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RESEARCH COMMUNICATION

Biochemical response of three *Vigna mungo* varieties (T9, RBU38 and VM4) under drought stress

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Abstract

Plants apply several strategies that are developed during their evolution and artificial domestication to overcome biotic and abiotic stresses. Among eminent environmental threats drought stress is a major factor that affects plants at physiological, biochemical and molecular level. Blackgram (*Vigna mungo*) is an important pulse crop but its productivity is adversely affected by drought. In the present work, different cultivars of blackgram *i.e.* T9, RBU38 and VM4 are taken to find out the effects of drought stress by the estimation of different biochemical parameters to better understand biochemical pathway modulations under stress and its possible mitigation. Damage to photosynthetic machinery as evident by decrease in chlorophyll content and loss of membrane integrity in the plants under drought stress. The adverse effects of drought on the plants were averted to a certain extent in RBU38 by activation of defence signalling through H_2O_2 at lower concentration, which proved damaging at high concentration for T9 and VM4 and a concurrent increase in proline content which may provide protection against oxidative stress. This study suggests that drought modulated biochemical parameters can be used as reliable indices for selection of genotypes with a better stress tolerance.

Keywords: *Vigna mungo*; drought stress; ROS

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Introduction

India is a developing country where a large population is vegetarian and food legumes, mainly the grains or pulses are an important constituent of the diet. Pulses are cheap and have a high nutrient content including starch, protein dietary fiber, oligosaccharides, phytochemicals and minerals (Borade *et al*., 1984). *Vigna mungo* (L.) Hepper, commonly known as blackgram, contributes 20% to overall world pulse production (Saravanakumar *et al*., 2007). In India it is the third important pulse crop (Arulbalachandran *et al*., 2010). However, production of blackgram is adversely affected by various environmental stress factors, especially drought that reduce yield (Souframanien and Gopalakrishna, 2006; Kundu *et al*., 2011; Pandey *et al*., 2014). Various strategies as drought escape, drought avoidance and drought tolerance are used by plants to complete their life cycle under stress. Plant responses are complex and diverse as they adapt to drought stress as evident in an immediate change in the phosphorylation status of a protein within minutes followed by modulation in gene expression. The response varies according to species and genotype of plants, length and severity of drought, age and stage of development of plants, organ and cell type (Bray, 1997; Alpert and Oliver, 2002; Walters *et al*., 2002). Plants try to restore the osmotic as well as ionic equilibrium of the cell (cellular homeostasis) and control the damages *via* decrease in the rate of ROS generation, accelerating ROS scavenging and increasing the recovery of damaged cellular components (Wang *et al*., 2002).

The aim of the present study is to screen different varieties of *Vigna mungo viz*., T9, RBU38 and VM4 at early growth stage for biochemical response to short-term drought condition. T9 is susceptible to MYMIV (Mungbean Yellow Mosaic India Virus) while VM4 is MYMIV-resistant; the characteristics of these varieties *vis-à-vis* water deficit are not known. RBU 38 is a widely cultivated variety in semi-arid regions of Rajasthan and is known for its drought tolerant quality. As VM4 is resistance to MYMIV,

its response to drought would enable to know its adaptability to drought stress for possible introduction.

Materials and Methods

Plant material and procedure

Three varieties of blackgram (T9, RBU38 and VM4) were used for the present work. Seeds of RBU-38 variety were obtained from Krishi Vigyan Kendra (KVK), Banasthali, Rajasthan. Seeds of T9 and VM4 varieties were obtained from Division of Plant Biology, Bose Institute, Kolkata, India.

Mature seeds of each variety were surface sterilized with 0.1% mercuric chloride (w/v) for 5min, washed thoroughly with sterile distilled water and soaked in distilled water to germinate in plant growth chamber at 28 ±2°C under a 16h photoperiod. The germinated seedlings were planted in plastic pots containing soil bed moistened with distilled water. The soil was obtained from the experimental plot of KVK, Banasthali. The soil properties are as follows: Soil contained nitrogen 220 $Kgha^{-1}$, phosphorus 14.50 $Kgha^{-1}$, potassium 240 $Kgha^{-1}$, sulphur 12.5 $Kgha^{-1}$, iron 24.5 $mgkg^{-1}$, copper 4.60 $mgkg^{-1}$, manganese 12.20 $mgkg^{-1}$, zinc 4.00 $mgkg^{-1}$, electrical conductivity 1.40, bulk density 1.68 mgm^{-3}, organic carbon 3.50 gkg^{-1} and pH 7.90 (data obtained from KVK).

