

EVALUATION OF THERMO-TOLERANCE POTENTIAL IN CUCUMBER GENOTYPES UNDER HEAT STRESS

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Summer vegetables production is enormously influenced by elevated temperature above threshold level which finally brings about a threat to food security in Indo-Pak region. Genetic variability is a significant tool to address this issue. The current study was intended to screen out heat tolerant cucumber genotypes and assessment of certain heat tolerant and sensitive genotypes for some water related, physiological and biochemical attributes. After one-month emergence of seedlings of twenty-five cucumber genotypes were subjected to elevated temperature (40°C/32°C day/night) for one week. Selection of cucumber genotypes for heat tolerance and sensitive was done based on several morphological, physiological and biochemical attributes. Substantial variation among genotypes was observed according to their capability to tolerate heat stress. Genotypes L3466 and Desi-cucumber (having electrolyte leakage of 50.5 and 46.5%, respectively) were found the most heat tolerant, while Suyo Long and Poinsett were found sensitive to heat stress (having electrolyte leakage of 52.3 and 54.5%, respectively). The significant difference was observed in water-related attributes, antioxidant activities, osmoprotectants and lipid peroxidation in leaves of tolerant (L3466 and Desi-cucumber) and sensitive (Suyo Long and Poinsett) genotypes under heat stress (40°C/32°C) when further analyzed. It was concluded that a few genotypes were more tolerant to elevated temperature (40°C) than others under study.

Keywords: Cucumber, heat stress, genetic diversity, physiology, biochemical changes.

INTRODUCTION

Climate change, global warming and uncertainty in weather conditions are burning concern regarding agriculture and crop production (Shakoor *et al.*, 2011). Heat stress is the elevation of temperature transient or permanently above threshold level (normally 10 to 15°C) is harmful for crops. The damaging effects depend on its intensity and duration (Wahid *et al.*, 2007). It has been observed in the past century that mean temperature of the globe had increased 0.5°C (IPCC, 2014). Predictions showed that after every ten years temperature would elevate by 0.3°C (Ahmad *et al.*, 2013) and would elevate approximately 1°C by the years 2025 and 3°C by the years 2100 leading to intensifying global warming situation (Porter, 2005). It is estimated that carbon dioxide contents have been elevated from 280 ppm to 380 ppm that absorb heat (Stern *et al.*, 2006). If carbon dioxide would be double, the temperature of air in atmosphere may rise by 1.1 to 6.4°C (Lobell and Field, 2007; Kim *et al.*, 2007). Anthropogenic actions are mainly responsible to release CO₂, CH₄, chlorofluorocarbons, NO and NO₂ as greenhouse gases are substantially growing slowly. So, global warming is changing growing season of agricultural crops. Early maturity, wilting, leaf and flower drop and decline in yield are major effects under heat stress (Porter, 2005). Moreover, changing of

physiological and biochemical process is affecting growth and development in such situation (Shaked *et al.*, 2004).

Heat stress is creating great problem for crop production. There are certain (maximum and minimum) limits of temperature within that limits plant can grow best (Fahad *et al.*, 2017). The ultimate outcome of heat stress is oxidative stress which happen because of active and reactive oxygen species (Hasanuzzaman *et al.*, 2013) as a result, defensive and adoptive mechanism starts (Shinozaki and Yamaguchi-Shinozaki, 2007) which lead to alterations in physiological and biochemical adoptive mechanisms of the plants by changes in gene expression (Moreno *et al.*, 2011) and generate heat shock proteins (HSPs), active oxygen species (AOS) as well as reactive oxygen species (ROS), dehydration-induced adaptations in phenological transitions to fight heat stress (Zandalinas *et al.*, 2017; Shahid *et al.*, 2017). Heat stress was given to seedling of different gerbera genotypes, it was noted that different genotypes behaved differently. Elevated temperature caused an increase of electrical conductivity; results also demonstrated that heat-stress tolerance is variable among gerbera cultivars (Kim *et al.*, 2016). Seedling stage is mostly affected by heat stress (Rasheed *et al.*, 2011; Ali *et al.*, 2018).

Cucumber (*Cucumis sativus* L.) although is warm season crop yet it is susceptible to heat stress (Zhang *et al.*, 2012). Its annual production is 71.7 MT globally (Khater, 2017).

Cucumber is native to subtropical and temperate zones and its best growth and development is observed at 15-32°C. However high temperature above threshold level deteriorating cucumber yield and quality (Zhao *et al.*, 2011). Characterization or screening of various genotypes of a species at seedling level against heat stress is need of the day (Shaheen *et al.*, 2016; Sita *et al.*, 2017) as heat stress is main hindrance in agriculture production (Schauberger *et al.*, 2017). To mitigate heat stress in cucumber is need of the day (Ding *et al.*, 2016; Ali *et al.*, 2018). Keeping in view the above-mentioned facts aim of the present research work was to screen out the cucumber genotypes for heat tolerance on morphological and physiological attributes and to further evaluated physiological and biochemical changes in heat tolerant and heat sensitive cucumber genotypes.

