardation of plant growth, variation in enzyme activity, reduction in synthesis of sugar and dry matter content in horse gram to overcome moisture stress possibly due to reduction in metabolic activity. The results presented in the study exhibit the participation of antioxidant enzymes in overcoming moisture stress.

Phenotypic data of irrigated and unirrigated plants (Table 5, 6) indicates the structural compaction and retardation of plant growth during soil moisture stress (Yasin *et al.*, 2013). Generally in other crops, deep root system and reduction in aerial system is found during moisture stress conditions to avoid

TABLE 2: Correlation coefficients for biochemical traits under moisture stress condition in Horse gram

	Pearson Correlation Coefficients						
	ao	ct	po	rw	tc	ca	cb
ct	-0.20952						
po	0.19352	-0.30148					
rw	0.31830	-0.30117	**0.54085****				
tc	0.24780	-0.33281	**0.58905****	0.43355			
ca	-0.02952	-0.04113	-0.20634	-0.43125	-0.23631		
cb	-0.28959	-0.03480	-0.51508	**-0.59459****	-0.40779	**0.89859****	
tc	-0.07782	-0.00016	-0.24716	-0.40264	-0.27365	**0.99049****	**0.90426****

Pearson test - * values are significantly different at p≤ 0.05, ** at p≤ 0.01
Ao- AAO ct- CAT po-PPO rw-RWC tc-total sugar ca-chlorophyll a
cb- chlorophyll b tc-total chlorophyll

TABLE 3: Principal components for POD, SOD and CAT on correlations

Number	Eigenvalue	Percent	Cumulative Percent
1	1.7162	57.206	57.206
2	0.7837	26.124	83.330
3	0.5001	16.670	100.000

Components 1 and 2 contributes 83.33% of the total variability

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TABLE 4: Principal component analysis for associated traits of Horse gram accessions

	Eigenvectors		
Parameter	component 1	component 2	component 3
AAO	-0.21367	0.30389	**0.56734**
CAT	0.02849	**-0.48827**	**0.71168**
PPO	-0.29472	**0.44519**	0.14603
RWC	**-0.35774**	0.18276	-0.24486
Total sugar	-0.28856	**0.41638**	0.14052
Chlorophyll a	**0.44678**	**0.35697**	0.18495
Chlorophyll b	**0.50178**	0.17915	-0.1111
Total chlorophyll	**0.45175**	0.32367	0.1551

Variance explained by each factor: component 1= 45.597% component 2 = 22.115% component 3 = 10.334%

evaporation and transpiration loss (Zhang and Kirkham, 1996), whereas, in horse gram under moisture stress, there is an extensive reduction in root length which is a not very common.

In crops like rice (Yu *et al.*, 2006) and sorghum (Zhang and Kirkham, 1996), the RWC goes down to create temporary wilting but in horse gram the RWC was unaffected due to osmoregulation coupled with anatomical changes to avoid stress. The results show that horse gram can compensate yield over survival to overcome prolonged moisture stress condition as yield loss is not a critical concern for a standalone fodder crop. Earlier reports of Gazanchian *et al.* (2007) in wheat grass describe the role of several oxidative stress tolerant enzymes *viz.*, ascorbate reductase, APX, SOD *etc.* in imparting tolerance to moisture stress. The variation in the enzymatic activity in the study is in accordance with the previous reports for the presence of antioxidant scavenging mechanism along with presence of a weak relationship of CAT and SOD in overcoming the stress situation in horse gram. As horse gram grows in extreme dry spells, moisture stress tolerance may be an inherent mechanism where SOD acts extensively to overcome stress and CAT may act during photoperiod stress and excess water availability conditions. The present findings are similar to studies by Palatnik *et al.* (1999) indicating complementary roles of antioxidants in concerted cell defence through ROS scavenging, re-establishing of the redox homeostasis and oxidative damage repair. The weak relationship of CAT and SOD as indicated by low R^2 value may be due to the presence of other complex mechanisms involved in the expression of these enzymes in genetically diverse material to overcome stress situation. The presence of excess water is also sensed as a stress condition by horse gram (Blumenthal and Staples, 1993) and the present results indicate excess of CAT over other anti-oxidant enzymes under excess water condition. Up-regulation of SOD (Gazanchian *et al.*, 2007, Xu and Huang, 2008) and CAT (Zhang and Kirham, 1994) under stress condition while a declining trend in activity of CAT has also been reported under moisture stress conditions (Abedi and Pakniyat, 2010). The POD activity remained unchanged in susceptible and tolerant accessions. In the present investigation, the relationships of AAO and CAT with PPO indicates lesser role of PPO in moisture stress tolerance, which otherwise has definite role during biotic stress tolerance (Nakayama *et al.*, 2000, Li and Steffens, 2002). In drought tolerant tomato plants, higher chlorophyll content, photosynthetic efficiency and reduced photo-inhibition were reported (Thipyapong *et al.*, 2004) along with suppressed PPO activity. A decrease in antioxidant enzymes SOD, CAT and POD under NaCl stress in soybean has also been reported (Amirjani, 2010).

