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Physiological, biochemical, and molecular responses of thermotolerance in moth bean (*Vigna aconitifolia* (Jacq.) Marechal)

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Abstract: Among several abiotic stresses, heat stress has a large negative impact upon agricultural productivity worldwide. Moth bean (*Vigna aconitifolia* (Jacq.) Marechal) attracts a great deal of attention due to its better performance under drought and heat stress conditions. An experiment was designed to find out the effects of heat stress on relative water content (RWC), membrane stability index (MSI), and proline, protein, and chlorophyll (Chl) contents using 10 genotypes of moth bean. Physiological and biochemical parameters indicated that RMO 40 followed by Jadia, IC 36157, Jwala, and Marumoth were heat-tolerant (HT) genotypes, and IC 121051, IC 36392, IC 39702, IC 472257, and IC 140725 were heat-susceptible (HS) genotypes of moth bean. Under heat stress, the HT genotypes showed minimal reduction in RWC, MSI, and protein as compared to the susceptible ones, revealing that the HT genotypes performed well under heat stress. Many heat shock proteins and dehydration proteins are synthesized in plants under heat stress. Expression analysis of three such genes was performed in ten genotypes of moth bean. The analysis revealed that the HT genotypes showed better performance under stress conditions for survival. The above studied parameters would be very useful in the identification of HT varieties for future breeding programs in moth bean and related *Vigna* species.

Keywords: Heat stress, membrane stability index, morphophysiological traits, relative water content

1. Introduction

Abiotic stresses such as heat, cold, moisture, and salinity are the most important environmental stresses that severely affect crop production in many areas of the world. All kinds of abiotic stresses are often interrelated and they cause morphological, physiological, biochemical, and molecular changes that negatively affect plant productivity and yield (Naya et al., 2007). Global warming predictions have suggested that temperatures would increase by another 2–6 °C by the end of this century (Peck and Teisberg, 1992). Heat stress is one of the most severe abiotic stresses that cause oxidative stress and generate reactive oxygen species that damage the cell membrane of the plants. Temperature plays a crucial role in plant growth, development, and yield (Mitter et al., 2012). Therefore, it is necessary to understand how plants adapt to extreme conditions. It is important to select stress-tolerant varieties that will be useful in the agricultural industry (Mahajan and Tuteja, 2005).

Vigna aconitifolia, commonly known as moth bean or matki bean, belongs to Fabaceae, the third largest plant family. Moth bean is one of the most important crops among all the pulse crops of the world (Chopra and Swamy, 1975). It has a high level of tolerance to drought and heat among all Asian *Vigna* species. It can survive at temperatures up to 40–45 °C in its harsh natural habitat as it has evolved a few morphological and physiological features. It is a herbaceous, short-day crop having a deep and penetrating root system. Moth bean is an important source of proteins, minerals, and vitamins and is also used in medicines and cosmetics. Therefore, it has been identified as one of the best food sources for the future. Moth bean, with its high tolerance to heat and drought, could be an excellent source of genes responsible for stress tolerance. There are no direct advanced technologies available that facilitate the crop production under extreme conditions. However, the development of stress-tolerant varieties and their inclusion in crop improvement and breeding programs might be an optimistic approach. The genotypes RMO 40 and Jadia were considered as heat-tolerant in earlier studies (Gurjar et al., 2014; Sharma et al., 2014; Harsh et al., 2016), although RMO 40 was reported to be drought susceptible by Sachdeva et al. (2016). Therefore, the genotypes with improved resistance

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and better performance under particular stress conditions need to be selected and studied for potentially important traits for use in breeding programs of moth bean. With respect to earlier reports, the present study was planned to identify heat-tolerant genotypes of moth bean on the basis of the physiological and biochemical adaptive approach, and expression analysis of heat stress genes was studied using qPCR.

