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Response of *Lablab purpureus* (Hyacinth bean) cultivars to drought stress

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ABSTRACT

Unpredictable rain patterns have forced farmers to cultivate drought resistant crops in primarily rainsfed areas. Dolichos lablab (Lablab purpureus L. sweet) a drought resistant plant, has several genotypes which are cultivated in different regions. Five extensively cultivated genotypes were subjected to drought stress and evaluated for their performance under drought stress. The effect was monitored in 10 day old seedlings over eight days under green house conditions. Stress indicators H_2O_2, lipid peroxidation, antioxidants like GSH, proline, ascorbic acid, and antioxidant enzymes such guaiacol peroxidase, catalase and glutathione reductase, metabolic enzymes like acid phosphatase and amylase were monitored. While some parameters showed similar trend in all five cultivars, others varied. Two cultivars, GL43 and HA4 showed elevation in glutathione reductase, guaiacol peroxidase and diminished catalase activity. An elevated level of amylase activity was observed in DL28, acid phosphatase showed increase in activity under stress. The results suggested that HA4 and GL43 as most resistant cultivars to drought.

Key words: Hyacinth bean, drought, oxidative stress, reactive oxygen species (ROS), antioxidants.

INTRODUCTION

Plants are constantly exposed to environmental challenges. Drought, salinity, flooding and heavy metal stresses, in single or in combination can affect plants. Drought stress is a serious problem for agriculture worldwide, affecting plant growth, productivity, and survival [1]. About four tenth of the worlds agricultural land is affected by drought. Drought is a condition of non availability of water, physiologically or due to water scarcity, leading to complex process involving numerous changes including attenuated growth, activation of genes, transient increase in ABA levels, accumulation of compatible solutes and protective proteins, increased levels of antioxidants and suppression of energy consuming pathways. On the cellular level, drought stress will affect vital metabolic functions and maintenance of turgor pressure. Cell expansion and cell wall formation are therefore especially sensitive to water limitation. In order to minimise water loss, plants respond to lower water availability with the closure of stomata, thereby affecting photosynthesis. Closure of stomata limits the gases exchange and there by bringing imbalance in light reaction and Calvin- Benson cycle [2], which results in reduction of electron carriers in chloroplast and mitochondria, producing reactive oxygen species by transfer of electrons to molecular oxygen [3]. Reactive oxygen species (ROS) thus generated are superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^\bullet$). Plants possess very efficient enzymatic and non-enzymatic antioxidant defence systems which work in concert to control the cascades of uncontrolled oxidation and protect plant cells from oxidative damage by scavenging ROS [4, 5].

As droughts are predicted to increase in both frequency and intensity due to climate change, a better understanding of drought response patterns and associated traits is essential for obtaining yield stability in water-liming environments. The activities of antioxidant enzymes and antioxidants under stress are usually regarded as indicators of tolerance of genotypes. Therefore, in order to develop drought resistant and high yielding plant species, the relationships between drought and antioxidant defense system must be clearly understood. *Lablab purpureus* (Hyacinth bean) is traditionally grown as a pulse crop for human consumption in South and Southeast Asia and

Eastern Africa. *Lablab* species are known to show drought tolerance when established. In order to evaluate the performance of five extensively cultivated *Lablab purpureus* cultivars, were subjected to drought stress and the results of which are presented herein.

MATERIALS AND METHODS

Plant material and Drought stress - Seeds of Hyacinth Bean *(Lablab purpureus)* genotypes, HA4 and GL43 were procured from University of Agricultural Science, GKVK, Bangalore; Genotypes DL4, DL21 and DL28 were procured from Tamilnadu Agricultural University, Coimbatore. Seeds were surface sterilized with 0.1% $HgCl_2$ for 10 seconds and washed repeatedly with distilled water. Overnight soaked seeds were germinated in acid-washed sand and vermiculite (1:1 w/w) and watered every 12 h in green house conditions, at 25°C, 70% humidity and 12 h light and 12 h dark photoperiod. Plants were grown for 10 days before inducing stress. Control plants were watered twice a day and stress was induced by withholding water. Leaf and root samples were collected at time intervals of 2, 4, 6 and 8 day after stress.

Estimation of antioxidants and other stress specific components

Hydrogen peroxide (H_2O_2): Hydrogen peroxide levels in stressed and control homogenate were determined according to the method of Velikova et al., [6]. 500 mg of leaf and root tissues, were homogenized in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10,000 rpm for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance was measured at 390 nm.