MATERIALS AND METHODS

The comparative study of twenty-five cucumber genotypes Beitalpha, L28293, Market more-76, Long green, Desi-cucumber, Indian Desi, Summer lot, Poinsett, L28322, Sweet success, Long green, Suvo Long, Market more, Poinsett 76, L3466, Tasty Jade, Straight Eight, Summer green, L28294, Green long, CMS 81, Heatmaster, L28390, Sumum, Green Wounder) against heat stress was performed. The germplasm was collected from Ayyub Agriculture Research Institute (AARI), National Agriculture Research Center (NARC), local varieties of a few countries along with a few national accessions. The experiment was performed in a growth room incorporated with mechanized units of heating, cooling, light (~12 000 lux) and humidifier/dehumidifier adjustment systems. Plants were grown in plastic pots having size of 12 cm × 6 cm in sand with weekly application of Hoagland's solution as seedling nutrition. Seedlings were grown under normal temperature (28°C/22°C day/night) and relative humidity of ~65% for thirty days. Then temperature was gradually increased by 2°C each day to avoid any osmotic shock to seedlings up to 40°C/32°C day/night. This temperature was maintained for seven days.

Morphological attributes: Data were recorded of agronomic traits viz. seedling shoot (cm) and root length (cm), mass of fresh seedling (g) and mass after drying (g) and number of leaves.

Physiological attributes: With the help of chlorophyll meter (CCM-200plus; Opti-Sciences, Hudson, NH, USA) SPAD values for chlorophyll contents were recorded. While electrolyte leakage was measured by taking leaf disks with method devised by Anderson *et al.* (1990) as ratio of initial readings taken by EC meter /final readings by EC meter (after autoclaving and incubation) expressed in percentage.

Gaseous exchange related parameters: The selected leaves of seedlings were placed in the jaws having chamber in the infrared gas analyzer (IRGA) (LCi-SD; ADC Bioscientific Ltd, Hoddesdon, UK) while these were still attached to parent

plant and data for gas exchange related characteristics i.e. photosynthetic rate (A) ($\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E) ($\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (G_s) (mmol s^{-1}) and sub-stomatal CO_2 (C_i) ($\mu\text{mol CO}_2 \text{mol}^{-1}$) were measured. Values for water use efficiency ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$) was measured by dividing photosynthetic rate (A) and transpiration rate (E).

After selection of two tolerant and two sensitive genotypes of cucumber against heat stress, these selected genotypes were grown again and given heat stress in equivalent way as in earlier and following attributes were calculated.

Leaf water related attributes: Water potential (Ψ_w) of fully expanded leaves was measured by cutting it by razor and placing it into gasket of pressure chamber (Model, 615, USA), same samples were frozen for a week (-20°C) after that these samples defrosted (thawed) at room temperature for one hour and then extracted sap of 10 μl by syringe and placed in osmometer (Ψ_s) (Wescor, Model-5500). Leaf turgor potential was calculated by equation ($\Psi_p = \Psi_w - \Psi_s$). Relative water contents (RWC) (%) of full expanded leaves of tolerant and sensitive genotypes were measured by method devised by Purcell and Sinclair (1994).

Biochemical attributes: Enzymatic antioxidants assays i.e. superoxide dismutase activity (SOD) in leaves, was measured by determination kit of Nanjing Jiancheng Bioengineering Institute (NJBI), China. Values for catalase (CAT) and peroxidase (POD) activities were determined by techniques explained by Chance and Maehly (1955) with a bit change. Lipid peroxidation was determined by malondialdehyde contents (MDA) following protocols of Cakmak and Horst (1991). Glycine betaine in leaves was determined following Grieve and Grattan (1983). Proline content were calculated applying acid ninhydrin technique (Deng *et al.*, 2011) with some modifications. Statistical analysis was performed by using Fisher's analysis of variance technique under CRD, and significance of heat tolerance ability of genotypes against heat stress was analyzed by using Tukey's honestly significant difference (HSD) test.

RESULTS

Morphological, physiological attributes and leaf gaseous exchange related attributes: On average genotypes revealed a significant variation ($p < 0.05$) of seedling shoot (cm) and root length (cm), mass of fresh seedling (g) and mass after drying (g) and number of leaves among different genotypes. It was also revealed that every genotype has different response in different growth attributes under elevated temperature regime (40°C/32°C day and night temperature) (Table 1).

