Original Research Article

Alleviating damage extent and enhancing yield through mild shading in microsperma and macrosperma genotypes of lentil (*Lens culinaris* Medikus) at terminal heat and combined stress condition.

Abstract

The threat of global climate change mainly in term of heat and combined stress is one of the utmost serious concerns for farmers, and have a negative impact on agriculture production and challenging food security worldwide. A field experiment was conducted during *Rabi* season at Pulse breeding Block, G.B Pant University of Agriculture and Technology, Pantnagar. The objective of this investigation was to effect of shading to mitigate the heat and combined stress as well as the comparison of heat and combined stress in microsperma and macrosperma genotypes. It was laid out in factorial random block design with three replications. The morpho-physiological and yield attributes of both genotypes under varied growth condition at different time interval were evaluated. The microsperma (PL-8) and macrosperma (PL-11) after shading changed their plant height (6% and 5%), NDVI value (5% and 4%), Membrane stability index (9% and 6%), days to maturity (7% and 6%) and total no of grain (15% and 37%) under heat stress while in combined stress it was changed by 8% and 10% in plant height,9% and 3% in NDVI value,14% in MSI,7% and 3% in days to maturityand13% and 23% in yield. The above finding proves the effect of shading mitigate the damage extent and also increase production of both lentil genotypes (PL-8) and (PL-11).

Keyword: Combined Stress, Shading,Microsperma and Macrosperma

Introduction

Over the past two decades, the threat of global climate change mainly due to high temperature and irregular precipitation pattern is one of the utmost serious concerns forfarmers, and researchers, as these factors have a negative impact on agriculture production and challenging food security worldwide**(Ranjan et al., 2023**).Due to the rapid and substantial release of greenhouse gases into the atmosphere, global warming is expected to exacerbate the global hunger index, including decreasing yields in lentil production. As per earlier findings, agriculture is deeply linked to climate change, and the main factor of abiotic stresses (heat, and drought) which decline the crop yield potential in range of about 30-70% worldwide**(De Santos *et al.,* 2022)**.The long -term data analysis of northern India showed that depleted soil moisture by long duration rice variety, altered precipitation pattern, delayed harvesting of crop and late sowing are the concerning reason of heat and water stress in lentil production**(Choukri et al., 2020).**

Terminal drought can suppress nearly all the processes of lentil growth and metabolism, damaged membranes by 21–40%, causing heavy yield losses, as it reduces flower production, pod number, and seed number **(Bhandari *et al.,* 2016).**Similarly,timing and intensity of exposure to high temperature are critical such as late sown pulses face heat stress at their reproductive stage, which damage the crop significantly**(Sita *et al*., 2017).**Among all stages, reproductive stage which determine the overall performance of crop is negatively impacted to terminal heat and water stress. There are several biochemical mitigation approaches has been used to reduce the biochemical and physiological impairment of crop at sensitive stage. To avoid the problem of high temperature and high intensity of solar radiation during late spring and summer period, growers reduce the incident radiation and alleviate some damage extent by using shading screens and shade cloth **(López-Marín et al., 2012).**

As the, previous studies suggested that shading helps to lessen the adverse effects of water stress by safeguarding the photosynthetic machinery from excess energy use, which supports higher photosynthetic rates even under water-stressed conditions **(DaMatta 2004)**. Some other process such as reduced leaf and air temperatures, lower vapor pressure deficits which helps to maintain plant water status, and diminished incoming solar radiation, which can ease oxidative stress **(Holmgren 2000)**.Artificial shading is one of the contemptible and amiable strategy to alleviate the damage extent of plant to continual rising temperature**(Zhang et al., 2021)**. However, very few studies are documented regarding shading in pulses, due to heat stress. Under shading condition, reduction of ambient heat of existing air, as the most of the energy are utilized by evaporation process, instead of going to sensible heat, therefore cool air temperature, known as evaporative cooling **(Dussadee*et al.,* 2018**).Shading improves the redistribution of storage dry matter from vegetative organs into grains. It has been demonstrated that grain filling relies on redistributed reserves from vegetative organs after anthesis **(Mensah et al., 2022).**

The objective of this research was to evaluate the effects of mild shading in comparison to widely followed standard conditions on microsperma and macrosperma genotypes of lentil under both heat and water-stressed environments. The study also aimed to the impact of terminal heat and water stress on yield losses and to determine mild shading could be a recommended strategy to mitigate these stresses.