Reductions in total sugar content reflect the declined photosynthetic rate in horse gram. Further, under prolonged stress, horse gram prefers to survive than to multiply thereby indicating presence of an efficient mechanism to conserve energy. In horse gram, reduction in total sugar content and retarded plant growth results in reduced ROS formation to avoid soil moisture stress. In concordance with the present results, Logan *et al.* (2006) reported, increase in antioxidant production cannot enhance resistance to ROS produced during photo-inhibition. Hence, overproduction of chloroplastic antioxidants in transgenic crops was not a suitable option to protect plants from stress condition (Logan *et al.*, 2006).

Plant responses to stress converge on cellular de-differentiation and reduction in protein synthesis (Dhindsa and Cleland, 1975; Grafi *et al.*, 2011)

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TABLE 5: Phenotypic variations of Horse gram accessions under MLT during 2010 and 2011 (under standard cultivation practices)

Accn. No.	Plant height (cm)	Days to 50% flowering	Pod length (cm)	Pod width (cm)	No. of pods/plant	No. of seeds/pod	Seed length (mm)	Seed width (mm)	100 seed weight	Seed yield/plant (g)	Days to maturity
D13	46.8 +1.56	86.2 +0.86	5.38 +0.09	1.62 +0.06	33.6+1.44	3.64+0.26	4.99+0.32	4.02+0.32	3.35+0.49	4.17+0.72	125.2+0.76
D14	**93.8 ±0.58**	**82.8 ±0.58**	**4.3 ±0.07**	**1.64±0.07**	**31.8±0.58**	**4.92±0.36**	**5.31 ±0.21**	**3.65±0.38**	**2.98±0.2**	**4.16±0.34**	**124.5±0.57**
D15	64.8 ±1.59	77.4 ±1.29	4.66 ±0.12	1.49 ±0.04	18.8±1.16	4.6±0.4	5.56±0.23	4.15±0.38	3.34±0.28	3.46±0.15	116.2±0.92
D3	85.8 +1.83	82.4 +0.93	4.93 +0.12	1.38 +0.05	26.6 +0.93	3.8 +0.2	5.12+0.34	4.65+0.22	3.37+0.56	4.28+0.13	146.2+2.14
D4	70.2 +1.85	82.4 +0.93	4.66 +0.05	1.28 +0.04	33+0.89	3.8+0.2	5.08+0.33	3.68+0.38	2.93+0.3	4+0.23	119.2+0.84
D5	61.6 +1.36	80.2 +0.73	4.52 +0.09	1.46 +0.05	22.8+1.59	5.24+0.37	5.47+0.22	4.17+0.38	3.16+0.44	4.05+0.08	119+0.73
D6	41.8 +1.59	79.4 + 1.5	4.65 +0.13	1.48 +0.04	19.2 +1.07	4.56+0.41	4.78+0.37	3.94+0.32	3.09+0.4	2.93+0.31	123.5+0.93
D7	65.8 +1.53	75.6 +1.29	4.39 +0.05	1.58 +0.04	43.2+0.86	4.92+0.45	6.06+0.32	4.07+0.32	3.96+0.25	8.3+0.34	117.7+0.67
D9	**72.2 ±1.36**	**80.8 ±1.07**	**4.54 ±0.05**	**1.58 ±0.07**	**31±0.84**	**3.6±0.25**	**6.04 ±0.32**	**3.73±0.37**	**3.13±0.35**	**4.26±0.22**	**119±0.73**

