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Physiological response of diverse maize genotypes to heat stress and simple sequence repeats markers-based genotyping in Pakistan

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Contribution | **Malik, S. I.** & **F. M. Watoo** conceived the idea, **S. Gohar** & **K. Adil** conducted the experiments, collected the data, and performed statistical analyses. **U. Farooq, A. Hussain**, **S. Sabahat**, **K. Javed** & **Z. Ahmed** collected the literature review, proofread and provided intellectual guidance.

ABSTRACT

The current study was designed to observe the response of maize genotypes under high-temperature stress conditions and to check their diversity using simple sequence repeats (SSR) markers in in-vitro conditions. Maize genotypes (n=10) were subjected to heat stress at ~45 °C for 72 h after growing into young seedlings. Their physiological responses were determined by measuring the changes in proline content (PC), chlorophyll content (CC), cell membrane stability (CMS), relative water content (RWC) and osmotic potential ($\Psi\pi$). The analysed data disclosed significant differences ($P \leq 0.01$) among all the characters. Comparison between control and heat-treated genotypes depicted a notable increase in PC, $\Psi\pi$ while a decrease in CC, RWC and CMS index in heat-treated plants. Maize genotypes viz. NCEV-1230, NARC-4, HN Gold and OPV-20 performed better under heat stress and also showed a strong genetic relationship for similarity index in dendrogram based on selected SSR markers related to abiotic stress tolerance. This study is beneficial for understanding the physiological behaviour of maize plants under heat stress and for further genetic studies on maize to select heat-tolerant lines for breeding programs.

Keywords: Climate change, thermal stress, plant physiology, genetic diversity, SSR Marker.

INTRODUCTION: Maize (*Zea mays* L.), a monoecious dicot that belongs to the family Poaceae having chromosome number ($2n$=20). It is also known as "Corn" in North America. The genome size of maize is 2,300 Mb and approximately 32,000 protein-encoding genes are estimated to be present in the maize genome (Schnable *et al.*, 2009). About 300 million people consume maize as a staple food in Africa (Vigouroux *et al.*, 2003). The USA is the largest producer of maize followed by China, Brazil, the European Union, Argentina, India, Ukraine, Mexico, Russia and Canada. World Maize production estimated by USDA was 1.23 billion metric tons in 2023-24. Annual production of maize in Pakistan was 11 million metric tons in 2022-23 which decreased 10.4% to 9.8 million metric tons in 2023-24 (Hussain, 2023). During 2022-23, the total cultivated area of maize in Pakistan was 1.7 million hectares which was a decline of 4.5% to 1.6 million hectares compared to 2023-24 (Hussain, 2023). There are limited opportunities to expand maize cultivation areas. Hence, there is a need to improve the maize production system to meet growing maize demand. Heat stress can reduce the grain yield in maize. This damage depends upon the duration, intensity of heat wave and plant sensitivity during crop developmental stages (Fahad *et al.*, 2017). During early developmental stages, high temperatures can affect seed germination and seedling vigour (Prasad *et al.*, 2017). At the vegetative stage heat stress affects plant metabolism by causing disturbance in carbon cycles, leaf pigments, photosynthetic activities, light perception and organic solutes translocation. It may cause pre-mature spore abortion, and mitotic defects at the flowering stage which triggers male sterility (Begcy *et al.*, 2019). Maize crop has the tremendous ability to endure brief exposures to high temperatures whereas, long spells of temperature ≥ 35 °C are not fit for crop development, especially for flowering and grain-filling stages (Tesfaye *et al.*, 2016). In maize, each degree rise in temperature above 30 °C, can reduce the yield 1-1.7% per day (Lobell *et al.*, 2011). Heat and light (combined stress) affect plant photosynthesis by reducing chlorophyll content, relative water contents, net photosynthetic rate, photochemical activity of photosystem II (PSII), stomatal conductance cell membrane stability in maize (Yadav *et al.*, 2018). In Pakistan, heat stress is more detrimental when the temperature rises up to 45 °C (Rahman *et al.*, 2013).