Material Methods

The field experiment was conducted during *Rabi* season of 2022 at the Norman Borlaug Crop Research Centre. G.B. Pant University of Agriculture and Technology, Pantnagar, district U.S. Nagar, Uttarakhand. Geographically, Pantnagar is situated at the foot hills of Himalayas at 29^0 North latitude and 79.5^0 East longitude and an altitude of 243.83 m above mean sea level.

Treatment Detail and Experimental Design

The experiment was conducted in factorial Randomized block design and were replicated thrice.It comprises 2 genotypes of lentil i.e., PL-8 (microsperma), PL-11 (macrosperma) and 4 treatments for each genotype, which was sown on 28 November and harvested in 17 April.For shading, shade tunnel was made just one week before initiation of flowering and theheight of shade tunnelwere 3.5 feet and direction are east to west maintained in the field.Control treatment known as standard practice in which lentil were grown in open condition with irrigation at 2 critical stages i.e., just before flowering and after 50 % of flowering.Thetotal 8 treatment combination were proposed in the experiment.

Soil and Weather Condition

The soil of Tarai region (Mollisols) has been developed from calcareous medium to moderately coarse textured parent material under the predominance of forest vegetation and poorly to moderately drained conditions. The climatic condition of experimental site experiences sub-tropical climate. The meteorological parameters for year 2020, namely minimum and maximum temperatures, has gone at the time of reproductive stage in the range of 12□-35□ during the experimental period were recorded from meteorological observatory .

Observations

Plant Height

Plant height was measured from soil surface of anchored root to tip of the main growing stem of 5 tagged plant in each treated plant with the help of meter scale. It was recorded at harvest under irrigated and rainfed conditions and expressed in cm.

Days to maturity

It was measured by regular supervision of field and count days to maturity in each replication of every treatment.

Membrane Stability Index

100mg fresh lentil leaf sample were collected from 2-3rd node from the top beneath to the flowers during 9 am to 11 am at initiation of flowering and 7 days after each spray of GABA. A 2 set of test tube, in which leaf sample and 10 ml of double distilled water was taken. Out of 2 set, one set of test tube will keep in water bath and heated at 40°C for 30 min. Thereafter, measure the electrical conductivity (EC) of the solution by conductivity metre, which is expressed as (C1). Similarly, the second set of test tube were kept in water bath at 100°C for 20 min and measure EC which is expressed in C2. The membrane stability Index (MSI) will measure by following formula:

$$MSI = [1 - (C1/C2)] \times 100$$

Normalized difference vegetation index (NDVI)

NDVI ratio is a common index used to evaluate vegetation cover were recorded by green Seeker instrument at flowering stage.It is calculated by following formula:

$$NDVI= (NIR-PAR)/(NIR+PAR)$$

Number of seeds per plant

The number of total seeds per plant were counted after harvesting of 5 randomly tagged plant of every replication and separate in labelled envelope.

Statistical Analysis

Data obtained from various observations was statistically analysed in two parts. Comparison of factor A (Genotypes) and factor B (Treatments) was done as per procedure of factorial random block design using standard techniques of analysis of variance (ANOVA) as per the procedures given by **Gomez and Gomez (1984).** The critical difference at 5% level of probability was calculated for testing the significance of difference between any two means wherever 'F' test was found significant.

Results

Plant height

In the present investigation as depicted in Fig.2. the plant height under heat stress and heat stress +water stress in both microsperma (PL-8) and macrosperma (PL-11) showed significant difference at harvest. In a comparison of growth conditions, under heat stress, microsperma (PL-8) has attained 7.4% increased height compared to combined stress (heat and water stress), while macrosperma (PL-11) has attained 3.5% more height compared to combine stress condition. After shading, under heat stress, microsperma (PL-8), plant height (48.6cm) increased to height (51.6cm), which is significantly taller by 6.2%, while in macrosperma (PL-11), plant height after shading (50.3cm), which is taller by 4.8%. Moreover, after shading in mirosperma (PL-8) and macrosperma (PL-11) plant height (41.6cm and 42.3cm) grown under combined stress (heat and water stress) was increased to height (45cm and 46.3cm),which was significantly taller by 8.2% and 9.5% respectively.

