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SEED YIELD LOSS IN MUNGBEAN IS ASSOCIATED TO HEAT STRESS INDUCED OXIDATIVE DAMAGE AND LOSS OF PHOTOSYNTHETIC CAPACITY IN PROXIMAL TRIFOLIATE LEAF

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Heat stress damages the photosynthetic capacity and ultimate crop yield; the genetic basis of which is scarcely known. Studies were conducted during the years 2014 and 2015 to understand the physiological basis of heat tolerance in seven mungbean [*Vigna radiata* (L.) Wilczek] varieties by monitoring changes in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents, photosynthetic pigments and gas exchange attributes in the trifoliate leaf in proximity to the pods as well as nutrient partitioning to the pods and developing seed by applying heat stress at the flowering stage. After 15 days of stress, the data for physiological and biochemical attributes were recorded while the data for seed yield and quality were taken at maturity. Heat stress caused a marked accumulation of H_2O_2 and MDA whilst decreasing net photosynthesis, stomatal conductance and water use efficiency, chlorophyll *a* and *b*, and total chlorophylls in the sensitive varieties, while increased carotenoids contents in the tolerant varieties. Seed yield per plant and 1000 seed weight were affected due to heat stress, leading to more reduced harvest index in the sensitive varieties. Heat stress also hampered the partitioning of nutrients such as nitrogen, sulfur and calcium to the developing seed. Interrelationships of different parameters revealed that heat stress damaged the leaf chlorophyll and leaf gas exchange due to enhanced production of H_2O_2 and MDA, while tolerant varieties (NM-2011 and NM-2006) exhibited better gas exchange properties and produced higher seed yield and harvest index in both the years. Seed yield was directly correlated to the partitioning of nutrients especially nitrogen, sulfur and calcium under heat stress. In conclusion, despite significant differences in the varieties, the yield loss in mungbean is associated to heat stress mediated oxidative stress on the leaf photosynthesis and reduced assimilate partitioning to the pod development and seed filling. Thus, selection based on greater rate of photosynthesis of the proximal leaf may be helpful for fetching better mungbean yield in relatively warmer environments.

Keywords: Assimilate partitioning, gas exchange, heat stress, seed yield, mungbean

INTRODUCTION

Plants growing in nature face abiotic stresses due to climatic changes and anthropogenic interventions. The land plants being sessile component on earth are more vulnerable to these stresses. Heat stress is perhaps the major amongst the cardinal climatic factors, which inhibits the leaf photosynthesis and causes severe loss of yield (Xu *et al*., 2016). Abiotic stresses, including heat stress, inhibit morphological, physiological and biochemical processes in plants (Zandalinas *et al*., 2017). Increase in average global temperature on land and ocean has increased by 0.85°C between 1880 and 2012 (IPCC, 2014). During the last ten years, there was ~0.2°C rise in ambient temperature, which along with increased concentration of greenhouse gasses resulted in global warming (Challinor *et al.*, 2014). Recently, a rise in ambient temperature has become a major threat to crop production, which also disturbs the growing season by shortening the life cycle and affecting the agricultural systems (Prodhan *et al.*, 2017; Zandalinas *et al*., 2017).

With an increase in ambient temperature, series of changes take place in plants, which cause abnormalities in growth and development associated with modifications at physiological and biochemical levels (Sita *et al.*, 2017). Of these, leaf photosynthesis and assimilate partitioning to developing sinks is quite sensitive to heat stress (Mathur *et al*., 2014). Reports show that heat stress reduced the expansion and enhanced leaf senescence primarily due to damaged photosynthetic machinery (Wahid *et al.*, 2007; Farooq *et al*., 2011). Heat stress resulted in excessive loss of water, which increased the plant susceptibility to oxidative damage (Ashraf and Harris, 2013). The disorganization of thylakoid membrane under heat stress caused the dysfunctioning of photosystems (Gururani *et al*., 2015; Nouri *et al*., 2015). A major effect of heat stress is the enhanced activity of chlorophyllase enzyme leading to the degradation of chlorophylls (Rossi *et al*., 2017).

In aerobic metabolism under normal environmental conditions, reactive oxygen species (ROS) are produced, which have essential role in the signaling of intracellular pathways (Wahid *et al.*, 2014; Sun and Guo, 2016). However, under high temperature stress, an imbalance in plant metabolism, leads to exaggerated production of ROS, which injures the thylakoid membrane, chloroplast and PSII along with the enhanced peroxidation of membrane lipids. At high temperature, stomata are closed and CO_2 availability to plants is reduced, which also results in excessive generation of free radicals and reduced photosynthesis. These events hamper the plant development and reduce the ultimate yield (Mittler, 2017). The stress tolerant plants show a higher and timely biosynthesis of such compounds, which can either suppress the production of ROS or their efficient scavenging shortly after their generation (Choudhury *et al.*, 2017; Chavez-Servin *et al.*, 2017).

Prevalence of heat stress during reproductive phase severely reduces the crop yield; a reason why reproductive stage is considered as a critical indicator for stress tolerance. Reduction in yield is associated with abnormal fertilization and low pollen viability at high temperature (Farooq *et al.*, 2011; Kaushal *et al*., 2013). Only few degrees increase in temperature may change the crop cycle, and result in accelerated flower and pods shedding, abnormal fruit formation and poor seed filling (Kaur *et al*., 2015; Kaushal *et al*., 2016). The grains show low amounts of nutrients because of early maturity. Heat stress also decreased the grain weight and total number of grains per plant (Fahad *et al*., 2017). However, there exist great differences in crops for the generation of ROS and heat tolerance at different growth stages (Wahid *et al*., 2007; Driedonks *et al*., 2016).

