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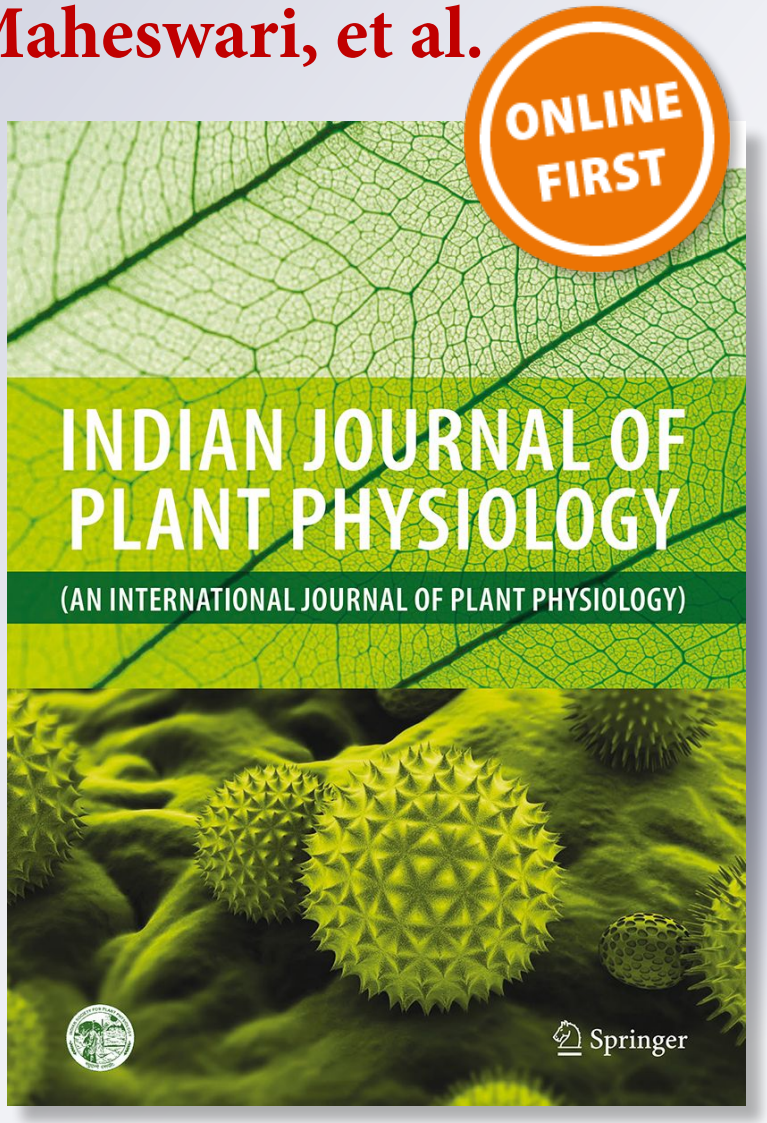

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SHORT COMMUNICATION

In vitro screening of *Vigna mungo* genotypes for PEG induced moisture deficit stress

S. K. Yadav · N. Jyothi Lakshmi · Vikram Singh · Amol Patil · Yogesh Kumar Tiwari · E. Nagendram · P. Sathish · M. Vanaja · M. Maheswari · B. Venkateswarlu

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Abstract Two black gram (*Vigna mungo* L. Hepper) genotypes LBG20 and PU19 were selected to study the impact of PEG induced drought stress on seed germination, metabolite concentration and activities of antioxidant enzymes. Stress caused considerable decrease in germination and fresh weight of seedlings of both the genotypes. It led to increase in protein concentration, contents of starch and total soluble sugars while decrease was observed in the activities of antioxidant enzymes, contents of free amino acids, reducing sugar and total phenols. SDS-PAGE analysis indicated accumulation of some proteins with the germination under stress conditions. LBG20 which showed increase in soluble sugars, starch, proteins and higher activities of antioxidant enzymes was observed to be relatively more tolerant to drought stress over PU19.