Proline (PRO): Proline content was determined according to the method of Bates et al., [7]. Free proline was extracted from 0.5 g of fresh tissue in 10 ml sulphosalicylic acid (3%) and the extract was filtered through Whatman no. 1 filter paper. A known quantity of the filtrate was mixed with 2 ml of acid ninhydrin reagent. The contents were boiled for 1 h in a boiling water bath and cooled rapidly on ice. The color was extracted in 4 ml toluene by vigorous shaking and the organic phase recorded at 520 nm against toluene as blank. Standard curve was prepared for different concentrations of proline.

Ascorbic acid (ASC): Ascorbic acid (AA) estimation was carried out according to the procedure of Sadasivam and Manickam [8]. The tissues was homogenized in 4% oxalic acid and centrifuged at 10,000 g for 10 min. The assay mixture consisted of 0.1 ml brominated sample extract made upto 3.0 ml with distilled water, 1.0 ml of 2% DNPH reagent 1-2 drops of thiourea. After incubation at 37 ^oC for 3 h, the orange red osazone crystals formed were dissolved by addition of 7.0 ml 80 % sulphuric acid and absorbance was read at 540 nm.

Glutathione (GSH): Glutathione (GSH) was determined according to Beutler [9]. The tissue was homogenized with 3% metaphosphoric acid. DTNB (5, 5'-dithiobisnitrobenzoic acid) was added to supernatants cleared by centrifugation. The formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm against reagent control.

Lipid peroxidation: Lipid peroxidation was determined by estimating malondialdehyde (MDA) content according to the method of Heath and Packer [10] with suitable modification. Briefly 0.5 g of fresh tissue was ground in 5.0 ml of 0.1% TCA containing 0.5% butylated hydroxytoluene containing 1.0% PVP. The homogenate was centrifuged at 7,000 g for 30 min. 4.0 ml of supernatant was mixed with 4.0 ml of substrate (0.5% thiobarbituric acid and 20% trichloroacetate). The mixture was boiled for 30 min, chilled on ice, and centrifuged at 12,000 g for 10 min. the absorbance of supernatant at 532 nm was measured and the non specific absorbance at 600 nm was subtracted. The MDA content was calculated from the extinction coefficient of 155 mM^{-1} cm^{-1}.

Assay of antioxidant enzymes

Plant material was homogenized with 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM PMSF, 5 mM β-mercaptoethanol and 1mM EDTA (Ethylenediaminetetraacetic acid). The homogenate was centrifuged at 10,000 rpm for 20 min at 4^oC. The supernatant was used as source of enzymes. All the steps in the preparation of the enzyme extract were carried out between 0 and 4^oC. Soluble protein was determined according to the method of Lowry et al., [11] with BSA as the standard.

Guaiacol peroxidase (GPOX) [E.C.No.1.11.1.1] activity was measured in a reaction mixture of 3.0 ml consisting of 50 mM phosphate buffer (pH 7.0) containing 20 mM guaiacol, 10 mM H_2O_2 and 100 µl of enzyme extract. The formation of tetraguaiacol was followed by an increase in absorbance at 470 nm [ε=26.6 mM^{-1} cm^{-1}] according to the method of Chance and Maehly [12]. One unit of peroxidase is defined as the amount of enzyme needed to convert 1µmol of H_2O_2, min^{-1} at 25^oC.

Catalase (CAT) [E.C.No.1.11.1.6] activity was assayed by following the decline in optical density of H_2O_2 at 240 nm (ε= 39.4 $M^{-1}cm^{-1}$) according to the method of Aebi [13]. The reaction mixture consisted of 50 µl of enzyme extract was used. The reaction was started by addition of H_2O_2, to a final concentration of 10 mM, and its consumption was measured for 2 min. unit of activity was defined as the amount of enzyme that catalyzes the oxidation of 1µmol H_2O_2, min^{-1} under the assay conditions.

Glutathione reductase (GR) [E.C.No.1.6.4.2] was assayed by monitoring the oxidation of NADPH at 340 nm (ε= 6220 $M^{-1}cm^{-1}$) according to the method of Carlberg and Mannervik [14]. The reaction mixture contained 50 mM Tris buffer (pH 7.5), 3 mM $MgCl_2$ 500 nM GSSG, 200 nM NADPH and 250 µl of enzyme extract in a total volume of 1.5 ml. One unit of enzyme is defined as the amount of enzyme that catalyzes the oxidation of 1µmol of NADPH min^{-1} under the assay conditions.