2. Materials and methods

2.1. Plant materials

The seeds of Jwala, Jadia, Marumoth, RMO 40, IC 121051, IC 36392, IC 39702, IC 472257, IC 140725, and IC 36157 were sown in 3 replicates in a vermiculite and sand mixture, in small pots inside a growth chamber maintained at 25 °C under 16 h light and 8 h darkness during 2016 at the National Bureau of Plant Genetic Resources, New Delhi. The experiment was designed with 3 replications for each genotype. Twenty-day-old plants with similar size were selected and divided into 2 sets. One set was kept in the oven (at 45 °C for 1 h) to induce heat stress, and the other set was used as a control for physiobiochemical parameters and for expression study. This temperature was decided with some modification based on a previous report on moth bean (Sharma et al., 2014). All the physiobiochemical and expression experiments were performed in 3 replications. Fresh leaves were used for determination of relative water content (RWC), membrane stability index (MSI), and proline, protein, and chlorophyll contents.

2.2. Determination of the physiobiochemical characteristics of plants

For the estimation of RWC (%), 100 mg of leaf samples in replicates were incubated in distilled water for 4 h at room temperature, and their turgid weights were recorded after an incubation period. Thereafter, the leaf samples were kept in an incubator at 60 °C for 72 h to determine their dry weight. The leaf RWC% was calculated by using the following formula (Gulen and Eris, 2003):

$$RWC\ (\%) = \{(FW - DW) / TW - DW)\} \times 100$$

(FW - Fresh weight, DW - Dry weight, TW - Turgid weight)

The MSI was assessed by measuring the electrical conductivity of the leaf samples by conductivity meter. The readings were taken after incubation of the leaf samples in double-distilled water at 40 °C for 30 min (C1) and thereafter at 50 °C for 15 min (C2) (Lutts et al., 1996). MSI was calculated as:

$$\text{Membrane stability index} = 1 - (C1/C2) \times 100$$

For proline estimation, the leaf samples were crushed in 3% sulfosalicylic acid and centrifuged at 5000 × *g* at 4 °C for 10 min followed by addition of 2 mL of ninhydrin and glacial acetic acid. After that samples were heated at 100 °C and the supernatant was used for proline estimation using a spectrophotometer (Bates et al., 1973). For determination of proteins, the leaf samples were homogenized in 50 mM phosphate buffer (pH 7.8) and centrifuged for 10 min at 15,000 rpm. The supernatant was separated and used for protein estimation (Bradford, 1976). For chlorophyll (total Chl) determination, a SPAD-502 meter (SPAD-502, Soil Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan) was used. It is a handheld device that is widely used for the rapid, accurate quantification of leaf chlorophyll content (Martinez and Guiamet, 2004).

2.3. Total RNA isolation, cDNA synthesis, and primer design

Leaves were simultaneously collected at the shoot apex from each control and stressed plant and were fixed in liquid nitrogen and stored at -80 °C for RNA extraction. The total RNA was isolated from control and stressed leaf samples in 2 technical replicates using the RNeasy Mini Kit (QIAGEN, USA) according to the manufacturer's protocol. First-strand cDNA was synthesized with 1 ng of the total RNA using a cDNA reverse transcription kit (Invitrogen, USA) according to the manufacturer's instructions. The Batch Primer3 program was used to design primers (Table 1) for stress-responsive genes from the transcriptome data of moth bean (unpublished).

2.4. Quantitative real-time polymerase chain reaction

Quantitative real-time PCR (qPCR) was used to identify the expression patterns of the three selected putative annotated stress-induced genes, including heat shock protein (*HSP*), dehydration responsive protein (*DRP*), and DNA-3-methyladenine glycosylase 1 (*Tag1*) in control and stressed samples. Each qPCR reaction was conducted in triplicate (three technical replicates). The housekeeping gene *actin* was selected as an endogenous control. Real-time PCR was performed in a LightCycler 480 (Roche, Switzerland) using SYBR Green qPCR Master Mix (KAPA SYBR FAST qPCR Kit Master Mix (2X) Universal). The qPCR program consisted of an initial step at 95 °C for 3 min to activate the Taq DNA polymerase, followed by 40 cycles of 95 °C for 30 s, 48 °C for 60 s, and 72 °C for 20 s, and a final melting curve analysis was performed. The relative expression of all genes was calculated by $_{\Delta\Delta}$CT method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

A two-way ANOVA between the ten moth bean genotypes and treatments was conducted to compare the effect of temperature on RWC, MSI, proline, protein, and chlorophyll. All effects were statistically significant at the 0.01 significance level (Table 2). All data are presented as means ± SE. The experiments were conducted in a randomized block design with three replications. The correlation analysis was carried out with GeneSTAT software, and PAST software was used for cluster analysis (Hammer et al., 2011).

