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Dissection of physiological traits for drought and heat stress tolerance in chilli (*Capsicum annum* L.)

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Abstract
Combined occurrence of drought and heat stresses becomes a major constraint in the era of 21st century. The present study was carried out in chilli K1, TNAU chilli hybrid CO1 and Ramanathapuram Gundu with six treatments of heat and drought to standardize the combined stress levels. The experiment to study the physiological basis of stress tolerance was conducted in open top chambers. The results showed that chilli could tolerate a combined stress up to a stress tolerance index of 0.501 based on the flowering and yield traits and this coincided with 60% pot capacity and ambient temperature + 3°C stress. Variety K1 had better tolerance to the combined stress with minimum yield reduction of 43.90% while, the Hybrid CO1 had poor performance with higher yield reduction of 57.23%. Irrespective of the genotypes, osmotic potential, osmotic adjustment and relative leaf water content (RLWC) were traits strongly associated with drought tolerance. Melaondialdehyde (MDA), plant height and leaf temperature were found to be associated with heat tolerance. The susceptibility of Hybrid CO1 was strongly correlated with severe reduction in RLWC and plant height and significant increase in MDA content under combined stresses

Keywords: chilli, drought, heat stress, reproductive stage, yield, pot capacity

1. Introduction

Increasing temperature and water scarcity had made agriculture production and productivity under threat. Combination of both water deficit and temperature occur simultaneously. Water deficit increases the atmospheric temperature and resulted in increased atmospheric drought (Rizhsky *et al.*, 2004) [37]. Climate change has negative impact on yield and quality improvement with estimated loss of 10-40% crop production in India by the year of 2080-2100 (IPCC 2014; Damatta *et al.*, 2010) [20, 9]. Water deficit and high temperature stress are major abiotic stresses directly affect the growth, development, reproduction and yield of crop plants (Bilal *et al.*, 2015; Lobell *et al.*, 2015) [6, 26]. The climate change scenario affects plants physiologically, leading to crop failure (Wahid *et al.*, 2007; Ziska *et al.*, 2011and DeBoeck *et al.*, 2012) [49, 52, 10].

Chilli (*Capsicum annum*), is the second most important vegetable next to tomato (Benson *et al.*, 2014). It also an important spice crop of solanaceae family with nutrient qualities like antioxidant properties, phenolic and carotenoid compounds including vitamin C and has important oleoresins and pungency compounds, capsaicin and capsanthin (Zhu *et al.*, 2012; Tian *et al.*, 2014) [51, 45]. In India, chilli crop has spread to an acreage of 0.364 M ha with production of 3.720 Million Mt and Tamil Nadu with 2197 ha with production of 30562.62 Mt (NHB, 2019; HAPIS 2019) [19, 18].

Among the different growth stages of crop, reproductive stage is more sensitive to the extreme temperature and water deficit (Wheeler *et al.*, 2000) [50]. Drought stress in chilli had negative effect on the growth and yield characters (Khan *et al*., 2008) [22]. One degree rise in temperature during growing period at field condition initially showed slight increase in the growth characters and yield of chilli, however it resulted in the negative relation to that of the harvest index and crop growth rate (Abhayapala *et al.*, 2018) [1]. Occurrence of both water deficit and heat stress is very much sensitive for chilli and more particular during flowering and reproductive stage considered as critical stage of chilli growth (Moreno *et al.*, 2003) [30].

An important physio-chemical mechanism by plant to acclimatize to abiotic stress is the changes in the osmotic potential of the cell with increased accumulation of osmolytes (Gonazalez-Chavira *et al.*, 2018) [15]. Osmotic adjustment maintains the turgor of the guard cell, leaf tissues and regulates the stomatal opening and closing under water stress in relation to the transpiration ratio (Ludlow and Muchow, 1990) [27]. Osmotic adjustment under water stress showed correlation with higher yield in various crops (Blum *et al.*, 2005) [7]. Relative Leaf Water Content (RLWC) determines the drought tolerance capacity without much hindrance to the normal physiological processes by maintaining the cell water contents. Decrease in RWC under abiotic stress results in reduced net CO_2 assimilation rate (Lawlor *et al.*, 2002) [25]. Wide transpiring leaf surface and elevated stomatal opening, has made *Capsicum annum* a susceptible crop to the water deficit (Delfine *et al.*, 2001) [11]. Combined stress of drought and heat, stomata experience antagonistic function. High temperature needs stomata to be open for lowering the leaf temperature whereas drought needs stomata to be closed for reducing the water loss (Rizhsky *et al.*, 2002) [36]. Interaction of water and high temperature stress negatively correlates with the plant height reducing the overall growth, number of leaves, leaf expansion and early completion of life cycle (Gunawardana and DeSilva, 2014) [16]. Malondialdehyde (MDA) content indirectly measures the damage to membranes caused by lipid peroxidation during heat stress (Vivitha *et al.*, 2017) [48].

Numerous studies on chilli revealed that, the effect of individual drought and high temperature stresses results in alteration of growth, physiological, biochemical and yield characters. However, the studies pertaining to the combined stress of drought and high temperature is very few. Hence, the combined stress effect of chilli need to be addressed under changing climatic conditions. This study will address the deleterious effects of combined stress during the reproductive stage of the chilli with reference to growth and yield parameters. Therefore, the objective of this study was standardizing the drought and heat treatments in chilli using Stress Tolerance Index (STI) to further study the physiological basis of stress tolerance, to investigate the physiology and yield traits of the chilli under the drought and increasing temperature, and to correlate the morpho-physiological traits with yield under stress.

2. Materials and Methods

2.1. Chilli genotypes

Two chilli varieties *viz.*, Kovilpatti 1 (K1) from Regional Research Station, Kovilpatti and local variety of Ramanathapuram Gundu (RG) type and a TNAU chilli hybrid CO1 (CO1), released from Tamil Nadu Agricultural University were selected for the study. The fruits of K1 and TNAU chilli hybrid CO1 are elongated and RG is round. The varieties were selected based on the yield and consumer preference along with recently released hybrid.

2.2. Drought and heat treatments

The study was carried out during 2019-2020 in Open Top Chamber facility located at Department of Crop Physiology, TNAU, Coimbatore. The open top chamber fabricated by polycarbonate sheet, with a dimension of 4 m × 4 m were used. Three open top temperature-controlled chambers, one for ambient and two for elevation were used for the study and temperature of one chamber was elevated by 3°C and other by 5°C compared to ambient chamber. The drought treatments were imposed by dry down gravimetric method (Durgadevi and Vijayalakshmi, 2020) [12]. Combined stress was given with imposing different levels of pot capacity under temperature elevation chambers.

