FIELD EVALUATION AND GENETIC DIVERSITY FOR HEAT TOLERANCE USING STRESS INDICES AND SSR MARKERS IN RICE

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ABSTRACT: High temperature is a major environmental stress factor limiting rice productivity. The present investigation was carried out to evaluate and identify heat tolerant rice genotypes on the basis of heat indices and SSR markers. Fifteen rice genotypes were designed in Randomized Complete Block Design (RBCD) with three replications under normal and heat stress conditions at two environmentally different sites (Sakha and New Vally) during 2018 and 2019 rice season. Parameters of heat indices were calculated based on grain yield under both conditions. The results indicated that the heat tolerant index (HTI) and mean productivity (MP) index had the highest correlation with yield under two the conditions, Giza178, Giza179, Giza182, Egyptian Yasmin, Egyptian Hybrid1 and N22 were the best genotypes based on indices values, representing high temperature tolerance of these genotypes. Results of variance analysis of yield and other traits in both conditions showed that there was a significant difference among genotypes and their traits. Egyptian hybrid 1 produced the highest mean values of grain yield/plant under normal and heat stress conditions (50.26 and 37.00 g/plant), meanwhile Sakha102 had the highest reduction (70.36%) and N22 had the lowest reduction (23.9%).All genotypes clearly grouped into two major clusters in the dendrogram at 58% similarity based on Jaccard's similarity index. The first cluster represents the Indica and Indica/Japonica tolerant rice genotypes for high heat, while the second cluster represents the Japonica sensitive genotypes for high heat.

Key words: Heat tolerance, Genetic diversity, SSR markers, rice.

INTRODUCTION

Rice is one of the important stable cereal crops feeding more than 3.5 billion global population (IRRI, 2017). Increasing rice production as a national goal could be achieved through increasing the production per unit area, but environmental stresses are limiting this goal. Heat stress in particular is affecting agricultural crops more frequently and more severely (Janni *et al.,* 2020 and Jagadish *et al.*, 2010) reported that rice responses to high temperature differ according to the developmental stage, with the highest sensitivity recorded at the reproductive stage. Temperatures > 35°C at anthesis and lasting for more

than (1 h) can lead to high sterility in rice. each degree-Celsius increase in global mean temperature would, on average, reduce global yields of Wheat by 6.0%, Rice by 3.2%, Maize by 7.4%, and Soybean by 3.1% (Liu *et al.,* 2017). The genetics of heat tolerance is poorly understood, because it's complex and controlled by multiple genes (Wahid *et al.*, 2007 and Xue *et al.*, 2012; Driedonks *et al.,* 2016). High temperature stress caused abnormal anther dehiscence and poor pollen germination which in turn increased spikelet sterility (Jagadish *et al.*, 2010). Heat stress during flowering stage negatively impacts both percent seed set and total grain yield (Bui *et al.*,

2014). The critical physiological parameter "spikelet with exerted anthers but with no ovule enlargement" was considered as the most sensitive stage with exposure to heat stress at important reproductive stages (Shi *et al*., 2015). Heat stress reduce the rice production significantly especially during grain-filling period in most parts of the world where the temperature becomes high during anthesis to maturity (grain-filling) stage of plant growth (Dhanda and Munjal 2009). Direct selection under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of such traits. Assessment of heat tolerance at the molecular level is more meaningful than at phenotypic level as the later involves data on morphological traits which are environmental dependent. Available genetic diversity in rice offers opportunity for the breeders to develop genotypes with wider adaptability having resistance to biotic and abiotic stresses by selection of recombinants of desired genes. The simple sequence repeats (SSR) markers can help breeders to select genotypes carrying gene(s) of

interest (Sadat *et al*., 2013), therefore, molecular maps based on these markers provide the breeders efficient strategies that may optimize time and resources and facilitate their manipulation in segregating plant breeding populations. These are powerful tools for many studies for genome characterization, detection of quantitative trait loci (QTL) for both abiotic and biotic stresses, evolutionary studies, and for marker assisted selection (MAS) (Chu *et al*., 2010 and Sadat *et al*., 2013). This study aimed to determination of genetic components for heat tolerance, determination of the best heat tolerant genotypes and identification of SSR markers linked to heat tolerance.

MATERIALS AND METHODS

Rice materials

Fifteen rice genotypes, including nine *Japonicas*, three *Indica* and three *Indica/Japonica* rice genotypes were selected for the current study. Seeds were obtained from genetic stock of Rice Research and Training Center (RRTC). The studied genotypes names, type and pedigree are presented in Table 1.

Table (1). list of Rice genotypes used in the experiment along with their type and pedigree.

No	Genotypes	Type	Parentage
1	Sakha 101	*Japonica*	Giza176/Milyang79
2	Sakha 102	*Japonica*	GZ4098-7-1/Giza177
3	Sakha103	*Japonica*	Giza 177/Suweon 349
4	Sakha104	*Japonica*	GZ4096-8-1/GZ4100-9-1
5	Sakha105	*Japonica*	GZ5581-46-3 / GZ4316-7-1-1
6	Sakha106	*Japonica*	Giza177/Hexi30
7	Sakha107	*Japonica*	Giza 177 /BLI
8	Sakha108	*Japonica*	Sakha 101/HR5824//Sakha 101
9	Giza 177	*Japonica*	Giza171/Yomjo No.1//PiNo.4
10	Giza178	*Indica/japonica*	Giza175/Milyang49
11	Giza179	*Indica/Japonica*	GZ6296 / GZ1368
12	Giza182	*Indica*	Giza181/IR65844-29-1-3-1-2
13	EgyptianYasmin	*Indica*	IR262-43-8-11 / KDML 105
14	Egyptian hybrid 1	*Indica/japonica*	IR69625A/Giza178
15	N22	*Indica*	Unknown

Field evaluation

The field experiments were done at two locations, i.e., at Sakha Research Station, Rice Research and Training Center (RRTC), Kafr EL-Sheikh Governorate and at New Valley Research Station, El-Kharga, New Valley Governorate, Agriculture Research Center (ARC), Egypt, during the two successive seasons 2018 and 2019. Air temperature (°C) and relative humidity (%) were averaged for every month during 2018 and 2019 rice growing seasons, at the two locations as shown in Table (2). Fifteen rice genotypes (varieties and/or lines) representing a wide range of diverse genetic materials for several agronomic, physiological characters and heat tolerance levels were selected for this study. Seeds of the studied genotypes were sown in the nursery and after 30 days from sowing, seedlings of each genotype were individually transplanted in the permanent field in 5 rows 20 cm between rows and hills. The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications according to Snedecor and Cochran (1967). All agronomic practice such as fertilization, irrigation, weeds and pests control were done recommended with rice crop during growing seasons of study. Data were collected on days to 50% heading (day), plant height (cm), number of panicles/plant, panicle length (cm), panicle weight (g), 1000-grain weight (g), sterility (%) and grain yield (g/p), according to the standard evaluation system for rice (RRTC, 2018). Heat tolerance indices were calculated by the following formulas in Table 3.

Table (2): The maximum and minimum temperature (°C) for months as well as relative humidity (%) at Sakha Agricultural Research Station and New Valley Agricultural Research Station during rice seasons 2018 - 2019.