Mungbean [*Vigna radiata* (L.) Wilczek] belongs to Fabaceae family, and is an important source of grain protein. It is used as a rich source of proteins for the teaming millions around the globe. In Pakistan mungbean is cultivated in spring and summer seasons and faces the episodes of heat stress during different stages of growth. This makes heat stress a major reason for low yield in mungbean. The leaves near the fruit (pod), referred to as proximal leaves, are the major contributor to the pod growth and eventual economic yield. Therefore, the photosynthetic capacity, the production and dousing of ROS in the proximal leaf to the pod may determine the final yield in mungbean. This may, however, depend upon the innate potential of the varieties to withstand heat stress. We surmise that heat stress induced oxidative damage may have great implications for net photosynthesis and final yield in plants. In this work, an attempt was made to find out the basis of reduction in seed yield based on extent of oxidative stress and the photosynthetic activity in the trifoliate mungbean leaf proximal to pod and their possible association with pod and seed yield using seven commercially released mungbean varieties.

MATERIALS AND METHODS

Seed material and experimental details**:** The experiment was conducted in the net house of the Department of Botany, University of Agriculture Faisalabad in summer from June to October 2014 and 2015 using seven commercial mungbean [*Vigna radiata* (L.) Wilczek] varieties namely NM-2011, NM-19-19, NM-2006, NM-98, NM-92, NM-13-1 and NM-20-21. The seed of the varieties was obtained from Plant Breeding and Genetics Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Seven seeds of each variety were sown on 15 June 2014 and 20 June 2015 in earthen pots lined with polythene sheets containing 10 kg of compost mixed loam soil (1:3 ratio). The design of the experiment was completely randomized factorial with three replications.

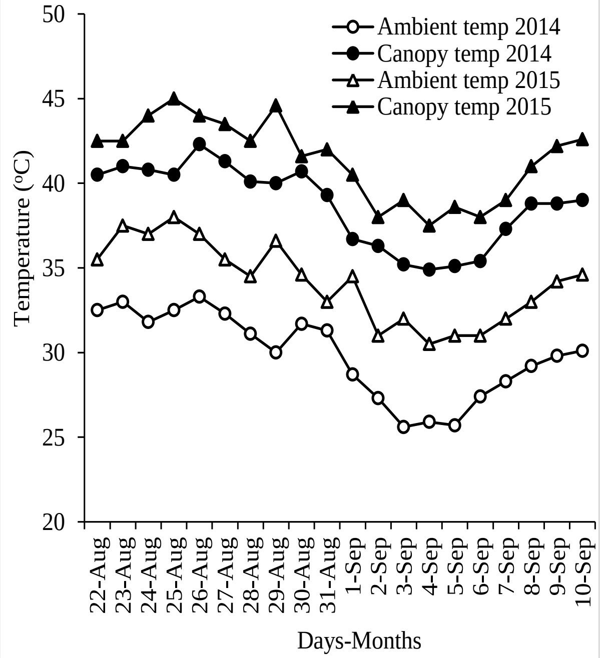

Figure 1. Changes in the net house and plexiglass canopy temperature during the years 2014 and 2015.

After germination the seedlings were thinned to three in each pot. The plants were normally grown up to flowering stage by placing pots in the net house. The plants were watered when required to avoid water deficit in the soil. In both the years, the plants of all the varieties flowered during the third week of August. Upon complete opening of most of the flowers, half of the pots were shifted to stainless steel frame fitted plexiglass canopies (1.5×1.5×2.0 m) with light transmission spectrum of 75–80% for the heat stress treatment. The canopy temperature during both the years was 7–10 °C higher than the net house temperature (Fig. 1). Data were recorded 15 days after shifting the plants into the canopy. However, one set of plants was kept for the determination of pod and seed yield characteristics at full crop maturity.

Oxidative stress**:** The H_2O_2 and MDA concentrations were measured in proximal trifoliate leaf as indices of oxidative stress using spectrophotometer (U-2001, Hitachi, Tokyo, Japan) with the methods of Velikova *et al.* (2000) and Heath and Packer (1968), respectively.

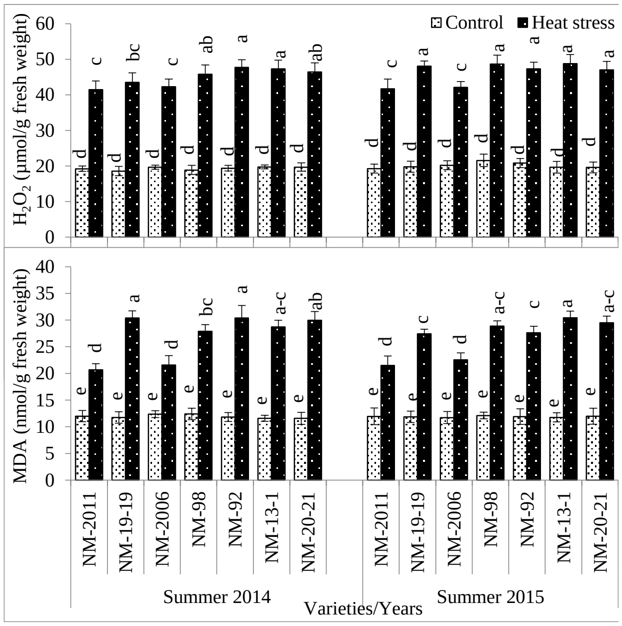

Figure 2. Changes in H_2O_2 and MDA content in shoot and root of mungbean varieties at flowering stage over the years 2014 and 2015.