Table 1. Primer sequences used for the qPCR validation of target genes in ten moth bean genotypes under control and heat stress conditions.

Transcript id	Primer's name	Tm (°C)	Sequence (5'-3')
trans_s_359	HSP_F	48	GAAGGTAATGGATGTGTCAT
	HSP_R	48	GAAGAGGAATTTGAAGCTG
trans_s_2705	TagI_F	48	ACACTCCTTAGCGGATTT
	TagI_R	48	CTCGACCTATTCTCAAAACT
trans_s_237	DRP_F	48	CCCATTATACAAAAGGTACG
	DRP_R	48	GACAAGAGTGGGTGATAGTC
Actin	Actin_F	48	GAAGTATCCTATTGAGCATGGC
	Actin_R	48	ACCCTTCATATATGGGCACC

Table 2. Combined analysis of the variance (F-value) of moth bean genotypes for relative water content (RWC), membrane stability index (MSI), proline, protein, and chlorophyll contents.

Source of variance	DF	RWC	MSI	Proline	Protein	CHL
Genotype	9	922.049**	2311.896**	6.928**	2.276*	583.984**
Treatment	1	1187.727**	1072.421**	87.897**	72.439**	486.274**
Genotype × treatment	9	51.441**	26.108**	4.485**	0.570^{ns}	30.461**

^{ns}: nonsignificant; *: $P < 0.05$; **: $P < 0.01$; DF: degrees of freedom.

3. Results

3.1. Effect of heat stress on RWC

In the present experiment, all the genotypes showed significant reduction in RWC under heat stress (Figure 1). The highest RWC was recorded in RMO 40 (83.11%), followed by Jadia, Jwala, and IC 36157 (75.12%, 72.92%, 68.56%), while the lowest RWC was noted in genotypes IC 36392 and IC 140725 (21.76%, 32.97% respectively), and Marumoth showed no significant reduction under stress condition.

3.2. Effect of heat stress on MSI

The MSI correlates with heat tolerance. After heat exposure, the least reduction in MSI was observed in RMO 40 (4.35%), followed by IC 36157 (6.16%), Jadia (6.71%), Jwala (12.20%), and Marumoth (13.49%), while the rest of the genotypes showed a higher reduction in MSI as compared to the control plants (Figure 1).

3.3. Effect of heat stress on proline, protein, and chlorophyll content

The percentage of increase in the proline content was significantly higher for RMO 40 (89.15%), Jadia (73.81%), IC 36157 (70.17%), and Marumoth (68.16%) under heat stress as compared to the other genotypes, while lower differences were found for genotypes IC 140725 (20.17%) and IC 39702 (33.33%). The minimum protein content decrease under stress conditions was found in IC 36157 (19.18%), followed by Jwala (26.36%) and RMO 40 (30.63%), while there was a significant difference in protein content of stressed and control samples for genotypes IC 39702 (67.96%) and IC 36392 (60.21%) (Table 3). Chlorophyll is an important component in plants for photosynthesis. Chlorophyll was measured after heat stress by using a SPAD chlorophyll meter. It is evident from Table 4 that the stress level resulted in increased SPAD level in all the genotypes of moth bean. Under stress conditions, higher SPAD units were observed in Jadia (51.55%), RMO 40 (49.95%), Marumoth (44.57%), and Jwala (40.41%) as compared to others, while the lowest chlorophyll SPAD readings were observed in genotypes IC 472257 and IC 121051 (29.11% and 33.13%, respectively).

