Improving the Productivity of *Camelina Sativa* L. Using Exogenous Application of Sulphur

Foliar spray of sulphur in heat stressed camelina

Tayyub Hussain[2,3], Abdul Shakoor[1,3,*], Umm e Hani[3,4], Safdar Ahmad[3], Ans Bin Abdul Razzaq[5], Ijaz Ahmad[6], Yousof Zahoor[3], Lubaba Komal[7], Laiba Kamal[8], Amina Iftkhar[3], Ali Raza[3], Samman Gul Vaseer[1]
[1] *Wheat Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan*
[2] *Maize, Sorghum and Millet Program, Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan*
[3] *Department of Agronomy, University of Agriculture Faisalabad Pakistan*
[4] *Department of Botany, University of Agriculture Faisalabad Pakistan*
[5] *Department of Agronomy, University of Agriculture Faisalabad Sub-Campus Burewal Pakistan*
[6] *Pulses Program Crop Sciences Institute, National Agricultural Research Centre, Islamabad Pakistan*
[7] *Institute of Botany, University of Punjab, Lahore Pakistan*
[8] *Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad Pakistan*
[*] Corresponding author’s email: *shakoor2914@gmail.com*

Abstract

Heat stress is a major detriment to crop growth and yield under the current patterns of climate change. The present study was aimed to compare the thermo-sensitivity of different camelina genotypes and optimizing exogenous sulphur to alleviate impacts of heat stress and to explore water relation attributes triggered regulations in yield related attributes of camelina. The experiment was conducted at University of Agriculture, Faisalabad in November 2019-20. The experimental design was used RCBD as split-split arrangement alongside three replications. The treatment comprised of heat stress T_1 = No stress (26^{o}C) and T_2 = Heat stress (32^{o} C) as main plot factor, two camelina genotypes G_{611} = Camelina 611 and G_{618} = Camelina 618 as split plot and exogenous sulphur application S_0 = 0 ppm and S_{1000} = 1000 ppm as split-split plot factors. Heat stress was induced at anthesis stage by covering the plants with transparent polythene sheet and exogenous application of sulphur was applied after imposition of heat stress. Heat stress badly affected the physiological parameters including water potential, osmotic potential and relative water contents. The traits related to water relations decreased by 36% under heat stress as compared to no stress. Yield related traits including no. of seeds, no. of branches, no. of siliques, 1000-seed weight and economical yield were negatively affected by heat stress. Exogenous application of sulphur proved helpful in reducing the heat stress effects in both genotypes of camelina. Sulphur at the rate of 1000 ppm enhanced the seed yield by 29% by improving the water potential and other physiological traits. The camelina genotype 618 performed better in heat stress condition as compared to camelina genotype 611. In crux, these findings indicated that thiourea as source of sulphur improved the heat stress tolerance in camelina, which might be attributed to maintenance of plant water status.

Key words: Camelina; Heat stress; Thiourea; Foliar spray; Genotypes

Introduction:

Camelina (*Camelina sativa* L.) is a substitute oilseed crop and cultivated globally (Schillinger, 2019). It is new crop and also recognized as false flax or gold of pleasure and grown mostly in northern temperate regions of North America and Europe as oil seed crop (Chaturvedi *et al*., 2018) that belong to family Brassicaceae (Righini *et al*., 2019). Camelina is a climate-resilient oilseed crop with a unique oil profile comprising omega-3 fatty acids (Morales *et al*., 2017) and up to 52% polyunsaturated fatty acids (Obeng *et al*., 2019), which is a rare characteristic in vegetable oils. Temperature and precipitation frequency changes caused by climate change are the most serious hazards to global food security (Yuan *et al*., 2017). The major extremes of climate change are high atmospheric temperature and drought, which have negative effects on crop growth as these stresses can reduce crop yields by up to 50% (Hasanuzzaman *et al*., 2020). Camelina is sensitive to day temperatures above 35°C, which results in a drastic reduction in photosynthetic rate, resulting in lower seed yield (Carmo-Silva and Salvucci, 2012).

Heat stress appears more sensitive to flowering than vegetative growth (Abeli *et al*., 2012). The extreme temperature also hampers the camelina's grain quality and yield (Wang *et al*., 2020). It affects the oil and linolenic acid contents of camelina which are greater in the cooler environment as compared to warmer (Obour *et al*., 2017). When the temperatures rise above 25^{o}C during the flowering and grain filling period of camelina, its saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs) increase while its polyunsaturated fatty acids (PUFAs) decrease. The reason behind this occurring is that heat stress during the flowering and grain filling period negatively affects the enzymes that synthesize PUFAs, but positively affects the enzyme that synthesizes SFAs and MUFAs (Singer *et al*., 2016).