Three week old plants (21 DAS) were used for the experiments. Plants were divided into two sets each with 12-15 replicate plants: set 1- healthy control plants which were waters regularly and set 2- water withhold from 24DAS to 27 DAS. All the biochemical parameters are studied after 3 days of short-term drought stress (27DAS) and 24hr after recovery (28DAS).

Chlorophyll content

Chlorophyll a, chlorophyll b and total chlorophyll content was determined using the method of Arnon *et al*., 1949. Fresh leaves (0.1g) were freeze dried in liquid nitrogen and homogenized in 2ml of chilled 80% (v/v) aqueous acetone and centrifuged at 10000g for 10 min at 4°C. The absorbance of the supernatant was measured at three wavelengths of 645, 652 and 663nm by using a spectrophotometer (Systronics – 2202).

Hydrogen peroxide (H_2O_2) content

H_2O_2 content was determined following the method of Alexieva *et al*. (2001) and calculated using the extinction coefficient (E=0.28 µmol cm^{-1}).

Total phenolic content

The method of Singleton *et al*. (1999) as modified by Chakraborty *et al*. (2008) was used to determine the total phenolic content and expressed as gallic acid equivalent (GAE).

Proline content

Proline content was determined by following the method of Bates *et al*. (1973).

Table 1: Effect of drought stress on chlorophyll contents (mg g^{-1} fw) of *V. mungo* leaves (*cv*. T9, RBU38 and VM4).

Treatments	Chl a	Chl b	Total Chl
	mg/g fw*	mg/g fw*	mg/g fw*
T9			
21DAS	0.96 ± 0.018^{d}	0.34 ± 0.006^{de}	1.29 ± 0.023^{e}
27DAS (D)	0.41 ± 0.009^{a}	0.34 ± 0.005^{de}	0.75 ± 0.015^{a}
28DAS (R)	0.51 ± 0.010^{b}	0.33 ± 0.001^{d}	0.84 ± 0.011^{b}
RBU38			
21DAS	1.43 ± 0.128^{e}	0.42 ± 0.032^{f}	1.85 ± 0.099^{g}
27DAS (D)	1.36 ± 0.065^{e}	0.36 ± 0.030^{e}	1.72 ± 0.041^{f}
28DAS (R)	1.40 ± 0.031^{e}	0.42 ± 0.008^{f}	1.82 ± 0.024^{g}
VM4			
21DAS	1.03 ± 0.003^{d}	0.26 ± 0.006^{c}	1.29 ± 0.008^{e}
27DAS (D)	0.77 ± 0.022^{c}	0.14 ± 0.014^{a}	0.92 ± 0.035^{c}
28DAS (R)	0.82 ± 0.007^{c}	0.20 ± 0.016^{b}	1.02 ± 0.022^{d}

* Values are mean±standard deviation (SD); data followed by same alphabets are not significantly different at p≤0.05 according to ANOVA and DMRT for each column. DAS: days after sowing, D: 3 days under drought stress, R, recovery.

Lipid peroxidation

Lipid peroxidation was determined following the method of De Vos *et al*. (1989) and calculated by using extinction coefficient (155 mM^{-1} cm^{-1}).

Statistical analyses

Analysis of Variance (ANOVA) - Duncan multiple range test (DMRT) is used to find out the significant differences between means ($p<0.05$) using SPSS software (16.0 SPSS Inc.).