Chlorophyll content, water use efficiency, electrolyte leakage explored significant differences ($p < 0.05$) among genotypes. It was also revealed that every genotype had different response in chlorophyll contents (SPAD units), water use

Table 1. Influence of heat stress (40°C/32°C day and night) on shoot length, root length, seedling fresh mass, seedling dry mass and number of leaves of 25 cucumber genotypes.

Genotypes	SL (cm)	RL (cm)	SFW (g)	SDW (g)	NL	CHL (SPAD)	EL (%)
Beitalpha	9.18±0.41E-H	6.73±0.11L-N	3.28±3.24DE	0.145±0.02C	3.25±0.18G	18.92±0.46E	62.0±1.42A
L28293	9.60±0.39E-H	10.88±0.17GH	3.18±3.6C-E	0.233±0.01C	4.08±0.32E-G	19.86±0.38E	46.3±1.45F-I
Market more-76	13.15±1.48F-H	11.38±0.07EFG	5.68±5.65BC	0.523±0.03BC	4.43±0.31B-G	25.23±0.54AB	64.5±1.91AB
Long green	9.28±0.35E-H	6.58±0.26M-O	3.13±3.11DE	0.271±0.03C	3.60±0.25FG	25.63±0.48AB	55.0±1.70CD
Desi-cucumber	9.55±0.50E-H	4.70±0.12P	12.64±11.94A	1.100±0.31AB	4.88±0.14B-F	27.62±0.97E	46.5±1.53F-I
Indian Desi	10.03±0.51D-H	5.38±0.09OP	10.60±10.68A	0.412±0.04C	4.25±0.26C-G	19.48±0.58FG	46.5±1.53F-I
Summer lot	10.20±0.47C-H	7.48±0.24K-N	3.91±3.61C-E	0.198±0.03C	3.44±0.25G	13.92±0.42H	47.3±1.79E-I
Poinsett	8.58±0.18H	6.41±0.10NO	3.35±3.31DE	0.120±0.05C	4.13±0.28D-G	8.60±0.49FG	54.5±1.67C-E
L28322	10.48±0.43C-H	7.78±0.10J-L	3.32±3.30DE	0.243±0.01C	4.38±0.24B-G	12.48±0.59C	50.3±1.66C-G
Sweet success	10.68±0.48C-F	12.38±0.24C-E	4.79±4.75CD	0.213±0.08BC	4.50±0.26B-G	18.89±0.21E	41.5±1.67I
Long green	10.58±0.45C-H	10.70±0.45F-H	4.72±4.36CD	0.275±0.01C	4.55±0.30B-G	14.66±0.59F	44.5±1.67G-I
Suyo Long	9.75±0.48E-H	9.50±0.17HI	2.18±2.10E	0.408±0.03C	4.31±0.22C-G	11.31±0.33GH	52.3±1.45C-F
Market more	10.23±0.61D-H	11.26±0.24E-G	3.73±3.60C-E	0.240±0.01C	5.00±0.29B-E	18.28±0.97E	55.8±1.45C
Poinsett-76	10.48±0.50C-H	12.20±0.28DE	3.29±3.35DE	0.231±0.01BC	5.25±0.29B-D	18.81±0.45E	46.3±1.73F-I
L3466	12.85±0.49B-D	13.68±0.12C	4.96±5.18B-D	0.442±0.04C	5.13±0.36B-E	23.04±0.48B-D	50.5±2.50C-G
Tasty Jade	10.28±0.35C-H	8.85±0.24IJ	4.17±4.17C-E	0.210±0.05AB	5.50±0.33BC	23.27±0.47BC	57.3±1.25BC
Straight Eight	14.08±0.41B	21.28±0.84A	7.13±7.19B	0.710±0.04C	5.00±0.24B-E	12.10±0.53FG	56.8±1.52BC
Summer green	13.78±0.45BC	7.70±0.17J-M	3.53±3.47DE	0.190±0.01C	4.50±0.24B-G	20.46±0.67DE	49.5±1.67C-H
L28294	26.25±0.63A	8.28±0.13JK	3.73±3.29DE	0.180±0.02BC	7.00±0.33A	12.83±0.25FG	53.0±1.89C-F
Green long	15.55±0.33B	12.38±0.60D-F	3.90±4.12C-E	0.328±0.03C	5.63±0.43B	18.97±0.77E	51.3±0.99C-G
CMS 81	10.00±0.58D-H	15.90±0.37B	3.10±3.12DE	0.301±0.05C	5.00±0.24B-E	19.10±0.82E	47.8±1.73D-I
Heatmaster	8.75±0.42GH	8.65±0.23I-K	4.11±4.36CD	0.338±0.04C	5.00±0.24B-E	18.72±0.87E	54.3±1.91C-E
L28390	11.73±0.45C-G	12.85±0.26CD	3.89±3.38DE	0.221±0.08C	4.25±0.29C-G	19.08±0.57C-E	47.3±1.91E-I
Sumum	11.90±0.31C-E	10.70±0.12GH	4.65±3.80C-E	0.343±0.02BC	3.93±0.25E-G	20.53±0.57E	56.0±1.25C
Green Wounder	11.28±0.42C-F	11.55±0.29E-G	4.92±4.67CD	0.398±0.04A	4.00±0.24D-G	19.69±0.52A	42.3±1.45HI
HSD value	2.86	1.36	2.14	0.32	1.29	2.75	7.66