***Photosynthetic characteristics*:** Before harvesting proximal trifoliate leaf was determined for leaf gas exchange attributes including net photosynthesis (*A*), transpiration rate (*E*), stomatal conductance (*gs*) and sub-stomatal CO_2 concentration (*Ci*) using Infrared Gas Analyzer (IRGA; LCA-4, ADC, Hoddesdon, Herts, UK). The IRGA leaf chambers condition were: leaf surface area, 11.35 cm^2; ambient CO_2 concentration, 354.4 µmol/mol; chamber temperature varied between ~31 to 38°C; leaf chamber gas flow, 392.8 mL/min; air-flow per unit leaf area was ~405 mol/m^2/s; ambient pressure ~99 kPa; chamber vapor pressure, 21-23 mbar and Q leaf was ~900-1100 µmol/m^2/s. The water use efficiency (WUE) was worked out as *A/E* ratio. The same leaf was used for the measurement of chlorophyll, a, b, their total with the method of Arnon (1949) while that of carotenoids with the method of Davies (1976).

***Pod and seed yield attributes*:** When dry and turned from green to dark brown in color, the pods were counted and handpicked. The aboveground dry matter (AGDM) yield was also taken. The number of seeds per pod was counted after carefully removing the seeds from pods, and weight of 1000 seeds was determined. For taking seed yield per plant, the seeds from all the pods on a plant were carefully removed and weighed. The harvest index (HI) was computed as:

HI (%) = (seed yield/plant) × 100/(AGDM).

***Seed nutrients*:** The seeds harvested during both the years were analyzed for macronutrients. Total nitrogen was measured with the Kjeldahl's method. For the analysis of phosphorus (P), potassium (K), calcium (Ca) and sulfur (S), the dried grinded seed material (0.5 g) was digested in concentrated nitric acid:perchloric acid (3:1 ratio) at 250°C for ~2.5 h or until the samples became clear. The final volume was made up to 50 mL and filtered. K and Ca contents were measured with flame photometer (Jenway, PEP-7, Staffordshire, UK). P was analyzed using molybdate-vanadate reagent method (Yoshida *et al.*, 1976). Sulfur was determined spectrophotometrically with the Gum-Arabic method of Tendon (1993).

Statistical analysis: The data for the physiological, nutrient and yield attributes described above were analyzed statistically for the analysis of variance sources using STATISTIX8 software. The least significant difference (LSD) test was applied (Steel *et al.*, 1996) and means having significant (P<0.05) difference were distinguished from each other by putting different letters on them. Pearson's correlations were established between different physiological, nutrient and seed yield variable.

RESULTS

***Oxidative stress*:** Data on H_2O_2 and MDA in the proximal trifoliate leaf showed that there was a general tendency of varieties to show increased production of H_2O_2 and MDA under heat stress, albeit great varietal differences were noticed. Data revealed that NM-2006 closely followed by NM-2011 indicated a lowest production of H_2O_2 during both the study years under heat stress. Likewise, MDA content although increased in all the varieties under heat stress, a minimum increase of 72 and 80% was recorded in NM-2011 during the respective years (Fig. 2).

***Photosynthetic pigments*:** In the years 2014 and in 2015, analysis of data for chlorophyll-*a*, chlorophyll-*b*, total chlorophylls, and carotenoids exhibited significant differences for varieties (P<0.01) and heat stress levels. In both the years, heat stress reduced the chlorophyll-*a*, *b* and their total chlorophyll in all the varieties; the year 2015 was more adverse (Fig. 1). The trend of changes in the photosynthetic pigments among the varieties was similar in both the years (Fig. 3). Among the varieties, a minimum decline in chlorophyll-*a* was noted in NM-2006 (3-13%) followed by NM-2011 (10-14%) while maximum one in NM-13-1 (39-49%) over respective controls in both the years. A minimum heat-induced damage to chlorophyll-*b* was observed in NM-2011 and (6%) and NM-2006 (3%) during 2014 and 2015, respectively while a maximum damage was recorded in NM-13-1 during 2014 (0%) and 2015 (63%). About total chlorophylls, NM-2006 performed better by showing 5 and 11% decline in 2014 and 2015, respectively while NM-13-1 exhibited a highest susceptibility (48 and 55% decline in 2014 and 2015, respectively). Mungbean varieties exhibited an interesting pattern of carotenoids accumulation. During 2014, NM-2011 indicated 7% greater carotenoids in 2014, although it