Nutrient application has been shown to improve heat stress tolerance in agricultural plants by altering numerous metabolic pathways in plants. Mineral nutrition is one of the different technologies in agricultural practice against abiotic stress (Ahmad *et al*., 2022). Application of different plant nutrients that are necessary for plant growth, metabolism, and stress regulation. Thiourea, as a sulphur source, is a significant stress-relieving chemical with the ability to scavenge hydroxyl or superoxide radicals (Sahu, 2017). It plays a critical role in the initiation of (ROS) reactive oxygen species foraging enzymes to enhance antioxidant defence under abiotic stresses (Bashir *et al*., 2015). It plays an important role in plant development because it is an important part of essential metabolic compounds such as glutathione, protein and amino acids (methionine and cysteine). It is importantly required in protein synthesis as well as in the development of chlorophyll in plants. Thiourea's water-soluble characteristics allow it to be easily absorbed, reducing stress damage and increasing crop output under stressful conditions (Devi *et al*., 2015). It increases the concentration of oil, glucosinolate and protein in the seed (Malhi *et al.*, 2007).

The study hypothesised that thiourea, a sulphur source, would improve growth and yield in camelina under heat stress. The specific objective of this study was to evaluate the performance of foliar applied sulphur in the form of thiourea to ameliorate the heat stress effect in camelina crop.

Material and Methods:

Experimental site:

A research experiment was executed in semi-arid climatic conditions during 2019-2020 at University of Agriculture Faisalabad, Punjab, Pakistan.

Plant Material:

Seed of camelina genotypes (611 and 618) were procured from the Stress Physiology Lab, Department of Agronomy, University of Agriculture, Faisalabad Punjab, Pakistan.

Treatments:

The experiment comprised of three variables (a) heat stress as a main plot factor; T_1 = No stress (26^{o}C), T_2 = Heat stress (32^o C), (b) Camelina genotypes G_{611} = Camelina 611, G_{618} = Camelina 618 as sub plot factor and (c) rate of sulphur exogenous applications (S_0: 0 ppm and S_{1000}: 1000 ppm) as a sub-sub plot factor.

Imposition of Treatments:

Heat stress was imposed using perforated and transparent polythene sheets (Kamal *et al*., 2017; Shahid *et al*., 2017a, b). Five plants were tagged in each experimental unit to determine the initiation of different terminal phenological stages and heat stress was imposed as per treatment when 50% of the plants reached the growth stage. Temperature of control and heat-stressed main plots was recorded during imposition of heat stress with help of digital temperature and humidity probe (Digital Multimeter-50302). The source of sulphur was thiourea (CH_4N_2S). Sulphur was foliar-applied using hand sprayer at rate of 300 L per hectare in all main plots after exposure of heat stress imposition at anthesis.

Experimental Design:

The experiment was laid out in Randomized Complete Block Design (RCBD) under split- split treatments' structure. Heat stress was randomized in main plots, camelina genotypes as split plot and foliar sulphur was applied as split-split plot. Treatments were replicated thrice.

Statistical Analysis:

Standard procedures were adapted to record response of various variables and significance of varying sources of variations was determined using Fisher analysis of variance technique while different means were compared using Tukey's Honestly Significant Difference (Tukey's HSD) test at 5% probability (Steel *et al*., 1997).

Agronomic Practices:

The crop was sown on November 25, 2019. Seed was used at the rate of 2.5 kg ha^{-1}. Pora method was used for sowing by keeping row-row distance 22.5 cm. According to the requirement of crop irrigation was done. The recommended dose (50, 30 and 60 kg ha^{-1}) of N, P and K respectively was applied. The nitrogen was applied in the form urea (46% N) while P and K was applied in the form of DAP (16% N and 46% P_2O_5) and sulphate of potash (50% K_2O) respectively. Precautionary measures were taken to protect the crop plants from insect pest. Imidacloprid @ 1.5 g L^{-1} of water was applied to control sucking insect. Harvesting was done manually, when colour of 90% of the pods of crop become brown. The crop was sun dried for one week in the field. At the end the threshing was done manually to get seed and yield parameters were recorded.