SL shoot length, RL root length, SFW seedling fresh mass, SDW seedling dry mass, NL number of leaves. Chl chlorophyll content, EC electrolyte leakage. Tukey HSD where ($p \leq 0.05$) showed significant. The values are means of four replicates \pm standard error (SE). Different lettering against mean values shows significant difference among means.

efficiency (%), electrolyte leakage (%) under elevated temperature regime (40°C/32°C) (Table 1). Similar findings were observed by IRGA records with significant variation ($p < 0.05$) in photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and related parameters i.e. transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$), leaf temperature ($^{\circ}\text{C}$), stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) and sub-stomatal CO_2 ($\mu\text{mol CO}_2 \text{mol}^{-1}$) among different genotypes. It was also revealed that every genotype has different response in different physiological attributes under heat stress of (40°C/32°C) (Table 2).

Selection criteria for heat tolerant and sensitive genotypes:

Ranking was done based on heat tolerance in cucumber genotypes in descending order i.e., L3466, Desi-cucumber, Indian Desi, Green long, Green Wounder, L28390, Summer green, Sweet success, Heatmaster, CMS-81, Market more-76, Straight Eight, Poinsett-76, Summer lot, Tasty Jade, L28294, L28293, Sumum, Market more, Beitalpha, Long green, Long green, L28322, Suyo Long, Poinsett, respectively. The ranking of genotype was done by giving maximum score to genotypes performing well in attributes studied and hence represented their heat tolerance potential. The parameters

whose higher values was desirable (shoot length, root length, seedling fresh mass, seedling dry mass, number of leaves, photosynthetic rate, water use efficiency, chlorophyll contents) was taken as positive parameters and genotype which was best in this parameter was given score 25 and minimum score was given 01. Those parameters whose minimum value was desired in stress situation i.e. transpiration rate, stomatal conductance, sub-stomatal CO_2 and leaf temperature, and electrolyte leakage, were taken as negative parameters and genotypes which was minimum value was given score 25 and maximum value was given score 01. Total score of genotypes was obtained by adding score of all parameters of each genotype. Then genotype with maximum score was considered most tolerant to heat and with minimum score was considered most sensitive to heat. So, genotypes L3466 and Desi-cucumber were found most heat tolerant while genotypes Suyo Long and Poinsett were found sensitive to heat stress (40°C /32°C day and night temperature).

Biochemical and water related attributes of selected genotypes: After selecting the tolerant and sensitive

Table 2. Influence of heat stress (40°C/32°C day and night) on photosynthetic rate, transpiration rate, leaf temperature, stomatal conductance to water and sub-stomatal CO₂ of 25 cucumber genotypes.