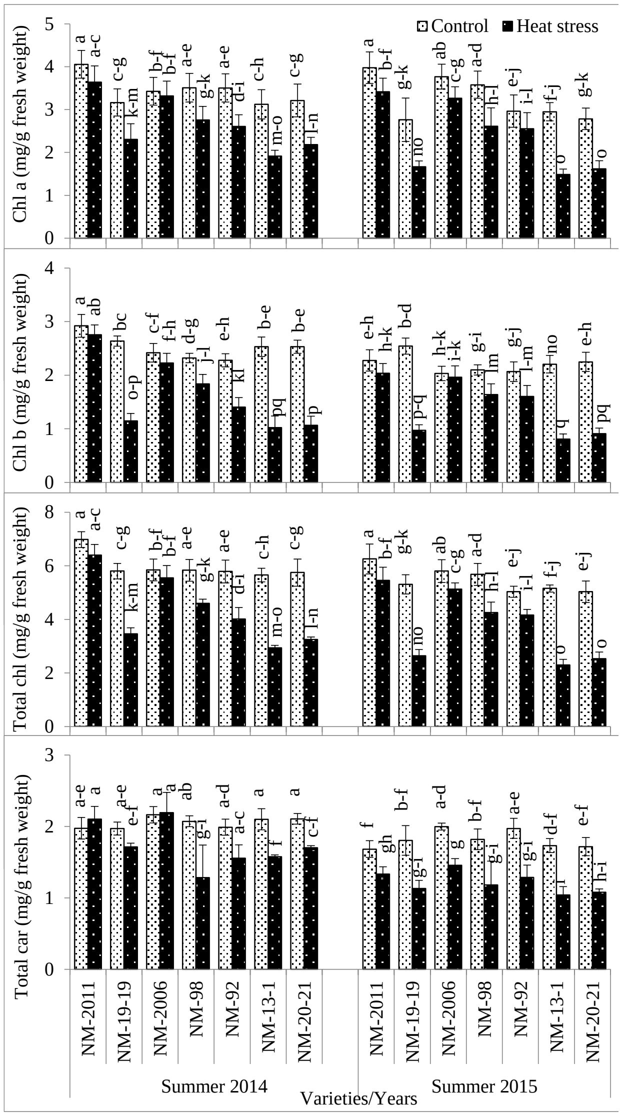

Figure 3. Changes in photosynthetic pigments content of mungbean varieties at flowering stage over the years 2014 and 2015.

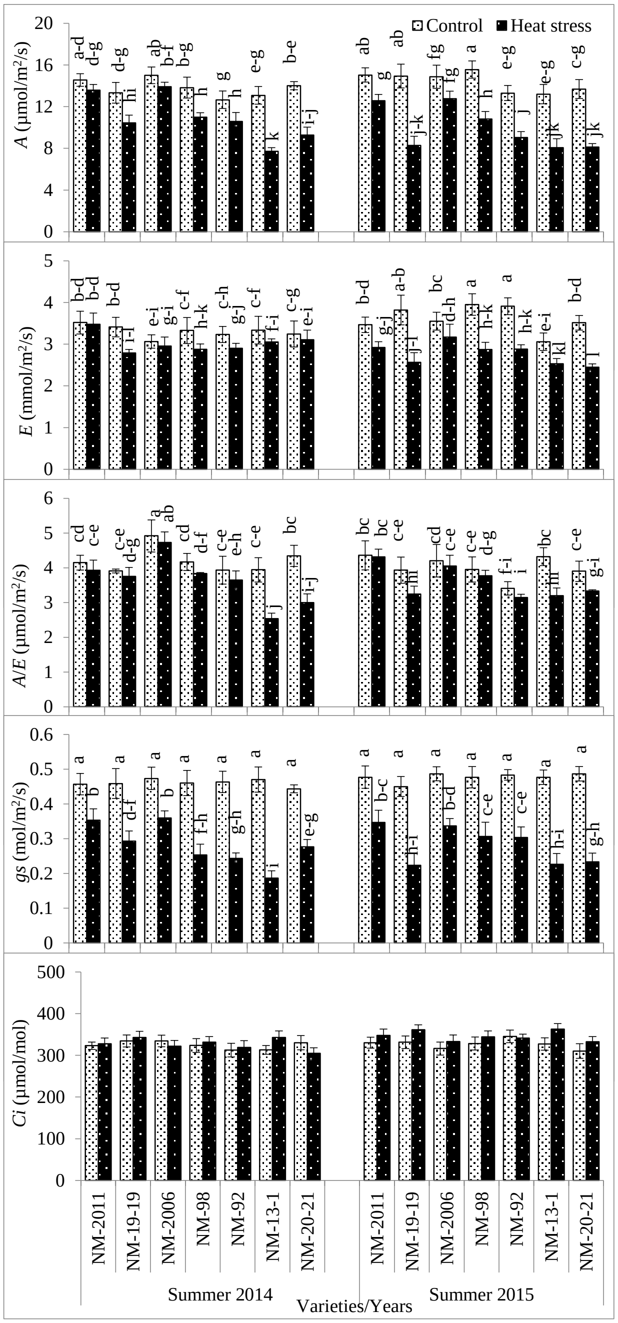

Figure 4. Changes in gas exchange characteristics of mungbean varieties at flowering stage over the years 2014 and 2015.

decreased by 22% in 2015. NM-98 during 2014 and NM-13-1 in 2015 indicated lowest carotenoids contents amongst all the varieties (Fig. 3).

***Gas exchange characteristics*:** Significant ($P<0.01$) differences were noted in all the gas exchange parameters of proximal trifoliate leaf except sub-stomatal CO_2 concentration ($P>0.05$) in the years 2014 and 2015 (Fig. 3). Among the gas exchange parameters, as expressed over respective controls, although *A* was reduced in all the varieties under heat stress, a lowest reduction (7%) was noticed in NM-2011 in 2014 and in NM-2006 (14%) in 2015, while NM-13-1 (41%) and NM-19-19 (45%) were the worst affected in 2014 and 2015, respectively. *E* decreased in all the varieties during 2014 and 2015 under heat stress. A maximum decline in *E* was observed in NM-1919 in 2014 (18%) and in 2015 (36%), while a minimal decline was noted in NM-2011 (2%) in 2014 and in NM-2006 (21%) in 2015. The *A*/*E* was affected much under heat stress in all the varieties during both the years. *A*/*E* was minimally reduced (~4%) in NM-2006 in both the years while it was maximally reduced in NM-31-1 by 36% in 2014 and by 26% in 2015.

Heat stress reduced the *gs* in all the varieties but a minimum reduction of 23 and 27%, was recorded in NM-2011 in both the respective years. Contrarily, NM-13-1 was the worst affected with a reduction of 60 and 52% *gs* in both the years. The *Ci* indicated no distinct trend of variation; some varieties indicated an increase while others showed a decrease in *Ci*. Nonetheless, NM-2006 during 2014 and NM-92 during 2015 indicated declines of 4 and 1%, respectively in *Ci* during both the years (Fig. 4).