Observation Recorded:

Physiological traits

The leaf water potential (Ψ_w) was calculated in the morning time from 8.00 am to 10:00 am using (Leaf Pressure Chamber instrument, PMS International Company, Model 600). The leaves which were used for water potential, placed at the refrigerator (-20°C) for 10 days to measure the osmotic potential/solute potential (Ψ_s). After 10 days these frozen leaves and crush using glass rod and sap was taken in eppendorf tubes with the help of syringes. Calibrated Cryoscopic osmometer (Osmomat 030-D, Cryoscopic osmometer printer, Genatec) was used to measure osmotic potential using cell sap. Leaf turgor potential is measured by using formula (Scholander *et al*., 1964).

$$(\Psi_p) = (\Psi_w) - (\Psi_s)$$

Samples of three leaves were taken early in the morning from each treatment. The fresh weight (FW) of each leaf was measured using digital balance (Shimadzu AW-320, Kyoto, Japan). After recording the fresh weight, disposable cups filled with distilled water were taken and leaves were dipped in these cups for whole night to attain turgor weight. After 24 hours the leaves were withdrawn from cups for recording the turgid weight (TW). After taking turgid weight, leaves were packed and placed in oven at 65°C for two days. At the end the dry weight (DW) of leaves were recorded using digital balance. Following formula was used to measure relative water content (Karrou and Maranville, 1995)

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

Yield and yield components

For calculation of number of siliques per plant four plants were harvested from each treatment and counted manually without personal biasness and then take average. To measure no. of seeds per silique, randomly ten siliques were harvested and threshed. Count number of seeds in harvested siliquas separately and then taken average. For calculating 1000-seed weight, 100 seeds were counted manually and then weighed using digital balance (Shimadzu AW-320, Kyoto, Japan) for each treatment and figured in grams. By using standard procedure 100 seeds weight was converted into 1000 seeds weight. The crop per plot was harvested and threshed manually to separate the seed. The biological yield, economical yield and harvest index were calculated per plot and converted into ton per hectare.

Results:

Physiological traits

Water relating traits including leaf water potential (LWP), leaf osmotic potential (LOP), leaf pressure potential (LPP) and relative water contents (RWC) of camelina genotypes (611 and 618) were significantly influenced by heat stress and sulphur application (**Figs.** 2-3). Heat stress reduces the LWP and RWC by 36% and 28% respectively as compared to no stress. Sulphur spray significantly improved the LWP and RWC under heat stress conditions. Sulphur application (S_{1000} = 1000 ppm) increased the water relations and RWC by 19% and 25% respectively as compared to no sulphur spray (**Figs.** 2-3). Camelina genotype (G_{618} = 618) performed better in all water relation traits as well as RWC by the foliar spray of sulphur under heat stress as compared camelina genotype (G_{611} = 611). Camelina genotype G_{618} proved heat stress tolerant as compared to camelina genotype G_{611}. Camelina genotype 618 performed better regarding (all water relation traits and RWC) as compared to camelina genotype 611 (**Figs.** 2-3).

Yield and yield components

All yield traits were significantly influenced by heat stress (**Fig.** 4, **Table** 1). Heat stress decreases the plant height, no. of siliquae per plant, no of seeds per plant and 1000 seed weight by 24%, 11%, 40% and 30% respectively as compared to no heat stress. Maximum No. of siliquae per plant (224), No. of seed per plant (15) and thousand seed weight (1.14 g) were observed with foliar application of sulphur (1000 ppm) as compared to no sulphur application (**Table** 1). The biological yield, economical yield and harvest index were also significantly affected under heat stress (**Figs.** 4 a, b & c). Heat stress diminished the biological yield and economical yield by 28% and 31% respectively. Foliar application of 1000 ppm sulphur improved the biological yield and seed yield by 22% and 29% respectively. Among both genotypes of camelina, higher seed yield was recorded in camelina genotype 618 as compared to camelina genotype 611 (**Figs.** 4 b).

Principal Component Analysis:

Principal component analysis was carried out around all variables under study for the determination of the influence of exogenous sulphur application on camelina subjected to heat stress (**Fig.** 1). Two components, PC1 and PC2 were subjected to principal component analysis. The total variance was shared in the range of 32.1 and 17.9% by PC1 and PC2, respectively.