Month	KafrEL-Sheikh Governorate			New Vally Governorate		
	Air Temp.2018-2019		RH%	Air Temp.2018-2019		RH%
	Max	Min		Max	Min	
May	32.6 - 31.9	25.3 - 23.8	59.7	40.5 - 42.3	21 – 23	23.5
June	34.2 – 33.0	25.4 – 25.3	61.7	40.8 – 41.5	26.3 – 25.8	23
July	33.9 – 33.5	25.2 – 25.4	66.8	43.2 – 41.5	25.8 – 26.4	27
August	32.8 – 34.2	23.5 – 25.2	66.9	42.1 – 42.4	27.2 – 26.7	28.6
September	32.6 – 32.4	25.3 – 23.5	65.7	38.2 – 38.6	22.9 – 23.1	33

Table (3). Heat tolerance indices

Index	Formula	Reference
Heat sensitive index (HSI)	$[(1 – (Ys / Yn)] / SI$	**Fischer and Maurer, 1978**
Stress tolerance index (STI)	$(Yn * Ys) / (Yn)^2$	**Fernandez 1992**
Mean productivity (MP)	$(Yn + Ys) / 2$	**Hossain *et al.*,1990 ;Mardeh *et al.* 2006**
Tolerance (TOL):	$Yn – Ys$	**Rosielle and Hamblin,1981**
Yield stability index (YSI)	Yn / Ys	**Bouslama and Schapaugh, 1984**
Yield index (YI)	$Ys/ Mean of Ys$	**Gavuzzi *et al.* 1997; Lin *et al.*,1986**

Which, Ys =mean yield under stress,
        Yn = mean yield under normal condition and SI= the stress intensity.

Genomic DNA isolation

Leaves were sampled from 15 days old seedling to extract genomic DNA for molecular screening of high temperature tolerance amongst the genotypes. Total genomic DNA was extracted after crushing in liquid nitrogen in microfuge tubes using CTAB method described by (Murray and Thompson 1988). The quantity and quality of DNA was assessed with 0.8% agarose gel electrophoresis using diluted uncut lambda phage DNA as size standard. The concentration of DNA was adjusted to approximately 15 ng / µl for PCR reaction.

PCR amplification and electrophoresis

Genetic diversity for 15 genotypes was screening using 16 SSR markers are linked to heat tolerance. Primers names, sequences, chromosome number and references are listed in Table 4. PCR amplification reactions was performed in 10 µl reaction mixtures, containing 1µl of template DNA, 2 µl of each forward and reverse primers, 3 µl ddH$_2$O and 5 µl of 2X *GoTaq* Green Master Mix (Promega, USA.). The reaction mixture was first denatured for 5 min at 95°C for, followed by 35 cycles of denaturation for 1 min at 94°C for, annealing at T$_m$ - 2°C for 30 seconds and elongation at 72°C for 1min, and a final extension at 72°C for 10 min. PCR amplification was loaded in 3% agarose gel containing Ethidium Bromide for electrophoresis in 1X TAE (pH 8.0). DNA ladder (50bp) was used for determination of size of amplicons. The gel was run at 60 volts (2.5V/cm) for 3 hrs and photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany)

Table (4). List of SSR markers sequences and chromosome number.

Primer name	Chromosome number	Sequence		reference
		Forward	Reverse	
RM3586	3	GAAGAGAGAGCCAGAGCCAG	ACACGATCGAGCTAGAAGACG	Bui Chi Buu *et al* (2013)
RM3735	4	GCGACCGATCAGCTAGCTAG	ATAACTCCTCCCTTGCTGCC	
RM6100	10	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC	kumar et (2014)
RM160	9	AGCTAGCAGCTATAGCTTAGCTGAGATCG	TCTCATCGCCATGCGAGGCCC	Buu et al 2014
RM310	8	CCAAAACATTTAAAATATCATG	GCTTGTTGGTCATTACCATTC	
RM3471	4	AGATCCCGACAGATGGTGAC	AACAGAGGGAGGGAGCAGAG	
RM5687	4	GATCGCTGGCGATTGATC	GACTTGTGGGGTGGTTTTTG	
RM471	4	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC	xiao et al 2011
RM190	6	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG	
RM225	6	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAGGC	
RM592	5	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA	Cao et al. (2015)
RM405	5	TCACACACTGACAGTCTGA	AATGTGGCACGTGAGGTAAG	Zhanget al. (2008)
RM148	3	CAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG	Chen et al. (2008)
RM153	5	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG	Zhao et al. (2016)
RM430	5	AAACAACGACGTCCCTGATC	GTGCCTCCGTGGTTATGAAC	Chenget al. (2012)
RM440	5	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG	Chenget al. (2012)

Data analysis

Scoring of amplified bands was done as present (1) or absent (0) for each genotype and primer pair. To identify the informative SSR marker, polymorphism information content (PIC) value for each SSR marker was analyzed using the software package, NTSYS-pc version 2.11 (Rolf, 1988). The total number of amplified bands, number of polymorphic alleles number of amplified alleles and polymorphism ratio (P %) and Polymorphism information content (PIC) were calculated to assess the diversity of alleles of marker locus (Anderson *et al.*, 1993). Genetic similarity coefficients were used to construct a dendrogram using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) sequential agglomerative hierarchal nested (SHAN) cluster.

RESULTS AND DISCUSSION

Analysis of variance:

The Analysis of variance under normal and heat stress conditions were revealed highly significant differences among rice genotypes for all studied traits Table 5. These results showed a wide range of variability among rice genotypes and this gives an opportunity for rice breeders to improve these traits through selection and hybridization to improve the desired traits and enable to select genotypes under heat tolerant genotypes. Similar results were found by kumar and Saravanan 2012 and Gaballa *et al.*, 2019.

Data in Tables 6 and 7 shows the mean performances of the 15 genotypes under normal and heat condition for the eight traits included days to heading , plant height, No. of panicles/plant, panicle length, panicle weight, 1000-grain weight, sterility % and grain yield/plant.

High temperature stress reduce plant duration via stimulate rice genotypes to early flowering, decrease plant height, and panicle length, reduce number of panicle/plant and produced the lowest panicle weight and 1000-grain weight. Moreover, the grain yield/plant was significantly reduced, whereas the minimum values (13.0 g/p) under heat stress, in compared to (50.3 g/p) under normal condition. The increment of temperature had significant negative effect on the grain productivity. On the other hand, high heat stress has increased sterility %. The normal condition under Sakha Research Station had given the desirable mean values for all genotypes performance for pervious mentioned traits. These results are in agreement with Abdallah, 2015 and Gaballa *et al.*, 2019. However the optimum temperature for the normal development of rice ranges from 27 to 32 °C (Yin *et al.,* 1996), and high temperature affects almost all the growth stages of rice, i.e. from emergence to ripening and harvesting. The developmental stage at which the plant is exposed to heat stress determines the severity of the possible damage to the crop (Wahid *et al.,* 2007). In addition, the most stages of development, which are considered to be sensitive to temperature in rice are flowering (anthesis and fertilization) and to a lesser extent the preceding booting stage (Satak and Yoshida 1978; Farrell *et al.,* 2006). IRRI 1976 stated that exposure to 41 °C for 4 h at flowering caused irreversible damage and plants became completely sterile. Whereas this high temperature (41 °C) had no effect on spikelet fertility at 1 day before or after flowering (Yoshida *et al.,* 1981).