***Yield attributes*:** Except for the number of pods per plant and aboveground dry matter yield ($P>0.05$), data revealed significant ($P<0.01$) difference among the varieties and heat stress for number of seeds per plant, 1000 seed weight, seed yield per plant, aboveground dry matter (AGDM) and harvest index (HI) during both the years (Table 1). Results revealed that the number of pods per plant was minimally reduced in NM-2011 in 2014 (14%) and in NM-2006 in 2015 (15%), while a greatest reduction was recorded in NM-13-1 in 2014 (31%) and in NM-98 in 2015 (17%) under heat stress. The number of seeds per plant although were reduced in all the varieties in both the years under heat stress, a minimum reduction was notable in NM-2011 and NM-20-21 in 2014 and 2015, respectively. Heat stress adversely affected 1000 seed weight in all the varieties but a minimal reduction (~31%) was recorded in NM-2011 and NM-2006 during both the years. Seed yield per plant was the most prominently affected parameter in all the varieties under heat stress in both the years but with a minimum reduction of 48% in NM-2011 while a maximum of 80 and 76% in the years 2014 and 2015, respectively. Although heat stress reduced the AGDM in all the varieties, a lowest decline was observed in NM-92 (17%) in 2014 and in NM-2006 (22%) in 2015 whilst a highest one in NM-19-19 (30%) and NM-13-1 (32%) in 2014 and 2015. Heat stress substantially reduced the HI but with great varietal differences. A lowest decline in HI was found in NM-2011 in both the years (35% in 2014 and 33% in 2015) while a highest decrease i.e., 73% in 2014 and 64% in 2015 in NM-13-1 (Table 1).

***Seed nutrients*:** Statistical analysis of data revealed significant ($P<0.01$) differences in the levels of seed N, P, Ca, K and S contents under heat stress in all the varieties in the years 2014 and 2015, although the year 2015 was relatively more adverse (Table 2). With large differences among the varieties, the seed N contents were maximally reduced in NM-19-19 and NM-13-1 in 2014 (~40%) and in NM-13-1 in 2015 (51%), whilst minimally in NM-2006 (25% in 2014 and by 34% in 2015) under heat stress. About seed P contents, NM-2006 displayed a lowest reduction of 8% in the year 2014

Table 1. Comparative changes in the yield and yield components of mungbean varieties under heat stress during summer months of the years 2014 and 2015.

Season	Treatment	Varieties	No. of pods per plant	No. of seeds per plant	1000 seed weight (g)	Seed yield (g/plant)	AGDM (g/plant)	Harvest index (%)
Summer 2014	Control	NM-2011	17.00±1.00	192.00±12.00a-c	48.22±1.48a	9.25±0.55a-c	17.62±1.22	52.60±2.52ab
		NM-19-19	17.67±1.15	200.67±23.67ab	47.70±1.14a	9.59±1.34ab	17.86±1.64	53.57±3.64a
		NM-2006	17.67±1.53	199.67±08.62ab	48.44±1.80a	9.68±0.66a	17.94±1.33	53.96±1.46a
		NM-98	16.67±0.58	183.00±11.53bc	48.68±1.46a	8.92±0.81a-c	18.06±0.34	49.35±3.61b
		NM-92	16.67±1.53	177.33±11.24c	46.63±1.52a	8.28±0.74c	16.45±0.76	50.29±3.09ab
		NM-13-1	17.00±1.73	192.00±10.39a-c	46.88±2.87a	9.01±0.92a-c	17.19±1.09	52.35±2.67ab
		NM-20-21	16.67±1.53	188.00±13.86a-c	47.68±2.55a	8.99±1.14a-c	17.64±1.43	50.89±3.88ab
	Heat Stress	NM-2011	14.67±2.08	145.67±14.36d-f	33.36±2.20b	4.84±0.19ef	14.10±0.48	34.34±1.62d
		NM-19-19	13.33±0.58	124.33±06.66g-i	20.27±1.54e	2.51±0.13j-l	12.50±0.57	20.14±1.27i
		NM-2006	13.67±0.58	132.33±13.28e-h	33.30±1.67b	4.41±0.49e-h	13.68±1.09	32.18±2.23d-e
		NM-98	12.67±1.15	118.00±09.17h-j	28.99±1.98c	3.42±0.27h-j	13.93±0.33	24.49±1.32gh
		NM-92	12.67±1.15	113.33±06.11ij	25.13±1.77d	2.85±0.34i-k	13.68±0.49	20.82±1.86hi
		NM-13-1	11.67±1.53	104.00±04.00j	17.17±1.00ef	1.78±0.08l	12.67±0.38	14.09±0.73j
		NM-20-21	12.33±1.15	114.67±04.04h-j	18.99±1.90ef	2.18±0.27kl	12.66±0.49	17.19±1.65ij
Summer 2015	Control	NM-2011	17.67±1.53	206.00±19.29a	48.04±1.34a	9.88±0.76a	19.29±1.27	51.24±1.67ab
		NM-19-19	17.33±1.53	190.00±14.00a-c	47.10±3.07a	8.96±0.97a-c	17.85±1.05	50.09±3.04ab
		NM-2006	17.33±0.58	190.67±06.35a-c	48.03±2.21a	9.17±0.69a-c	18.02±1.54	50.91±1.92ab
		NM-98	17.67±1.15	200.00±11.53ab	48.60±2.00a	9.74±0.94a	18.31±1.01	53.08±2.30ab
		NM-92	16.67±1.15	188.67±11.37a-c	47.52±2.31a	8.97±0.79a-c	18.06±0.70	49.63±2.84b
		NM-13-1	16.33±1.53	179.00±13.53c	48.10±3.31a	8.60±0.70bc	16.01±0.24	53.66±3.59a
		NM-20-21	15.67±1.53	150.33±05.51de	47.49±2.57a	7.15±0.59d	16.30±1.34	43.91±3.02c
	Heat Stress	NM-2011	14.67±0.58	156.33±07.77d	33.05±2.64b	5.18±0.67e	14.97±0.97	34.52±2.61d
		NM-19-19	13.67±1.15	140.33±04.62d-g	26.78±1.64cd	3.76±0.29g-i	13.62±0.72	27.60±1.36fg
		NM-2006	14.67±0.58	141.67±07.64d-g	32.82±2.17b	4.64±0.12e-g	14.04±0.74	33.13±2.21de
		NM-98	14.67±1.53	140.67±04.93d-g	27.32±1.84cd	3.84±0.32f-i	13.03±0.34	29.49±1.76ef
		NM-92	14.33±1.53	132.67±03.79e-h	25.95±1.01cd	3.45±0.23h-j	12.93±0.06	26.64±1.68fg
		NM-13-1	13.67±1.15	127.33±09.29g-i	16.51±1.15f	2.10±0.10kl	10.85±0.39	19.37±1.64i
		NM-20-21	13.67±0.58	127.67±11.59g-i	18.51±0.72ef	2.36±0.23kl	11.61±0.38	20.32±1.35i