Keywords Antioxidant enzymes · Germination · Metabolites · *Vigna mungo*

Environmental stresses adversely affect plants growth, metabolism, and yield. Drought is one of the major environmental stress that affect crop production and sustainability. The severity of drought stress depends on the stage of the crop and duration of stress which affects plant performance (Reddy et al. 2004). Drought severely affects various plant physiological and biochemical parameters. At physiological level, it causes decrease in the water potential, relative water content in leaves, seed germination and amount of chlorophyll, carotenoid, chl a/b ratio. Damage done by drought varies considerably between the species and also within the species. At biochemical level drought causes lipid peroxidation, membrane injury, protein degradation, enzyme inactivation and change in carbohydrate, protein and lipid metabolism. Plants have evolved different strategies to cope up with these damages. Plant generates ROS at stress condition as defense mechanism. The rapid removal of these toxic oxygen radicals is of prime importance in any defense mechanism. Plant synthesizes different non-enzymatic and enzymatic antioxidant systems such as carotenoids, ascorbic acid, α-tocopherol, flavones, anthocyanins, carotenoids, and ascorbic acid, peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DAR) and catalase (CAT) to quench the ROS generated under under stress. A direct relationship of antioxidant enzyme generation and stress tolerance was observed in pigeonpea (Kumar et al. 2011), *Vicia faba* (EL-Tayeb 2006), *Vigna aconitifolia* (Soni et al. 2011) and *Vigna mungo* (Gupta et al. 2009). In the present study, we have examined the effect of PEG induced drought stress on seedling germination and associated physiological and biochemical changes such as antioxidant activity, metabolite concentration and accumulation pattern in two popular black gram (*Vigna mungo* L. Hepper) genotypes LBG20 and PU19.

Twenty seeds of both the cultivars were germinated in petri dishes (110 mm) on two layers of wet filter paper at 37 °C for 3 days under two moisture conditions for each set of observations. Drought stress was imposed by using

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PEG-6000 solution to achieve water deficit level of −0.5 MPa. Other set was irrigated normally which served as control. Samples were drawn, washed with double distilled water and analyzed for different physiological and biochemical parameters at 24 h interval. For estimation of alcohol soluble metabolites, 1.0 g material was homogenized in 80 % ethanol and the clarified supernatant was used for estimation of total soluble sugars, reducing sugars, phenolics and free amino acids and contents were expressed as μg mg^{-1} FW. Total soluble sugars were estimated by the method of Dubois et al. (1956). Reducing sugars were estimated by DNS method (Miller 1972). Free amino acids were determined by ninhydrin method (Colowick and Kaplan 1967). Total phenols were estimated by the method