Plants maintain their homeostasis and redox levels in cells by modifying their antioxidant system and by releasing various compatible solutes under stress (Hasanuzzaman *et al.*, 2013). Accumulation of different solutes including sodium, potassium, chloride, (Farouk, 2011) complete solvent sugars, organic and inorganic solutes, free amino acids, glycine betaine, and proline are seen as key elements to achieve osmotic balance in plants under stress conditions (Zahid *et al.*, 2016). Osmotic adjustment incorporates tolerance against stress in plants in numerous ways. Plants achieve higher plasma membrane stability and integrity to minimize electrolyte leakage and preserve their water content to normalize their physiological functioning under abiotic stress by an osmotic adjustment system (Babu *et al.*, 2004). Proline acts as an osmoprotectant and plays a vital role in radical scavenging and redox balancing in plants under stress conditions (Oraki and Aghaalikhana, 2012). High proline contents increase tolerance against heat stress (Kishor *et al.*, 2005). Genes *PhHsf19, PhHsf21,* and *PhP5CR* are closely associated with the upregulation of heat shock transcriptional factors (Hsfs) and change in proline levels, indicating they play a vital role in tolerating heat stress during heat periods in petunia (Yue *et al.*, 2019). Overexpression of gene *OsProDH* causes a reduction in the proline content and makes plants more susceptible to heat stress (Guo *et al.*, 2020).

In molecular marker-assisted breeding programs, SSR markers are highly recommended to select parental lines for genetic improvement in maize crops (Prasanna *et al.*, 2010). The SSR markers are widely used by researchers to study heterosis, combining ability and genetic diversity in maize (George *et al.*, 2004), because they are easily detectable with PCR analysis (Polymeropoulos *et al.*, 1991). High allelic variation, robust, abundant and co-dominant nature make SSR markers distinct from other genetic markers (Senior *et al.*, 1998). Considering the current climate change scenario, the main target of plant breeders is to develop heat-tolerant varieties to achieve the goal of food security. This can be accomplished through conventional and molecular-assisted breeding with the knowledge of the biochemical and physiological mechanisms of plants to incorporate heat-tolerant genes in the running blood (Tiwari and Yadav, 2019). Present experiment estimating the response of different maize genotypes under high-temperature stress conditions to check the diversity among them using simple sequence repeats (SSR) markers for further genetic studies on maize for the selection of heat-tolerant lines for maize breeding programs.

OBJECTIVES: The objectives of the study were to (1) evaluate maize genotypes for heat tolerance based on physiological parameters, (2) establishing phylogenetic relationships and assessing genetic diversity based on SSR markers.

MATERIALS AND METHODS: This experiment was conducted in the biotechnology laboratory and glasshouse of the Department of Plant Breeding and Genetics (PBG), Pir Mehr Ali Shah (PMAS) Arid Agriculture University, Rawalpindi (AAUR). Maize genotypes viz.

NARC, NARC-2, NARC-4, NCEV-1230, OPV-6, NCEV-4, NCEV-1270, HN Gold, NCEV-3 and OPV-20 were obtained from the National Agricultural Research Centre (NARC), Islamabad, Pakistan and were grown in loamy soil and manure in proportion (2:1) using a completely randomized design (CRD) with three repeats for both the control and treatment groups under optimal conditions in the glasshouse. Fourteen-days old seedlings (n=10) with 3 repeats (only treatment group) were subjected to heat stress at ~45 °C for 72 h while, the control group was kept under normal growing conditions.

Physiological parameters: Proline content (PC): Leaf samples (0.25 g) of treated and normal plants were homogenized with 5 mL of 3% aqueous sulfosalicylic acid independently and left on the bench for 72 h. Samples were placed at 45 °C for 10 min. and cooled at room temperature followed by centrifugation at 18,000 g for 5 min. Filtrate (2 mL) was transferred into fresh test tubes containing glacial acetic acid (2mL) and ninhydrin (2mL). Tubes were heated for 1 h at 95 °C and cool down on ice. Toluene (4 mL) was added to each test tube containing the sample and vortexed for 15-20 sec. The upper layer was decanted while a light pink lowered layer was poured in a glass cuvette to determine absorbance at 520 nm (UV-visible) using a spectrophotometer. Toluene was used as blank. Proline content was determined (Bates *et al.*, 1973).

$$\mu moles\ proline/g\ of\ fresh\ weight\ plant\ materia = [(\mu g\ proline/ml \times ml\ toluene) \div 115.5\ \mu g/\mu mole] \div [(g\ sample)/5]$$

Chlorophyll content (CC): Chlorophyll content was measured by using a portable chlorophyll meter (OPTI-SCIENCES CCM-200 plus) from leaves of both heat-treated and normal plants in three repeats using three independent plants for each group (Al-Shatti *et al.*, 2014).