Means sharing the same letter in a column differ non-significantly ($P>0.05$).

Table 2. Changes in seed nutrient content of mungbean varieties under heat stress.

Season	Treatment	Varieties	Concentration (mg/g dry weight)				
			N	P	Ca	K	S
Summer 2014	Control	NM-2011	56.48±4.09a	3.89±0.29de	1.67±0.14ab	7.03±0.61a-e	5.92±0.34a
		NM-19-19	53.14±2.64a	4.26±0.35b-d	1.83±0.13a	6.57±0.53d-g	5.65±0.44a
		NM-2006	54.44±3.54a	4.15±0.29cd	1.63±0.12bc	6.42±0.08e-h	5.71±0.45a
		NM-98	54.55±3.70a	3.10±0.57g-i	1.75±0.07ab	6.92±0.08b-f	5.66±0.23a
		NM-92	53.77±4.18a	3.56±0.39e-g	1.70±0.10ab	6.68±0.45d-g	5.83±0.18a
		NM-13-1	56.64±4.32a	3.25±0.32g-i	1.76±0.15ab	6.42±0.42e-h	5.80±0.54a
		NM-20-21	56.31±2.27a	3.33±0.29f-h	1.73±0.10ab	6.42±0.38e-h	5.78±0.23a
	Heat stress	NM-2011	41.17±3.17b	4.25±0.39b-d	1.48±0.12cd	6.39±0.25f-i	4.94±0.17b
		NM-19-19	31.652.79±e-h	2.95±0.20h-j	1.10±0.04g	4.51±0.21l	3.65±0.32ef
		NM-2006	40.90±1.88b	3.81±0.08d-f	1.37±0.13de	6.21±0.40g-j	4.59±0.18bc
		NM-98	38.59±4.39bc	2.90±0.15h-k	1.34±0.09de	5.75±0.30jk	4.05±0.28de
		NM-92	36.41±2.05b-e	3.23±0.38g-i	1.30±0.10ef	5.86±0.34h-k	4.21±0.39cd
		NM-13-1	34.22±3.35c-f	2.43±0.22k-m	1.04±0.10g	4.29±0.25l	3.23±0.21f
		NM-20-21	35.24±1.16c-e	2.75±0.25i-k	1.04±0.05g	4.48±0.25l	3.31±0.21f
Summer 2015	Control	NM-2011	57.64±3.68a	4.67±0.22ab	1.70±0.18ab	7.59±0.42a	5.87±0.30a
		NM-19-19	55.09±2.41a	4.52±0.27a-c	1.60±0.17bc	7.06±0.55a-d	5.74±0.33a
		NM-2006	57.19±4.25a	4.56±0.27a-c	1.74±0.08ab	7.51±0.61ab	5.96±0.37a
		NM-98	54.42±2.18a	4.31±0.18b-d	1.76±0.15ab	7.54±0.29a	5.81±0.21a
		NM-92	57.81±3.76a	4.64±0.39a-c	1.73±0.03ab	7.31±0.54a-c	5.85±0.42a
		NM-13-1	55.64±3.03a	4.81±0.23a	1.69±0.05ab	7.50±0.28ab	5.86±0.44a
		NM-20-21	56.50±4.85a	4.57±0.46a-c	1.73±0.08ab	7.04±0.36a-e	5.75±0.32a
	Heat stress	NM-2011	37.64±1.01b-d	3.33±0.21f-h	1.37±0.11de	7.06±0.28a-d	4.95±0.41b
		NM-19-19	29.26±1.05f-h	2.19±0.16lm	1.10±0.05g	5.29±0.44k	3.31±0.20f
		NM-2006	38.02±2.92bc	3.31±0.14f-h	1.35±0.17de	6.86±0.37c-f	4.61±0.44bc
		NM-98	32.76±3.62d-g	2.58±0.23j-l	1.16±0.04fg	6.17±0.25g-j	3.46±0.35f
		NM-92	33.64±2.84c-g	2.23±0.25lm	1.13±0.04fg	5.78±0.29i-k	3.58±0.14ef
		NM-13-1	27.31±0.79h	2.04±0.20m	1.03±0.12g	4.50±0.35l	3.21±0.24f
		NM-20-21	29.00±1.53gh	2.41±0.36k-m	1.04±0.08g	4.50±0.42l	3.35±0.29f

Means sharing same letter in a column differ non-significantly ($P>0.05$).

but that of 28% in the year 2015, while a highest reduction was noticed in NM-19-19 (31%) in 2014 and in NM-13-1 (58%) in 2015. The seed Ca contents although decreased in all the varieties under heat stress, a greatest reduction was detected in NM-13-1 by 41 and 40% in 2014 and 2015, respectively while a lowest decline was evident in NM-2011 by 11 and 19% in the respective years. Seed K was relatively less affected due to heat stress albeit the varietal difference was evident. A lowest decline in seed K was found in NM-2006 (3%) in 2014 and in NM-2011 (7%) in 2015, whereas a highest reduction was found in NM-13-1 in both the years. Although reduced due to heat stress in all the varieties, the seed S was affected the least in NM-2011 (~16%) while it decreased highly (~44%) in NM-13-1 in both the years (Table 2).