Table 1 : Plant Height (cm)

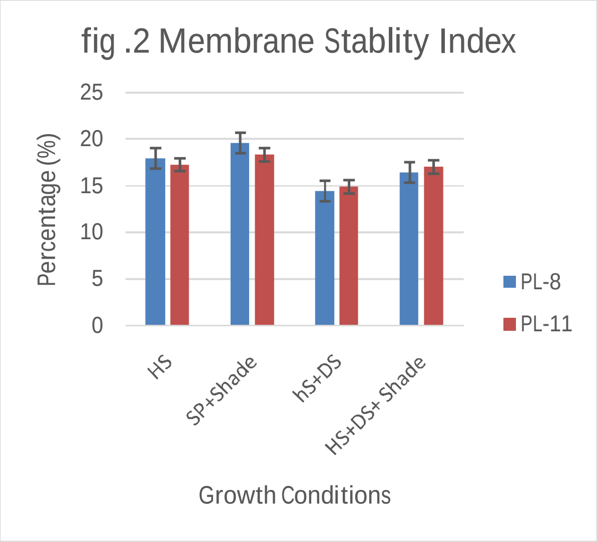
	PL-8	**% Change**	**PL-11**	**% Change**
Heat Stress	48.60		48.00	
Heat Stress+25% Shade	51.67	↑6%	50.33	↑5%
Heat stress+ Water stress	41.67	↓19%	42.13	↓12%
Heat stress+ Water stress+ 25% shade	45.00	↑8%	46.33	↑10%
Mean	46.75		46.70	
	Genotype		Treatment	G*T
SE(m)	0.273		0.386	0.546
C.D (0.05)	NS		1.183	NS

Membrane Stablity Index

In the present investigation as depicted in Fig.3. the membrane stability index under heat stress and heat stress +water stress in both microsperma (PL-8) and macrosperma (PL-11) showed significant difference at the time of flower initiation. In a comparison of growth conditions, under heat stress, membrane stability index in microsperma (PL-8) was increased by 26% compared to combined stress (heat and water stress), while macrosperma (PL-11) increased their stability index by 14% compared to combine stress condition. After shading, under heat stress, microsperma (PL-8), the membrane stability index (17.97) increased to (19.6), which is significantly by 9%, while in macrosperma (PL-11), after shading membrane stability index was 18.37 which is higher by 6%. Moreover after shading, microsperma (PL-8) and macrosperma (PL-11), grown under combined stress (heat and water stress) was increased their the membrane stability index (16.4 and 17.03), which was significantly higher by 14% each , after shading.

Table 2 : Membrane Stablity Index

	PL-8	**% Change**	**PL-11**	**% Change**
Heat Stress	17.97		17.28	
Heat Stress+25% Shade	19.60	↑9%	18.37	↑6%
Heat stress+ Water stress	14.47	↓26%	14.93	↓14%
Heat stress+ Water stress+ 25% shade	16.44	↑14%	17.03	↑14%
Mean	17.12		16.90	
	Genotype		Treatment	G*T
SE(m)	0.112		0.158	0.224
C.D (0.05)	NS		0.484	0.685

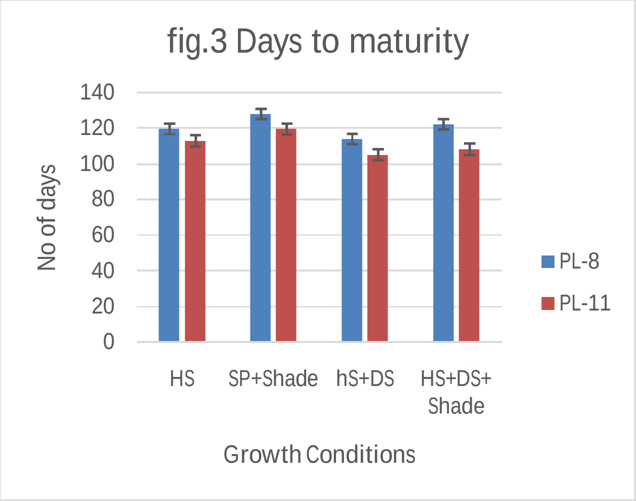


Days to maturity

In the present investigation as depicted in Fig.3. the days taken to maturity under heat stress and heat stress +water stress in both microsperma (PL-8) and macrosperma (PL-11) showed significant difference at harvest. In addition, there was a significant difference between between microsperma (PL-8) and macrosperma (PL-11) in maturity. In a comparison of growth conditions, under heat stress, the days taken to maturity in microsperma (PL-8) was increased by 11% compared to combined stress (heat and water stress), while macrosperma (PL-11) has taken days to attain maturity was increased by 12% compared to combine stress condition. After shading, under heat stress, microsperma (PL-8), the total days taken to maturity (119 days) increased to (128 days), which is significantly by 7%, while in macrosperma (PL-11), days taken to maturity after shading (119days), which is taller by 6%. Moreover, after shading in microsperma (PL-8) and macrosperma (PL-11), total days taken to maturity (114 days and 105days) grown under combined stress (heat and water stress) was increased to grain no (122 and 108 days), which was significantly higher by 7% and 3% respectively.