Cell membrane stability (CMS): Leaf samples (10 discs) from treated group were heated at 45 °C for 8 h with 10 mL double distilled water whereas, the control group leaf samples were placed at a normal temperature of 25 °C for 8 h. The electrical conductivity (EC) of the solution was measured by using a conductivity meter. Tubes were autoclaved for 15 min. at 121 °C. The EC of all samples was measured and CMS was calculated (Tripathy *et al.*, 2000).

$$CMS = \frac{1 - T_1/T_2}{1 - C_1/C_2} \times 100$$

T1= treated sample conductance (before autoclaving), T2= treated sample conductance (after autoclaving), C1= controlled sample conductance (before autoclaving), and C2= controlled sample conductance (after autoclaving).

Leaf osmotic potential (Ψπ): Leaf samples of both control and treated plants were immersed for 5 min. in 1.5 mL microtubes containing liquid nitrogen and then frozen at –80 °C for 2 days. The samples were crushed and centrifuged at 15000g for 10 min. and cell sap (50 µL) was used to measure osmatic potential (Ψπ) with the aid of an osmometer (Blum, 1989). The readings were recorded in mmol/kg and expressed as Mega Pascal (MPa) according to Vant's Hoff equation:

$$OP\ (-Mpa) = (R \times T \times Osmometer\ reading)$$

R is the gas constant (0.008314), T = laboratory temperature.

Relative water content (RWC): Leaf samples from both control and treated plants were placed in vials and weighted (W) which was the fresh weight. Then samples were hydrated for 4 h at room temperature. After 4 h the samples were taken out from water and weighed immediately to obtain fully turgid tissue weight. Then samples were oven dried at 80 °C for 24 h and weighed to get dry weight. RWC of leaf samples was calculated using the formula (Kuhad *et al.*, 1989).

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

FW = fresh weight, DW = dry weight, TW = turgid weight.

The SSR marker analysis: Total genomic DNA was extracted using the Cetyl trimethylammonium bromide (CTAB) method (Doyle, 1991) and confirmed on agarose gel (1%). The SSR primer pairs (table 1) were used to study genetic diversity in maize. For the amplification, PCR was performed by using DNA as a template and SSR markers primers. The master mix (15.6 µL DEPC water, 2.5 µL 10X Taq buffer, 1 µL $MgCl_2$ (50 mM), 0.5 µL dNTP,s mix (10 mM each), 2.0 µL forward and 2.0 µL reverse primers, 1 µL DNA template and 0.4 µL Taq polymerase) for a total reaction volume of 25 µL. The PCR reaction conditions were initial denaturation at 95 °C for 4 min., denaturation at 94 °C for 45 sec., annealing at 50-60 °C for 45 sec., and extension at 72 °C for 1 min. The PCR was set for 30-35 cycles for sufficient amplification of target regions. The final extension was performed at 72 °C for 10 min and samples were analysed on 3% agarose gel using gel documentation system.

Statistical analysis: Physiological data was analysed by using the statistical software Statistix 8.1 while charts were made using MS Excel 2016. Analysis of variance (ANOVA) was performed to note statistical differences among maize genotypes on the basis of physiological parameters in plants under heat stress and normal conditions. Cluster analysis on the basis of molecular data was performed using the software Minitab 19.

Sr. No.	Name		Sequence (5´ - 3´)	GC (%)	T_m (°C)	Ann. Temp (°C)
1	Umc 1630	F	CAGACCTTCGAGGGCAAGAACT	55	61	55
		R	AGTTTTGGCTTCTTCTCCCAAGTC	46	60	
2	Umc 2013	F	GGGACGAGAGTCTGTTGTTGTTG	52	61	55
		R	GTTGATGCATGTGACTCTGGAAAC	46	60	
3	Umc 1069	F	AGAGAATCCCCAAGCAAACAAAC	43	58	53
		R	CTTCATCGGAGCCATGGTGT	55	58	
4	Bnlg 1810	F	ATGCTCCTCCTCTCCTCCAT	55	58	52
		R	GCGATGATGAGCTGCAAGTA	50	56	
5	Bnlg 1189	F	CGTTACCCATTCCTGCTACG	55	58	50
		R	CTTGCTCGTTTCCATTCCAT	45	54	
6	Phi 053	F	CTGCCTCTCAGATTCAGAGATTGAC	48	62	56
		R	AACCCAACGTACTCCGGCAG	60	60	

Table 1: List of primer pairs for SSR markers.
Forward "F", Reverse "R", Guanine and Cytosine "GC", Melting temperature "T_m".