Results and Discussion

The cultivars of blackgram *i.e.* T9, RBU38 and VM4 that were subjected to short-term drought stress of three days showed a rapid loss in chlorophyll a and total chlorophyll content (Table 1). Chlorophyll content of RBU38 plants

was least affected when compared to plants of T9 and VM4. Similar decrease in chlorophyll content also observed by Baisak *et al*. (1994), Alam *et al*. (2013) and Pandey *et al*. (2014) under drought stress. On recovery, chlorophyll content increased in all varieties of blackgram but was less than that of respective control plants. All photosynthetic components were damaged under stress conditions *i.e.* ETC of thylakoid, carbon reduction cycle and tissue (Apel and Heribert, 2004; Quan *et al*., 2008) it is harmful for plants and sometimes lead to cell death (Trippi *et al*., 1989; Smirnoff, 1993). At lower concentration it acts as signal molecule against stress conditions and work for plant defence mechanisms and at higher concentration or its long persistence can damage the plant cells. As RBU-38 plants are tolerant to drought as compared to T9 and VM4, lower H_2O_2 content was observed whereas, in T9 and VM-4

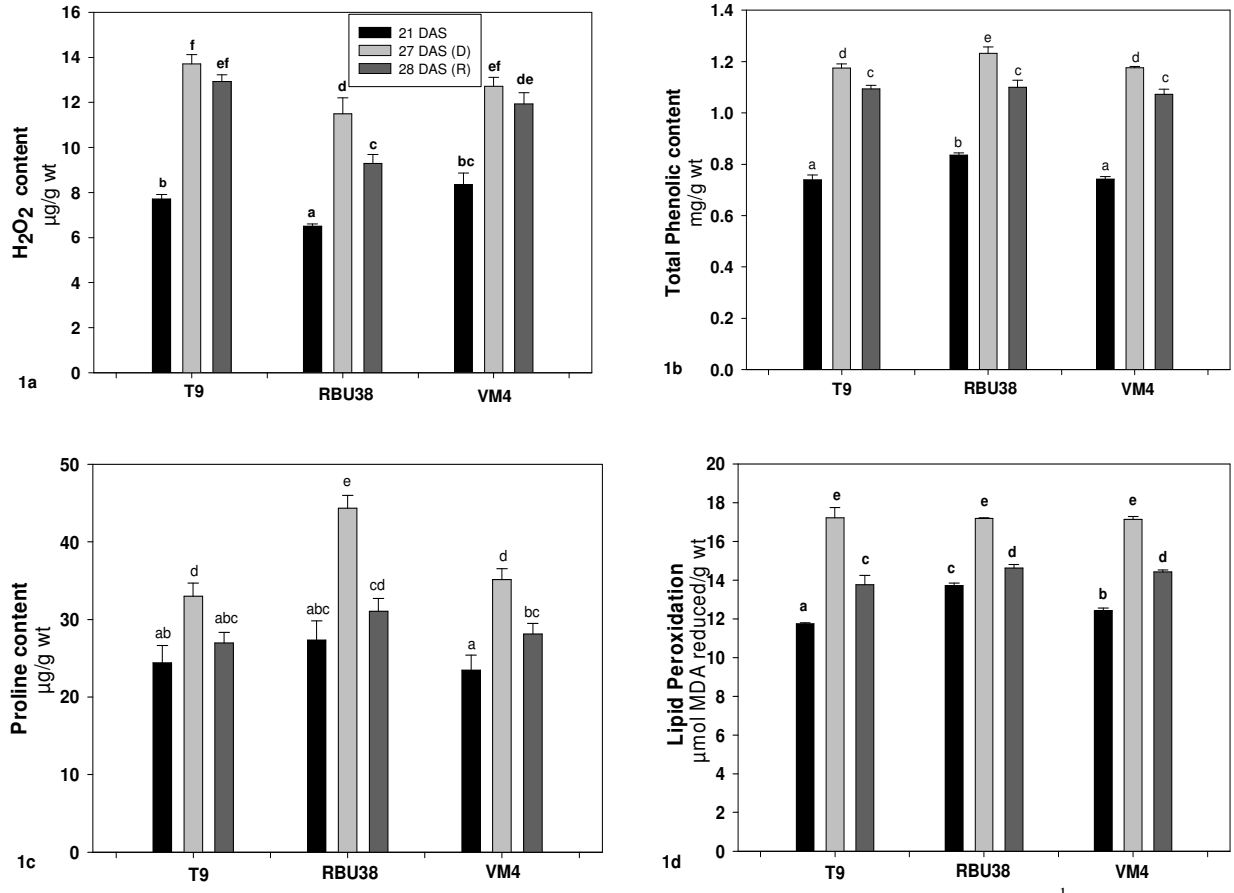

Fig. 1. Effect of drought stress on biochemical parameters of *V. mungo* leaves. a: H_2O_2 content (µg g^{-1}wt.); b: total phenolic content (mg g^{-1} wt.); c: Proline content (µg g^{-1}wt.); lipid peroxidation (µmol MDA reduced g^{-1} wt.)
Bars are mean ± SD; bars followed by same alphabets are not significantly different at p≤0.05 according to ANOVA and DMRT. DAS: days after sowing, D: 3 days under drought stress, R, recovery.