Table (5). Analysis of variance for studied traits under normal and heat stress.

S. O. V.	df	Days to heading(day)		Plant height(cm)		Panicles/plant		Panicle length(cm)	
		N	S	N	S	N	S	N	S
Replications	2	0.69	1.69	0.27	4.84	6.02	1.40	7.14	0.43
Genotypes	14	130.36**	163.36**	264.91**	343.46**	16.31**	10.20**	7.87**	8.44**
Error	28	0.88	1.09	1.12	1.16	0.86	1.11	0.42	0.67
S. O. V.	df	Panicle weight(g)		1000-grain weight (g)		Sterility%		Grain yield/plant (g)	
		N	S	N	S	N	S	N	S
Replications	2	0.02	0.00	0.05	0.27	2.94	11.89	1.81	2.55
Genotypes	14	1.42**	0.54**	21.70**	48.77**	51.46**	414.87**	104.00**	211.58**
Error	28	0.01	0.01	0.37	1.05	0.75	8.96	0.64	0.81

N : normal condition S: heat stress
Mean performance

Table 6. Mean performance of days to heading, plant height, panicles and panicle length for the studied genotypes under normal and heat stress.

Genotypes	Days to heading (day)		Plant height (cm)		Panicles/plant		Panicle length (cm)	
	N	S	N	S	N	S	N	S
Sakha101	102.0	90.0	92.3	72.9	23.0	12.7	24.3	13.9
Sakha102	92.0	78.0	98.0	82.0	19.0	13.0	24.0	13.0
Sakha103	92.0	74.7	99.0	69.0	20.7	13.0	20.3	13.9
Sakha104	100.0	78.0	101.0	83.0	24.7	13.7	23.0	13.5
Sakha105	92.0	79.0	98.3	73.3	23.0	12.0	21.0	14.3
Sakha106	94.0	76.0	104.0	78.0	20.0	16.0	21.3	16.0
Sakha107	94.0	73.3	102.3	80.0	19.0	12.0	21.0	15.3
Sakha108	107.0	84.0	95.0	78.2	22.3	14.0	23.0	14.6
Giza177	90.3	77.0	99.0	70.0	18.0	14.0	22.0	13.3
Giza178	100.0	79.0	97.0	76.0	23.0	18.0	23.0	17.3
Giza179	91.7	71.0	93.7	80.3	23.0	16.7	21.0	18.0
Giza182	94.3	75.0	98.0	69.9	24.0	15.0	23.0	17.0
E. Yasmin	112.0	96.0	109.7	83.0	25.0	14.0	25.0	17.0
E,Hybrid 1	106.0	76.0	107.7	83.0	25.0	15.0	26.0	16.0
N22	100.0	92.7	131.0	113.7	24.0	17.0	21.0	17.1
Grand mean	98.2	79.9	101.7	79.4	22.4	14.4	22.5	15.3
Minimum value	90.3	71.0	92.3	69.0	18.0	12.0	20.3	13.0
Maximum value	112	96.0	131.0	113.7	25.0	18.0	26.0	18.0
LSD 0.05 %	1.30	1.45	1.47	1.49	1.28	1.47	0.90	1.14
0.01 %	1.89	2.11	2.14	2.17	1.87	2.13	1.31	1.66

Table 7. Mean performance of Panicle weight, 1000-grain weight, Sterility % and Grain yield/plant for the studied genotypes under normal and heat stress.

Genotypes	Panicle weight (g)		1000-grain weight (g)		Sterility (%)		Grain yield/plant (g)	
	N	S	N	S	N	S	N	S
Sakha101	4.1	1.0	28.7	14.0	9.6	40.0	47.8	17.9
Sakha102	3.6	1.2	28.5	11.0	9.8	43.3	43.9	13.0
Sakha103	3.0	1.5	23.5	13.0	8.1	41.0	35.6	17.0
Sakha104	3.9	1.7	24.8	15.0	6.3	38.0	42.7	15.0
Sakha105	3.7	1.2	27.3	13.0	8.0	37.0	46.4	19.0
Sakha106	3.9	1.7	28.6	16.0	8.2	49.1	46.2	20.0
Sakha107	3.1	1.4	27.0	13.0	7.8	45.0	37.2	20.0
Sakha108	4.3	1.2	28.4	14.0	9.4	47.0	48.2	16.0
Giza177	3.7	1.0	27.5	11.0	8.0	65.2	38.3	14.0
Giza178	4.1	2.4	24.1	20.0	7.7	24.0	46.5	34.2
Giza179	4.0	1.9	27.3	21.0	12.0	29.0	48.9	36.0
Giza182	3.7	1.9	24.0	21.0	13.4	32.0	47.8	33.0
E. Yasmine	5.3	2.1	28.3	23.0	9.2	30.0	30.6	22.0
E,Hybrid 1	5.5	1.9	26.5	22.0	22.7	33.0	50.3	37.0
N22	3.3	1.9	20.7	17.0	5.1	18.0	38.1	29.0
Grand mean	3.95	1.60	26.15	16.27	9.69	38.11	43.23	22.87
Minimum value	3.0	1.0	28.7	11.0	5.1	18.0	30.6	13.0
Maximum value	5.5	2.4	20.7	23.0	22.7	65.2	50.3	37.0
LSD 0.05 %	0.13	0.12	0.84	1.42	1.20	4.16	1.11	1.25
0.01 %	0.19	0.17	1.23	2.07	1.74	6.05	1.62	1.82

Mean performance and heat stress indices for grain yield.

Grain yield is the ultimate objective of any crop improvement program. Data in Table 8 showed that yield performance under normal (Yn) and heat stress conditions (Ys), decline %, parameters of heat stress indices (HSI, HTI, MP, YI and YSI) to study heat tolerance and susceptibility in rice genotypes. All the indices were calculated on the basis of the mean of grain yield for the genotypes under normal and stress conditions. The mean of grain yield for the tested genotypes ranged from 50.26 to 30.57g/plant under normal condition Table 9 and Figure 1. Egyptian Yasmin possessed minimum yield potential (30.57 g/plant), while Egyptian Hybrid1 had maximum grain yield with 50.26g/plant, whereas grain yield ranged

from 13.00 g/plant for Sakha103 to 37.00 g/plant. Egyptian Hybrid1 had high yield under normal and heat stress. Mean comparison in Figure 1 illustrated that all genotypes gave high yielding under normal condition while under heat stress five genotypes gave the highest grain yield (Egyptian Hybrid1 followed by Giza179, Giza178, Giza182, and N22.Two rice genotypes gave moderate, Egyptian Yasmin and Sakha107and the other genotypes gave lowest grain yield. Depending on the specific stages of rice development, heat stress reduces number of tillers/ panicle, decrease number of grains /panicle and lower grain weight, thus negatively affecting yield formation results observed by Abdel-Hafez *et al.,* 2016 and Xu *et al.,* 2020.Yield reduction % and the comparison of yield under normal and heat stress conditions

and decline % are presented in Table 9 and Figures 2 & 3. Sakha102 showed maximum yield reduction 70.36%, while N22 recorded minimum yield reduction 23.90%. Clearly, heat stress caused a significant reduction (45.9%) in grain yield. The highest reduction was found in Sakha102 (70.36%) and the lowest reduction recorded for N22 (23.90%). Generally all genotypes under heat stress produced low grain yield. Data in Table 2 show a rise of temperature during three month July, August and September ranged from 38.5 to 45 ^{0}C during initial reproductive and ripening stage explained and confirmed previous results in this study. The reduction in mean values of grain yield under heat stress condition studied by Thapa *et al.*, 2020 and reported that increased temperature leads to rapid completion of rice life cycle resulting in poor expression of yield contributing traits and consequently lowers the grain yield. Nakagawa *et al.*, 2003 reported that temperatures higher than the optimum induced floret sterility and thus decreased rice yield. When heat stress occurred during initial growth phases, i.e., booting and flowering, Aghamolki et *al.*, 2014 noticed that greater yield reduction due to reduction in all the important yield components excluded effective tillers, i.e., fertile spikelets, 1000 grain weight and increase in sterile and aborted spikelets per panicle. In the initial reproductive stage usually infertile spikelets increase due to pollen infertility and anther dehiscence, accordingly the number of pollen scattered on stigma and the fertility and fertilization of pollens declined markedly.