Genotypes	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Gs ($\text{mmol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	LT C (Celsius)	C _i ($\mu\text{mol CO}_2 \text{mol}^{-1}$)	WUE (A/E)
Beitalpha	2.34±0.18B-E	0.191±0.01B-D	2.78±0.18F-H	31.43±0.64A-C	1054±38L	0.425±0.04A-D
L28293	1.44±0.10F-H	0.246±0.01A-D	3.67±0.23B-D	31.43±0.39A-C	1144±34L	0.159±0.03CD
Market more-76	1.28±0.16G-I	0.195±0.01B-E	3.25±0.11C-G	30.05±0.40A-C	1173±34KL	0.242±0.04CD
Long green	1.55±0.05F-H	0.175±0.02F-H	3.56±0.23B-E	30.85±0.60A-C	1285±26J-L	0.421±0.03CD
Desi-cucumber	2.78±0.17B	0.115±0.01F-H	2.90±0.07E-H	28.44±0.73C	1311±25I-K	1.015±0.16A-D
Indian Desi	2.00±0.12C-F	0.113±0.01E-H	3.03±0.07D-H	30.17±0.13A-C	1153±34L	0.823±0.08CD
Summer lot	1.69±0.07E-H	0.125±0.012E-H	3.13±0.142C-H	30.63±0.68A-C	1347±38H-J	0.611±0.03CD
Poinsett	1.42±0.20F-H	0.110±0.01F-H	2.90±0.09E-H	32.64±0.37AB	1355±25H-J	0.243±0.05CD
L28322	0.75±0.04I	0.112±0.01AB	3.50±0.22C-F	31.76±0.23AB	1306±38I-K	0.214±0.06D
Sweet success	1.57±0.27F-H	0.205±0.01B-F	4.24±0.19AB	31.45±0.66A-C	1406±33G-J	0.231±0.06CD
Long green	1.05±0.07HI	0.165±0.02A-C	3.77±0.17BC	32.32±0.48AB	1443±18F-I	0.273±0.05D
Suyo Long	2.48±0.11B-D	0.206±0.01D-F	4.23±0.22AB	32.80±0.42A	1472±24E-H	0.608±0.05CD
Market more	1.88±0.12D-G	0.145±0.01D-F	3.72±0.16B-D	31.04±0.57A-C	1536±21D-G	0.532±0.03CD
Poinsett-76	1.37±0.19F-I	0.145±0.01F-H	3.46±0.15C-F	31.23±0.91A-C	1597±36C-E	0.174±0.04CD
L3466	1.34±0.05G-I	0.105±0.01C-F	2.45±0.12H	29.66±0.46BC	1635±23B-D	0.450±0.04CD
Tasty Jade	1.51±0.24F-H	0.155±0.02E-G	3.64±0.16B-D	31.42±0.95A-C	1646±38B-D	0.234±0.07CD
Straight Eight	1.38±0.08F-I	0.131±0.01F-H	3.31±0.16C-G	32.00±0.54AB	1718±35B-D	0.487±0.02CD
Summer green	2.58±0.11BC	0.105±0.01GH	3.05±0.08C-H	31.58±0.57AB	1518±29D-G	0.938±0.12B-D
L28294	1.39±0.08F-I	0.081±0.01A	2.61±0.16GH	31.81±0.59AB	1822±32A	0.149±0.05CD
Green long	2.25±0.06B-E	0.106±0.01F-H	2.85±0.12E-H	32.33±0.15AB	1741±34A-C	0.809±0.10B-D
CMS 81	2.01±0.10C-F	0.110±0.01F-H	3.11±0.14C-H	31.63±0.99AB	1760±34AB	0.646±0.09CD
Heatmaster	2.32±0.04B-E	0.075±0.01H	2.42±0.08H	32.59±1.01AB	1840±35A-C	1.058±0.16A-D
L28390	4.88±0.14A	0.110±0.01F-H	3.22±0.13C-G	32.27±1.23AB	1855±29AB	1.503±0.32AB
Sumum	5.28±0.14A	0.210±0.02AB	4.90±0.22A	31.42±0.83A-C	1563±23D-F	1.259±0.18A-C
Green Wounder	5.34±0.21A	0.130±0.01E-G	3.44±0.09C-F	32.54±0.35AB	1565±26D-F	2.084±0.34A
HSD value	0.65	0.054	0.73	3.03	144	0.59

A photosynthetic rate, Gs stomatal conductance, E transpiration rate, C_i sub-stomatal CO₂, WUE water use efficiency. Tukey HSD±0.05 where (p≤0.05) showed significant difference. The values are means of four replicates ± standard error (SE). Different lettering against mean values shows significant difference among means.

genotypes were further studied regarding water related attributes viz osmotic potential, water potential, turgor potential, relative water contents and activity of antioxidants in leaves i.e. superoxide dismutase (SOD) (U mg⁻¹ Protein), peroxidase (POD) (U mg⁻¹ Protein), catalase (CAT) (U mg⁻¹ Protein) and protein (mg/ml).

Water potential (Ψ_w) (-MPa), osmotic potential (Ψ_π) (-MPa) and relative water contents (RWC) (%) were high in tolerant genotypes (L3466 and Desi-cucumber) and low in sensitive genotypes (Suyo Long and Poinsett) while turgor potential (Ψ_w) (MPa) were high in tolerant genotypes (L3466 and Desi cucumber) low in sensitive genotypes (Suyo Long and Poinsett) under consideration. There was significant variation among tolerant and sensitive genotypes for these antioxidants and protein. The value of water potential was high in L3466 (0.56) and Desi-cucumber (0.52) as compared to Suyo Long (0.46) and Poinsett (0.42). The value of osmotic potential was high in L3466 (0.44) and Desi-cucumber (0.40) as compared to Suyo Long (0.35) and Poinsett (0.37). The value of turgor potential was high in L3466 (0.12) and Desi-cucumber (0.13)

as compared to Suyo Long (0.10) and Poinsett (0.05) under high temperature (40°C/32°C day and night temperature) described in Figure 1 (A,B,C,D).