β-Amylase (AMY) [E.C.No.3.2.1.1] activity was assayed according to the method of Bernfeld [15]. The reaction mixture consisted 0.5 ml of 2% starch solution prepared in 50 mM phosphate buffer and 0.5 ml of enzyme extract.

Acid phosphatase (AP) [E.C.No.3.1.3.2] activity was assayed according to the method of Hoerling and Svensmark [16], employing α-naphthyl phosphate or p-nitro phenyl phosphate as substrates. Each unit of activity is defined as the number of µmoles of α-naphthol or p-nitro phenol released min^{-1}.

Statistical analysis

All data are expressed as means of triplicate experiments. Comparisons of means were performed using GraphPad prism 5.0 software. The mean differences were compared by lowest standard deviations test. Differences in mean values were considered significant if the pooled variance $p<0.05$.

RESULTS AND DISCUSSION

Production of ROS is the early response that plants show towards dehydration stress [17]. Drought stress caused varied responses in five varieties of *Lablab purpureus.* All genotypes showed an elevation in H_2O_2 under stress (Table Ia & Ib). HA4 was quick to respond to dehydration stress in terms of H_2O_2 levels in both leaves and root tissues, wherein leaf showed higher H_2O_2 levels per gram tissues. Similar effects were observed in GL43 genotype, which showed the onset on 4th day in roots and 2nd day in leaves, with higher amount of H_2O_2 in leaf. Other genotypes did not show much alternation in H_2O_2 levels, except a sharp rise in root H_2O_2 levels in DL21 at 8th day after drought stress. The varied response suggested varied ability of genotypes to respond to applied stress. Elevated H_2O_2 in response to drought stress have been reported in cotton (*Gossypium hirsutum*) cultivars during combined drought and heat induced oxidative stress [18] and in *Triticum durum* after water stress [19].

Elevated levels of H_2O_2 and, or other ROS is accompanied by lipid peroxidation under stress, coinciding with H_2O_2, MDA (product of lipid peroxidation) was elevated in HA4 and GL43 genotypes indicating a correlation between H_2O_2 levels and extent of lipid peroxidation (Table Ia & Ib). The HA4 cultivar showed higher levels of lipid peroxidation than GL43 in roots, but both the varieties had similar range of MDA in leaves. Interestingly, the levels of MDA in leaves were much lower than those in roots, suggesting a stronger antioxidant mechanism in leaves. These results are in agreement with drought stressed cucumber seedling [20], wheat [21] and genus Avena [22].

Stressed plants have been shown to produce antioxidants to overcome oxidative damage. ASC and GSH are major antioxidants which together can effectively reduce ROS by scavenging. GSH protects biological macromolecules either by forming adducts directly with reactive electrophiles or by acting as a proton donor in presence of ROS yielding GSSG [23, 24]. The level of ASC under oxidative stresses depends on the balance between the rates of Asc biosynthesis and turnover related to antioxidant demand [25]. GSH levels in the tested cultivars (Table Ia & Ib) were not affected to any significant levels under drought. However, two of the cultivars were distinct in possessing 3-4 fold higher GSH levels than other three cultivars. The data suggested that HA4 and GL43 are endowed with greater ability to destroy ROS than other genotypes.

In both, leaves and root tissue, ASC levels increased under applied stress (Table Ia & Ib). HA4 differed from other genotypes in having lower levels of ASC. In other four genotypes, ASC levels fluctuated with slight increase upto 4th day and declined to initial levels followed by rise after 6th day of stress. Among these four varieties, DL21 exhibited higher ASC levels at all stages of stress. These results are in agreement with drought stressed *Withania somnifera* [27] and almond [28]. The increase ASC pool in three cultivars under stress suggests its role as a powerful reducing agent, in free radical scavenging. The changes in ascorbate pool may be due to change in glutathione pool that has been implicated in recycling of ascorbate [29]. However cultivars with elevated ASC did not show much variation in GSH content on the corresponding days of stress.

Proline, another non enzymatic molecule is known to accumulate under different types of abiotic stress. PRO has been proposed to act as an osmoprotectant, a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation, and OH^- and $_1O^2$ scavenger [30,31]. All genotypes showed increased proline levels under stress relative to control (Table Ia & Ib), except DL4, which showed significantly lower proline. DL28 exhibited relatively higher PRO. Root tissue of GL43 and HA4 exhibited greater levels of proline, and progressively increased in extended duration of stress (5-fold). However, the other genotypes had negligible PRO content. These results are in agreement with PRO level elevation observed in genotypes of *Abelmoschus esculentus* [32], sugar cane [33], wheat [34] and corn cultivars [35] under drought stress.