3.4. Correlation matrix and cluster analysis

Determination of the correlations by using GeneSTAT between different traits, especially RWC and MSI, allows the identification of interactions among affected traits in moth bean. The RWC was positively and significantly correlated with MSI ($r = 0.880$). In the same manner, the protein was significantly correlated with MSI ($r = 0.482$). The highest correlation coefficient was observed between the RWC and MSI ($r = 0.880$). The protein showed a

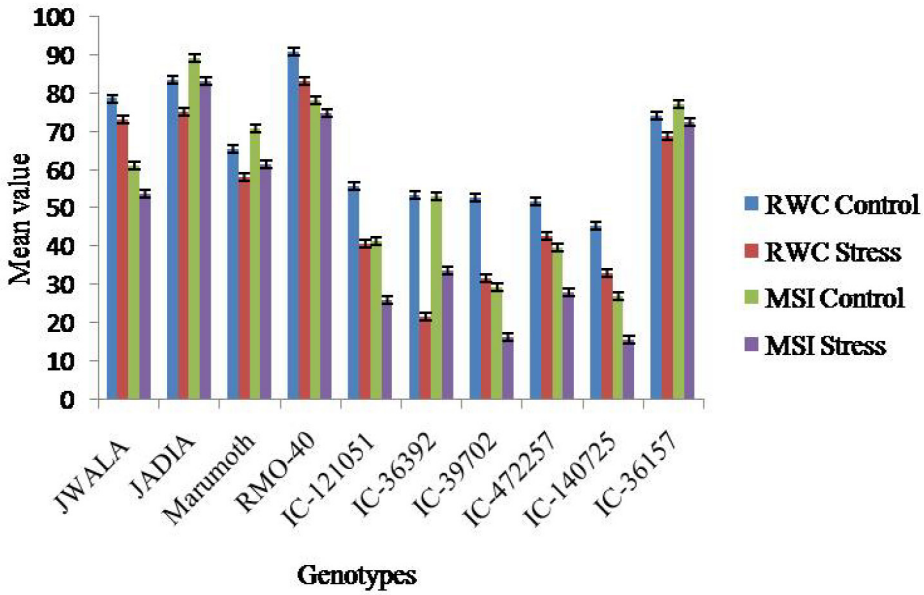

Figure 1. Effect of heat stress on RWC and MSI in moth bean genotypes. Values are ± standard error.

Table 3. Effect of heat stress on protein content (µg/mL) and proline content (µg/g) in ten moth bean genotypes under control and stress conditions.

Moth bean	Protein content			Proline content		
	Control	Stress	% Decrease	Control	Stress	% Increase
Jwala	4.39	3.24	26.36	1.68	4.07	57.72
Jadia	4.93	2.86	41.98	2.67	4.64	73.81
Marumoth	4.28	2.67	37.61	2.23	3.75	68.16
RMO 40	4.44	3.08	30.63	2.49	4.71	89.15
IC 121051	3.28	1.46	55.47	1.68	2.74	63.08
IC 36392	2.79	1.11	60.21	1.69	2.79	65.5
IC 39702	4.12	1.32	67.96	1.68	2.24	33.33
IC 472257	3.09	1.46	52.75	1.88	2.59	37.76
IC 140725	2.74	1.13	58.75	2.28	2.74	20.17
IC 36157	4.69	3.79	19.18	2.28	3.88	70.17

negative correlation with proline (r = -0.206). The MSI in comparison to RWC represented a larger proportion of chlorophyll content (r = 0.771 and 0.638, respectively) (Table 5). Under heat stress, tolerant and susceptible genotypes were grouped in separate clusters. The first cluster was constituted by tolerant genotypes (Jwala, Jadia, Marumoth, RMO 40, IC 36157), whereas the second cluster constituted heat-susceptible genotypes (IC 121051, IC 36392, IC 39702, IC 140725, IC 472257) (Figure 2).

3.5. Quantitative real-time PCR analysis

For the present study, three differentially expressed genes with known functions were selected for real-time analysis. The transcripts genes encoding heat shock protein (HSP), dehydration responsive protein (DRP), and DNA-3-methyladenine glycosylase 1 (Tag1) were chosen for primer design for qPCR. The RT-PCR analysis indicated up- and downregulation of genes after heat stress treatments in different genotypes of moth bean. The heat stress produced the expected expression for each gene. For each sample only one housekeeping gene, *actin*, was used for the normalization.