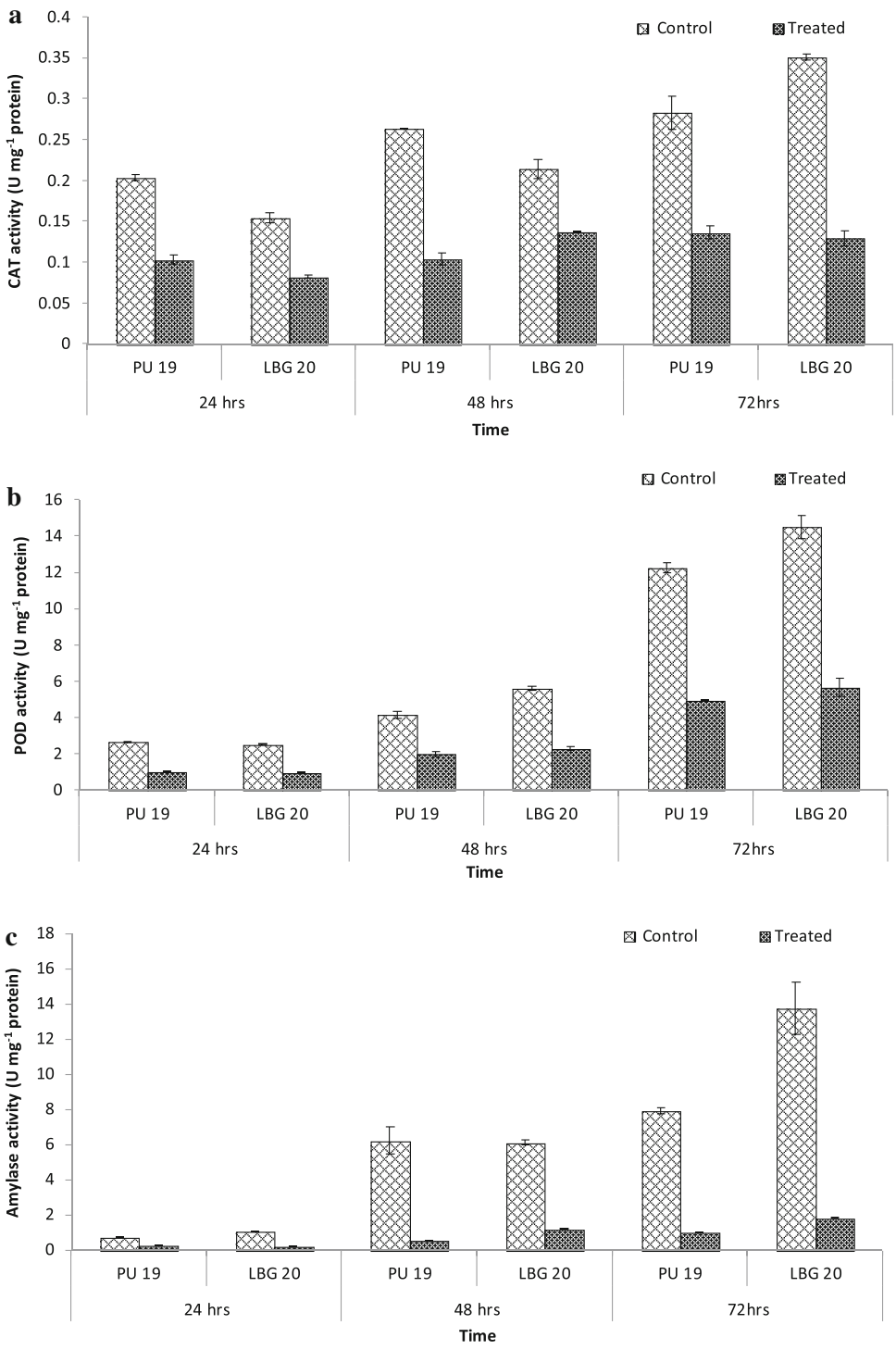


Fig. 1 Effect of PEG induced drought stress on CAT (**a**), POD (**b**) and amylase (**c**) activity of *Vigna mungo* seedlings

of Swain and Hillis (1959). The pellet after extraction with 80 % ethanol, was washed with 80 % ethanol till the washing does not give colour with anthrone reagent and then dried. Water and perchloric acid (52 %) at the ratio of 1:1 was added into pellet and centrifuged. The process was repeated twice and obtained supernatant was used for measurement of starch content. Starch content was determined by anthrone method (Scott and Melvin 1956). Enzyme extract for assay of peroxidase (POD, E.C. 1.11.1.7) and catalase (E.C. 1.11.1.6) was prepared by homogenizing 0.5 g material in 50 mM Tris-buffer (pH 7.5) containing 1 % soluble polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 10,000*g* for 10 min at 4 °C. Catalase activity was measured according to the method of Beers and Sizer (1952), with minor modifications and quantified by its molar extinction coefficient (ε 36 M cm^{-1}). POD activity was determined by Chance and Maehly (1995) method and was quantified by its molar extinction coefficient (ε11.3 mM cm^{-1}). For amylase assay, 0.5 g material was homogenized in 2 M NaCl and centrifuged at 15,000 rpm, 4 °C for 12 min. Amylase was assayed as per DNS method (Miller 1972) and expressed as U mg^{-1} protein. Protein concentration in the enzyme extracts was determined according to Lowry et al. (1951), using bovine serum albumin as a standard. SDS-PAGE analysis was carried out as per Laemmli (1970) method with 15 % separation gel and 5 % stacking gel using pre-stained molecular weight marker (range 20–148 kDa, Gene direx). Proteins were visualized by staining with Coomassie brilliant blue. The experimental data were statistically analyzed using three-way analysis of variance (ANOVA).