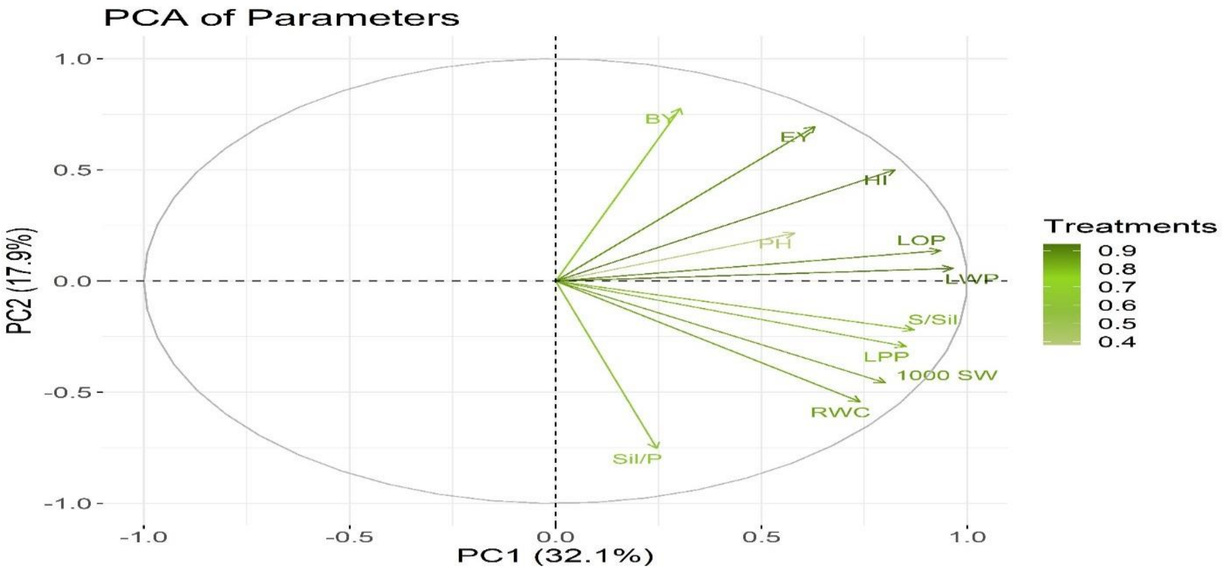

Figure 1: Principal component analysis of the various parameter under study

LOP = Leaf osmotic potential; LWP = Leaf water potential; LPP = Leaf pressure potential; RWC = Relative water contents; 1000 SW = 1000 seed weight; EC = Economical yield; BY = Biological yield; HI = Harvest index

Discussion:

Crop yields are influenced by the changing climatic conditions including temperature uncertainties. High temperature stress restrained the development and yield credits by increasing osmotic pressure that prompted morphophysiological changes in plants and reduction water relations and gas trade ascribes. For the effective development, plants have advanced different versatile systems to reclaim the warmth stress harms, for example, they regulate their digestion by the exogenously applied development advertisers that assisted them with contending heat stress (Kumar *et al*., 2020). The result revealed the negative impact of heat stress on water relations and RWC which as a result negatively influenced the yield -aactivities and did not permit camelina plant to perform well (**Figs.** 2-3, Ahmed *et al*., 2021). The overrun of ROS and lessened antioxidative activities causes reduction in the metabolic efficiency in addition to oxidative damage to the plant cells assemblies due to heat stress (Hasanuzzaman *et al*., 2020). Heat stress causes reduction in net photosynthetic rate, CO_2 uptake rate and leaf water potential in leaves (Abeli *et al*., 2012), leading to lessened sources of photosynthate, hampering the production of additional source sinks. Result revealed that Camelina 611 highly affected by heat stress than Camelina 618 which is hardly affect by increased temperature (heat stress). Camelina plants that observed more heat stress had more lower water potential, osmotic potential, pressure potential and relative water contents (**Figs.** 2-3, **Table** 1) This study is correlated with the Ashraf and Hafeez (2004) who revealed that stomata close due to higher temperature that diminished the photosynthesis which is severely affected the intracellular CO_2. Other scientist also stated that increased temperature limited the stomatal activity. Heat shock result in lessening of photosynthetic pigment (Wang *et al*., 2009). Sulphur foliar spray play role in maintaining and regulating the morphophysiological traits including water potential, relative water contents and yield traits in camelina genotypes under increased temperature (**Figs.** 2-3, **Table** 1). Freeha *et al.* (2008) revealed that RWC is a rundown of water status of tissue and foliar usage of Thiourea (sulphur) at critical period of the yield expressively additionally created water output by keeping up with higher leaf water status and controlling stomatal opening, which allowed plants to use water under tension conditions. Similarly, Abbas *et al*. (2015) revealed that S scarcely activate the antioxidant defence system of plant result in increasing the water potential. Further, Camelina genotype 618 show better physiological traits as compared to camelina genotype 611 due to heat tolerant (**Figs.** 2-3). This is aligned with Heidari (2009) who revealed that highest antioxidant activity exhibited by tolerant crops that as contrasted to sensitive crop produce increased yield.