***Correlations*:** Since heat stress damages the biological membranes and photosynthetic apparatus is more prone to the generation of ROS, the Pearson's correlation coefficients were drawn of MDA and H_2O_2 with leaf gas exchange properties and photosynthetic pigments. Data revealed that under ambient temperature none of the photosynthetic pigments and gas exchange parameters was correlated with leaf MDA and H_2O_2 in both the years. However, under heat stress, negative correlations were observed of both MDA and H_2O_2 with all the leaf pigment contents, net photosynthesis and stomatal conductance, while, additionally, A/E was negatively correlated with MDA and H_2O_2 during 2015 only. Similarly, 1000 seed weight and seed yield per plant indicated strong negative correlations with both MDA and H_2O_2 during both the years (Table 3). About seed nutrient data, no relationship could be found of the seed yield attributes (1000 seed weight, seed yield per plant and harvest index) with seed nutrients (N, P, Ca, K and S) under control temperature during both the study years. However, under heat stress, all the seed yield attributes exhibited strong positive correlation with the entire seed nutrients in both the years (Table 4).

DISCUSSION

During critical reproductive stages, terminal heat stress and resulting physiological alterations are recognized as major determinants of final yield (Fu *et al.*, 2017; Moshatati *et al.*, 2017). The heat stress damages the cellular membranes with the production of ROS; H_2O_2 and MDA as an indicator of membrane damage (Shahid *et al.*, 2017). During reproductive

Table 3. Correlation coefficients of oxidative stress attributes with photosynthetic pigment contents, gas exchange and seed yield parameters of trifoliate leaf of mungbean varieties under control and heat stress conditions (n = 7).

Independent variable	**Dependent variable**	**2014**		**2015**	
		Control	**Heat stress**	**Control**	**Heat stress**
Leaf malondialdehyde	Hydrogen peroxide	-0.236ns	0.903**	0.432ns	0.953**
	Chlorophyll *a*	0.458ns	-0.883**	0.068ns	-0.865*
	Chlorophyll *b*	-0.266ns	-0.920**	0.023ns	-0.826*
	Total chlorophylls	0.193ns	-0.906**	0.083ns	-0.846*
	Carotenoids	0.234ns	-0.784*	-0.334ns	-0.876**
	Net photosynthesis	0.550ns	-0.832*	0.339ns	-0.868*
	Transpiration rate	-0.263ns	0.621ns	0.559ns	-0.749ns
	Water use efficiency	0.547ns	-0.621ns	-0.339ns	-0.836*
	Stomatal conductance	0.401ns	-0.758*	0.008ns	-0.788*
	Substomatal CO_2 level	0.250ns	0.043ns	0.121ns	0.291ns
	1000 seed weight	0.518ns	-0.894**	-0.136ns	-0.908**
	Seed yield per plant	0.461ns	-0.914**	0.065ns	-0.934**
Leaf hydrogen peroxide	Chlorophyll *a*	-0.110ns	-0.776*	0.097ns	-0.812*
	Chlorophyll *b*	-0.131ns	-0.762*	-0.547ns	-0.757*
	Total chlorophylls	-0.139ns	-0.773*	-0.100ns	-0.785*
	Carotenoids	0.650ns	-0.790*	0.564ns	-0.825*
	Net photosynthesis	0.173ns	-0.829*	0.245ns	-0.837*
	Transpiration rate	-0.526ns	-0.392ns	0.698ns	0.674ns
	Water use efficiency	0.424ns	-0.706ns	-0.539ns	-0.833*
	Stomatal conductance	0.165ns	-0.908**	0.153ns	-0.754*
	Substomatal CO_2 level	-0.294ns	-0.119ns	0.336ns	0.442ns
	1000 seed weight	0.200ns	-0.796*	0.097ns	-0.849*
	Seed yield per plant	0.179ns	-0.763*	-0.007ns	-0.826*

Significant at ** P<0.01; * P<0.05; and ns non-significant.

Table 4. Correlation coefficients of seed yield attributes with seed nutrient contents of mungbean varieties under control and heat stress conditions (n = 7).