The effect of drought stress on enzyme activity showed non-significant difference between the genotypes. PEG induced drought stress caused considerable decrease in germination and fresh weight of seedlings in PU 19 and

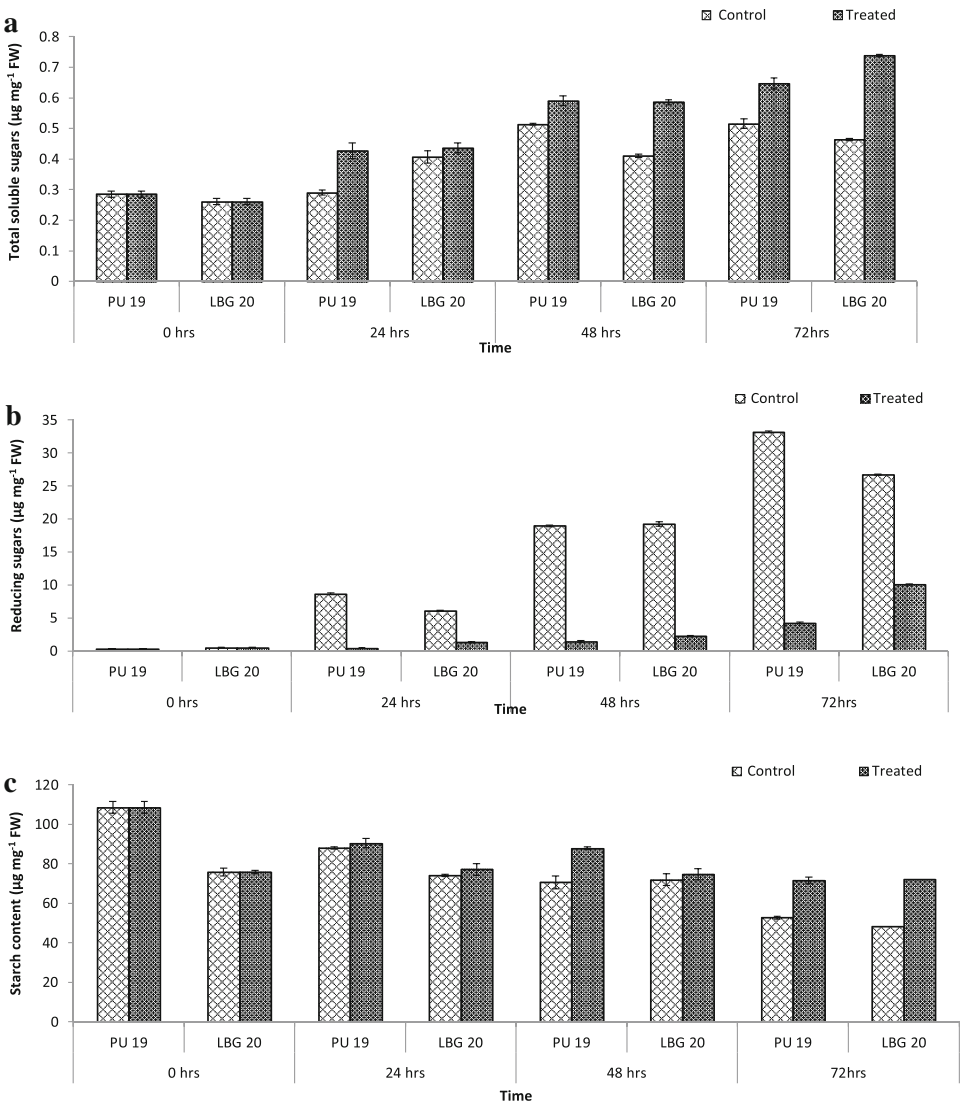