Table (8): Mean performance of 15 rice genotypes for grain yield /plant, decline % and five heat stress indices.

Heat susceptible genotype	Grain yield (normal)	Grain yield (stress)	Decline %	HSI	HTI	MP	TOL	YSI
Sakha 101	47.83	17.90	62.50	1.261	0.458	56.78	29.93	0.37
Sakha 102	43.87	13.00	70.36	1.418	0.305	50.37	30.87	0.30
Sakha103	35.57	17.00	52.09	1.052	0.324	44.07	18.57	0.48
Sakha104	42.73	15.00	64.89	1.308	0.343	50.23	27.73	0.35
Sakha105	46.44	22.00	52.62	1.061	0.547	57.44	24.44	0.47
Sakha106	46.23	21.00	54.57	1.100	0.519	56.73	25.23	0.45
Sakha107	37.15	20.00	45.78	0.993	0.397	48.15	17.15	0.54
Sakha108	48.24	16.00	66.80	1.347	0.413	56.24	32.24	0.33
Sakha177	38.25	14.00	63.39	1.278	0.287	45.25	24.25	0.37
Heat tolerant genotypes	Grain yield (normal)	Grain yield (stress)	Decline %	HSI	HTI	MP	TOL	YSI
Giza178	46.53	34.16	26.50	0.576	0.850	63.61	12.38	0.73
Giza179	48.85	36.00	26.30	0.570	0.941	66.85	12.85	0.74
Giza182	47.79	33.00	30.90	0.670	0.844	64.29	14.79	0.69
Egyptian Yasmine	30.57	22.00	28.03	0.607	0.360	41.57	8.57	0.72
E. hybrid 1	50.26	37.00	26.30	0.571	0.995	68.76	13.26	0.74
N22	38.14	29.00	23.90	0.519	0.592	52.64	9.14	0.76

HSI = Heat Sensitive Index;   HTI = Heat   Tolerance   Index;   MP = Mean   Productivity;
TOL = Tolerance   and YSI = Yield Stability Index.

Table (9). Correlation between yield with heat stress indices for eight agro-morphological traits in 15 rice genotypes under normal and heat stress condition.

Traits	Yn	Ys	HTI	TOL	YSI	MP	YI	HIS
Yn	1.00							
Ys	.379	1.00						
HTI	.615*	.960**	1.00					
TOL	.329	-.749**	-.540*	1.00				
YSI	-.065	.894**	.735**	-.959**	1.00			
MP	.887**	.763**	.908**	-.144	.400	1.00		
YI	.377	1.000**	.959**	-.751**	.895**	.762**	1.00	
HIS	.095	-.862**	-.699**	.948**	-.974**	-.363	-.863**	1.00
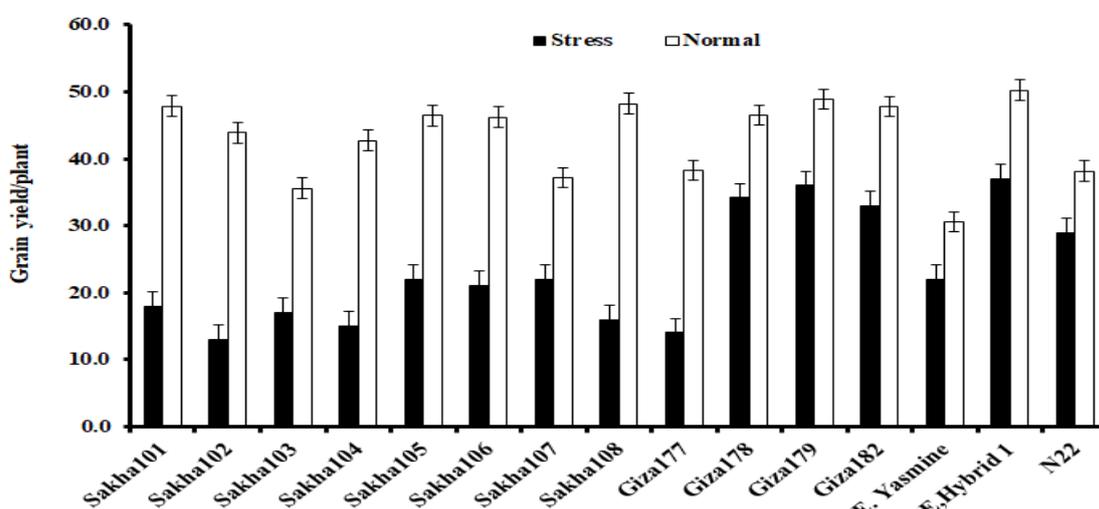
Figure (1): Mean performance for grain yield under normal and heat stress condition.
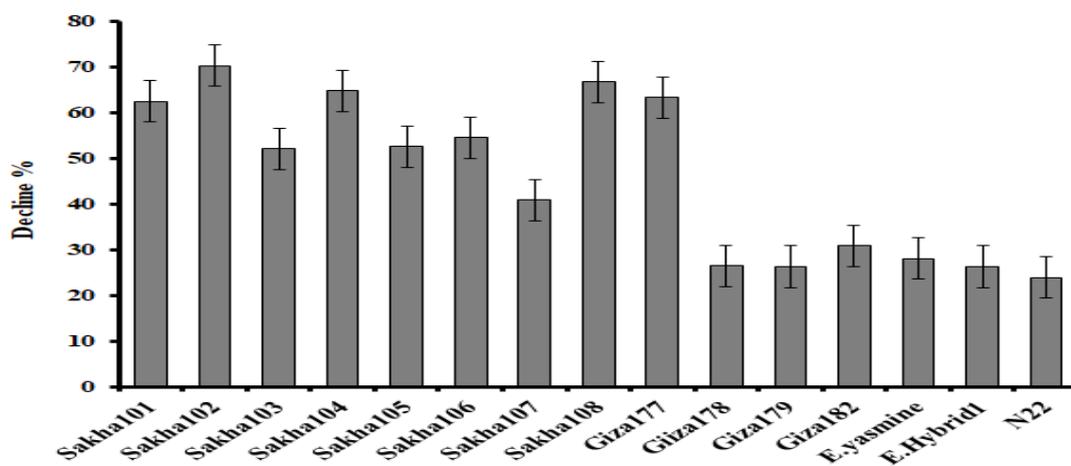
Figure (2): Decline % for grain yield under heat stress condition.
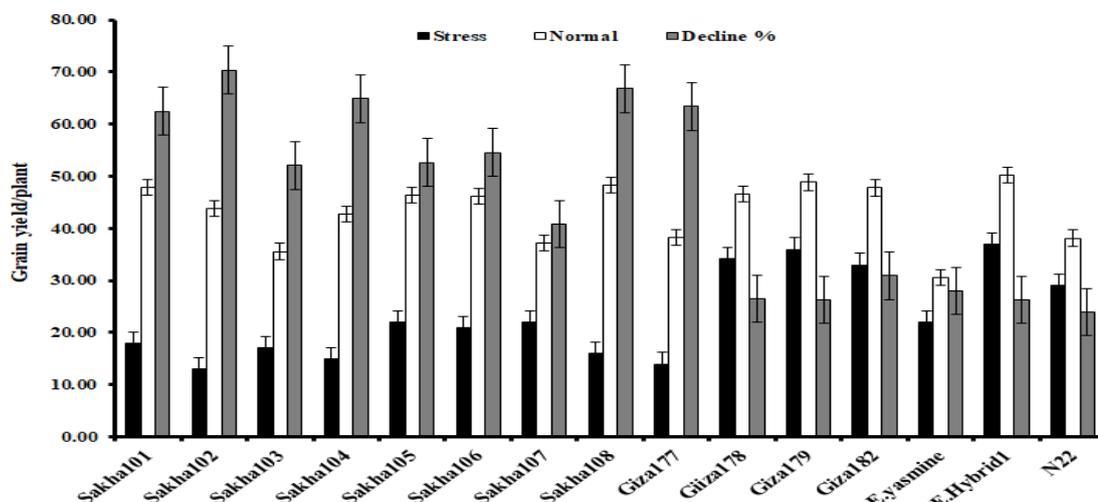
Figure (3): Comparison of yield under normal and heat stress condition and decline %