Under drought stress, antioxidant enzymes like guaiacol peroxidase, catalase and glutathione reductase are induced to overcome oxidative stress [36]. Antioxidant enzymes like GPOX are associated with many important biosynthetic processes, including lignifications of cell wall, degradation of IAA, biosynthesis of ethylene, wound healing, and defense against abiotic and biotic stresses. GPOXs are widely accepted as stress "enzymes." GPOX can function as effective quencher of reactive intermediary forms of O_2 and peroxy radicals under stressed conditions [37]. All cultivars of *Lablab purpureus* tested showed a common pattern in GPOX levels, with root GPOX levels 10 fold higher than leaf. In the leaf tissue, HA4 cultivar exhibited better GPOX levels in both control and stressed seedlings. Stressed seedlings exhibited greater levels of GPOX with progress of stress and 3 fold enhancements was observed after 8 days of stress (Fig 1a). Root tissue of HA4 and GL43 showed higher levels of GPOX over the entire period of stress, the stressed tissues showed 10-15% reduction in GPOX activity as noticed in all varieties (Fig 1b). Similar decrease in GPOX has been observed in common vetch (*Vicia sativa L.*) [38] and Triticum durum [19] under drought stress. On the contrary, increase in GOPX activity was observed in drought stressed cultured plants of *Macrotyloma uniflorum* [39], and wheat varieties (*Triticum aestivum* L.) [34].

Glutathione reductase is a NAD(P)H-dependent enzyme, involved in the ASC/GSH cycle that operates in chloroplast, cytoplasm, mitochondria as well as peroxisomes [35]. GR catalyzes the reduction of GSSG to GSH and thus, maintains high cellular GSH/GSSG ratio. Hyacinth bean cultivars differed in their response to drought stress in terms of GR levels (Fig 2a & 2b). While HA4 cultivar exhibited higher GR activity in leaf and root tissue over the entire period of stress, all other cultivars showed a decline in GR in the first half of stress and a marginal increase towards second half in leaf and slight increase in first half and decline in second half in root. Enhanced levels of GR have been found associated with better tolerance to stress in oilseed Rape [41] and melon seedlings [42].

CATs are ubiquitous tetrameric heme-containing enzymes which catalyzes the dismutation of two molecules of H_2O_2 into water and oxygen. Peroxisomes are the major sites of H_2O_2 production, CAT scavenges H_2O_2 generated in this organelle during photorespiratory oxidation and β-oxidation of fatty acids [43, 35]. The cultivars of Hyacinth bean differed in CAT levels, while HA4 and GL43 exhibited greater levels of CAT. HA4 showed more than 3-fold higher CAT activity, in both control and stressed seedlings. Similarly, GL43 had more than 2-fold excess of CAT in both leaf and root tissue. Overall, all the cultivars exhibited a decrease in CAT activity under applied stress (Fig 3a & 3b). The reduction in CAT activity indicated that CAT may not be involved in antioxidant defence against drought stress in the Hyacinth bean cultivars. The protective action of CAT is limited because it has relatively poor affinity for its substrates and is sensitive to light-induced inactivation compared with other antioxidant enzymes [44]. Also, as CAT has a rapid turnover, conditions inhibiting its synthesis will lower the steady-state level of this enzyme [43]. The increase in hydrogen peroxide and lipid peroxidation, decrease of catalase activity in our findings correlated with findings of Chakraborty and Pradhan [34] on wheat cultivars in response to drought stress. Accumulation of GSH under CAT deficiency has been linked to upregulation of biosynthetic pathways [45]. Work in barley revealed a marked stimulation of sulphate incorporation into organic compounds when catalase was genetically decrease or biosynthetically inhibited [46]. Negligible accumulation of GSH in *Lablab purpureus* cultivars suggested that GSH production is not linked to sulphur sinks. The countervailing levels of CAT and GPOX suggested the efficient balance in antioxidant function of these varieties.

In addition to antioxidant enzymes the role of metabolic enzymes such as amylase and acid phosphatase are important to maintain metabolic homeostasis during stress. Acid phosphatase is known to maintain the level of inorganic phosphate under stress, which can be co-transported with H^+ along a gradient of proton motive force. AP activity increased in both leaf and root tissue during drought stress (Fig 4a & 4b). Leaf tissue of HA4 and GL43 showed higher levels of expression compared to other genotypes. Root tissue of DL28 showed higher AP activity towards the later stages of drought. However the control tissue also had considerable activity. Our observations are in consonance with results of drought stressed pig weed [47]. This stimulation is believed to increase the orthophosphate (Pi) availability. Free soluble Pi plays a vital role in many biological processes including photosynthesis, respiration, enzyme regulation, energy transfer, metabolic regulation and nucleotide phosphorylation [48] and may help the plant to survive longer under stress conditions.