This study indicated that *HSP* was upregulated under stressed conditions in both tolerant as well as susceptible genotypes. *HSP* was highly upregulated in

Table 4. Effect of heat stress on chlorophyll content in moth bean genotypes under control and heat stress conditions.

Moth bean genotypes	Chlorophyll content		% Increase
	Control	Heat stress	
Jwala	41.55	47.30	40.41
Jadia	52.63	56.34	51.55
Marumoth	45.63	48.35	44.57
RMO 40 IC 121051	51.12 34.19	59.60 35.98	49.95 33.13
IC 36392	40.80	43.97	39.72
IC 39702	38.64	41.40	37.56
IC 472257	30.37	38.17	29.11
IC 140725	37.42	35.57	36.64
IC 36157	45.84	55.83	44.62

tolerant genotypes Jwala, RMO 40, Jadia, and IC 36157 (6-fold, 4-fold, 2.7-fold, and 2-fold, respectively), while genotype Marumoth was slightly upregulated under heat stress. Hence, these genotypes are expected to exhibit better tolerance upon exposure to high temperature. The susceptible genotypes IC 121051, IC 36392, and IC 140725 showed slight upregulation for the HSPs under extreme temperature condition. *HSP* gives an idea of the survival mechanism in susceptible genotypes under extreme conditions. In addition, *HSP* was highly downregulated in IC 472257 and IC 39702 (Figure 3). The *DRP* gene was significantly upregulated in 5 of the 10 genotypes. The *DRP* gene showed a higher upregulation in genotype RMO 40 (6-fold), followed by Jwala and Jadia (4-fold and 2.6-fold, respectively), while IC 36157 had over 2-fold expression for *DRP* as compared to the control, and *DRP* was also upregulated in the genotype Marumoth (1.5-fold). Interestingly, the expression of *DRP* was slightly higher in 2 susceptible genotypes, IC 121051 and IC 36392, while other susceptible genotypes, IC 472257, IC 39702, and IC 140725, exhibited a significant downregulation (Figure 3). The upregulation/downregulation of these genes in tolerant and susceptible genotypes confirmed the activity of these genes under heat stress. DNA-3-methyladenine glycosylase 1 (also known as *Tag1*) is a part of the helix-hairpin-helix DNA repair glycosylase superfamily. The expression of this gene was found to be upregulated especially in the tolerant genotypes, and it was significantly downregulated in other genotypes when compared to their respective controls (Figure 3).

4. Discussion

Drought, salinity, and high temperature are kinds of abiotic stress that cause severe damage to plants. Heat stress exhibits a negative impact on productivity and growth of plants. A change in the expression of heat-associated genes, RWC, MSI, and proline accumulation in plant cells is an indicator of oxidative stress. The present study revealed changes in RWC, MSI, proline accumulation, protein, and chlorophyll content among ten moth bean genotypes under heat stress. According to the present findings, RMO 40, Jadia, Jwala, IC 36157, and Marumoth exhibited better performance under heat stress as compared to IC 121051, IC 36392, IC 39702, IC 472257, and IC 140725. The RWC measures the water status of tissue under stress conditions. It is an indicator of the condition of water balance in plants. It is considered as one of the important agricultural parameters under changing temperatures that can be used for screening of plants for resistance (Mazorra et al., 2002; Kumar et al., 2004). A reduction in the RWC under heat stress was reported in *Lotus creticus* (Banon et al., 2004) and tomato (Morales et al. 2003; Katerji et al., 2004). Heat stress causes water loss from plants, which severely damages the membrane structure and function. The damaged cell membrane becomes more porous for

Table 5. Pearson correlation coefficients among the physiological and biochemical traits, RWC, MSI, and proline, protein, and chlorophyll contents in moth bean genotypes.