2.2.1. Experiment 1: Standardizing drought and heat treatments in chilli based on STI for physiological analysis

Nursery of the three selected chilli genotypes were raised in the protrays. The crop was transplanted in pots during 2019 and grown with all package of practices recommended. 35 days old seedlings were transplanted to the pots with red soil and two seedlings were maintained per pot of 18 kg capacity with 15 kg of soil. The individual pots were maintained for control and other treatments in their respective chambers. The plants were grown normally till flowering stage and the treatments were imposed. The treatments include T1- Control (100% Pot Capcity (PC) + Ambient Temperature), T2- 80 % PC and A+ 3°C, T3- 80% PC and A+ 5°C, T4- 60% PC and A+ 3°C, T5- 60% FC and A+ 5°C, T6- 40% PC and A+ 3°C and T7- 40% PC and A+ 5°C. Then the pots at 50% flowering were subjected to elevated temperature treatment under the Open Top Chambers. After 15 days of stress treatments, the plants were shifted to normal condition. The flowering and yield data were collected from the tagged plants and flowers which are exposed to stress for a period of 10 days. The data related to maximum temperature and relative humidity during the treatment period of stress treatments are given in Table 1**.**

2.2.2. Experiment 2: Morpho-physiological traits of chilli genotypes under combined drought and heat stress condition

Based on experiment 1, 60% PC and A+ 3°C was considered as optimum level of combined stress treatment to study the morpho-physiological mechanism of chilli. The lower level of PC combined with high temperature had higher deleterious effect while, higher PC had less effect on growth, physiology and yield of chilli. Three varieties Kovilpatti 1 (K1), TNAU chilli hybrid CO1 (Hybrid CO1) and Ramanathapuram Gundu (RG) were taken for the study and the stress treatments include i) Control - (100% Pot Capcity (PC) + Ambient Temperature), ii) Drought - 60 % PC with Ambient Temperature, iii) High temperature - 100% PC and A+ 3°C, iv) Drought + High temperature - 60% PC and A+ 3°C. At the time of flowering, the pots of all varieties were shifted to respective chambers of ambient and high temperature. Reproductive stage stress was imposed for 10 days and data were recorded during stress on 7th day and after one week of stress recovery. Recorded maximum temperature at the time of flowering ranged from 36°C to 43°C in the elevated chamber.

2.3. Methods

2.3.1. Stress tolerance index

Stress tolerance index (STI) of chilli was calculated based on the yield data collected from stressed and non-stressed plants using the formula, of STI = $(Yp \times Ys)/Yp^2$ where Yp is the yield under control condition, Ys is the yield under stress condition (Fernandez, 1992) [14].

2.3.2. Osmotic potential and osmotic adjustment

The penultimate fully expanded leaf on the main stem was cut, wrapped in a plastic bag and soaked in water in the refrigerator for 24 hours to rehydrate the tissue. The rehydrated leaf was placed in aluminium foil, frozen with liquid nitrogen for 30 seconds to stop the physiological function of its cells and stored in a -80°C freezer. The sap was collected by squeezing the leaf sample with the help of a sterile syringe and the osmolality (mmol kg^{-1}) of the expressed sap was determined using a vapour pressure osmometer (Vapro Model 5520 Wescor Inc., Logan, UT, USA). Osmotic potential (ψ_s) was calculated as, $\psi_\pi = -c\ RT$, where c is concentration, R is the universal gas constant (0.0832) and T is the temperature in degrees Kelvin (310° K). The following conversion equation was used to compute osmotic potential (in MPa).

Osmotic potential = [(Osmolality mmol kg^{-1}) (0.0832) (310)] / 10000

Osmotic adjustment was calculated as the difference between the turgid potential in the well-watered treatment and stress treatment (Babu *et al.*, 1999) [3].

Osmotic adjustment (MPa) = Osmotic potential in control - osmotic potential in drought

2.3.3. Relative Leaf water content

Fully expanded third leaf was collected early in the morning.

Leaf discs of equal size were soaked in water for four hours and turgid weight was measured. Then the leaf bits were kept in hot air oven at 60°C and dry weight was recorded (Barrs and Weatherly, 1962) [4]. RLWC were calculated using following formula and expressed as per cent.
RLWC (%) = [(Fresh weight – Dry weight) / (Turgid weight – Dry weight)] x 100

2.3.4. Plant height
The plant height was measured using the meter scale from the lower shoot region to the highest position of plant. The plant height was measured in cm.

2.3.5. Leaf Temperature
Leaf temperature were measured using Fluke 59 mini IR thermometer and expressed in °C

2.3.6. Malondialdehyde
The lipid peroxidation level was determined by quantifying the MDA equivalents using 2- Thiobarbituric acid (TBA) and expressed in µmol g^{-1} of fresh weight (Hodges *et al.*, 1999) [17]. For this 0.25 g of leaf tissue was homogenized in 4 ml of 1% TCA solution (w/v) and centrifuged at 10,000 rpm for 10 min. Supernatant was transferred to a fresh test tube and 1 ml of 0.5% TBA in 20% TCA was added and incubated in boiling water for 30 min. Then, the samples were re-centrifuged at 10,000 rpm for 5 min and the absorbance values of the supernatants were measured at 532 nm. The values for non-specific absorption at 600 nm were subtracted. MDA concentration was calculated by the formulae: MDA content (µmol g^{-1}) = Δ (*A*532 – *A*600) / 1.56 × 10^5.

2.3.7. Yield parameters
The yield parameters like individual fruit weight, No. of fruits per plant ant fruit yield at 1st picking were taken after fruit tagging during stress. Each sample was taken with proper replicates to minimize the error and mean values were given in the table.

2.4. Statistical analysis
The data on various parameters were subjected to univariate analysis of variance using IBM SPSS statistics 21. ANOVA was performed with proper replications to minimize error and to study the physiological differences between the treatments and genotypes of chilli. Experiments were arranged in a Factorial completely randomized design (FCRD), with four and five replications in Experiment 1 and Experiment 2 respectively. The collected data were presented with the respective standard errors of means and the least significant difference (LSD 0.05). The software ORIGIN Pro 8 software was used to draw the graphs. Pearson Correlation was done using IBM SPSS statistics 21.