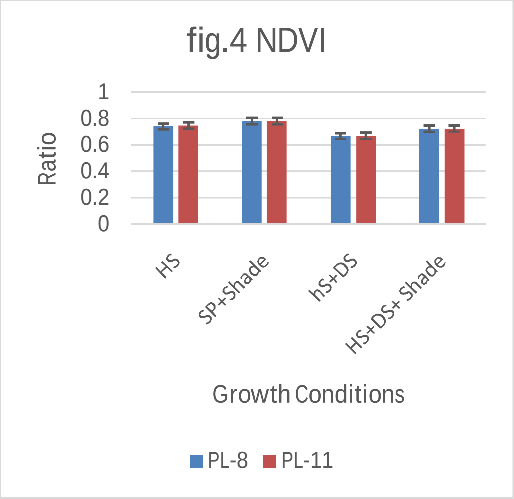
Table 3 Days to maturity

	PL-8	% Change	PL-11	% Change
Heat Stress	119.7		113	
Heat Stress+25% Shade	128.0	↑7%	119.667	↑6%
Heat stress+ Water stress	114.0	↓11%	105.333	↓12%
Heat stress+ Water stress+ 25% shade	122.3	↑7%	108.333	↓3%
Mean	121.0		111.583	
	Genotype		Treatment	G*T
SE(m)	0.54		0.764	1.08
C.D (0.05)	1.654		2.339	3.308



Normalized Difference vegetation Index

In the present investigation as depicted in Fig.3. the NDVI values under heat stress and heat stress +water stress in both microsperma (PL-8) and macrosperma (PL-11) showed significant difference at the time of pod initiation. In a comparison of growth conditions, under heat stress, NDVI values in microsperma (PL-8) was increased by 9 % compared to combined stress (heat and water stress), while macrosperma (PL-11) increased their NDVI values by 3% compared to combine stress condition. After shading, under heat stress, microsperma (PL-8) and macrosperma (PL-11), the NDVI ratio(0.74 and 0.75) increased to (0.78cm), which is significantly by 4% and 5% respectively. Moreover, after shading, microsperma (PL-8) and macrosperma (PL-11), grown under combined stress (heat and water stress) was increased their the NDVI values to 0.731 and 0.733, which was significantly higher by 9% and 8% respectively.



Total no of grain/plant

In the present investigation as depicted in Fig.3. the total no of grain per plant under heat stress and heat stress +water stress in both microsperma (PL-8) and macrosperma (PL-11) showed significant difference at harvest. In addition, there was a significant difference between between microsperma (PL-8) and macrosperma (PL-11). In a comparison of growth conditions, under heat stress, the total grain no per plant in microsperma (PL-8)was increased by20% compared to combined stress (heat and water stress), while macrosperma (PL-11) has total grain no per plantincreased by 23% compared to combine stress condition. After shading, under heat stress, microsperma (PL-8), the total grain no per plant (147) increased to (169), which is significantly by 15 %, while in macrosperma (PL-11), total grain no per plant after shading (156), which is higher by 37%. Moreover, after shading in microsperma (PL-8) and macrosperma (PL-11), total grain no per plant (114 and 87) grown under combined stress (heat and water stress) was increased to grain no (129 and 107), which was significantly higher by 13% and 23% respectively.

Table 4 Total no of grain/plant

	PL-8	% Change	PL-11	% Change
Heat Stress	147		114	
Heat Stress+25% Shade	169	↑15%	156.667	↑37%
Heat stress+ Water stress	114	↓20%	87.333	↓23%
Heat stress+ Water stress+ 25% shade	129	↑13%	107.667	↑23%
Mean	139.75		116.417	
	Genotype		Treatment	G*T
SE(m)	1.507		2.131	3.014
C.D (0.05)	4.615		6.526	9.229