Yield parameters including no. of siliquae/ plant, 1000-seed weight and seed yield were influenced by heat stress (**Fig.** 4, **Table** 1). All these parameters perform better in no stress condition as compared to heat stress. Increased temperature caused reduction in these traits. Ahamed *et al*. (2010) who stated that yield and yield components are affected by heat stress due to impedance in growth.

Foliar sulphur 1000 ppm caused increased in no. of siliquae, 1000-seed weight and seed yield as compared to no sulphur (**Fig.** 4, **Table** 1). The numbers of siliqua per plant in mustards increased after foliar application of 0.1 percent Thiourea (S source) (Premi *et al*., 2006). These outcomes are also in line with the findings of Imran *et al*. (2015), who found that the number of pods per plant increases as sulphur levels rise. Ghosh *et al*. (2000) observed that foliar applying S at various levels increased the number of siliquae per plant in canola cultivars. These results are related to those of Malik *et al.* (2004) and Imran *et al.* (2015), who found a substantial difference in the number of seeds per capsule due to S levels. Sulphur increased the availability of photosynthates to pods, allowing seeds to reach their full potential, as evidenced by an apparent increase in 1000-seed weight (**Fig.** 4, **Table** 1, Malhi *et al*., 2002). These findings are also consistent with those of Begum *et al.* (2012) and Sattar *et al.* (2011), who found that when S levels were increased, yield increased. Camelina 618 genotype showed higher yield performance as compared to camelina 611 genotype. Variations in yields between these genotypes might be due to genetic makeup and absorption of nutrients.

Conclusion:

Sulphur had key role to adjust plant defensive system against heat stress in camelina. The foliar application of sulphur helped to mitigate the heat stress affect and improved yield traits by improving physiological traits (water relation attributes and RWC) under heat stress. Foliar spray 1000 ppm sulphur may improve the yield traits of camelina. Camelina genotype 618 was least affected by heat stress as compared to camelina 611. Hence, camelina genotype 618 was more heat tolerant as compared to camelina genotype 611.

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Table 1: Influence of exogenous application of sulphur on yield traits of heat stressed camelina genotypes

Heat Stress	Camelina Genotypes	Sulphur (S) application	Plant height (cm)	No. of siliquae per plant	No. of seeds per siliquae	1000-grain weight (g)
No heat stress	G_{611}	S_0 = 0 ppm	99.03 a	216.7 cd	13 c	1.007 bcd
		S_{1000} = 1000 ppm	104.23 a	224.0 b	15 b	1.143 abc
	G_{618}	S_0 = 0 ppm	102.37 a	221.0 bc	16 b	1.243 ab
		S_{1000} = 1000 ppm	100.16 a	229.3 a	20 a	1.37 a
Heat stress	G_{611}	S_0 = 0 ppm	75.13 a	200.3 g	6 e	0.843 d
		S_{1000} = 1000 ppm	78.73 a	210.7 ef	8 d	0.847 d
	G_{618}	S_0 = 0 ppm	80.47 a	207.0 f	10 d	0.867 cd
		S_{100} =1000ppm	83.43 a	215.7 de	13 c	0.930 cd

Any two means not sharing a letter in common and differ significantly at significance level ($p \leq 0.05$)

Figure 2: Influence of exogenous application of sulphur on (a) leaf water potential (-MPa) and (b) leaf osmotic potential (-MPa) of heat stressed camelina genotypes