3. Results
3.1. Experiment 1: Standardizing drought and heat treatments in chilli based on STI for further physiological analysis
Number of flowers per plant at 50 % flowering and days to 50 % flowering shows significant difference (P<0.05) among stress treatments and genotypes (Table 2). Severity of stress decreased the number of flowers and days for 50% flowering. Among the six stress treatments, T4 (60% PC+ 3°C) attained 50% flowering nearly 10 days earlier in all genotypes compared to control plants irrespective of variety/hybrid. Treatment with 60% PC+ 3°C had better physiological relevance to abiotic stress with 50% tolerance nature. Early flowering of chilli was seen in Hybrid CO1 (104.0, 89.33 days) RG (105.0, 89 days) and K1 (104.33, 92. 67 days) under 60% PC+ 3°C and 40% PC+ 5°C treatments respectively.
Among the genotypes, Hybrid CO1 recorded higher number of flowers (43.33) compared to other varieties in control, while the same resulted in higher flower reduction under stress condition. Among the different stress treatments, T2 (80% PC+ 3°C) exhibited lower reduction in numbers of flower while the highest was recorded in T7 (40% PC+ 5°C) compared to control (100% Pot Capcity (PC) + Ambient Temperature) while, the treatment with 60% PC+ 3°C had optimum level of flower reduction compared to control. Variety K1 had higher number of flowers (22.67) under 60% PC+ 3°C stress condition followed by RG (21.33) and hybrid CO1 had minimum flowers (19.33).
Chilli genotypes are morphologically varies with one another as they have elongated and rounded chilli types. Yield characters of chilli showed significant variation (P<0.05) between treatments and varieties (Table 3). As occurrence of stress gradually decreases the average fruit weight, number of fruits per plant irrespective of genotypes, the lowest yield was recorded in treatment with 40% PC and A+ 5°C. The average fruit weight of Hybrid CO1 was 7.23 g and K1 was 5.93 g followed by RG chilli with 4.47 g under control (100% Pot Capcity (PC) + Ambient Temperature) condition. Under 40% PC+ 5°C stress condition, the Hybrid CO1 had higher weight loss over control. 60% PC and A+ 3°C had significant weight reduction over control irrespective of genotypes.
The number of fruits per plant showed significant difference (P<0.05) among the treatments and genotypes (Table 3.). Stress treatments showed decreasing trend and drastically reduced the number of fruits per plant under severe stress condition. Moderate stress of 60% PC and A+3°C had higher reduction of fruits compared to control (100% Pot Capacity (PC) + Ambient Temperature) and lesser reduction over severe stress condition (40% PC and A+ 5°C). Hybrid CO1 had significantly reduced number of flowers (10.33) followed by K1 (12.00) under severe stress condition of 40% PC and A+ 5°C while RG had higher number of fruits.

3.1.1. Stress tolerance index
STI showed significant (P<0.05) difference between drought and heat stress treatments and genotypes. Among the treatments, control (100% Pot Capacity (PC) + Ambient Temperature), T2 (80% PC+ 3°C), T3 (80% PC+ 5°C), T4 (60% PC and A+ 3°C) had higher level of stress tolerance (above 0.5) whereas, the other treatments had STI lesser than 0.5. This results showed that treatments with mild stress had higher tolerance and intense stress had lesser tolerance capacity (Fig. 1). The treatment, 60% PC+ 3°C with 0.501 STI had moderate level of stress tolerance and above 0.5 and it was selected to study the further physiological changes in chilli under combined heat and drought stress.

3.2. Experiment 2: Morpho-physiological traits of chilli under individual and combined stress conditions
3.2.1. Osmotic potential and Osmotic Adjustment
Osmotic potential and adjustment exhibited highly significant

Variations ($P<0.05$) among the genotypes under different stress conditions (Fig. 2). Among the treatments, combined stressed plants (D+HT) showed maximum osmotic potential and osmotic adjustment followed by drought treatment (D). Combined stressed plants of K1 (-2.35 MPa; 0.83), Hybrid CO1 (- 2.21 MPa; 0.80) and RG (-2.23 MPa; 0.80) had higher osmotic potential and adjustment. Drought stress plants (D) have second higher osmotic potential and osmotic adjustment with values of -1.99 MPa; 0.47in K1, -1.91 MPa; 0.49 Hybrid CO1 and -1.98 MPa; 0.55 RG respectively. The variety K1 exhibited plasticity to combined stress and individual drought stress, by maintaining higher osmotic potential and adjustment.

Under stress recovery, the osmotic potential and osmotic adjustment got decreased irrespective of treatment and genotypes and no variations were found under interaction effect of treatment and Varieties. The osmotic potential of combined stress of drought and heat got decreased to -1.86 (K1), -1.80 (Hybrid CO1) and -1.88 (RG) compared to stressed plants. Among the different stress treatments, combined stress and individual drought stress influenced the synthesis of osmolytes to mitigate stress and exhibit tolerance in chilli.

3.2.2. RLWC

Significant variations on RLWC were observed between genotypes and treatments (Fig. 3). The RLWC values were maintained above 55% and the treatments with combined stress of drought and heat (D+H) and drought (D) had minimum RLWC compared to heat stress (HT). Hence, K1 had higher RLWC and lower percent reduction among the stress treatments and showed relative tolerance compared to other genotypes as sustained by maintenance of better water status. With regard to stress recovery, the treatments and genotypes showed significant difference ($P<0.05$). Chilli plants regains its turgidity and recorded higher RLWC over stress treatments and maintained the RLWC above 75% irrespective of treatments and genotypes.

3.2.3. Plant height

Irrespective of the stress condition and genotypes, the plant height showed significant variation (Fig. 3). Among the treatments, combined stress (D+HT) and drought stress (D) treatments have reduced height whereas the high temperature treatment had increased height or maintained the height over control. The treatment with D+HT had higher reduction of 31.14%, 23.86% and 26.31% over control in K1, Hybrid CO1 and RG respectively. An increase in plant height was recorded during the stress recovery stage.

3.2.4. Leaf Temperature

A highly significant difference was observed between the chilli variety/hybrids and treatments during stress and stress recovery treatments (Fig. 4.). The treatment of combined stress D+HT and high temperature (H) showed higher leaf temperature of 35.57, 36.07 and 35.94 and 32.87, 35.73, 34.95°C in K1, Hybrid CO1 and RG genotypes respectively. While the drought stress treatment had lower temperature compared to heat and combined stress. Chilli variety of K1 had lower leaf temperature during stress treatments and maintained cooler canopy over other genotypes.

Stress recovery of chilli showed no variation while treatments and interaction had significant difference ($P<0.05$). The leaf temperature decreased to average of 31°Cover stress recovery among all the genotypes.

3.2.5. MDA content

The present study revealed that drought, heat and combined drought and heat stress had significant variation on MDA content in chilli (Fig. 4). Considering the treatments, combined drought and heat stress (D+HT) registered maximum MDA content in Hybrid CO1 (2.92 µmol g^{-1}) followed by RG (2.66 µmol g^{-1}) and minimum MDA content was recorded in K1 (2.54 µmol g^{-1}) while the same recorded 2.57 µmol g^{-1}, 2.35 µmol g^{-1} and 2.14 µmol g^{-1} under stress recovery condition. The heat stress treatment had higher MDA content with higher degree of membrane damage due to lipid peroxidation next to combined drought and heat stress (D+HT). 2.48, 2.31, 2.29 µmol g^{-1} were the hierarchy of higher to lower MDA content expressed by Hybrid CO1, RG and K1. The stress recovery MDA content decreased to 2.11 µmol g^{-1} in Hybrid CO1, 1.94 µmol g^{-1} in RG and 1.77 µmol g^{-1} in K1. Drought and control treatments had lower level of MDA content compared to heat and combined drought and heat stress.