Observations recorded at 50% flowering under field condition (data are mean of five replicates ± SE)

thereby triggering a quiescent state of the cell, which retards overall growth of the plant as observed in the present study. The anatomical, physiological and biochemical response to drought stress may interfere at several levels resulting in a unique way to affect plant development (Szucs *et al.*, 2010). The observed difference in anatomy confirms the presence of mechanisms other than antioxidant enzymes. Anatomical changes under drought conditions are similar to heat stress condition (Wahid *et al.*, 2007) *viz.*, reduction in cell size, curtailed water loss, increased stomatal and trichotamous densities and greater xylem vessels (Aono *et al.*, 1995). RWC remained unaltered during moisture stress condition; however alteration in the plant structure was observed (Fig. 1f). Similar results like impact of stress on mesophyll cells to increase plasma membrane permeability have also been reported (Enstone *et al.*, 2003; Zhang *et al.*, 2005; Reinhardt and Rost, 1995). The overall effect of all anatomical changes due to reduction in available soil moisture level results in retardation of plant growth and productivity.

The mechanism of adaptation to different stress varies with crop plants including a series of enzymatic and non-enzymatic detoxification system to counteract antioxidant system to protect cells from oxidative damage (Sairam and Tyagi, 2004). Similar results were observed in the present study. Multiple signalling pathways regulate the stress responses of plants (Knight and Knight, 2001) and gene expression overlaps in response to induced stress conditions (Chen *et al.*, 2002). The contrasting expression of D9 (9, 18 in Fig. 5) and D14 (2, 11 in Fig. 5) facilitates the selection of these two accessions for our future breeding programme. The accession D13 under irrigated and

FIG. 2: Weak association of CAT and SOD under irrigated and unirrigated conditions

un-irrigated conditions shows the ability to survive under both conditions without significant difference in enzyme activity conclusively indicating the presence of other mechanism to detoxify the ROS produced due to moisture stress (Fig. 6). The accessions selected from this study are being multiplied for the future breeding programme and for gene bank submission.

Horse gram is a crop which can grow in varied conditions of drought and is found to grow from south to north of India with a tremendous diversity and drought tolerance. Thus, horse gram diversity can be a potential source for identification of various moisture tolerance mechanisms using genomics and proteomics approach and be established as a model crop for such studies.

TABLE 6: Phenotypic variations of Horse gram accessions under glass house conditions

	Plant height		Branches		Leaf area		Root length	
Accession	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated
D3	19.93 ± 0.74	43.67± 6.17	2.67± 0.33	5.33± 0.88	7.13± 0.12	14.7± 0.17	2.23± 0.09	25.33± 2.19
D4	20.03± 2.48	68± 9.81	3± 0.58	7± 1.15	6.76± 0.14	16.53± 0.87	2.4± 0.06	23.67± 0.88
D5	26.93± 1.39	55.67± 8.95	4± 0.58	7.67± 2.03	5.9± 0.06	25.32± 0.66	2.6± 0.06	32.33± 2.96
D6	18.17± 0.44	45± 5.13	3.33± 0.33	5.33± 0.33	10.3± 0.4	18.17± 0.44	4.83± 0.03	23± 0.58
D7	24.67± 3.38	60.33± 7.69	2.33± 0.33	7.67± 0.33	7.63± 0.3	18.46± 0.44	2.6± 0.06	32.33± 0.88
D9	**17.1± 2.4**	**35± 4.04**	**2.67± 0.67**	**6.33± 0.88**	**9.7± 0.55**	**13.8± 0.15**	**5.7± 0.12**	**16.67± 1.2**
D13	20± 0.76	26.33± 1.76	2.67± 0.33	3± 0.1	5.75± 0.18	14.98± 1.04	3.77± 0.18	14.67± 1.76
D14	**31± 2.65**	**99.33± 1.76**	**5± 0.2**	**6.67± 0.67**	**7.3± 0.26**	**21± 1.04**	**2.5± 0.06**	**16± 1.53**
D15	24.17± 0.73	47.5± 5.48	3.67± 0.33	7± 0.58	8.59± 0.2	9.857± 0.2	2.47± 0.18	16± 1.53

Observations recorded on 65th days after germination under glass house condition at National Phytotron Facility (data are mean of three replicates ± SE)

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FIG. 3: PCA: Loading plot with PC 1 and PC 2 for associated traits of horse gram accessions.