RESULTS: Analyses of variance (ANOVA) showed highly significant differences ($P \leq 0.01$) among all genotypes for different physiological parameters in both normal and heat-stressed plants. Mean square values from ANOVA, grand means and coefficient of variance (CV) for all physiological traits in control and stressed plant groups was presented in table 2.

Proline accumulation under heat stress: Analysis of variance (ANOVA) for PC showed a highly significant difference among maize genotypes for both control ($F_{2,9}$=37.8) and heat-treated ($F_{2,9}$=86.3) groups ($P \leq 0.01$) (table 2). An increase in PC was observed in all genotypes under stress conditions. The high PC value was observed in genotypes NARC-4 (0.171 µmol/g), OPV-20 (0.166 µmol/g) and NCEV-1230 (0.165 µmol/g) under heat stress showing their better response to heat stress as compare to all other genotypes Whereas, the genotypes NCEV-1270 and NCEV-3 showed lowest PC values of 0.072 µmol/g and 0.062 µmol/g, respectively (figure 1) were marked as heat susceptible.

Osmotic potential (Ψπ): ANOVA revealed highly significant variation for $\Psi\pi$ in both control ($F_{2,9}$=38.1) and stressed ($F_{2,9}$=61.9) (Table 2) maize plants at $P \leq 0.01$. A remarkable increase in $\Psi\pi$ was observed in all genotypes under heat stress conditions when compared to the control group of plants (Figure 1). Values for $\Psi\pi$ were relatively higher in NCEV-1270 (-0.145 Mpa) and HN Gold (-0.118 Mpa) after heat stress treatment (figure 1), while, OPV-20 (-0.06 Mpa) and NCEV-1230 (-0.04 Mpa) retained less water obvious from low $\Psi\pi$. Hence, based on $\Psi\pi$ two genotypes viz., NCEV-1270 and HN Gold were identified as more tolerant to heat stress at the seedling stage while OPV-20 and NCEV-1230 were marked as susceptible genotypes.

Relative water content (RWC): ANOVA of ten maize genotypes for RWC revealed highly significant variation in the control group ($F_{2,9}$=11.1, $P \leq 0.01$) and treated group ($F_{2,9}$=51.8, $P \leq 0.01$) (table 2). A decline in leaf RWC was observed in all heat-treated plants when compared to the plants kept in normal growing conditions. The highest RWC were retained in genotypes OPV-20 (83.34%), NCEV-1270 (72.15%) and NARC (68.55%) after heat treatment which determines tolerance against heat stress in these genotypes while, for these genotypes, the base values for RWC under normal conditions were 86.24%, 82.57%, and 76.89%, respectively (figure 1). The genotypes NCEV-1230, NCEV 4 and NCEV-3 were designated as relatively heat-susceptible based on measured RWC after exposure to heat stress. RWC in genotypes OPV-6 and HN Gold was moderate under heat stress conditions when compared to normal conditions (figure 1). Hence, OPV-6 and HN Gold were considered moderately tolerant genotypes for this parameter.

Cell membrane stability (CMS): For CMS, ANOVA highly significant differences ($F_{2,9}$=18.6, $P \leq 0.01$) among all genotypes before and after heat treatment were found (table 2). The genotypes which showed maximum CMS values were NCEV-1230 (51.9%), OPV-20 (51.46%) and HN Gold (50.64%), were marked as heat tolerant whereas, NARC-2 showed 32% membrane stability under high-temperature

stress (figure 1) was considered as heat susceptible genotype.

Chlorophyll content (CC): Reduction in leaf CC was observed in all maize genotypes in stress conditions when compared to plants in normal conditions but the level of reduction significantly varied (figure 1). ANOVA revealed highly significant variation among maize genotypes in both control ($F_{2,9}$=3.34, P≤0.01) and treated ($F_{2,9}$=3.99, P≤0.01) groups for chlorophyll content (table 2).