3.2.6. Yield characters of chilli under combined stress conditions

Fruit weight, number of fruits and total weight of chilli showed significant difference ($P<0.05$) between stress treatments and genotypes. The combined drought and heat stress (D+HT) had recorded lower yield with reduced fruit weight and number of fruits per plant followed by heat stress (Table 3). Hybrid CO1 registered higher number of flower reduction of 59.25% over control followed by K1 with 50.39% under combined stress condition. The heat and drought had reduction percentage (51.23% and 27.77% in Hybrid CO1; 41.02 and 17.94% in RG; 37.79 and 15.74% in K1). Higher yield reduction was recorded in hybrid CO1 (57.23%) followed by RG (44.03%) and minimum reduction was registered in K1 (43.90%) over control under combined stress (D+HT). Heat stress also had yield reduction of 36.93% 25.42% and 24.33% respectively for Hybrid CO1, RG and K1 whereas drought stress experienced comparatively lower yield reduction with 21.50% (Hybrid CO1), 13.08 % (RG) and 14.65% (K1). Among the genotypes, hybrid CO1 had higher fruit and yield reduction under all stress conditions compared to control.

4. Discussion

Reproductive stage stress is most sensitive to drought and high temperature in various crops (Abhayapala *et al.*, 2018) [1]. Stress Tolerance Index (STI) was used to standardize the combined heat and drought treatments based on flowering and yield characters under control and stressed condition. STI was calculated by comparing the yield potential of control and stressed plant. Among the six treatments, the tolerance level of three treatments (T2, T3 and T4) were found to be above 50% STI. T4 (60% PC and A+3°C) was standardized to study the physiological basis of combined treatment to drought and heat.

Generally, all the stress treatments caused a decrease in number of flowers and days taken for 50% flowering, fruit weight and fruit numbers in all the varieties taken for the study. In the present study, six treatments of combined drought and heat stress imposed at flowering had higher flower reduction over control.

Occurrence of stress during flowering resulted in reduced number of flowers and yield in Hybrid CO1 and also shortened the flowering period by early flowering. Combined stresses were found to have retarded growth and flower abscission. Chilli crop exposed to high temperature or drought or combined stresses during vegetative and reproductive stage have reduced growth, flower and fruit abscission (Saha *et al.*, 2010) [40]. Elevated temperature at reproductive stage resulted in sterility of the flower, flower drop, ill-filled distorted fruits and time taken for flowering in bell pepper (Erickson and Markhart, 2001) [13]. Severity of the drought stress reduced the number of flowers in *Capsicum* (Malika *et al.*, 2019) [29].

Reduced fruit size and number of fruits were observed in the present study. Among the genotypes, Hybrid CO1 had higher yield reduction over control (Table 2.) with less number of fruits per plant and reduced fruit weight, while variety K1 had lower reduction. Combined drought and heat stress had lower yield with reduced grain weight in wheat (Urban *et al.*, 2018) [46]. In line with the above findings, flower drop and sterility during heat stress due to pollen abortion, reduced fertilization, malformation of the floral characters and abnormal seed set were seen in crops like tomato, maize, bell pepper, canola, soybean, flax and brassica (Erickson and Markhart, 2001; Pagamas and Nawata, 2008) [13, 32]. Similarly reduction in number of fruits per plant, fruit length and diameter, individual fruit weight, fruit yield per plant and fruit dry weight per plant were recorded under water deficit condition in chilli (Khan *et al.*, 2008; Malika *et al.*, 2019; Rosmania *et al.*, 2019) [22, 29, 39].

An important physio-chemical mechanism by plant to acclimatize the abiotic stress is the changes in the osmotic potential of the cell with increased accumulation of synthesis of osmolytes [18]. In this study, combined and individual drought stress had higher level of osmotic potential with accumulation of osmolytes and decreased the water potential of plant less than the soil to absorb more water from the deeper layers. Variety K1, was found to have better tolerance towards the stress with higher accumulation of osmolytes and hence had better level of adjustment towards drought. Osmotic potential and osmotic adjustment had positive relation with total yield of chilli (Table 4.). Osmotic adjustment maintains the turgor of the guard cell, leaf tissues and regulates the stomatal opening and closing under water stress in relation to the transpiration ratio (Ludlow and Muchow, 1990) [27]. In line with our findings, combined drought and heat recorded higher osmotic potential than individual drought and heat stresses in lentils (Sehgal *et al.*, 2017) [43] and wheat (Sattar *et al.*, 2020) [42]. Individual drought stress had higher level of osmotic adjustment in Thai chilli (Sato *et al.*, 2003) [41]. Heat stress alone accumulated osmolytes to decrease the osmotic potential in wheat (Argentel-Martinez *et al.*, 2019) [2]. Osmotic adjustment and potential were the traits that strongly associated with drought stress, which could be used to characterize the drought scenario and tolerance of chilli genotypes.

The plant water status under abiotic stress condition were measured with relative leaf water content of the crop. Maintaining of internal water status is much important to have normal functioning of plants and other metabolic activities. RLWC is found as index for measuring the abiotic stress, especially the low water stress. Study in chilli showed a decreasing trend in RLWC under combined and drought stresses (Fig. 3b). Drought stress showed positive correlation with RLWC and yield potential in chilli (Table 4). Among the genotypes, K1 was found to have higher RLWC during stress condition irrespective of treatments. Among the treatments, combined stress (D+HT) recorded higher RLWC reduction of 29.76 %, 35.82% and 30.77% followed by drought (D) treatment with 24.96%, 29.34% and 26.85% over control in K1, hybrid CO1 and RG respectively. Earlier findings also revealed that tolerant plants maintain higher levels RLWC to get higher yield under drought condition (Malika *et al.*, 2019; Okunlola *et al.*, 2017) [29, 31]. Interaction of drought and heat stress had higher impact over leaf water potential with decreased photosynthetic activity in wheat (Shah and Paulsen, 2003) [44]. Hydration capacity of plants decides the drought tolerance capacity of plants. Heat stress had lower effect on water relations in sorghum as it directly impacts the soil moisture status (Machado and Paulsen, 2001) [28]. The water deficit in plants lowered the water potential and turgidity of cell by closing stomata and reduced cell expansion (Jaleel *et al*., 2009) [21].