	MSI	Proline	RWC	Chl	Protein
MSI	1**				
Proline	0.341	1**			
RWC	0.880**	0.279	1**		
Chl	0.771**	0.617**	0.638	1**	
Protein	0.482	-0.206	0.523	0.124	1**

Significant correlations are indicated by **P < 0.01. Chl: Chlorophyll, MSI: membrane stability index, RWC: relative water content.

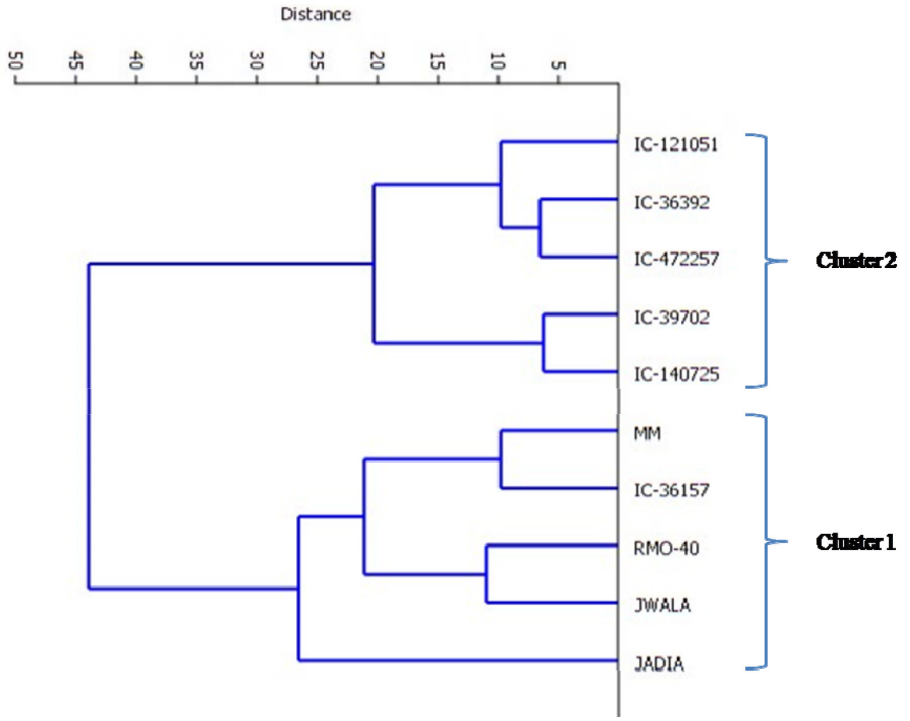

Figure 2. Dendrogram based on the Euclidean distance and morphophysiological and biochemical traits in ten moth bean genotypes.

ions, causing electrolyte leakage (Senaratana and Kersi, 1983). This study showed a significant reduction of MSI in all the genotypes under heat stress. Significant changes in MSI were reported earlier in wheat cultigens under heat stress at 30 °C for 2 h (Kumar et al., 2012). Recently it has been reported that MSI might be correlated with several physiological and biochemical parameters (Garty et al., 2000). The tolerant genotypes had less electrolyte leakage, while the susceptible genotypes had a higher leakage of electrolytes compared to the control (Figure 1).