Heat Stress Indices

To study heat tolerance and susceptibility in rice genotypes, we used five stress indices i.e., heat susceptible index (HIS), heat response index, stress tolerance, yield stability index and yield index. All the indices were calculated on the basis of the mean of grain yield of the genotypes under normal and stress conditions.

Heat susceptible index (HIS) data in Figure 4. The genotypes with low HSI< 1 value are heat stress tolerance and the genotypes with high positive HIS values are susceptible for higher temperature (Reynolds *et al.,* 1994 and fisher and maurer, 1978). The results showed that the genotypes Giza178, Giza179, Giza182, Egyptian Yasmin, Egyptian Hybrid1 and N22 had low values of HSI for yield trait. While, rice genotypes Sakha101, Sakha102, Sakha103, Saha104, Sakha105, Sakha106, Sakha108 and Giza177 gave high HSI values. Heat susceptible index (HIS) for the important index can be utilized for selection for tolerant genotypes. *Heat response index (HRI)* also calculated to confirm the above results. Maximum HRI value is the better indicator of the superior performance of genotype under heat stress environment (Fernandez 1992,

Mardeh *et al.,* 2006). The rice genotypes Egyptian hybrid1 followed by Giza 179, Giza178, Giza182 and N22 with high HRI values indicating the tolerance heat stress while, genotype Sakha101, Sakha102, Sakha 103, Saha104, Sakha105, Sakha107, Sakha106, Sakha108, Egyptian Yasmin and Giza 177 showing susceptibility to heat Figure 5. Concerning Stress Tolerance (TOL), the genotypes with low TOL values are more stable in two different Conditions and suitable for the screening of breeding materials for heat tolerance). variability were found amongst the genotypes for tolerance (TOL) and rice genotypes Egyptian Yasmin, N22, Giza178, Giza 179, Giza 182 and Egyptian Hybrid 1 exhibited the lower TOL values and the genotypes intermediate Sakha107and the other genotypes were higher TOL values indicating non sensitive for the heat conditions. Figure 6. Similar results were recorded by several workers for selections based on these indices Ouk *et al.,* 2006 and Mardeh *et al.,* 2006. Mean Productivity Index (MPI), the genotypes with high values of MPI is more desirable index. Data in Figure 7 illustrated that Egyptian Hybrid 1, Giza179, Giza178 and Giza182 had desirable high MPI values and high tolerant to high temperature.

For Yield Stability Index (YSI), maximum values are the better indication of the superior performance of genotypes under heat stress environment. Differences were found amongst the genotypes for YSI and as in case of HSI the genotype N22 had the highest YSI followed by Egyptian Hybrid1, Giza179, Giza178, Egyptian Yasmin and Giza.182 exhibited stability to stress while, the rest of genotypes had lower values exhibited instability under stress except Sakha107 genotype which was intermediate in nature Figure 8. Similar studies on these indices were carried out by many authors (khan *et al.*, 2016 and himanshu *et al.*, 2017). In case of Yield Index (YI); the rice genotype with high values of Yield index (YI) is suitable for heat condition. The genotype had >1 value considered tolerant while, the genotypes having <1 value denoted as sensitive one. The rice genotypes Egyptian Hybrid1, Giza179, Giza178, Giza182 and N22 showing higher values as in case of STI and MPI. Similarly lower values of YI were noted in the genotypes Sakha101, Sakha102, Sakha103, Sakha104, Sakha108 and

Giza177 exhibited sensitive to high heat and Egyptian Yasmin. Sakha107, Sakha106, Sakha105 were intermediate (Figure 9). It is clear from heat tolerance indices that indica and indica/japonica genotypes are more tolerant to high temperature than japonica genotypes, these results confirmed by Barakat *et al.*, 2011and Shah *et al.*, 2011. Among the examined indices HTI (0.615) and (0.960) and MP (0.887) and (0763) have positive and high significant correlation with grain yield/plant under normal and stress conditions, respectively Table 9 illustrated suitability of these indexes for rice selection. Positive and high significant correlation YI index with yield under heat condition but this index has low correlation with yield under normal condition. While, under stress condition data revealed that highly significant and positive correlation with HTI (0.959), YSI (0.895) and MP (0.762) and high significant negative correlation between HSI and HTI (-0.699). Many similar studies dissussed by Khan *et al.*, 2016 Garg *et al.,* 2017; Thapa *et al.*, 2020 and Saha *et al.,* 2020.
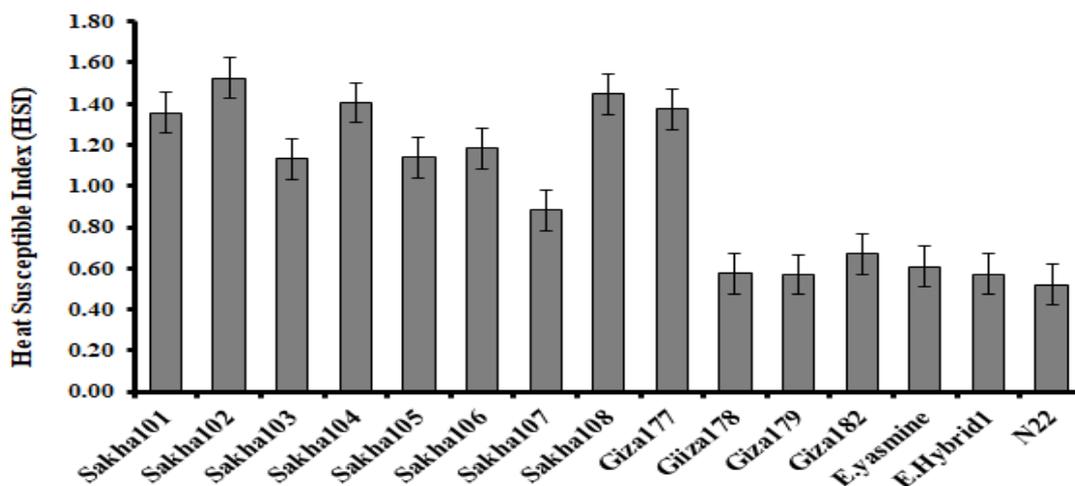