The combined effect of drought and high temperature had negative effect on the plant height compared to that of single stress because of its additive effects (Prasad *et al.*, 2008) [35]. Plant growth and development is coupled with number of metabolic reactions. Under stress condition, the growth of the plant is hampered as an adaptation strategy (Rollins *et al.*, 2013) [38] and result in reduced plant height, number of leaves and leaf expansion (Gunawardena and DeSilva *et al.*, 2014) [16]. In the line with above findings, plant height was severely reduced under combined heat and drought treatments. Contrary to the above findings, heat stress increased the plant height (0.09%) in Hybrid CO1. Individual drought stress and heat stress had decreased the plant height in chilli. Plant height was found to reduced under individual drought and heat stress treatments in chilli and sorghum (Prasad *et al.*, 2006; Khan *et al.*, 2012) [34, 23]. In accordance with the results of present study, interaction of drought and heat had experienced a negative effect over plant growth in chilli Gunawardena and DeSilva *et al.*, 2014) [16].

Occurrence of drought and heat together has greater impact over individual stress effects. Leaf temperature was found to be an important indicator of abiotic stresses such as drought and heat stress in chilli plants as it had negative relation with yield which is evident in Table 4. ((Malika *et al.*, 2019; Rollins *et al.*, 2013) [29, 38]. Decreased leaf temperature during drought and heat stresses was found to have improved stress tolerance. The variety K1 chilli with lower leaf temperature had tolerance to the combined and individual stresses (Fig. 4). Decreased leaf temperature regulates transpirational cooling, maintains stomatal movement, evaporation rate, gas exchange properties and WUE during stress and imparts tolerance in chilli. Increased leaf temperature increases the heat stress intensity and reduced the stomatal conductance in rape seed. Heat stress combines with the drought and occurs simultaneously, as the increased leaf temperature had photosynthetic impairment in crop plants and depletes the soil water (Lamaoui *et al.*, 2018) [24].

Lipid peroxidation of membrane under stress results in higher malondialdehyde content. Combined stress (D+HT) followed by heat stress had higher level of membrane damage with the measure of MDA content in all variety/hybrid. Membrane is the main structure which get affected first under the temperature stress because it alters the membrane fluidity. This impairs the membrane based physiological process (Blum, 1988) [8]. Measure of MDA content is a reliable indicator of heat stress tolerance in chilli (Vivitha *et al.*, 2017) [48]. MDA content of chilli had negative relation with that of yield in chilli (Table. 4). Variety K1 had minimum lipid peroxidation (50.39%) over hybrid CO1 (58.94%) and RG (58.27%) compared to control in interactive stress effect. Likewise K1 had lower percent increases of 44.97% and 17.10% in individual heat and drought stress respectively. Combined stress as well as heat stress showed sensitivity over membrane integrity resulting in membrane fluidity and leaky resulting in impairment of photosynthesis in barley (Rollins *et al.*, 2013) [38]. Heat stress in rice showed increased membrane damage by measuring higher MDA content (Vivitha *et al.*, 2017) [48].

The fruit yield of chilli has positive correlation and significant (P<0.01, P<0.05) among number of fruits, plant height, osmotic adjustment, osmotic potential and RLWC and negative correlation with MDA content, leaf temperature (Table. 4). This indicate that under stress condition, plant height, number of fruits, osmotic potential and RLWC contributes maximum to the yield of chilli. Varieties with higher fruit numbers, plant height and higher level of osmolyte synthesis and relative leaf water content had better yield while, MDA and leaf temperature decrease the yield potential of chilli. Similar results were reported in chilli by correlating the yield with plant height, osmolytes synthesis, RLWC, leaf temperature and MDA content in *Capsicum annum* (Patel *et al.*, 2009; Vani *et al.*, 2007) [33, 47].

Table 1: Temperature and RH in Open Top Chamber during the experimental period

Date	Ambient		Ambient+3°C		Ambient+5°C	
	Temperature (°C)	Relative Humidity	Temperature (°C)	Relative Humidity	Temperature (°C)	Relative Humidity
18/2/2019	36.22	82.0	39.08	79.3	41.23	75.2
19/2/2019	34.60	79.2	37.59	78.5	39.19	77.1
20/2/2019	32.76	81.2	36.25	80.9	38.17	80.6
21/2/2019	32.97	81.5	35.65	84.7	37.74	82.1
22/2/2019	32.81	83.6	36.50	83.4	38.22	82.0
23/2/2019	32.38	83.1	36.32	82.6	37.96	80.6
24/2/2019	33.01	80.6	37.32	78.5	38.16	77.5
25/2/2019	32.13	80.8	36.20	79.6	38.52	78.3
26/2/2019	35.53	83.9	38.61	82.9	40.76	81.5
27/2/2019	35.00	85.6	39.25	83.6	41.75	84.9
28/2/2019	35.43	83.5	39.80	83.0	41.52	82.6
1/3/2019	36.19	81.9	39.92	81.5	41.21	80.7
2/3/2019	36.24	80.9	40.36	78.1	42.59	79.6
3/3/2019	36.59	79.6	39.58	79.0	41.02	78.4
4/3/2019	36.52	84.2	39.32	82.6	41.54	81.8

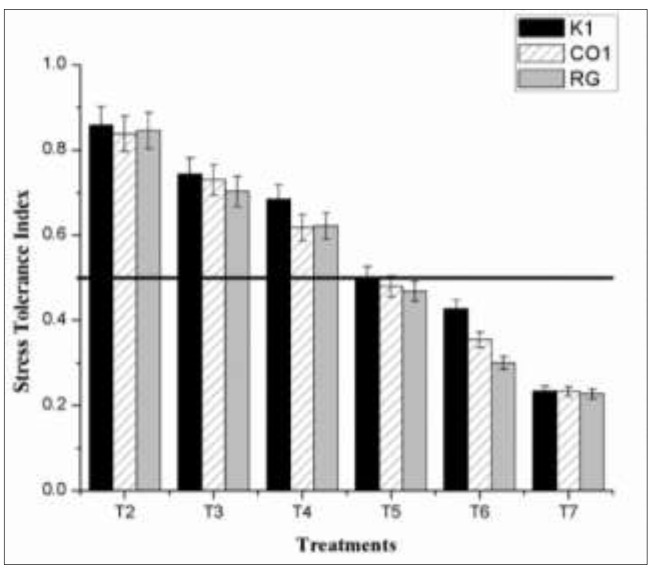

Fig 1: Stress tolerant index of chilli under combined drought and heat stress treatments

Table 2: Effect of combined drought and heat stress on flowering and yield characters in chilli