Table I a- Levels of stress markers in leaves and roots of drought stressed *Lablab purpureus* (Hyacinth bean)

Strain		DL4				DL21				DL28			
Time	Parameter	Leaves		Root		Leaves		Root		Leaves		Root	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
2DAS	H_2O_2[a]	16.6±0.29	16.1±0.34	3.45±0.03	8±0.11	18.2±0.68	16.4±.089	11±1.15	8.6±0.46	12.8±0.56	21.1±1.17	9.99±0.51	12.5±0.58
4DAS		21.5±0.49	36.8±1.44	3.86±0.06	4.86±0.08	24.5±2.6	39.6±0.6	5.1±0.13	5.79±0.35	44.5±1.54	38.5±1.16	4.55±0.42	6.76±0.52
6DAS		28.1±1.16	47.6±1.25	9.1±0.14	9.38±0.09	44.4±1.75	32.4±1.75	10.7±0.58	8.1±0.44	31.8±0.72	40.4±1.17	7.59±0.34	4.81±0.09
8DAS		48.7±.087	37.2±1.16	6.62±0.11	2.62±0.11	50.3±0.9	60.5±1.93	3.5±0.269	50.1±3.29	34.2±3.07	46.6±2.01	2.2±0.088	2.76±0.11
2DAS	ASC[a]	97.2±1.25	116.1±1.55	74.25±1.67	120.15±1.3	199.58±1.7	208.5±3.3	70.2±5.87	105.3±1.7	222.75±1.2	274.56±3.5	87.5±1.98	132.3±2.14
4DAS		209.25±0.78	243±3.75	151.48±1.7	175.5±2.3	210.6±2.1	166.05±1.2	79.65±1.5	113.4±1.6	234.5±1.16	261.9±1.74	132.3±2.32	184.95±1.9
6DAS		102.6±1.61	113.4±1.48	62.1±1.3	75.6±1.88	182.25±1.4	230.85±0.96	64.8±1.32	110.7±2.5	147.15±1.1	134.8±0.84	74.25±1.02	86.4±2.35
8DAS		179.55±1.71	284.85±2.3	210.36±1.2	216±1.45	197.1±1.08	355.05±1.04	176.85±2.04	345.6±2.0	315.9±1.19	380.25±0.7	193.05±1.9	226.8±2.51
2DAS	GSH[a]	63.21±0.86	55.86±0.87	62.47±1.18	76.44±1.03	88.93±1.34	76.44±1.6	60.27±0.8	82.32±1.7	54.39±1.25	72.76±1.29	56.59±1.35	78.64±1.43
4DAS		96.29±0.63	63.94±1.24	63.94±1.16	127.15±1.1	82.32±2.35	44.1±1.91	42.36±1.2	96.59±0.9	55.86±1.35	85.26±1.59	63.21±1.34	122.01±1.1
6DAS		352.8±3.85	53.66±1.51	43.56±0.91	54.39±1.17	69.09±2.23	38.22±1.2	7.35±0.8	44.1±1.8	50.71±1.12	57.33±1.33	54.39±1.95	58.8±0.86
8DAS		31.605±0.69	97.02±1.58	41.16±0.74	97.02±1.04	61.005±1.2	38.95±1.6	47.04±1.3	126.4±0.8	34.54±1.18	49.24±0.88	57.26±4.79	139.65±3.1
2DAS	PRO[a]	183.56±0.85	169.05±0.9	71.66±1.21	103.56±1.3	135.99±1.3	180 ±1.15	102.9±1.6	58.8±1.55	439.19±1.2	617.4±1.17	75.35±1.6	218.6±6.44
4DAS		31.99±0.73	60.63±0.99	33.07±1.53	84.52±1.13	371.18±0.8	617.4±0.7	33.0±1.48	62.1±1.68	556.7±1.07	900.9±1.88	9.18±0.48	38.58±1.06
6DAS		82.69±0.99	158.5±1.18	38.58±1.13	161.7±1.75	259.0±1.16	479.5±1.7	18.3±0.99	42.2±1.25	396.9±1.17	586.1±1.07	88.2±1.28	108.4±1.06
8DAS		21.135±0.93	260.7±1.19	22.48±0.52	689.0±3.29	294.1±1.85	313.8±1.1	53.2±1.12	79.0±1.75	834.7±1.14	987.8±1.21	190.5±1.26	231.5±1.03
2DAS	MDA[b]	1.06±0.02	1.63±0.07	1.58±0.12	1.32±0.128	0.027±0.009	0.053±0.002	0.06±0.006	0.061±0.003	1.41±0.01	1.92±0.02	0.91±0.08	1.32±0.09
4DAS		1.25±0.11	2.59±0.11	1.28±0.104	1±0.45	0.085±0.002	0.178±0.002	0.05±0.001	0.042±0.001	1.25±0.02	1.02±0.02	1±0.33	1.16±0.25
6DAS		1.46±0.18	1.44±0.118	1.68±0.115	1.19±0.047	0.166±0.004	0.202±0.002	0.22±0.013	0.161±0.001	1.31±0.023	1.36±0.04	1.27±0.1	1.31±0.18
8DAS		1.47±0.14	3.42±0.112	1.57±0.09	1.81±0.06	0.278±0.003	0.406±0.004	0.18±0.001	0.242±0.002	1.6±0.18	1.32±0.08	1.01±0.1	1.16±0.09