There was significant variation among tolerant and sensitive genotypes for these antioxidants and protein. The value of SOD was high in L3466 (8.45) and Desi-cucumber (6.68) as compared to Suyo Long (5.28) and Poinsett (4.38). The value of POD was high in L3466 (8.23) and Desi-cucumber (6.68) as compared to Suyo Long (4.65) and Poinsett (5.28). The value of CAT was high in L3466 (0.14) and Desi-cucumber (0.12) as compared to Suyo Long (0.10) and Poinsett (0.10). The value of total protein was high in L3466 (40) and Desi-cucumber (37) as compared to Suyo Long (28) and Poinsett (32) under high temperature (40°C/32°C) as described in Figure 1 (E,F,G,H).

The values of glycine betaine (μmol g⁻¹ FW) was high in L3466 (4.72) and Desi-cucumber (4.08) as compared to Suyo Long (3.32) and Poinsett (3.25). Proline contents (μmol g⁻¹ FW) in leaves were also high in L3466 (7.33) and Desi-cucumber (6.52) as compared to Suyo Long (5.37) and

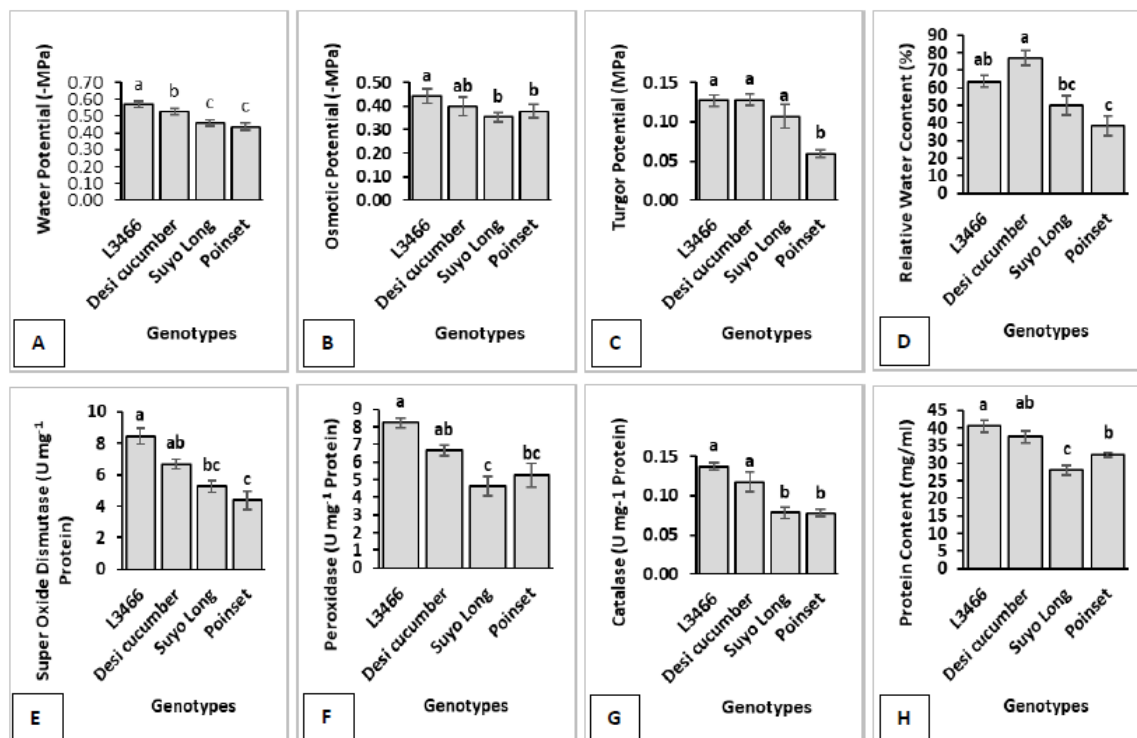


Figure 1. Response of (A) Leaf water potential (Ψ_w), (B) Leaf osmotic potential (Ψ_s), (C) Leaf turgor potential, (D) Relative water content (%) (RWC), (E) Superoxide dismutase activity, (F) Peroxidase activity, (G) Catalase activity and (H) protein content (mg/ml) of tolerant and sensitive genotypes of cucumber to elevated temperature (40°C/32°C day and night). Different lettering above error bars shows significant difference among means of genotypes following Tukey HSD where ($p \leq 0.05$) showed significant difference.