	Days to 50% flowering (Days)			No. of flowers per plant @50% flowering (Number)			Individual fruit weight (g fruit^{-1})			No of fruits plant^{-1}		
Treatments	KI	CO1	RG	KI	CO1	RG	K1	CO1	RG	K1	CO1	RG
T1	116.33	110.33	115.00	38.00	43.33	36.00	5.93	7.23	4.47	22.67	26.33	20.00
T2	110.00	108.33	113.33	34.00	35.00	32.00	5.20	6.83	4.10	21.67	23.67	19.00

T3	108.67	100.33	110.33	29.33	27.67	25.00	5.33	6.37	3.50	18.00	21.33	18.00
T4	104.33	104.00	105.00	22.67	19.33	21.33	5.50	6.03	3.60	17.33	21.00	15.67
T5	101.00	95.67	100.67	16.00	16.00	14.33	5.30	5.50	3.40	15.67	14.67	13.67
T6	96.33	90.67	92.33	18.00	14.00	16.33	5.07	5.10	3.30	13.00	11.67	15.00
T7	92.67	89.33	89.00	16.00	13.33	14.67	4.77	4.90	3.30	12.00	10.33	14.00
	G	T	G×T	G	T	G×T	G	T	G×T	G	T	G×T
SE(d)	0.866	0.371	2.598	0.376	0.1614	1.129	0.029	0.013	0.088	0.241	0.103	0.722
CD (P <0.05)	1.750**	0.750**	5.250 NS	0.761**	0.326**	2.283**	0.059**	0.025**	0.178**	0.487**	0.209**	1.460**
NS- Non significant *-significant **-Highly significant												
T1- Control (100% Pot Capcity (PC) + Ambient Temperature), T2- 80 % PC and A+ 3°C, T3- 80% PC and A+ 5°C, T4- 60% PC and A+ 3°C, T5- 60% PC and A+ 5°C, T6- 40% PC and A+ 3°C and T7- 40% PC and A+ 5°C.												

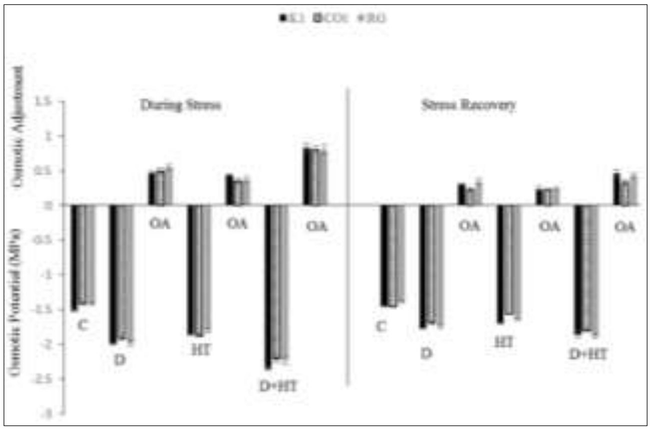

Fig 2: Effect of heat and drought stress on osmotic potential and adjustment of chilli

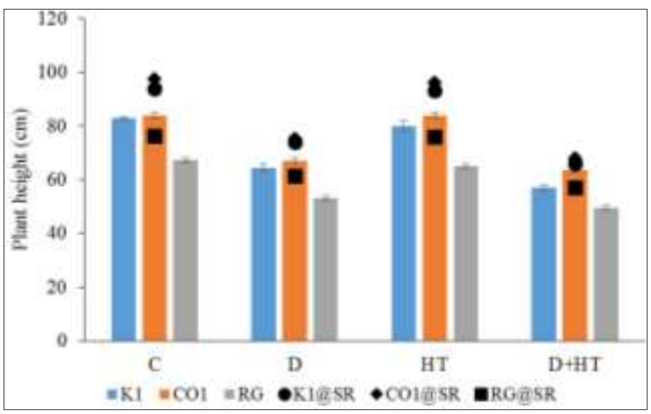

Fig 3a: Plant height of chilli genotypes under combined, heat and drought stress and stress recovery (SR) conditions

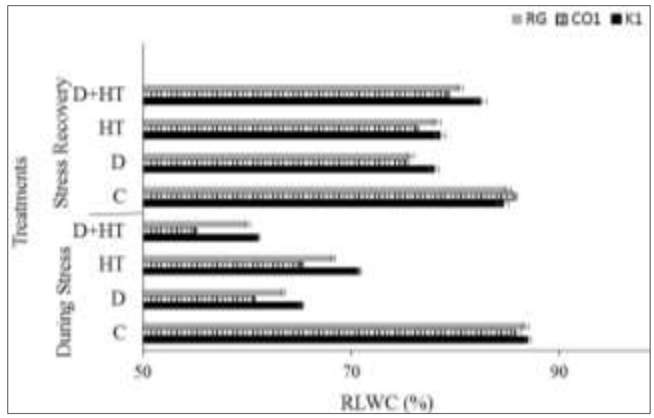

Fig 3b: RLWC of chilli genotypes under different stress conditions

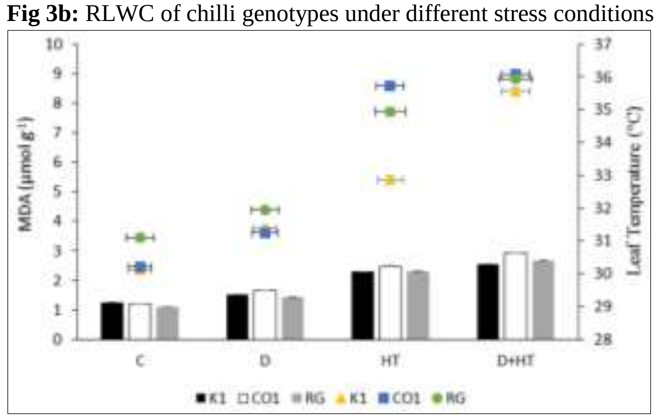

Fig 4: Influence of heat and drought stress on MDA content and Leaf temperature in chilli

Table 3: Yield characters of chilli under heat and drought stress conditions

	Individual fruit weight (g)			No of fruits plant $^{-1}$			Fruit yield (g plant $^{-1}$)		
Treatments	K1	CO1	RG	K1	CO1	RG	K1	CO1	RG
C	5.9	7.06	4.46	25.4	32.4	23.4	143.10	188.13	125.99
D	5.44	6.28	3.64	21.4	23.4	19.2	121.65	147.80	109.51
HT	5.36	6.38	3.26	15.8	15.8	13.8	108.27	118.64	93.96
D+HT	5.12	5.46	2.76	12.6	13.2	12.2	80.27	80.46	70.51
	G	T	G×T	G	T	G×T	G	T	G×T
SE(d)	0.021	0.016	0.065	0.164	0.123	0.494	0.462	0.346	1.387
CD (P <0.05)	0.043**	0.032**	0.131**	0.332**	0.249**	0.99**	0.931**	0.698**	2.795**
NS- Non significant *-significant **-Highly significant									
Control - (100% Pot Capcity (PC) + Ambient Temperature), Drought (D) - 60 % PC with Ambient Temperature, High temperature (HT)- 100% PC and A+ 3°C, Drought + High temperature (D+HT)- 60% PC and A+ 3°C									