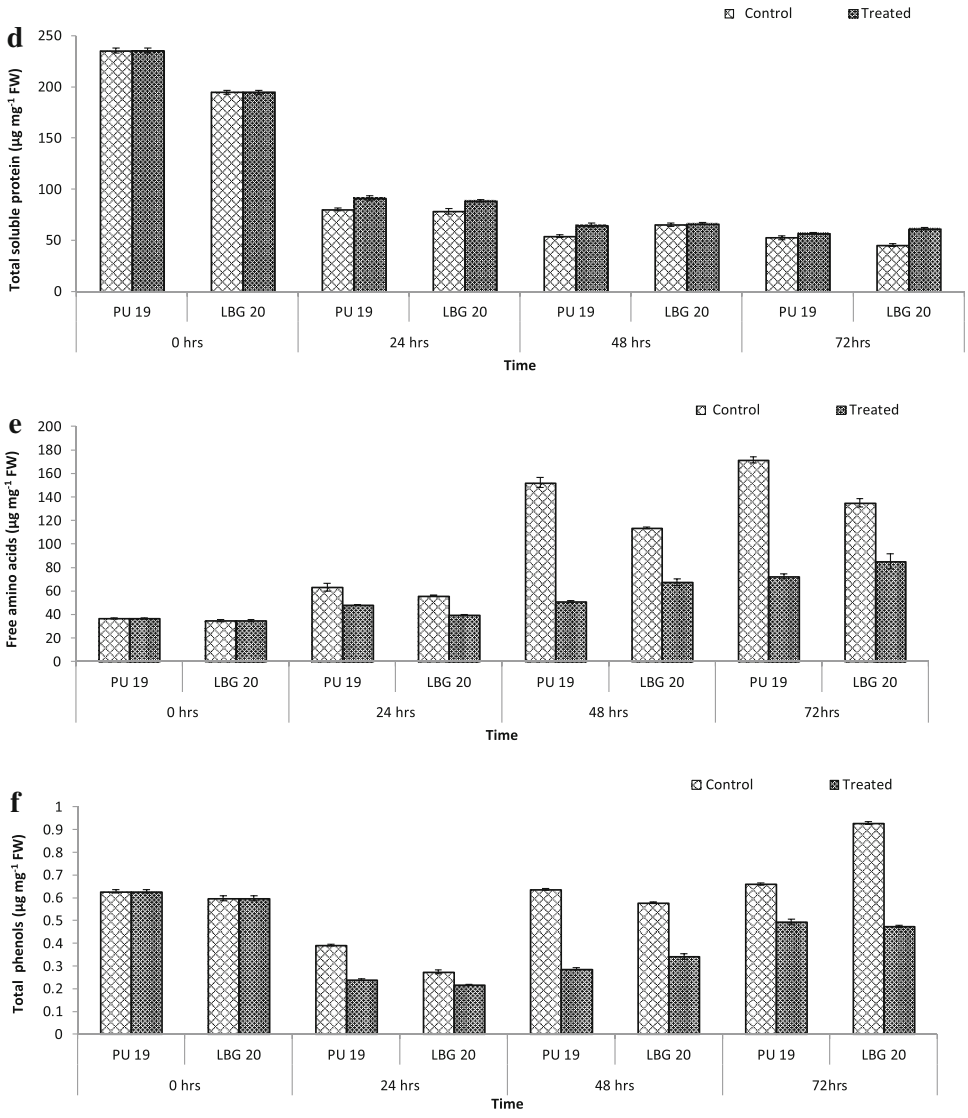


Fig. 2 Effect of PEG induced drought stress on metabolites: total soluble sugars (**a**), reducing sugars (**b**), starch (**c**), total soluble protein (**d**), free amino acids (**e**) and total phenols (**f**) of *Vigna mungo* seedlings