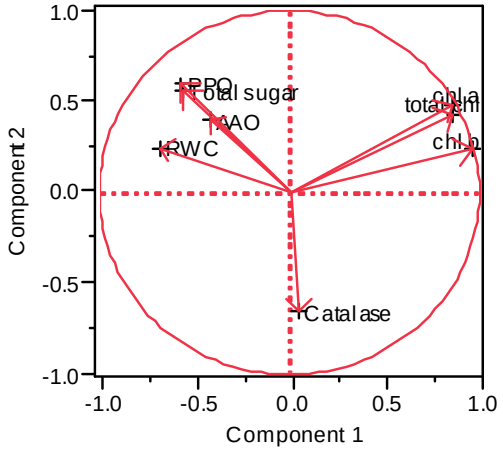


FIG. 4: PCA: Loading plot with PC 1 and PC 2 for CAT, SOD and POD of horse gram accessions.

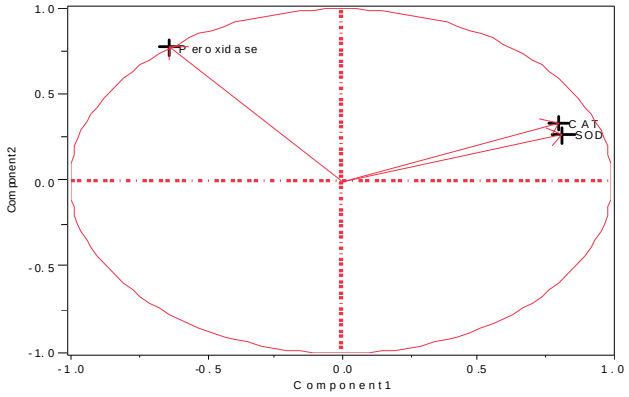


FIG. 5: Loading plot for PC1 and PC2 for CAT, SOD and POD of horse gram accessions

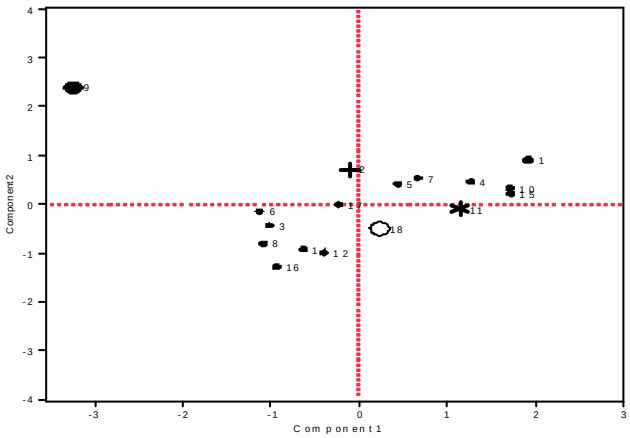
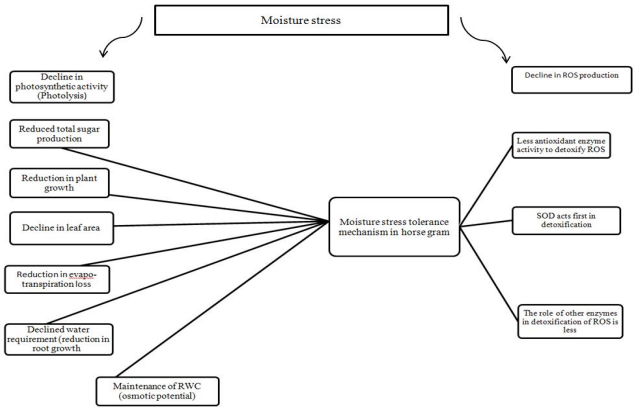


FIG. 6: Possible mechanism operating moisture stress tolerance in horse gram based on the present investigation



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