Table 4: Correlation of yield with physiological parameters of chilli under heat and drought stress conditions

Yield/physiological traits	Fruit yield (g plant^{-1})	No. of fruits plant^{-1}	MDA (µmol g^{-1})	Leaf temperature (°C)	Plant height (cm)	Osmotic adjustment	Osmotic potential (MPa)	RLWC (%)
Fruit yield	1	0.962**	-0.792**	-0.823**	0.651*	0.089	0.798**	0.669*
No. of fruits plant^{-1}		1	-0.884**	-0.905**	0.523	0.146	0.799**	0.739**
MDA			1	0.952**	-0.281	-0.281	-0.768**	-0.739**
Leaf temperature				1	-0.386	-0.235	-0.740**	-0.704*
Plant height					1	-0.302	0.676*	0.602*
Osmotic adjustment						1	0.074	0.048
Osmotic potential							1	0.924**
RLWC								1

**-Correlation is significant at the 0.01 level (2-tailed) *- Correlation is significant at the 0.05 level (2-tailed)

5. Conclusion

Drought of 60% PC and elevated temperature of + 3°C based on STI was standardized as is the optimum level to identify the physiological mechanism underlying stress tolerance in chilli. This treatment clearly differentiated the contrasting genotypes for susceptibility/tolerance to combined stress. The parameters like osmotic potential, osmotic adjustment and RLWC were used as reliable trait to identify the drought tolerance mechanism. Plant height, leaf temperature, MDA content were observed to indicate the degree of heat stress treatments. Higher the stress level, greater was the impact on yield of chilli. Among the genotypes, Kovilpatti 1 (K1) followed by Ramanathapuram Gundu (RG) had better survival under combined stress with heat and drought.

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References

1. Abhayapala R, De Costa J, Malaviarachchi W, Kumara A, Suriyagoda L, Fonseka R.. Exploitation of differential temperature-sensitivities of crops for improved resilience of tropical smallholder cropping systems to climate change: A case study with temperature responses of tomato and chilli. Agriculture, Ecosystems and Environment, 2018; 261:103-114.
2. Argentel-Martínez L, Garatuza-Payan J, Yepez EA, Arredondo T, de los Santos-Villalobos S. 2019. Water regime and osmotic adjustment under warming conditions on wheat in the Yaqui Valley, Mexico. *PeerJ* 7:e7029 DOI 10.7717/peerj.7029
3. Babu, RC, Pathan, MS, Blum A, Nguyen HT. Comparison of measurement methods of osmotic adjustment in rice cultivars**.** Crop Science, 1999; 39**:**150-158.
4. Barrs HD, Weatherley PE. A re-examination of the relative turgidity technique for estimating water deficit in leaves. Australian Journal of Biological Sciences, 1962; 15**:**413-428.
5. Benson GAS, Obadofin AA & Adesina JM. Evaluation of plant extracts for controlling insect pests of pepper (*Caspicum spp*.) in Nigeria humid rainforest. New York Science Journal. 2014; 7(1):39-43.
6. Bilal M, Rashid RM, Rehman SU, Iqbal F, Ahmed J, Abid MA, Ahmed Z, Hayat A. Evaluation of wheat genotypes for drought tolerance. Journal of Green Physiology Genetics and Genomics, 2015; 1:11-21.
7. Blum A. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? Australian Journal of Agriculture Research, 2005; 56:1159-1168.
8. Blum A. Plant Breeding for Stress Environments. CRC Press, Boca Raton, FL, USA. 1988
9. DaMatta FM, Grandis A, Arenque BC, Buckeridge MS. Impacts of climate changes on crop physiology and food quality. Food Research International, 2010; 43:1814-1823.
10. DeBoeck HJ, Kimball BA, Miglietta F, Nijs I. Quantification of excess water loss in plant canopies warmed with infrared heating. Global Change Biology, 2012; 18: 2860–8
11. Delfine S, Loreto F, Alvino A. Drought-stress effects on physiology, growth and biomass production of rainfed and irrigated bell pepper plants in the Mediterranean region. Journal of American Society of Horticulture Science, 2001; 126(3):297-304.
12. Durgadevi R, Vijayalakshmi D. Mulberry with increased stomatal frequency regulates gas exchange traits for improved drought tolerance. Plant Physiology Reports, 2020, 1-9.
13. Erickson AN, Markhart AH. Flower production, fruit set, and physiology of bell pepper during elevated temperature and vapor pressure deficit. Journal of American Society of Horticulture Science. 2001; 126(6):697-702.
14. Fernandez GCJ. Effective selection criteria for assessing stress tolerance. In: Kuo CG (Ed) Proceedings of the International Symposium on Adaptation of Vegetables and Other Food Crops in Temperature and Water Stress. Tainan, Taiwan, 1992.
15. González-Chavira MM, Herrera-Hernández MG, Guzmán-Maldonado H, Pons-Hernández JL. Controlled water deficit as abiotic stress factor for enhancing the phytochemical content and adding-value of crops. Scientia Horticulturae, 2018; 234:354-360.
16. Gunawardena MDM, De Silva CS. Identifying the impact of temperature and water stress on growth and yield parameters of chilli (*Capsicum annuum* L.). OUSL Journal, 2014; 7: 25-42
17. Hodges DM, Delong JM, Forney CF, Prange RK. Improving the thiobarbituric acid reactive substances assay for estimating lipid peroxidation in the plant tissues containing anthocyanin and other interfering compounds. *Planta*, 1999; 207:604-611.
18. Horticulture area production information system, 2018-2019. https://www.aps.dac.gov.in/