Independent variable	**Dependent variable**	**2014**		**2015**	
		Control	**Heat stress**	**Control**	**Heat stress**
1000 seed weight	Seed N	-0.024ns	0.918**	-0.211ns	0.906**
	Seed P	0.181ns	0.877**	-0.223ns	0.806*
	Seed Ca	-0.243ns	0.966**	0.650ns	0.914**
	Seed K	0.392ns	0.962**	0.860*	0.961**
	Seed S	-0.343ns	0.955**	0.491ns	0.813*
Seed yield per plant	Seed N	-0.052ns	0.887**	-0.020ns	0.906**
	Seed P	0.674ns	0.942**	-0.230ns	0.806*
	Seed Ca	0.022ns	0.935**	-0.001ns	0.914**
	Seed K	-0.193ns	0.915**	0.728ns	0.961**
	Seed S	-0.370ns	0.963**	0.448ns	0.813*
Harvest index	Seed N	-0.046ns	0.860*	-0.306ns	0.903**
	Seed P	0.827*	0.947**	0.047ns	0.824*
	Seed Ca	-0.091ns	0.909**	-0.035ns	0.925**
	Seed K	-0.361ns	0.889**	0.767*	0.975**
	Seed S	-0.037ns	0.951**	0.458ns	0.824*

Significant at ** P<0.01; * P<0.05; and ns non-significant.

phases in mungbean and many other plant species, the leaf proximal to the flower or developing fruit makes a major contribution to the final yield (Wahid and Rasul, 2005). In this study analysis of trifoliate proximal leaf revealed that heat stress resulted in the enhanced production of H_2O_2 and MDA (Fig. 2), showing a distinctive loss to the thylakoid lamellae as evident from the loss of photosynthetic pigments (Fig. 2). The extent of heat damage was relatively more during 2015 due to higher temperature inside the plexiglass canopy compared to 2014 (Fig. 1). The photosynthetic pigments are an integral part of photosystems installed in the thylakoid lamellae, their loss, especially that of chlorophyll *b*, was more important. Retention of high carotenoids contents but with significant differences among the varieties was noted (Fig. 3).

It is reported that photosystem-II and photosynthetic pigments are quite labile to the oxidative damage (Sun and Guo, 2016; De Silva and Asaeda, 2017). Tight correlations of MDA and H_2O_2 during both the years in heat stressed trifoliate leaf of varieties (Table 3) indicated that heat stress initially leads to ROS generation, which damages the thylakoid membrane, as noted form enhanced MDA contents, which eventually leads to the loss of photosynthetic pigments. In this study, the tight negative correlations between MDA, H_2O_2 and the photosynthetic pigments during both the years revealed the innate heat tolerance potential of mungbean (Table 3). Among the varieties, NM-13-1 and NM-19-19 were highly sensitive to heat damage while NM-2011 and NM-2006 were the most tolerant to heat stress. It is worth noting that tolerant varieties could maintain greater carotenoids contents under heat stress, which carries significance in view of the fact that carotenoids can efficiently eliminate the free radicals associated with excited electrons due to photo-oxidation (Young and Lowe, 2018).

Regulated stomatal oscillation is important for concentrating the CO_2 for its fixation via Calvin cycle (Taiz *et al.*, 2015), while heat stress impinges adverse effect on the guard cell function (Urban *et al.*, 2017). Measurement of the trifoliate leaf for the gas exchange parameters revealed that heat stress reduced the *A*, *E*, *A*/*E* and *gs*, but did not influence the *Ci* (Fig. 4). Nonetheless, under heat stress, NM-2011 and NM-2006 performed better in comparison with other varieties in both the years. Among the gas exchange characters, it was noted that net photosynthesis and stomatal conductance had close negative correlations with the H_2O_2 and MDA contents of the leaves (Table 3). This suggested that the production of both the metabolites is damaging not only to the photosystem but also to the absorption and assimilation of CO_2 in the leaf in the photoassimilates production (Asthir, 2015).

In mungbean, trifoliate proximal leaf is a major contributor to the developing and seed filling pod and seed (Shah and Prathapasenan, 1991; Islam, 2015). From the enhanced oxidative damage, loss of photosynthetic pigments and leaf gas exchange properties, it is clear that heat stress impeded the plant performance by restricting the light harvesting and CO_2 assimilation by Rubisco (Kaur *et al*., 2015; Patriyawatya *et al*., 2018). Determination of yield and yield components revealed differential behavior of the varieties as found in terms of yield and yield components (Table 1). With large differences amongst the varieties, the seed yield related parameters showed negative response to heat stress. It was of immense importance to find that 1000 seed weight and seed yield per plant were more explicitly reduced during both the years, since the canopy temperature during 2015 was higher than in 2014 (Fig. 1). The differences in reduction in seed yield and harvest index of the varieties points to the fact that reduction in photosynthetic capacity of proximal trifoliate leaf directly impacted the photoassimilates partitioning to the developing pod; but the tolerant varieties (NM-2011 and NM-2006) were on a greater advantage. However, from the strong negative correlations of 1000 seed weight and seed yield per plant against leaf MDA and H_2O_2 (Table 3), heat stress induced oxidative stress not only affected gas exchange but also appeared to hamper the photoassimilate partitioning to the developing pod in exhibiting final seed yield.

Assimilates partitioned from leaves to the developing reproductive organs include bulk of sugars, nutrients, hormones etc. (Wahid and Rasul, 2005; Taiz *et al*., 2015). In this research the harvested seed determined for some major nutrients revealed that N was partitioned in major quantity followed by K and S. Also, there was great difference in the experimental years for the partitioning of these nutrients (Table 2). The nutrient data when correlated with 1000 seed weight, seed yield per plant and harvest index, revealed that all the nutrients were not correlated under control condition but tight positive correlations were noted under heat stress in both the years (Table 3), implying that there exists differential tendency of the mungbean varieties in the assimilate partitioning. In view of differences in the mungbean varieties, the breeding/selection for heat tolerance specifically focusing on the proximal trifoliate is possible in this crop species.

***Conclusion*:** The heat-mediated enhanced production of ROS and damage to photosynthetic system in the proximal trifoliate leaf are the main reasons for reduced production and partitioning of photoassimilates for reduced seed yield and yield components. In view of the significant differences among the varieties for heat stress induced oxidative damage, breeding and selection of materials showing high photosynthesis of proximal trifoliate leaf under heat stress may be quite beneficial for fetching higher mungbean yield in relatively warmer areas.

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