	PC		OP ($\Psi\pi$)		RWC		CC		CMS
	Normal	Stressed	Normal	Stressed	Normal	Stressed	Normal	Stressed	Stressed
Genotypes	0.004**	0.001**	1.05**	0.002**	136.36**	387.71**	4.31**	4.0**	175.53**
Grand Means	0.071	0.121	0.0365	0.085	73.66	63.39	11.83	8.1427	43.016
CV (%)	8.74	6.03	4.55	7.75	4.75	4.32	9.61	12.30	7.13

Table 2: Mean square values, grand means and CV (%) form ANOVA for all physiological parameters of ten maize genotypes under normal and heat-stress conditions.

** Highly significant (P≤0.01), *significant (P≤0.05), NS non-significant (P>0.05), proline content "PC", chlorophyll content "CC", cell membrane stability "CMS", relative water content "RWC", osmotic potential "OP", coefficient of variance "CV"

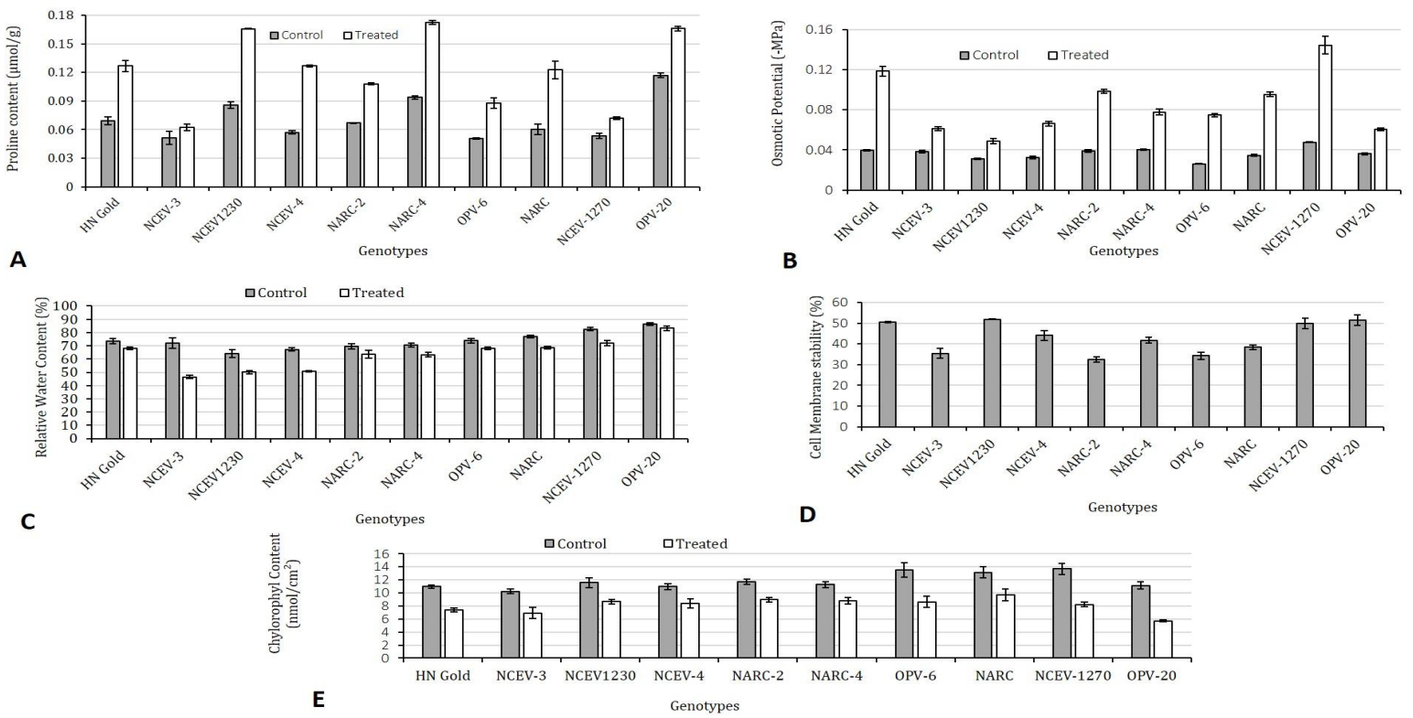

Figure 1: Comparison of maize genotypes under normal and stressed conditions for A: Proline content, B: Osmotic potential, C: Relative water content, D: Cell membrane stability, E: Chlorophyll content.