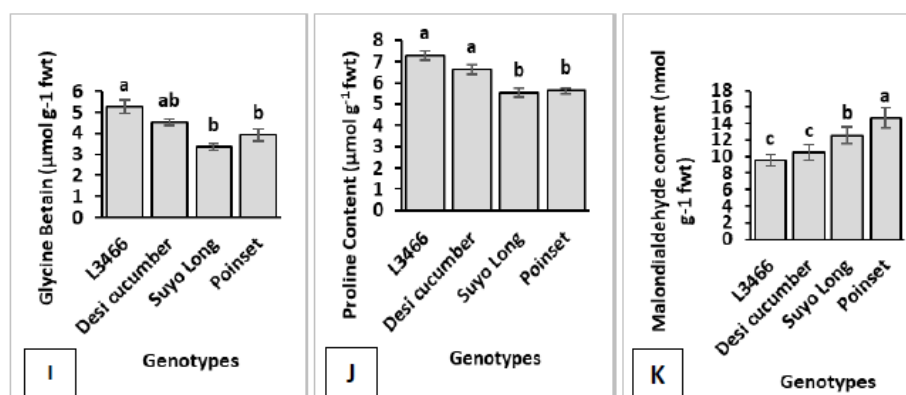


Figure 2. Response of (I) Glycine betaine, (J) Proline and (K) Malondialdehyde content in leaves of tolerant and sensitive genotypes of cucumber to elevated temperature (40°C/32°C day and night). Different lettering above error bars shows significant difference among means of genotypes following Tukey HSD where ($p \leq 0.05$) showed significant difference.

Poinsett (5.64). Malondialdehyde contents (nmol g⁻¹ FW) were high in L3466 (9.98) and Desi-cucumber (10.50) as compared to Suvo Long (12.53) and Poinsett (14.05) under high temperature (40°C/32°C day and night temperature) described in Figure 2 (I,J,K).

DISCUSSION

Genotypes under consideration behaved contrastingly for growth, physiological and biochemical attributes under heat stress, which depicted that heat tolerance varies with

genotypes of each crop (Hussain *et al.*, 2016; Saeed *et al.*, 2007). This contrasting behavior of different genotypes is already observed by Abdelmageed *et al.* (2009). In one of the experiment screening of different genotypes of gerbera against heat stress was found valuable (Kim *et al.*, 2016).

Present study signifies genotypic response towards growth characters with some cucumber genotypes revealing more growth than others while some showed less growth during heat stress according to ability to tolerate heat stress. Reminiscent results were observed by Khater (2017), who reported that at high temperature (29°C) revealed negative growth responses (shoot diameter, shoot length, leaf area etc.) as compared to low temperature at (26°C) in seedlings of grafted cucumber. It might be due to fact that seedling stage is more sensitive to heat stress which reasons a reduction in cell division lowering meristematic function in different parts of the plants, especially in leaves (Zhou *et al.*, 2016). It could also capture elongation of the cell wall and hampers cell differentiation resulting seize in growth of the seedling (Potters *et al.*, 2007). Heat stress at 40°C decrease the seedling dry mass, shoot length and chlorophyll contents in cucumber seedlings (Khan *et al.*, 2012). Comparable, outcomes were also stated in several tomato genotypes under heat stress (Naika *et al.*, 2005). This positive association of root fresh mass with water and nutrient uptake efficiency in plant growth would enhance growth. More number of leaves specifies more net carbon assimilation and finally rises in dry mass of both shoot and root. These enormous differences disclose the capacity of heat-tolerant genotypes to produce more biomass under a high-temperature regime as compare with sensitive genotypes.

Seedlings are more prone to effect than mature plants and the reduction in plant during heat stress and cause modifications in physiology (Wollenweber *et al.*, 2003). So, a significant variation was seen photosynthetic rate among various cucumber genotypes studied, since photosynthesis is most heat-sensitive physiological processes (Guilioni *et al.*, 2003) having three key sites in photosynthetic apparatus that are susceptible to heat stress i.e. both photosystems, primarily photosystem II is mostly influenced with its oxygen-evolving complex, the ATP-generating and carbon integration mechanisms (Nishiyama *et al.*, 2006; Mohanty *et al.*, 2007). Initially alteration pre-dominates for photosystem II, which is also responsible for acclimation and rescue process under elevating temperature (Murata *et al.*, 2007).

Heat stress causes inhibition of process of electron transporter and RuBisCO activation state inhibition leading to the inhibition of the photosynthetic CO₂ assimilation rate (Salvucci and Crafts-Brandner, 2004). Reduction in photosynthetic rate was observed when temperature was enhanced, possibly owing to decreased stomatal conductance. Those genotypes which were found tolerant to heat stress were found higher CO₂ assimilation rate due to their well-organized photosynthetic apparatus (Camejo *et al.*, 2005). It

was observed that prosthetic apparatus was highly damaged by heat stress in cucumber plants grafted on luffa when exposed to heat stress (Li *et al.*, 2016). So, present study depicted higher CO₂ assimilation rate in heat-tolerant genotypes as compared to heat-sensitive genotypes (Camejo *et al.* 2005). These alterations conducted changes in capacity of mesophyll cells to perform normal photosynthetic process. Results for sub-stomatal CO₂ indicated a significant impact among genotypes, which indicated that there is additional CO₂ is gathered in the leaf. Moreover, such situation produces alterations in stomatal conductance which increases resistance to carbon dioxide diffusion through stomata (Camejo *et al.* 2005).