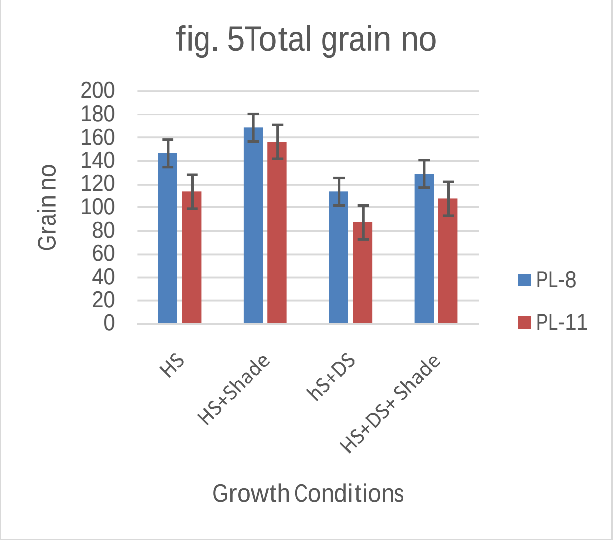


fig. 5Total grain no

Discussion

The growth of plants depends on how effectively it harnesses available growth factors, particularly nutrients, to thrive.In the present investigation, the plant height of both genotypes of lentil were shorter under heat and combined stress at harvest, suggesting that reduced height during stress is correlated with suppressed expression of growth-related metabolism, as well as disruptions to essential physiological processes like cell elongation and growth rate, leading to stunted plant growth (Rollins *et al*., 2013).A similar reduction has been also observed in chickpea (**Awasthi *et al*.,2014),** faba bean (Siddiqui *et al*., 2015), and common bean **(Beebe *et al*., 2013).**

Under shading condition, one of the foremost physiological responses is increases in plant height which is normally associated with their ability to intercept light efficiently and same was observed in our study (Fig 2). Our research finding indicates that low light was inversely correlated to heat stress and water accessibility, such that plants can grow effectively at mild shade conditions because of the negative trade-off created between shade and heat stress tolerances which is in line with **Youn *et al.,* (2021).**In the combined stress condition, MSI in both of the genotypes (PL-8 and PL-11) was significantly reduced compared to heat stress due to reduction in water potential gradient between soil Vs plant, and may bedue to director indirecteffects of extreme heat involving oxidation. which is consisted with previous finding (**Sita *et al.,* 2023)**.In the current investigation during heat stress,theMSIdecreaseddue to oxidative stress which severely affects the cellular activity, which is also consisted with the previous findings inlentil,*Cicer arietinum* under heat stress**(Sita *et al.,* 2017)**. The 25 % mild Shading condition protects membrane disintegration by reduce the degradation of D1protein configuration and loss of cellular functioningwhich maintain a cooler environment and minimize excessive water evaporation **(Dussadee et al., 2018)**.Higher NDVI values indicate greater leaf greenness and more green leaf biomass. A huge significant reduction in NDVI ratio under heat stress in both genotypes might be ue to increased activity of chlorophyllase and chlorophyll-degrading peroxidase increases and the content of chl is severely reduced (**Hu et al., 2020)**. In the current investigation, NDVI ratio was significantly less compared to control due to combined (heat and water) stress, plants reduce their chlorophyll content by closing their stomata. This process helps limit water loss through evaporation but also increases resistance to the intake of atmospheric CO_2, which is essential for photosynthesis**(Thakur *et al.,*2020).**Under shading condition, highest NDVI ratio has been observed, could be at low PAR value increased chlorophyll content and rate of CO_2 fixed as explained in the previous studies **(Kappel *et al.,* 1983).**Under water stress condition, due to scarcity of abundant photosynthate and other factor, quicker survival mechanism has followed, and produced seed quickly and matured. Moreover, drastic hastening of reproductive phase during heat stress due to forced maturity in extreme temperature also evidenced in finding of **(Lamichaney et al., 2021).**Under heat stress condition, due to scarcity of abundant photosynthate and other factor, quicker survival mechanism has followed, and produced seed quickly and matured. Moreover, drastic hastening of reproductive phase during combined stress due to forced maturity in extreme temperature also evidenced in finding of **(Lamichaney et al., 2021).** After shading condition, ambient temperature has been faced by plant, which could divert the energy to vegetative phase and modulator SVP stability was increased, hence plant more and gently translocate the photosynthate to grain (**Jin et al., 2022).**

Basically, yield attributesare the cumulative result of all physio- biochemical and molecular aspects of both source and sink strength for photo assimilates as well as nutrients over the course of seed development. Due to accelerated maturity under heat stress, there was not proper grain filling, resulting significantly less no of grain.However, drastic accelerated maturity and severe loss of nutrient due to water stress hampered the food translocation in pod. Hence severe reduction grain no per plant. After shading in both stress gradually and proper translocation of food due to abundant photosynthetic pigments which is also supported by similar to **Alon et al., (2022).**

Conclusion

In conclusion, the results of the present investigation inferred that combined stress damaged more crop compared to heat stress. Moreover, microsperma (PL-8) has more tolerance to heat and combined stress compared to macrosperma (PL-11). After shading, the damage extent has been ameliorated which directly correlated to increased grain no per plant.

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