Seedlings of Lablab purpureus genotypes were drought stressed and individual seedlings were analyzed for determination of H_2O_2, ASC, GSH, PRO and MDA. Results are mean ± SD, obtained from three replicates.
[a] µg/g fresh weight tissue.
[b] m moles/g fresh weight tissue.

Table I b - Levels of stress markers in leaves and roots of drought stressed *Lablab purpureus* (Hyacinth bean)

Strain		GL43				HA4			
Time	Parameter	Leaves		Root		Leaves		Root	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress
2DAS	H_2O_2[a]	12.4±1.07	14.1±0.57	2.3±0.08	48±1.45	75.33±1.52	71.67±1.21	31.07±1.07	29.3±1.45
4DAS		11.9±0.47	113.2±1.19	3.1±0.14	51±0.88	86±1.71	112.7±1.84	30.08±1.3	29.87±1.34
6DAS		18.9±1.45	156.1±0.6	2.8±0.05	62±1.45	106.8±1.01	165.4±1.89	30.56±0.72	32.31±1.18
8DAS		23±1.23	270.8±1.17	3.1±0.12	68±1.45	109.73±1.4	194.5±1.22	29.21±0.85	40.63±0.86
2DAS	ASC[a]	17.24±0.98	18.36±1.09	18.32±0.98	19.52±1.12	49.3±0.92	56.63±0.79	37.37±1.19	44.13±1.79
4DAS		21.71±1.18	32.44±1.74	21.05±2.22	15.74±1.64	42.3±1.29	50.3±0.97	34.68±0.59	53.73±1.21
6DAS		25.11±1.04	38.74±1.52	20.11±2.29	15.98±1.64	44.43±1.54	54.29±1.22	58.21±2.34	73.9±2.19
8DAS		26.45±1.52	37±1.18	18.41±1.04	9.98±1.53	42.44±1.98	59.73±2.16	57.59±2.92	80.19±0.9
2DAS	GSH[a]	480.21±2.2	478.54±1.34	30.21±1.1	41.24±1.15	324.3±3.52	331.5±0.58	109.7±0.97	157.3±1.09
4DAS		464.17±1.3	481.54±1.24	31.27±0.89	47.12±0.55	316.5±1.42	376.1±1.64	133.2±1.49	313.3±1.41
6DAS		490.54±1.1	501.21±0.95	34.21±0.6	52.78±1.33	354.7±1.46	446.2±2.01	177.3±0.87	260.4±0.82
8DAS		500.14±1.2	624.11±1.91	37.75±1.25	67.47±1.55	373.4±1.4	461.3±1.41	165.7±1.4	191.8±2.05
2DAS	PRO[a]	482.42±1.2	512.36±1.05	387.64±3.8	401.5±2.86	671.6±1.66	473±1.45	771.8±1.28	576.3±1.55
4DAS		491.7±1.61	521.17±1.73	321.6±1.88	479.1±1.83	586.2±2.88	528.05±2.3	662±1.2	585.9±2.6
6DAS		500.1±2.58	587.39±8.72	394.4±2.25	517.15±1.4	616±1.45	613.01±0.9	703.4±1.7	734±2.64
8DAS		492.03±1.2	618.23±1.25	412.0±1.39	678.44±1.5	601.0±0.46	710.0±1.16	983.3±1.82	1043±3.17
2DAS	MDA[b]	4.6±0.17	5.1±0.11	3.8±0.14	4.1±0.17	5.46±0.14	6.2±0.12	6.48±0.22	10.95±0.08
4DAS		4.9±0.17	5±0.288	3.5±0.17	4.4±0.14	4.598±0.26	5.47±0.21	10.1±0.11	11.56±0.966
6DAS		5.12±0.09	6.5±0.18	3.7±0.11	4.6±0.17	4.412±0.13	5.81±0.177	10.15±0.23	19.47±1.69
8DAS		5.3±0.33	6.8±0.23	3.6±0.17	4.8±0.2	5.29±0.08	6.896±0.17	10.75±0.55	23.28±1.03