Relatively high chlorophyll retention was recorded in genotypes NARC and NARC-2 with values recorded as 9.7 nmol/cm^2 and 8.96 nmol/cm^2, respectively showing their tolerance to heat stress because these genotypes showed a minimum reduction in CC as compared to other genotypes under investigation. However, the lower retention of chlorophyll content was recorded in genotypes OPV-20 (5.7 nmol/cm^2) and NCEV-3 (6.93 nmol/cm^2), respectively showing their susceptibility to heat stress (figure 1).

Evaluation of genomic DNA: Agarose gel electrophoresis of DNA samples showed DNA bands for all genotypes under UV light by using a gel documentation system (figure 2) suggesting the presence of an ample amount of DNA without degradation for PCR analysis. Further usefulness of genomic DNA for finding genetic diversity was assessed by polymerase chain reaction (PCR) using maize *Actin* primer pair. PCR was performed by using maize *Actin* primer pair (50 °C annealing temperature) to confirm the ability of DNA to yield PCR products. Agarose gel electrophoresis revealed 500 bp PCR products when separated by agarose gel electrophoresis and visualized under UV light in the gel documentation system (figure 2).

Analysis of genetic diversity: Analysis of genetic diversity was assessed in ten maize genotypes by using six reported pairs of SSR primers. Four primer pairs umc2013, bnlg1189, bnlg1810 and phi053 showed successful amplification during PCR. Polymorphism was observed in the gel pictures of SSR markers umc2013 (figure 3) and bnlg1810 (figure 3). These two primers were considered useful for assessing genetic diversity in maize genotypes. The primers bnlg1189 and phi053 yielded approximately 200 bp monomorphic amplicons at 50 and 56 °C annealing temperatures respectively (figure 3). The rest of the two primers umc1630 and umc1069 failed to produce any amplicon. Hence, the results suggested that these primers have no utility in assessing genetic diversity in maize genotypes under investigation.

Cluster analysis: Cluster analysis revealed the genotypic similarities and differences based on SSR markers polymorphism among ten maize genotypes (figure 4). Cluster A includes two further subgroups 1A and 2A. The genotype HN Gold showed 81.67% similarity with NCEV-1230 (sub-group 1A) and 63.33% similarity with genotype NARC-4 (sub-group 2A) in the graph.

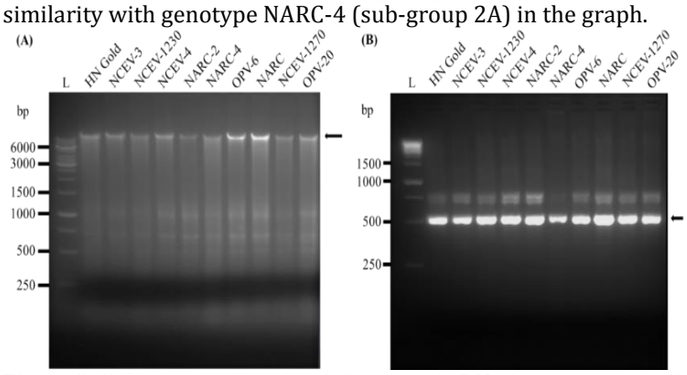

Figure 2: Gel pictures showing (A): genomic DNA bands of ten maize genotypes on 1% agarose concentration, B: PCR amplification of *g*DNA from maize genotypes by *ZmActin* primers on 3% agarose concentration, L represents 1 kb DNA Ladder

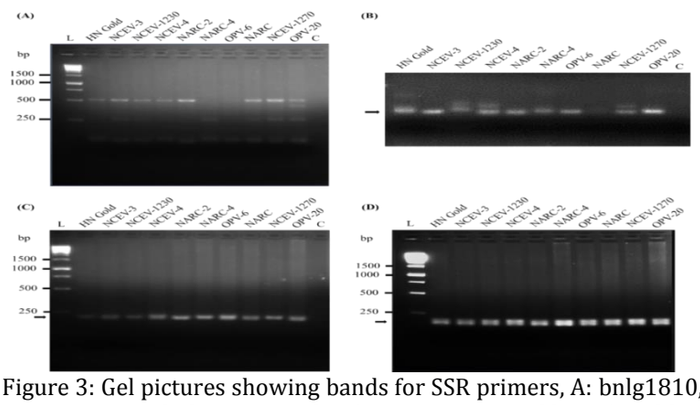

Figure 3: Gel pictures showing bands for SSR primers, A: bnlg1810, B: umc2013, C: bnlg1189, D: phi053. L represents 1 kb DNA Ladder, Agarose concentration for all gels = 3%, C represents negative control of PCR.