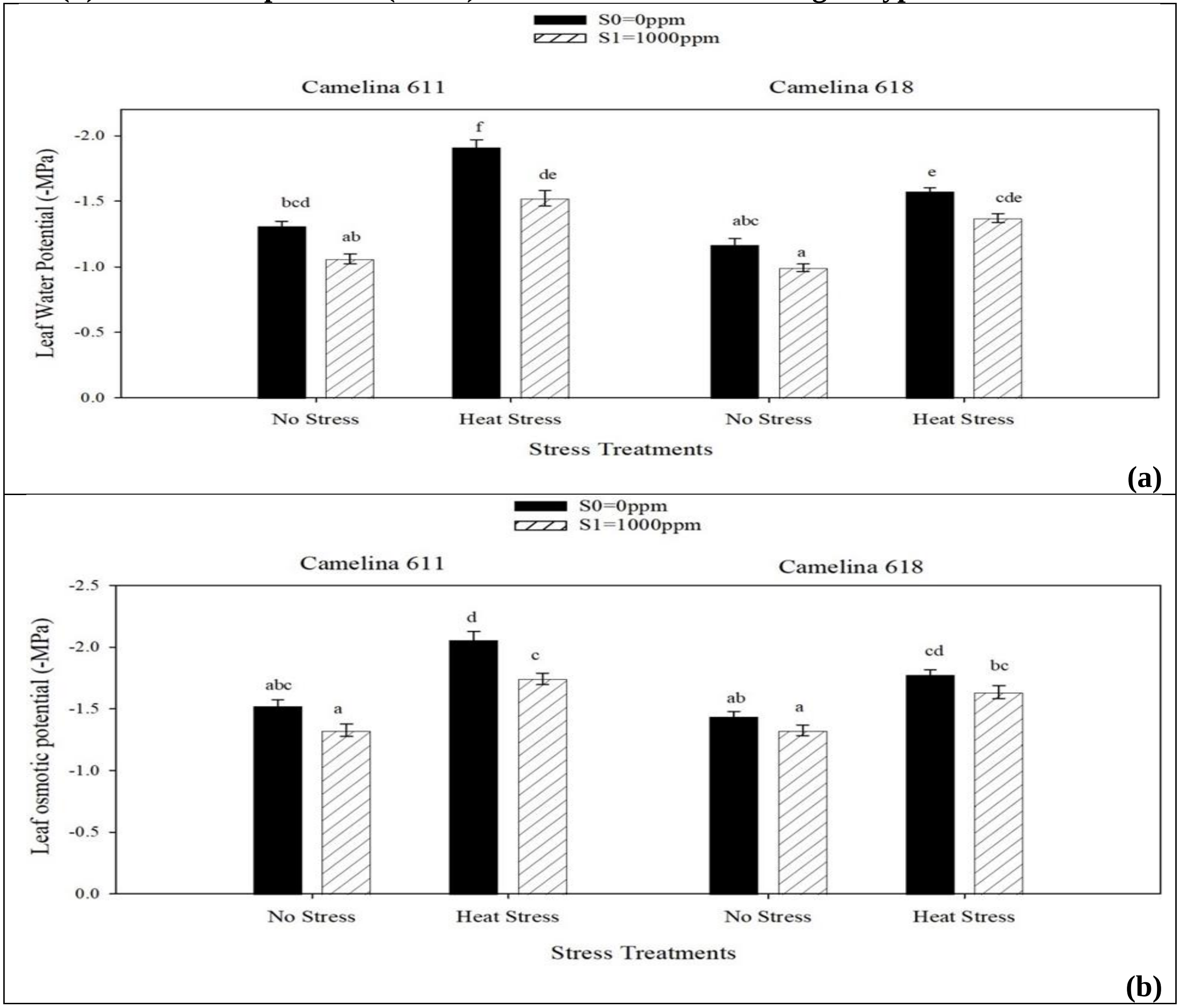


Figure 3: Influence of exogenous application of sulphur on (a) leaf pressure potential (-MPa) and (b) relative water contents (%) of heat stressed camelina genotypes