Proline plays an important role in plant defense against abiotic stresses such as drought, heat, and salinity. Previous studies have indicated the mechanism of proline accumulation in plants under different stress condition (Molinari et al., 2004; Kishor et al., 2005; Gosami et al., 2014). In higher plants, proline accumulation is a well-known phenomenon in response to various environmental conditions such as water and heat stress. It is considered to be an important component of plants for testing the level of heat stress (Kuo et al., 1986). Proline accumulation under high temperature was found higher in the heat-tolerant genotypes of moth bean as compared to the control (Table 3). The intensity of proline accumulation revealed the tolerance level of the tolerant and susceptible genotypes under heat stress. Chlorophyll is an important chloroplast component for photosynthesis in plants (Krause and Wei, 1991). SPAD readings have been interpreted to reflect chlorophyll contents. The chlorophyll SPAD values may differ from the plant genotypes reported by Sibley et al. (1996). The results indicated that the changes in chlorophyll SPAD values in moth bean genotypes were indicative of genotypic response to heat stress (Table 4). Some previous findings also support that abiotic stresses like drought stress significantly increase the SPAD value in sugarcane (Silva et al., 2007) and sesame (Mensah et al., 2006). Reduction in chlorophyll content is a sign of oxidative stress and chlorophyll degradation. However, this result revealed higher chlorophyll content under heat stress, indicating an early recovery of photosynthesis in moth bean plants. Arunyanark et al. (2008) suggested that SPAD readings could be used as a tool for fast assessment of chlorophyll status in peanuts. The present study showed a correlation between the morphophysiological traits and heat adaptive capabilities and indicated that RWC, MSI, and proline, protein, and chlorophyll contents could be used as reliable indicators in the selection of heat-tolerant genotypes. All these indicators are directly or indirectly related to heat stress. Previous studies also found significant changes in physiobiochemical responses such as MSI, proline, and protein content under short-duration heat stress in moth bean (Sharma et al., 2014; Harsh et al., 2016).

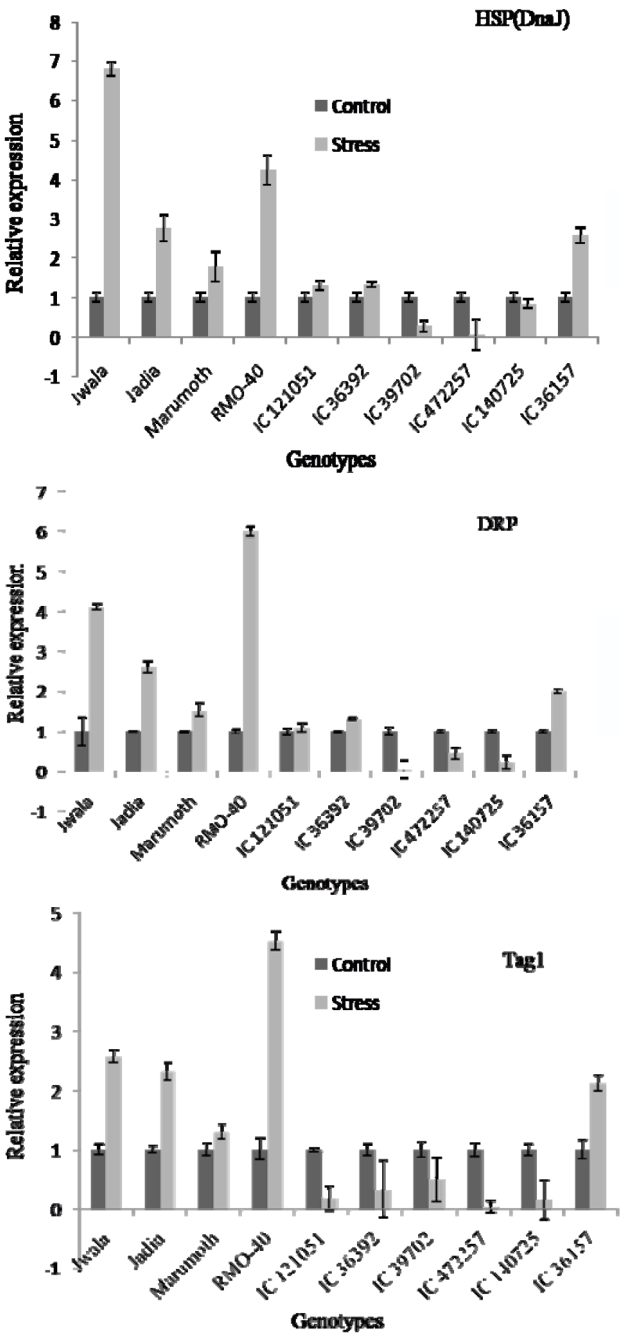

Figure 3. Relative expression of stress genes (*HSP*, *DRP*, *Tag1*) in heat-tolerant and heat-susceptible genotypes of moth bean under control and heat stress.