Figure (4). Heat Susceptible Index (HSI) on the basis of grain yield of the studied genotypes under normal and heat stress conditions.
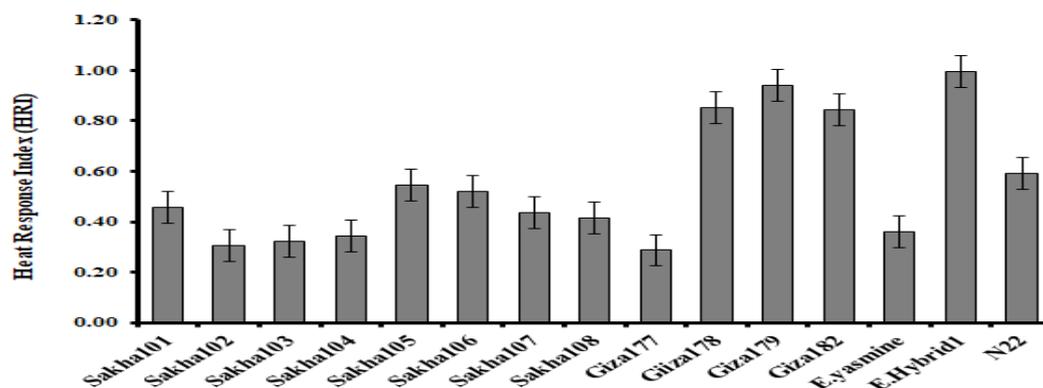
Figure (5). Heat response index (HRI) on the basis of grain yield of the studied genotypes under normal and heat stress condition.
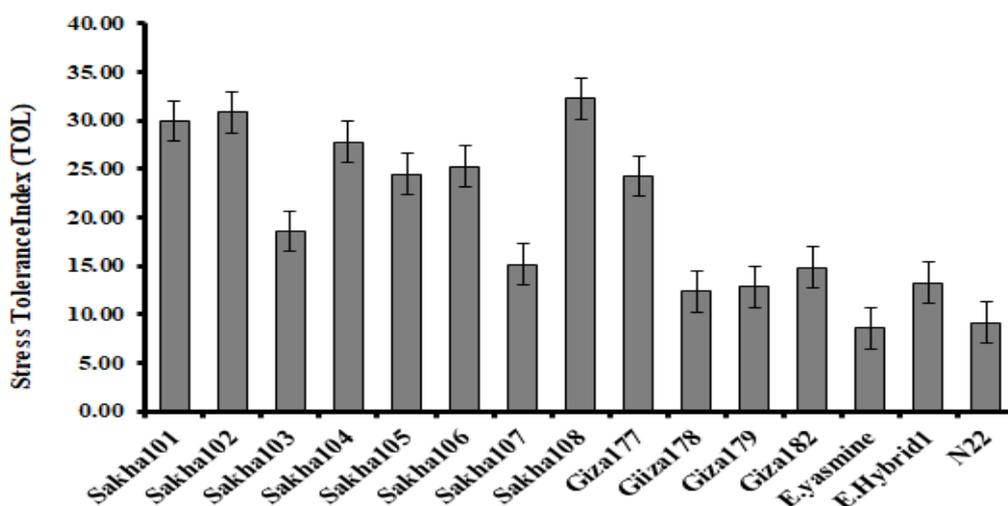

Figure (6): Stress Tolerance index (TOL) on the basis of grain yield of the studied genotypes under normal and heat stress condition.
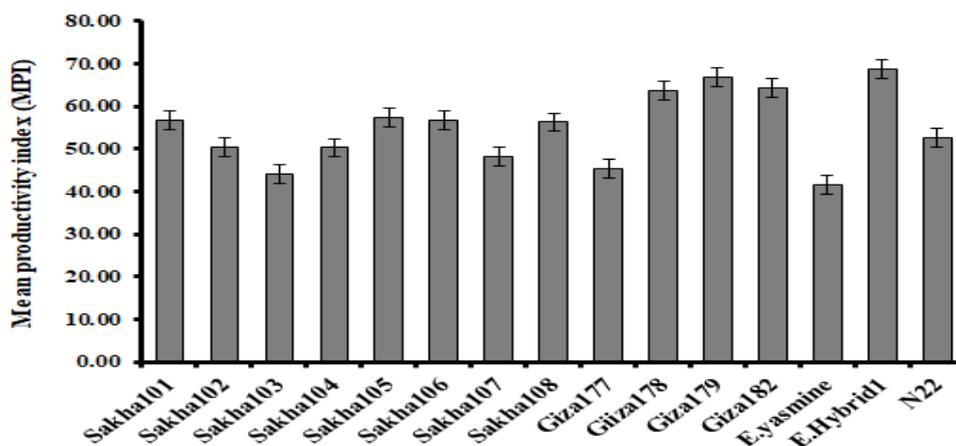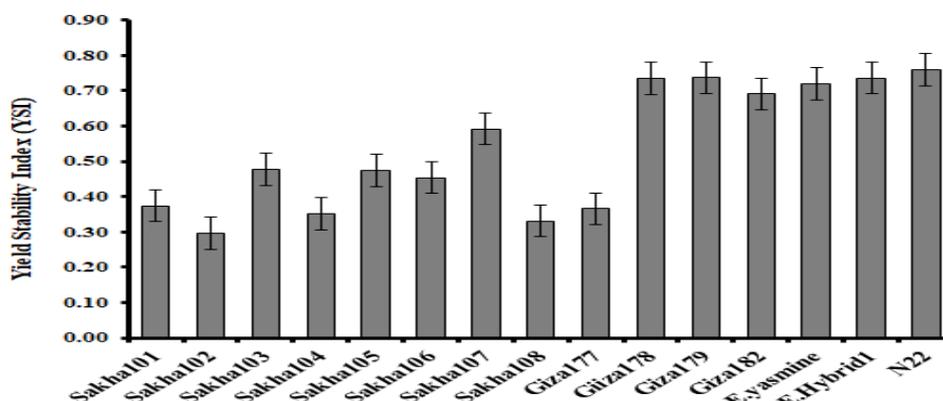

Figure (7): Mean productivity index (MPI) on the basis of grain yield of the studied genotypes under normal and heat stress condition.