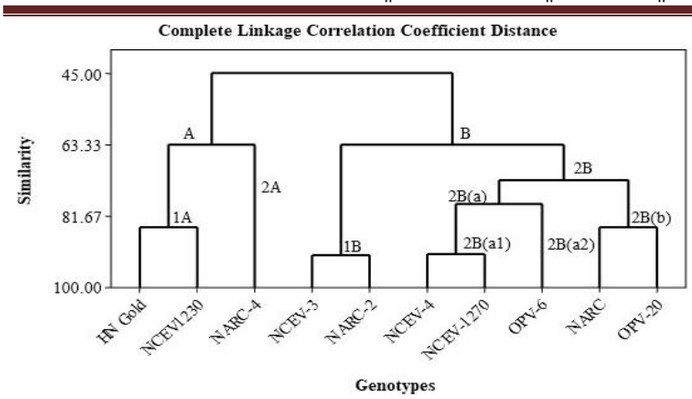

Figure 4. Dendrogram showing similarity among diverse maize genotypes based on SSR markers.

It shows that they are genetically closer relatives of each other than other groups. Hence, Genotypes in cluster A were genetically more similar to each other but genetically distinct from genotypes of group B. Genotypes HN Gold and OPV-20 were placed in distinct groups in the dendrogram. So, this position indicated maximum genetic distinctness between these genotypes.

DISCUSSIONS: Worldwide, heat stress is one of the major problems in cereal crops which is considered for lowering the yields. Maize is a major and widely grown crop in Pakistan that is adversely affected by heat stress. Plants alter their physiological and biochemical mechanisms and cell metabolic processes for their survival under heat stress (Waqas *et al.*, 2015). So, these experiments were conducted to better understand the physiological behaviour of maize genotypes under heat stress and to know genetic diversity based on SSR markers. The study results supported the defined general mechanisms in plants under heat stress which have previously been reported in different studies conducted by scientists (Ali *et al.*, 2020). Proline acts as an osmoprotectant because it is responsible for the movement of water into the cell by activating the process of osmosis and helps to survive plants under stress conditions (Ali *et al.*, 2008). Plants with higher proline contents show tolerance against stress. In this study, the genotypes NARC-4, NCEV-1230, and OPV-20 followed by HN Gold showed a relatively higher accumulation of proline contents under heat stress conditions as compared to the control group. Previous studies reported, an increase in proline content, total free amino acids and total soluble sugars while a significant decrease was observed in activities of nitrate reductase and total soluble proteins in maize genotypes under heat as well as in normal conditions (Ayub *et al.*, 2021). Hence, the highest values of PC showed that these genotypes are more heat-tolerant than other genotypes for this parameter. Whereas, the lowest mean values were observed in genotypes NCEV-3 and NCEV-1270 for proline under stress conditions which indicates the susceptibility of genotypes to heat stress.

Osmotic balance is regulated in plants by the accumulation of solutes, for example, sodium, potassium, chloride, complete solvent sugars, organic and inorganic solutes, free amino acids, glycine betaine, and proline under stress conditions (Zahid *et al.*, 2016). An increase in Ψπ is responsible for enhancing osmotic adjustments in plants. Plants with higher PC show an increase in Ψπ that indicates higher osmotic adjustment under stress. Accumulation of PC is not only the factor responsible for osmotic adjustment in plants under stress conditions but also many factors are involved in the improvement of osmotic adjustment in plants under stress conditions. Mean values-based comparison among all selected genotypes for Ψπ (-MP) revealed that genotypes NCEV-1270 and HN Gold exhibit high Ψπ (-MP) under stressed conditions. However, lower values for Ψπ (-MP) were observed in genotypes OPV-20 and NCEV-1230 which indicates less osmotic adjustment in plants during the heat period rendering these genotypes as heat susceptible. Genotypes OPV-20 and NCEV-1270 retained the highest RWC under heat stress, whereas, the genotypes NCEV-1230 and NCEV-3 showed lower values for RWC. Hence, the genotypes OPV-20 and NCEV-1270 were considered more heat-tolerant because they retained more water during the heat period. The genotypes with lower RWC were considered as heat susceptible for this parameter because they showed more water loss under high temperatures. Cell membrane stability is known as one of the main indicators to judge a plant's capability to survive under heat stress (Huang *et al.*, 2001). This study also revealed the genotypes with high cell membrane stability index under raised temperatures. Analysis for this parameter revealed well-performing genotypes NCEV-1230 and OPV-20 followed by HN Gold with maximum membrane stability index under stress conditions. However, the genotypes with the lowest membrane stability index among the genotypes were NARC-2 and OPV-6 under stress conditions.