stomatal regulation of CO_2, together with an increased accumulation of carbohydrates, lipid peroxidation (LP) and disturbance of water balance (Allen and Ort, 2001). In plants tolerant to drought like RBU38, the photosynthetic machinery may be protected from damage mainly by biochemical modulation specifically the antioxidant mechanism as noted in the subsequent results.

In the present study higher level of H_2O_2 content was observed in T9 plants at 27 DAS (D) and 28 DAS (R) among all three cultivars (Fig. 1a). He *et al*. (2011) observed higher level of H_2O_2 in sensitive genotypes when compared with tolerant genotypes in wheat. Although, H_2O_2 is an important indicator and signalling molecule for stressed plants higher H_2O_2 content contributed to oxidative stress which was aggravated under drought.

All plants of blackgram showed an increase in total phenolics at 27 DAS (D) and on recovery, a decrease was observed but remained higher than that of the control plants (Fig. 1b). Least increase in total phenolics was observed in RBU38 plants while T9 and VM4 plants showed almost same pattern under short-term drought stress as well as on recovery. Phenolic compounds provide protection to plants to biotic and abiotic stress (Chakraborty *et al*., 2008; Mandal *et al*., 2010; Jha *et al*., 2013; Pandey *et al*., 2014).

Increased proline content was observed for all three varieties against three days of short-term drought stress and higher accumulation was observed in RBU38 plants when compared with T9 and VM4 plants (Fig. 1c). After recovery, decreased proline content was observed but it remained higher for each variety when compared with the control plants of the same variety. Studies of plants under drought stress indicate proline accumulation as a protective behaviour (Alam *et al*., 2010; Keyvan, 2010; Yazdanpanah *et al*., 2011; Alam *et al*., 2013). Turkan *et al*. (2005) observed higher proline content in drought tolerant plants in comparison to sensitive plants. Higher accumulation of proline in RBU38 plants is indicative of its drought tolerant property and may be responsible for limited damage to the photosynthetic machinery and preserve membrane integrity.

Production of ROS under drought stress damages the structure of protein, DNA and lipids (Apel and Heribert, 2004) estimated in terms of malondialdehyde (MDA; volatile aldehyde). All plants of blackgram showed an increase in MDA content at 27 DAS *i.e.* under drought stress, the least being in RBU38 and highest in T9. On recovery decrease was observed but content was more than that of relative control plants (Fig. 1d), which indicates that the values may eventually decrease to the levels of the control. Least lipid peroxidation was observed in RBU38 plants among all blackgram varieties under drought stress as well as on recovery. Higher level of lipid peroxidation is observed in drought sensitive plants when compared to tolerant plants (Turkan *et al*., 2005).

Conclusion

The present study shows that the performance of RBU38 plants under short-term drought stress is better in comparison to T9 and VM4 plants. The biochemical parameters studied indicate a specific pattern in the RBU8 which may be correlated with its drought tolerant property *viz*., least reduction in chlorophyll content and lipid peroxidation. Preservation of the photosynthetic machinery as well membrane integrity, which faces the maximum and the most visible damage under drought stress mainly due to the activation of antioxidant machinery in RBU38 can be attributed to its drought tolerant character. This is evident by the rapid increase in proline content in the RBU38 plants which plays a protective role. Similarly, a modulation in the H_2O_2 content, which is generally implicated to increased oxidative damage to cell, is also noted. It is to be noted that despite causing oxidative stress to plants, H_2O_2 at physiologically permissible concentration is considered to be a vital signalling molecule that initiates several protective molecular mechanisms in the cell and may play an important part in the drought tolerant character of RBU38. These parameters may be utilised to screen potential varieties and incorporated in plant breeding experiments to increase the performance of legume plants in general and blackgram in particular in terms of yield under stress conditions.

Competing interests

The authors declare that they have no competing interests.

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