Heat stress caused a significantly increased temperature of leaves among different cucumber genotypes. It is vital to study because at optimum leaf surface temperature plant can perform normal metabolic processes. Parallel findings are reported previously which indicate sensitive genotypes induce high leaf surface temperature, while tolerant genotypes resist enhancing leaf surface temperature which ensure high transpiration rate in sensitive genotypes (Nkansah and Ito, 1995). High transpiration caused more water loss in sensitive genotypes which might brought about tissue and organ dehydration lead to wilting of seedlings (Mazorra *et al.*, 2002). It was also observed by Din *et al.* (2016) that cucumber seedlings exposed to heat shock caused excessive high transpiration rate and high stomatal conductance.

Selected sensitive genotypes revealed low turgor potential compared with tolerant ones as heat stress cause osmotic stress which limited root hydraulic conductance lead to diminish the uptake of water and organic solutes from roots and translocation of ions through xylem vessels confines photosynthesis and enhance transpiration which ultimately reduces leaf osmotic potential and chlorophyll fluorescence etc. (Huve *et al.*, 2006; Taiz and Zeiger, 2002). These conditions would lead toward closure of stomata and lessen the water potential in tissues (Wahid *et al.*, 2007).

Present research exposed significant variation regarding chlorophyll content among selected cucumber genotypes at seedling stage due to degradation of chlorophyll probably since transformations in plants' microscopic structures in heat stress. (Semenova, 2004; Kreslavski, *et al.*, 2008). Sensitive genotypes showed more alterations than tolerant genotypes, as tolerant genotypes can keep relatively their micro-bodies structure normally, reminiscent results were reported by Baninasab and Ghobadi (2011), when they exposed cucumber seedlings to heat stress in 40°C which injured relative chlorophyll content and chlorophyll fluorescence ratio. Higher SPAD value indicates greater photosynthetic ability of plants and ultimately enhanced growth. Wang *et al.* (2018) stated that after heat treatment, the chlorophyll content decreased rapidly, and the degree of the modification in heat-resistant plants was lesser than that in non-resistant varieties.

Electrolyte leakage was found an important indicator to quantify heat stress injury. This indicator is much helpful for assessing cell membrane stability (Bajji *et al.*, 2001). So, results indicate that genotypes varied significantly in their ability to maintain normal cell membrane structure. This is since reactive oxygen species are involved in damaging the cell membrane structure. It was noted that heat tolerant genotypes produced more enzymatic antioxidants than sensitive ones. Heat stress finally results in oxidative stress owing to production of reactive oxygen species (ROS), these free radicals include OH^\cdot , H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$. Superoxide dismutase (SOD) detoxifies these ROS and further it is scavenged by peroxidase (POD) and catalase (CAT) (Wahid *et al.*, 2012). During heat stress plant showed adoptive mechanism through the accumulation of osmolytes and compatible solutes. Present research work showed high proline and glycine betaine contents in their leaves as compared with sensitive ones. Such findings have been reported when concentrations of compatible osmolytes such as sugars, sugar alcohols, glycine betaine, proline, soluble protein, quaternary ammonium, and tertiary sulphonium compounds changed under heat stress (Sairam and Tyagi, 2004). The accumulation of osmolytes may be involved in osmotic adjustment, and it is an important adjustment tool in many plants in similar situations (Hasanuzzaman *et al.*, 2013). Further production of ROS cause degradation of proteins (Wang *et al.*, 2018) and causes cell membrane injury, becomes a cause to produce ROS that mainly attacks photosystem II and respiratory pathways (Goraya *et al.*, 2017). MDA content were enhanced under heat stress as it is previously reported that these can be used to measure the heat injury of plants (Mittler, 2002; Al meselmani *et al.*, 2006).

Conclusion: It was observed that genotypes L3466 and Desi cucumber were found comparatively most heat tolerant while genotypes Suyo Long and Poinsett were found comparatively sensitive to heat stress. These consequences indicated that morpho-physiological and biochemical attributes were genotype dependent, revealing significant variation in genetic diversity (within a specie genotypes). These observations in cucumber possibly could be valuable to initiate a program on breeding in cucumber introducing heat tolerance ability under global warming scenario.

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