The present study concludes that RMO 40, followed by Jadia, IC 36157, Jwala, and Marumoth, were able to maintain significantly higher RWC, MSI, and chlorophyll, protein, and proline contents under heat stress conditions; therefore, they can be grouped as relatively heat-tolerant. IC 121051, IC 36392, IC 39702, IC 472257, and IC 140725 showed a significant decline in all morphophysiological parameters under high temperature and hence they can be reported as susceptible genotypes. It is obvious that tolerant and moderately tolerant genotypes will perform better in morphophysiological and biochemical processes in response to heat stress.

Plant response to heat stress and other abiotic stresses, such as drought and salinity, are similar in nature; hence, analyses of responses to heat stress are likely to help in deciphering related molecular mechanisms. Heat shock proteins (HSPs) act as molecular chaperones to prevent denaturation and protect organisms under heat stress. HSPs protect plant cells against the adverse effects of heat stress (Parsell and Lindquist, 1993). HSPs are categorized into different classes on the basis of their molecular weight (HSP100, HSP90, HSP70, HSP60, HSP40) (Kotak et al., 2007; Hartl et al., 2011). Several low-molecular-weight HSPs such as HSP40, and small HSPs, are induced upon exposure to high temperature. HSP40 (DnaJ), also called J-domain-containing protein (J-protein), is a co-chaperone component of the HSPs (Kampinga and Craig, 2010). Previous reports have reported the role of HSP40 in plant immunity in soybean and tomato (Soellick et al., 2000; Liu and Whitham, 2013). In the present study the expression profiles of *HSP40* (*DnaJ*) revealed that this gene is highly expressed in heat-tolerant genotypes as compared to the susceptible genotypes under heat stress (Figure 3). A similar pattern of gene expression was observed in tolerant and susceptible varieties of rice (Chen et al., 2014). The expression of HSPs is also observed in the moth bean heat-tolerant genotype RMO 40 under heat stress (42 °C for 30 min) (Gurjar et al., 2014) and these studies also validated the present observation.

Another gene chosen for the expression studies was DNA-3-methyladenine glycosylase 1, also known as *Tag1*. The activity of *Tag1* protects or sensitizes cells to the toxic effects of DNA alkyl lesions (Michael et al., 1999). In addition, *Tag1* showed upregulation under heat stress. The expression level of *Tag1* was higher in all the tolerant genotypes as compared to the susceptible ones. The expression level of this gene was four times higher in the tolerant genotype RMO 40 (Figure 3).

When plants suffer from heat stress, various dehydration proteins activate and generate defense mechanisms. Dehydration-responsive element-binding protein plays a crucial role in regulating the expression of genes in response to various abiotic stresses, such as moisture and heat stress (Dubouzet et al., 2003; Agarwal et al., 2006). The present study clearly shows an elevated expression of dehydration-responsive protein under heat stress (Figure 3). Earlier reports also reported the involvement of dehydration-responsive genes under abiotic stress in plants. The role of higher expression of dehydration-responsive element-

binding protein (DREB2) under drought and heat stress tolerance have been reported in several crop plants, such as soybean (Mizoi et al., 2013) and sugarcane (Wahid and Close, 2007). Some reports also revealed that the expression of dehydration-responsive proteins led to improved tolerance against various abiotic stresses such as dehydration, high salinity, and heat stress (Liu et al., 1998; Sakuma et al., 2002), which is substantiated by the present findings. Moreover, it was revealed that a rapid induction of dehydration-responsive proteins occurred by various abiotic stress.

In conclusion, the tolerant and susceptible moth bean genotypes responded differently at the physiological and molecular levels when exposed to heat stress. The tolerant genotypes could be a good source of efficient alleles for various stress-tolerant pathways or mechanisms. The increase in RWC and decrease in MSI are associated with heat tolerance in moth bean. The tolerance and susceptibility were also confirmed by the expression studies of three genes involved in the stress tolerance mechanism. This study has identified moth bean genotypes RMO 40, Jadia, IC 36157, Jwala, and Marumoth as heat-tolerant, which can be used in breeding programs to develop improved heat-tolerant varieties.

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