Under high-temperature stress, reduction in chlorophyll content (CC) was observed in the plants due to either reduced biosynthesis of the chlorophyll or due to its degradation (Dutta *et al.*, 2009). Scientists reported a significant reduction in chlorophyll content, chlorophyll fluorescence and cell membrane stability under elevated temperatures in sensitive inbred lines of maize (Yadav *et al.*, 2018). In current studies, more chlorophyll reduction was observed in maize genotypes OPV-20, NCEV-1270, and OPV-6 among all genotypes, which showed heat susceptibility of these genotypes for this parameter. The genotypes NARC-4 and NCEV-4 were observed better for the stay-green attribute with the lowest reduction in CC under heat stress and considered heat tolerant.

SSR markers based genetic diversity was also assessed for all selected genotypes. Scientists have been widely used SSR markers in maize for the selection of parental lines, to study heterosis and combining ability, and for assessing genetic diversity (George *et al.*, 2004). A study conducted on maize genotypes in Pakistan suggested that SSR markers are reliable and useful for assessing genetic diversity (Bibi *et al.*, 2010). In the present molecular studies of maize, two SSR primers umc2013 and bnlg1810 showed polymorphism and were found useful to assess genetic diversity in maize genotypes. The primers bnlg1189 and phi053 showed monomorphism for all genotypes and couldn't show any diversity among genotypes. A cluster analysis based on polymorphic molecular markers divided the genotypes into groups and showed similarity and distinctness among genotypes. According to molecular data analysis, the genotypes HN Gold, NCEV-1230, and NARC-4 were found genetically more similar to each other and kept in the same group. The rest of the genotypes were placed in another group which showed distinctness to other groups. Genetic differences were observed between the genotypes of both groups. Decisively, the analysis based on physiological parameters revealed that the genotypes NCEV-1230, NARC-4, HN Gold, and OPV-20 are heat-tolerant. These genotypes performed well under warm conditions for parameters such as PC, Ψπ, CMS, and RWC. Especially, the genotype NARC-4 showed greater values for parameters CC and PC and performed satisfactorily for all other selected parameters. Hence, this genotype is considered as moderately heat-tolerant under stress. Cluster analysis based on SSR markers also revealed the maximum genetic similarities in genotypes NCEV-1230, NARC-4, and HN Gold. These genotypes were placed in the same group (A) in the dendrogram. Whereas, OPV-20 and NCEV-1270 were placed in group (B) and showed a great distance from group (A) in the dendrogram which indicates that they are not genetically similar to the genotypes of group (A). When we compared the molecular data with physiological data for selected maize genotypes, we found a strong association between them.

CONCLUSION: The analysis based on physiological parameters revealed that the genotypes NCEV-1230, NARC-4, HN Gold, and OPV-20 are heat-tolerant. These genotypes performed well under warm conditions for parameters such as PC, Ψπ, CMS, and RWC. Especially, the genotype NARC-4 showed relatively higher proline content and chlorophyll content and also performed satisfactorily for all other selected parameters. Hence, this genotype is considered as moderately heat-tolerant under stress. This study also revealed a strong association between molecular and physiological data for selected maize genotypes. The genotypes performed well under heat stress and also showed genetic similarity as revealed by SSR markers and dendrogram.

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LIFE SCIENCE REPORTING: In current research article no life science threat was reported.

ETHICAL RESPONSIBILITY: This is original research, and it is not submitted in whole or in parts to another journal for publication purpose.

INFORMED CONSENT: The author(s) have reviewed the entire manuscript and approved the final version before submission.

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