LBG 20 which could be due to stress induced dormancy. Pratap and Sharma (2010) observed PEG mediated decrease in germination, fresh weight and moisture content of black gram seedlings. Catalase activity showed increased trend as the germination progressed but under stress condition it decreased for both the genotypes. Approximately 52 and 63 % decrease in CAT activity was observed for PU19 and LBG20 of 72 h old stressed seedlings (Fig. 1a). The POD activity also responded significantly to water stress. The POD activity was high in controlled seedlings as compared with seedlings exposed to water stress. PEG decreased the POD activity by 60 % in PU19 and 61 % in LBG20 after 72 h soaking (Fig. 1b). POD and CAT activities steadily increased in LBG 20 and became higher at 72 h under control and could have a role in imparting enhanced drought tolerance over PU 19. Similar findings have been noticed in *P. acutifolius* and *P. vulgaris* under glycol mediated water stress by Turkan et al. (2005). The amylase activity in seedlings under the influence of PEG decreased approximately by 87 % compared to control after 72 h soaking for both the genotypes (Fig. 1c). This could lead to decline in mobilization of reserved materials in the treated plants under stress conditions compared with control seedlings. The accumulation of sugars either actively or passively is an important adaptation mechanism for plants in response to osmotic stress like water deficit (Hoekstra et al. 2001). The increase in content of total soluble sugars under PEG induced stress in both the genotypes is in conformity with above findings.

Imposition of moisture stress led to significant changes in metabolite content in both the genotypes (Fig. 2a–f). Total soluble sugar content increased by ($*P < 0.05$) 25 % (PU 19) and 49 % (LBG20) under stress as compared to respective control after 72 h germination (Fig. 2a). Higher accumulation of total soluble sugars in LBG 20 over PU 19 might result in enhanced drought

Fig. 2 continued



tolerance. Mohammadkhani and Heidari (2008) observed similar trend in maize varieties. The reducing sugar content in germinating seedlings also showed increasing trend with the germination time. High concentration of reducing sugars was observed under control conditions as compared to stress. PEG decreased the reducing sugar content by 87 % in PU19 and 63 % ($^{**}P < 0.01$) in LBG20 after 72 h of germination (Fig. 2b). Starch content was found to be higher in stressed plants of both genotypes. It increased by 35 % in PU19 and 50 % in LBG20 as compared to their respective controls at 72 h after germination (Fig. 2c). It could be due to starch mobilization getting affected due to PEG induced drought stress. Though a significant increase in protein concentration was observed under drought stress over irrigated control, a steady decrease in protein concentration for both LBG20 and PU19 ($^{**}P < 0.01$) was observed with the progress of germination. The amount of protein was slightly higher in LBG20 as compared with PU19 at 72 h (Fig. 2d). Protein being key towards osmotic balance under drought stress and is generally higher in stress tolerant plants (Huang et al. 2000). High levels of protein could enable the plant to maintain low water potential. Higher protein in LBG 20 under stress condition might impart better drought tolerance. Drought induced decrease in the free amino acid content in both genotypes while their content increased with the germination time. After 72 h of germination under stress conditions, ~58 and 37 % decrease in levels of free amino acid was observed in PU19 and LBG 20 respectively, ($^{**}P < 0.01$) (Fig. 2e). Higher free amino acid levels in LBG 20 could have provided better osmoregulation, which can stabilize antioxidative enzymes resulting in higher protection under stress condition. Grag et al. (2001) observed higher content of free amino acids under water stress leading to better drought tolerance in moth bean. The initial total phenols concentration was less in both the genotypes, however, an increase was observed with the progress of germination. Stress condition led to decrease in total phenols concentration by ($^{*}P < 0.05$) 25 % in PU19 and 49 % in LBG20 at 72 h germination (Fig. 2f). Increased phenols have been observed to be associated with drought tolerance in *V. radiata* and *Glycine max* (Fernandez-Orozco et al. 2008).