Seedlings of Lablab purpureus genotypes were drought stressed and individual seedlings were analyzed for determination of H_2O_2, ASC, GSH, PRO and MDA. Results are mean ± SD, obtained from three replicates.
[a] µg/g fresh weight tissue.
[b] m moles/g fresh weight tissue.

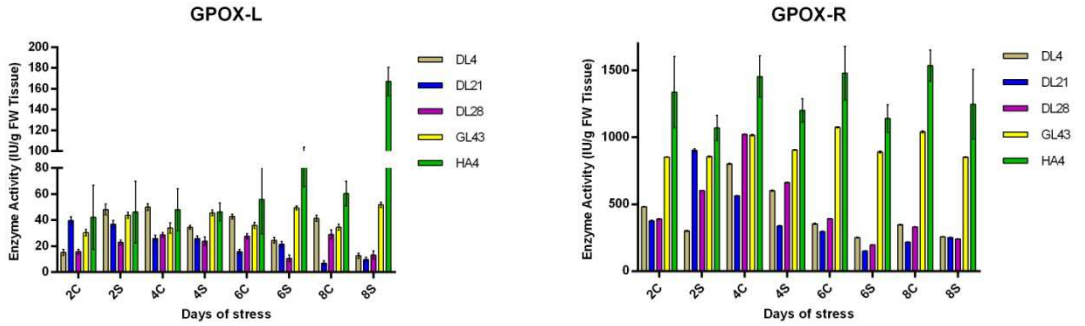

Figure 1. Effect of drought stress on GPOX activity in leaves (L) and roots (R) of *Lablab purpureus* genotypes
Results are mean ±SD (P<0.005), obtained from three replicates

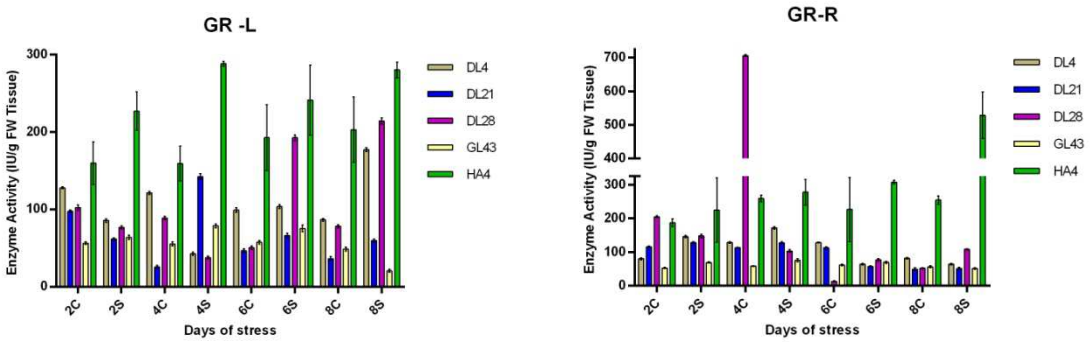

Figure 2. Effect of drought stress on GR activity in leaves (L) and roots (R) of *Lablab purpureus* genotypes
Results are mean ±SD (P<0.005), obtained from three replicates

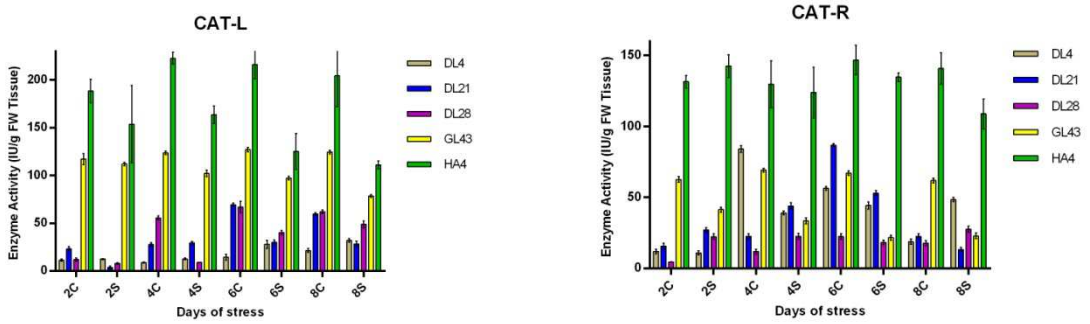

Figure 3. Effect of drought stress on CAT activity in leaves (L) and roots (R) of *Lablab purpureus* genotypes
Results are mean ±SD (P<0.005), obtained from three replicates