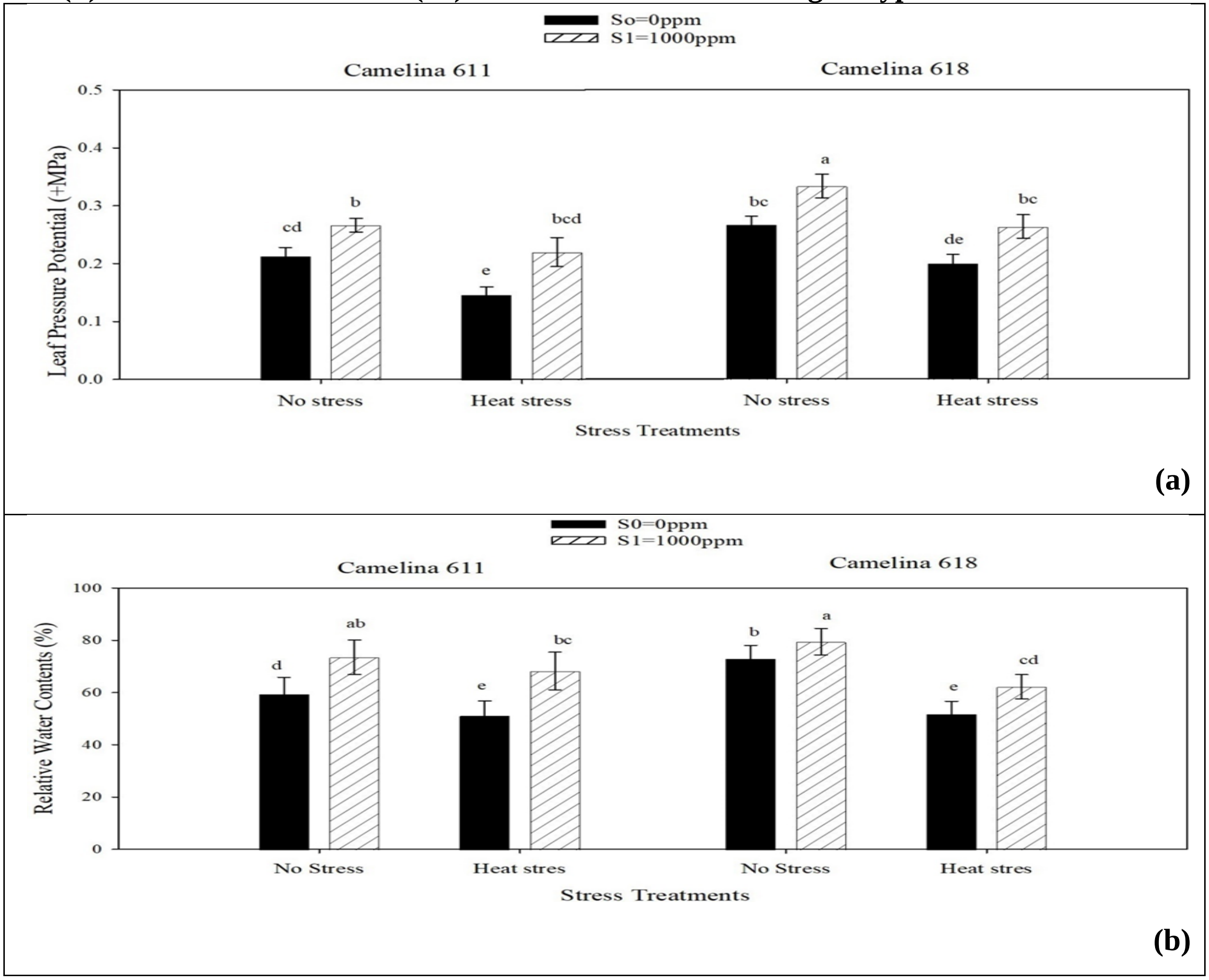
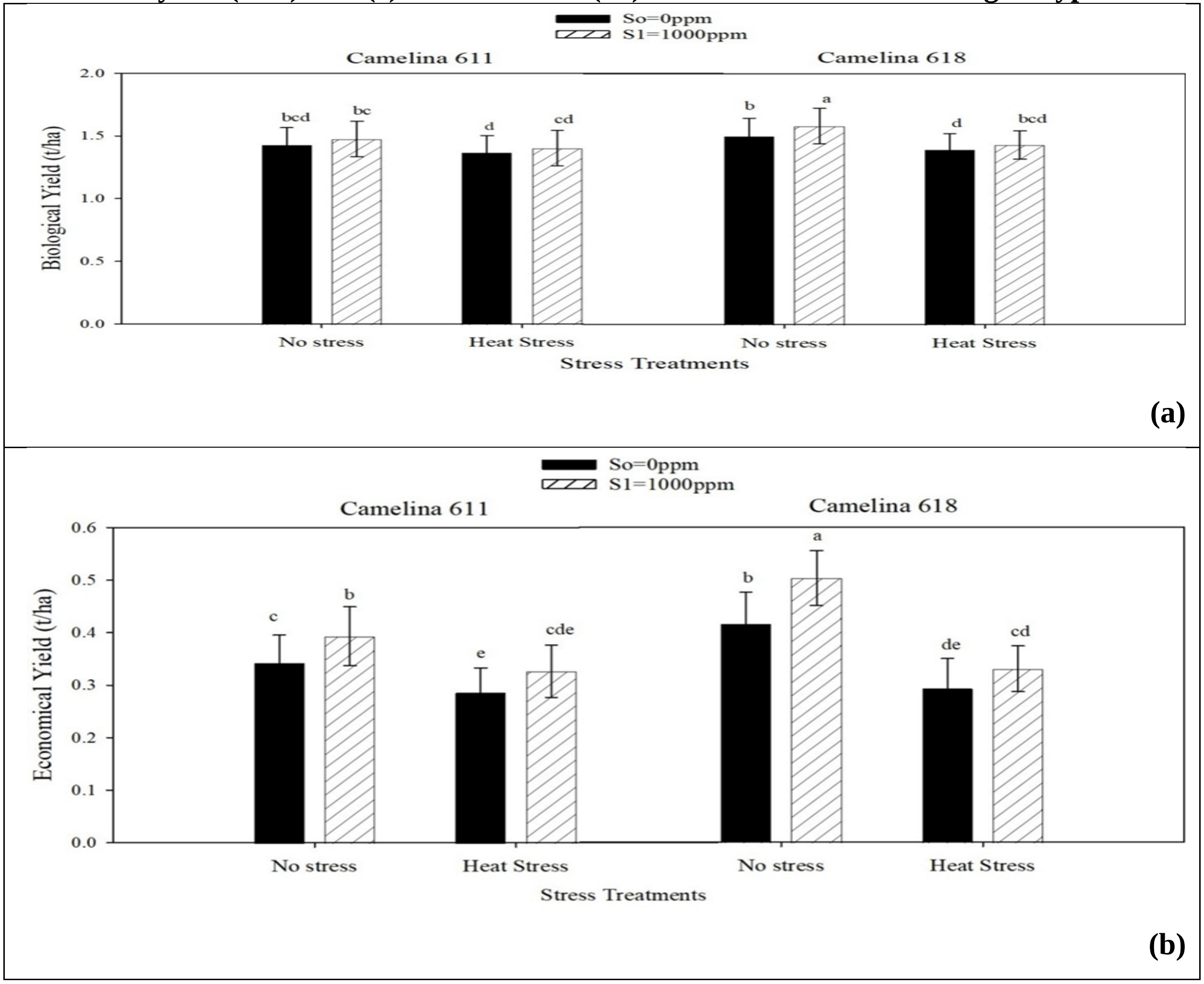
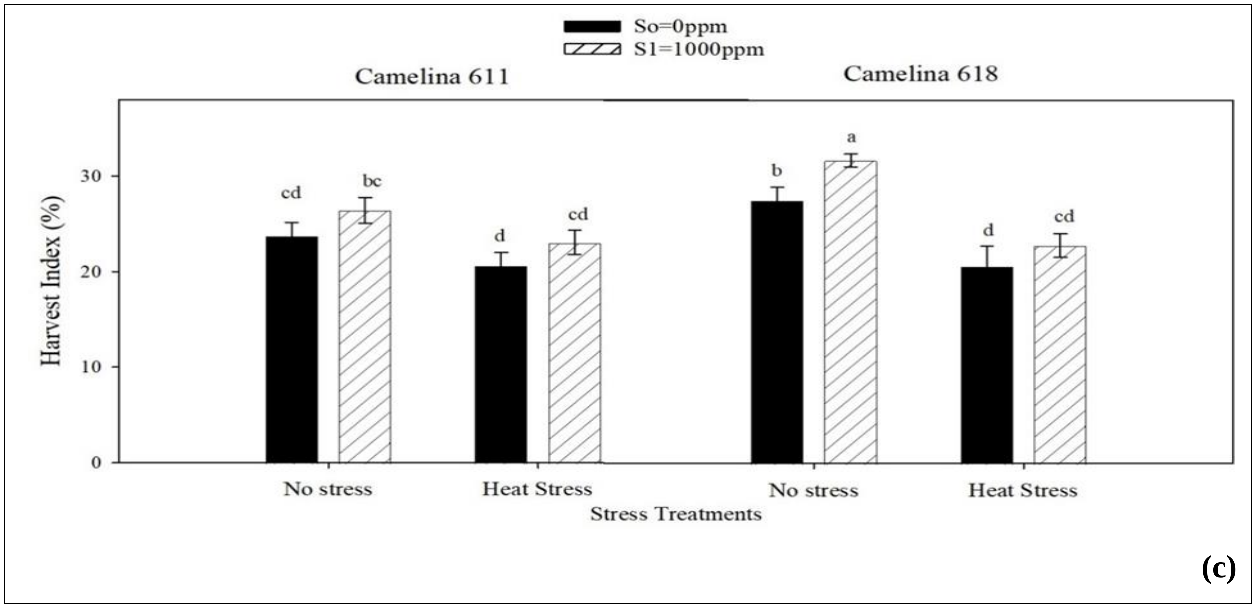


Figure 4: Influence of exogenous application of sulphur on (a) biological yield (t/ha), (b) economical yield (t/ha) and (c) harvest index (%) of heat stressed camelina genotypes





▬ So=0ppm
▨ S1=1000ppm
Camelina 611
Camelina 618
Harvest Index (%)
0
10
20
30
cd
bc
d
cd
b
a
d
cd
No stress
Heat Stress
No stress
Heat Stress
Stress Treatments
(c)