Figure (8): Yield Stability Index (YSI) on the basis of grain yield of the studied genotypes under normal and heat stress condition.
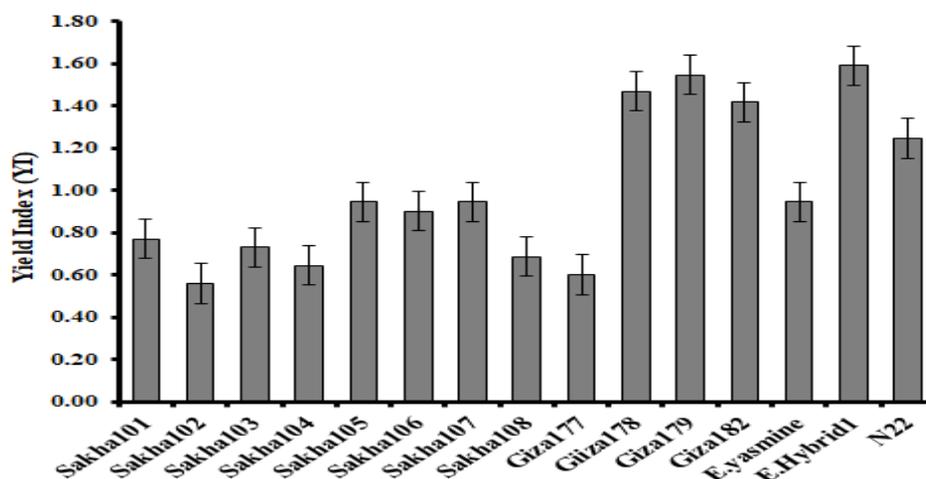

Figure (9): Yield Index (YI) on the basis of grain yield of the studied genotypes under normal and heat stress condition.

Molecular analysis

The results obtained in Table 10 based on the analysis of the 15 rice genotypes using polymorphic SSR loci to detect genetic variability under the molecular level. Among the sixteen SSR markers used for genotyping, four markers (RM3735, RM190, RM405 and RM471) were monomorphic, while 12 SSR markers generated polymorphic alleles among the studied genotypes and showed different levels of polymorphism. Based on the banding sizes and patterns, a UPGMA dendrogram was generated to elucidate the genetic relationships between the tested genotypes. A total of 241 amplified bands with an average of 14.18 amplified bands/primer. The number of alleles varied from 1 to 2 alleles / locus, and 29 amplified alleles were found to be polymorphic which were amplified by 12 SSRs with an average 1.71.the polymorphic alleles play important roles for variety differentiation, diversity characterization and conservation potential parents (Pradhan,2016; Singh *et al.*, 2016 and Ravindr *et al.*, 2019.Polymorphism information content (PIC) values reflection of allele diversity and frequency among genotypes also varied

from locus to another and provide an estimate of the discriminating power of the marker (Nagy *et al.*, 2012). PIC value among the polymorphic markers varied from 0.00 for RM3735, RM471, RM190, RM592 and RM471 to 60 for RM225 with an average of 0.28. The detected polymorphism reflects the amount of diversity among the tested genotypes and thus the possibility of genetic improvement using such a set of genotypes in breeding programs since genetic diversity is the prerequisite for successful such programs. Genetic similarity coefficient values for the studied genotypes are presented in Table 11. The coefficient of similarities based on data among the studied genotypes ranged from 0.22 to 1.0 with an average similarity index of 0.61 indicating wide genetic diversity present in studied genotypes. The lowest genetic similarity was observed between the pure Indica variety N22 and the pure Japonica variety Sakha105 (0.22), while the highest similarity was observed between both Japonica varieties Sakha102 and Sakha103 and Sakha105 also within Sakha 105 with Sakha103 also Giza178 with Egyptian hybrid1. Among Japonica genotypes, genetic similarity coefficients ranged from 0.50 and 1.0 with an average of 0.75. The lowest similarity coefficient was observed between Sakha102, Sakha103, Sakha105 and the high yielding rice variety Sakha101. The genetic similarity among tested rice genotypes are presented in a dendrogram based on Jaccard's similarity index and UPGMA method Figure 10 provided a clear resolution of relationships among studied variety. At 36% similarity, the UPGMA cluster diagram clearly showed two major groups with additional sub-cluster with in

each group. This dendogram showed that the cultivars derived from genetically similar type clustered together. Group A involved the high heat tolerant Indica and Indica/Japonica rice genotypes, while the group B involved the high sensitive Japonica rice genotypes (heat susceptible). The main group A was further divided in two sub clusters A_1 and A_2 at 54% similarity, cultivar in this group is interest to rice breeders because some of them are used as a donors for breeding heat tolerance. Similarly, the main group (B) was further separated into two sub clusters, B_1 and B_2 at about 57% similarity. The sub clusters A_1 included the Indica rice variety Giza182 and the wide spread Indica/Japonica rice variety Giza178 and E. hybrid1,but Giza179 didn't group any sub cluster, while the sub cluster A_2 included the both Indica rice genotypes Egyptian Yasmine and N22.On the other hand, the sub cluster B_1 included both rice genotypes Sakha105 and Sakha104. The Japonica genotypes Sakha102, Sakha3 and Sakha105 were found in sub cluster B_2. while the genotypes Sakha 107, Giza177, Sakha108 and Saka106 didn't group any sub cluster. Finally, clustering genotypes in the constructed dendogram was largely dependent on their genetic background. The ability of cluster analysis to divide rice genotypes according to their genetic background and origin were summarized in different studies. El-malky, 2007; Mazal, 2014 and Ramadan *et al.*, 2017 and found that all japonica Egyptian rice genotypes were grouped in one cluster while all Indica Egyptian rice genotypes were grouped another cluster. Representative banding pattern for 15 rice genotypes are shown in Fig 11.

Table (10): Summary of Molecular analysis for tested genotypes using SSR markers.

SSR marker	# of amplified bands	# of amplified alleles	# of polymorphic alleles	Polymorphism %	PIC value
RM148	15	2	2	100	0.48
RM5687	15	2	2	100	0.44
RM430	15	2	2	100	0.48
RM440	14	2	2	100	0.49
RM3586	13	2	2	100	0.23
RM3735	13	1	0	0	0
RM6100	14	2	2	100	0.24
RM160	13	2	2	100	0.35
RM310	15	2	2	100	0.23
RM3471	15	2	2	100	0.44
RM471	15	1	0	0	0
RM190	15	1	0	0	0
RM225	11	2	2	100	0.60
RM592	15	2	2	100	0.48
RM405	15	1	0	0	0
RM153	13	2	2	100	0.35
RM471	15	1	0	0	0
Total	241	29	24		
Average	14.18	1.71	1.41	70.59	0.28