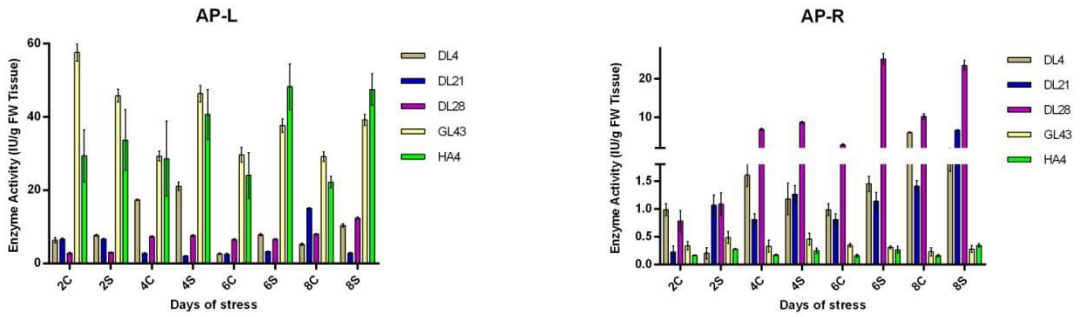

Figure 4. Effect of drought stress on Acid phosphatase activity in leaves (L) and roots (R) of *Lablab purpureus* genotypes
Results are mean ±SD (P<0.005), obtained from three replicates

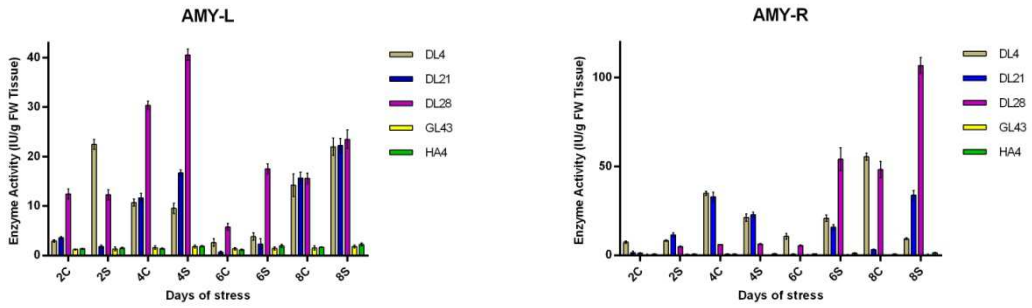

Figure 5. Effect of drought stress on Amylase activity in leaves (L) and roots (R) of *Lablab purpureus* genotypes
Results are mean ±SD (P<0.005), obtained from three replicates

β –amylase is a metabolic enzyme which plays a major role in cell survival. Hydrolysis of starch by β-amylolytic pathway represents the predominant pathway of starch degradation in leaves under normal growth conditions and may also be involved in stress induced starch hydrolysis. β -amylases produce maltose from glucans. In the cytosol, maltose is converted to glucose and, subsequently, fructose and sucrose are formed [49, 50]. Cultivars of *Lablab purpureus* showed variable levels of β –amylase under stress and non stress conditions. DL28 and DL4 showed higher levels of amylase when compared to HA4 and GL43 which had negligible β –amylase activity (Fig 5a & 5b). Increase in amylase activity in response to stress can be related increase in sugars that accumulate in response to stress, can function as osmolytes to maintain cell turgor and have the ability to protect membranes and proteins from stress damage [52, 53].

CONCLUSION

From the data obtained for enzymatic and non enzymatic markers of tolerance to drought stress, it is observed that all five cultivars which are extensively cultivated differ in their ability to respond to drought stress. Of the five chosen cultivars, HA4 and GL43 showed greater tolerance as indicated by the parameters. Thus, these two cultivars have acquired distinct physiological/ biochemical mechanisms to adapt to harsher conditions of drought stress. From the above results, it can be concluded that HA4 and GL43 are ideal cultivars for tropical drought prone regions.

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