The SDS-PAGE analysis for both *Vigna mungo* genotypes showed approximately similar banding pattern (Fig. 3). The accumulation of proteins in the range of ~170–190 kDa was recorded under drought stress condition for both the genotypes. Disappearance of some of the proteins in the range of ~34–154 kDa was also observed with the germination for both controlled and stressed condition. Interestingly, a 22 kDa (approx.) protein was found to be expressing only under drought stress condition for both the genotypes indicates its possible involvement in drought stress tolerance. Soni et al. (2011) have reported a expression of 22 kDa protein under PEG induced drought stress in *Vigna aconitifolia*.

In conclusion, based on the results observed in the present study black gram genotype LBG20 was found to be relatively

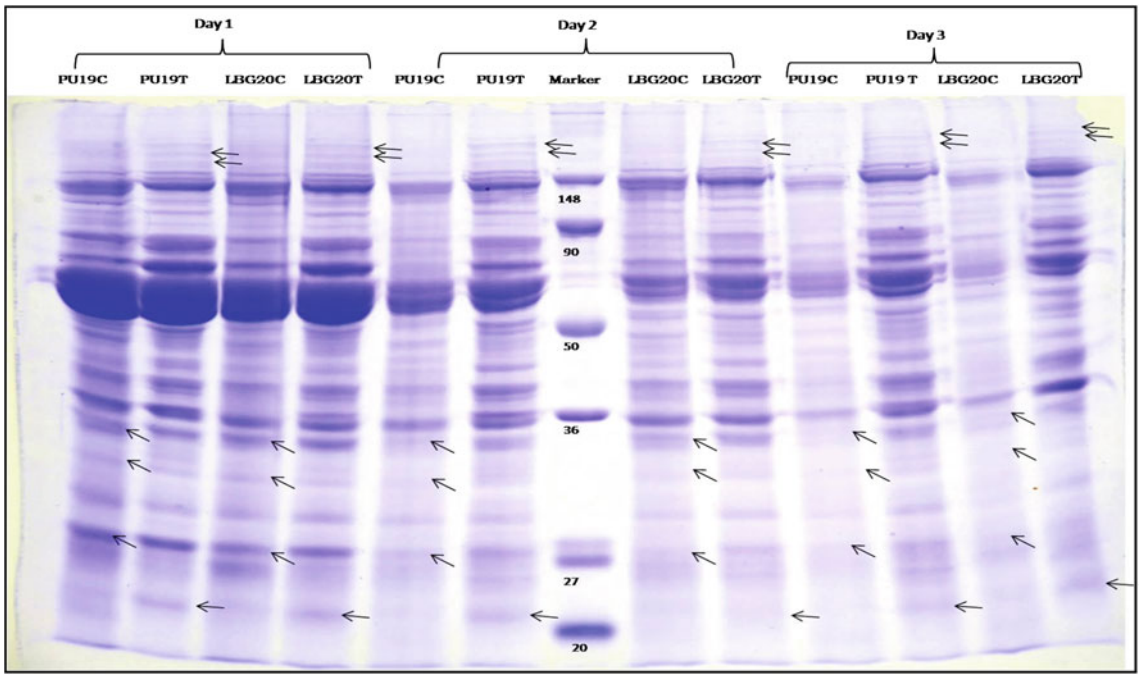


Fig. 3 SDS-PAGE analysis of *Vigna mungo* genotypes. *Straight arrow* represents new protein, *Tilted arrow* represents repressed protein *C* control seedling, *T* treated seedling

more tolerant to drought stress than PU19. Better tolerance to drought of LBG20 could be related to its higher activity of antioxidant enzymes such as CAT and POD, resulting in lower ROS production and lipid peroxidation and higher membrane stability. Higher total soluble sugars and free amino acids might be playing a role to stabilize antioxidant enzymes such as CAT and POD, resulting in their higher activity in LBG20 under drought stress. The study concludes that genotypes possessing higher antioxidant activity and osmolyte accumulation might be useful in improving the adaptive responses of water stress.

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