19. Indian Horticulture Database. National Horticulture board. 2018-19. Ministry of Agriculture Government of India.
20. IPCC. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Eds. R.K. Pachauri and L.A. Meyer (IPCC, Geneva, Switzerland), 2014, 151.
21. Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJA, Somasundaram R, Pameerselvam R. Drought stress in plant: A review on morphological characteristic and pigment composition. International Journal of Agriculture and Biology, 2009; 11:100-105.
22. Khan MAI, Farooque AM, Haque MA, Rahim MA, Hoque, MA. Effects of water stress at various growth stages on the physio-morphological characters and yield in chilli. Bangladesh Journal of Agricultural Research. 2008; 33(3):353-362.
23. Khan MAI, Hoque, MA, Farooque AM, Habiba U, Rahim MA. Physio-morphological features of chilli accessions under moisture stress conditions. Bangladesh Journal of Agricultural Research. 2012; 37(2):263-269.
24. Lamaoui M, Jemo M, Datla R, Bekkaoui F. Heat and drought stresses in crops and approaches for their mitigation. Frontiers in Chemistry, 2018, 6:26.
25. Lawlor DW, Cornic G. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell and Environment, 2002; 25:275-294.
26. Lobell DB, Hammer GL, Chenu K, Zheng B, McLean G, Chapman SC, *et al*. The shifting influence of drought and heat stress for crops in northeast Australia. Global Change Biology, 2015; 21:4115-4127.
27. Ludlow MM, Muchow RC. A critical evaluation of traits for improving crop yields in water-limited environments. In Academic Press Advances in agronomy, 1990; 43:107-153.
28. Machado S, Paulsen GM. Combined effects of drought and high temperature on water relations of wheat and sorghum. Plant and Soil. 2001; 233(2):179-187.
29. Malika LY, Deshabandu KT, DeCosta WJM, Ekanayake S, Herath S, Weerakoon WW, *et al*. Physiological traits determining tolerance to intermittent drought in the *Capsicum annuum* complex. Scientia Horticulturae, 2019; 246:21-33.
30. Moreno MM, Ribas F, Moreno A, Cabello MJ. Physiological response of pepper (*Capsicum annum* L.) crop to different trickle irrigation rates. Spanish Journal of Agricultural Research, 2003; 1:65-74.
31. Okunlola GO, Olatunji OA, Akinwale RO, Tariq A, Adelusi AA. Physiological response of the three most cultivated pepper species (*Capsicum spp.*) in Africa to drought stress imposed at three stages of growth and development. Scientia Horticulturae, 2017; 224:198-205.
32. Pagamas P, Nawata E. Sensitive stages of fruit and seed development of chili pepper (*Capsicum annuum* L. var. Shishito) exposed to high-temperature stress. Scientia Horticulturae, 2008; 117:21-25.
33. Patel PN, Fougat RS, Sasidharan N. Studies on genetic variability, correlation and path analysis in chillies (*Capsicum annuum* L.). Field Crops Research. 2009; 10(3):626-631.
34. Prasad PVV, Boote KJ, Allen LH. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain sorghum [*Sorghum bicolor* (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. Agricultural and Forest Meteorology, 2006; 139: 237-251.
35. Prasad PVV, Staggenborg SA, Ristic Z. Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. In: *Response of Crops to Limited Water* (Ed. LR Ahuja, VR Reddy, SA Saseendran & Q Yu; Understanding and Modeling Water Stress Effects on Plant Growth Processes, Adv. Agric. Syst. Model. 1. ASA, CSSA, SSSA, Madison, WI), 2008, 301-355.
36. Rizhsky L, Liang H, Mittler R. The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiology, 2002; 130:1143-1151.
37. Rizhsky L, Liang H, Shuman J, Shulaev V, Davletova S, Mittler R, *et al*. When defense pathways collide: the response of Arabidopsis to a combination of drought and heat stress. Plant Physiology, 2004; 134:1683-1696.
38. Rollins JA, Habte E, Templer SE, Colby T, Schmidt J, Von Korff M, *et al*. Leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley (*Hordeum vulgare* L.). Journal of Experimental Botany. 2013; 64(11):3201-3212.
39. Rosmaina, Parjanto, Sobir, Yunus A. Screening of *capsicum annuum* L. Genotypes for drought tolerance based on drought toleranc indices. SABRAO Journal of Breeding and Genetics, 2019, 51(3).
40. Saha SR, Hossain MM, Rahman, MM, Kuo CG, Abdullah S. Effect of high temperature stress on the performance of twelve sweet pepper genotypes. Bangladesh Journal of Agricultural Research. 2010; 35(3):525-534.
41. Sato S, Moreshet S, Takagaki M, Shinohara Y, Ito T. Effects of drought stress on sap flow, stomatal conductance, and leaf water potential of pepper cultivars (*Capsicum annuum* L.). Japanese Journal of Tropical Agriculture. 2003; 47(2):61-69.
42. Sattar A, Sher A, Ijaz M, Ul-Allah S, Rizwan MS, Hussain M, Cheema M.A. Terminal drought and heat stress alter physiological and biochemical attributes in flag leaf of bread wheat. Plos one. 2020; 15(5):e0232974.
43. Sehgal A, Sita K, Kumar J, Kumar S, Singh, S, Siddique, KH, Nayyar H.. Effects of drought, heat and their interaction on the growth, yield and photosynthetic function of lentil (*Lens culinaris* Medikus) genotypes varying in heat and drought sensitivity. Frontiers in Plant Science, 2017; 8:1776.
44. Shah NH, Paulsen GM. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and Soil. 2003; 257(1):219-226.
45. Tian SL, Lu BY, Gong ZH, Muhammad-Shah SN. Effects of drought stress on capsanthin during fruit development and ripening in pepper (*Capsicum annuum* L.). Agricultural Water Management, 2014; 137:46-51.
46. Urban O, Hlavacova M, Klem K, Novotna K, Rapantova B, Smutna P, *et al*. Combined effects of drought and high temperature on photosynthetic characteristics in four winter wheat genotypes. Field Crops Research, 2018; 223:137-149.

47. Vani SK, Sridevi O, Salimath PM. Studies on genetic variability, correlation and path analysis in chilli (*Capsicum annuum* L.). Annals of Biological Research, 2007; 23(2):117.
48. Vivitha P, Raveendran M, Vijayalakshmi D. Introgression of QTLs controlling spikelet fertility maintains membrane integrity and grain yield in improved white Ponni derived progenies exposed to heat stress. Rice Science. 2017; 24(1):32-40.
49. Wahid A, Gelani S, Ashraf M & Foolad, MR. Heat tolerance in plants: An overview Environ. Exp. Bot, 2007; 61:199-223.
50. Wheeler TR, Craufurd PQ, Ellis RH, Porter JR, Prasad PVV. Temperature variability and the yield of annual crops. Agriculture, Ecosystems and Environment, 2000; 82:159-67
51. Zhu JJ, Qiang PENG, Liang YL, Xing WU, Hao WL. Leaf gas exchange, chlorophyll fluorescence, and fruit yield in hot pepper (*Capsicum anmuum* L.) grown under different shade and soil moisture during the fruit growth stage. Journal of Integrative Agriculture. 2012; 11(6):927-937.
52. Ziska LH, Blumenthal DM, Runion GB, Hunt ER, Diaz-Soltero H. Invasive species and climate change: an agronomic perspective. Climatic Change, 2011; 105:13-42.