Table (11): Similarity matrix among tested rice genotypes

|	Sk101	Sk102	Sk103	Sk104	Sk105	Sk106	Sk107	Sk108	G177	G178	G179	G182	Eyasmin	H1	N22
Sk101	1.00														
Sk102	0.50	1.00													
Sk103	0.50	1.00	1.00												
Sk104	0.78	0.57	0.57	1.00											
Sk105	0.50	1.00	1.00	0.57	1.00										
Sk106	0.60	0.65	0.65	0.68	0.65	1.00									
Sk107	0.52	0.94	0.94	0.60	0.94	0.68	1.00								
Sk108	0.60	0.74	0.74	0.68	0.74	0.60	0.78	1.00							
G177	0.52	0.83	0.83	0.60	0.83	0.68	0.88	0.78	1.00						
G178	0.33	0.43	0.43	0.39	0.43	0.28	0.45	0.45	0.39	1.00					
G179	0.32	0.48	0.48	0.43	0.48	0.32	0.50	0.57	0.43	0.65	1.00				
G182	0.30	0.35	0.35	0.30	0.35	0.30	0.36	0.36	0.30	0.76	0.55	1.00			
Eyasmin	0.45	0.27	0.27	0.45	0.27	0.33	0.28	0.39	0.28	0.52	0.57	0.50	1.00		
H1	0.33	0.43	0.43	0.39	0.43	0.28	0.45	0.45	0.39	1.00	0.65	0.76	0.52	1.00	
N22	0.33	0.22	0.22	0.39	0.22	0.23	0.23	0.28	0.23	0.60	0.57	0.43	0.60	0.60	1.00
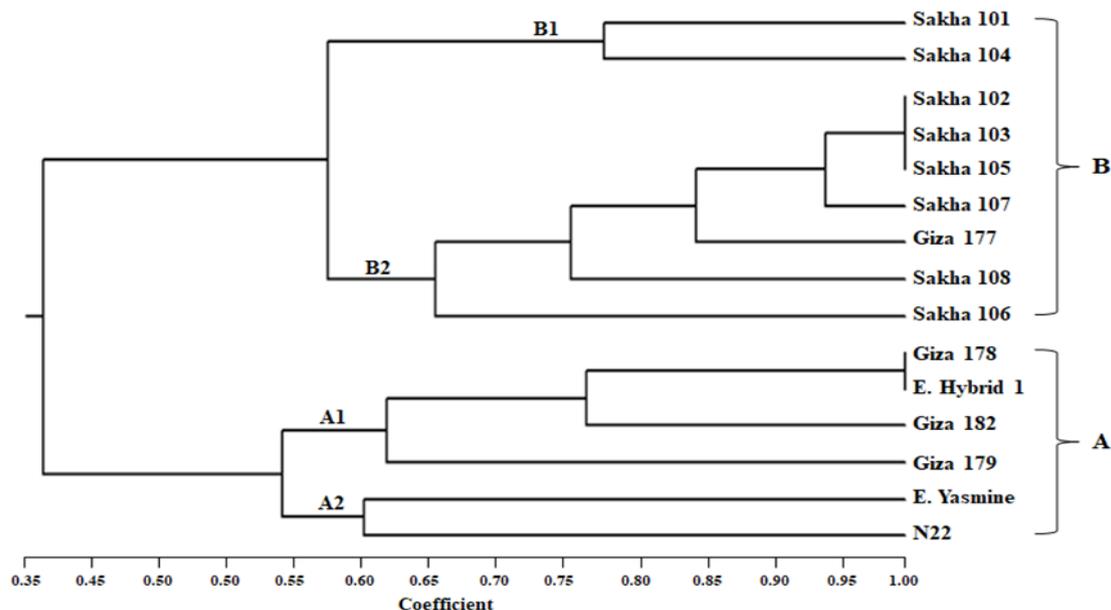
Figure (10): Dendrogram explaining the genetic relationships among tested genotypes using SSR markers employing UPGMA method.
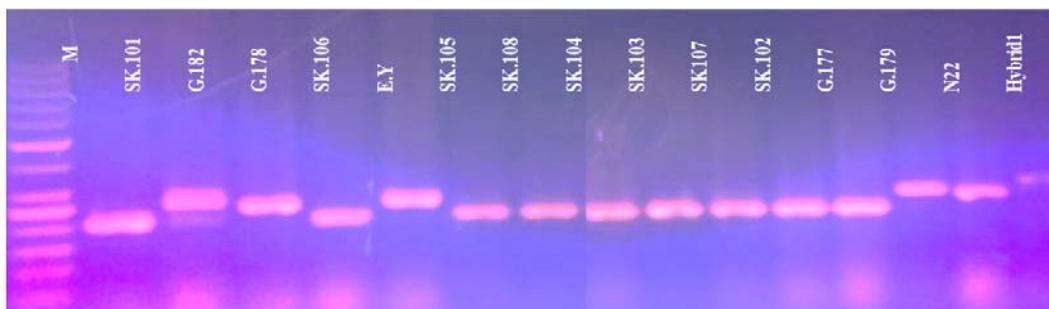

Fig. 11: The banding pattern of RM148 obtained from 15 rice genotypes (Lane 1 to 15) M=100 bp ladder.

Conclusion

High temperature is the most important environmental stresses reduce the performance of field crop. Generally all genotypes under heat stress produced low grain yield, the highest reduction was found in Sakha102 with value 70.36% and the lowest reduction recorded for N22 with 23.90%. Among different heat indices were evaluated HRI, and MP have high correlation with grain yield/plant under normal and heat stress conditions indicting more suitability of these indices for selection of tolerant genotypes. These indices could identify Giza178, Giza179, Giza182, Egyptian Yasmin, Egyptian Hybrid1 and N22 as the best tolerant genotypes under heat condition. As well as, these genotypes were heat tolerance placed in the same cluster. The study revealed that simple sequence repeat (SSR) markers facilitated the classification of these cultivars according to their genetic background. The study also noticed that these rice cultivars had a higher genetic diversity and therefore very useful for proper identification and selection of appropriate parents for use in breeding

programs, including gene mapping, and for application of marker assisted selection (MAS).

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التقييم الحقلى والوراثى للتحمل للاجهاد الحرارى لبعض أصناف الأرز باستخدام دلائل الإجهاد ومعلمات SSR

تهانى محمد عبدالله مظال

باحث بقسم بحوث الأرز – محطه البحوث الزراعيه بسخا– مركز البحوث الزراعيه – الجيزه– مصر .

الملخص العربى

أجريت هذه الدراسه خلال موسمى ٢٠١٨ و ٢٠١٩ فى موقعين، الموقع الأول بمركز البحوث والتدريب في الأرز – بمحطة البحوث الزراعية بسخا حيث تم التقييم تحت الظروف العادية .والموقع الثاني في محطة البحوث الزراعية بالوادي الجديد تحت ظروف الحرارة العالية .وكان الهدف من هذه الدراسه تقييم بعض الاصناف المصريه تحت ظروف الاجهاد الحراره وتحديد الاصناف المتحمله للحراره باستخدام مقاييس التحمل للإجهاد وإستخدام المعلمات الجزئيه وتحديد المناسب من هذه المعلمات واستخدامها بعد ذلك فى التربيه للتحمل للاجهاد الحرارى.حيث تم تقييم ١٥ تركيب وراثى من الأرز باستخدام تصميم القطاعات الكامله العشوائيه فى ثلاثه مكررات وتم تسجيل البيانات على طول النبات و عدد الآيام حتى التزهيرو طول السنبله و وزن السنبله ووزن الألف حبه و نسبه العقم و وزن الحبوب /النبات و كذلك تم استخدام ١٧ معلم وراثى.أظهر تحليل التباين وجود تباينات عاليه المعنويه للتراكيب الوراثيه المستخدمه مع البيئات لكل الصفات المدروسه. من بين مقاييس التحمل للاجهاد أظهر **MP index** عالى الإرتباط مع المحصول مما يدل على اهميته فى تحديد التراكيب الوراثيه المتحمله للحراره.

تم الحصول على أفضل متوسط قيم فى المحصول تحت الظروف العاديه والحصول على أفضل تركيب وراثى تحت ظروف الإجهاد الحرارى فى الأصناف الهجين مصرى ١ وجيزه١٧٩ وجيزه١٧٨ وجيزه N22,١٨٢ والياسمين المصرى.تشير النتائج الى أهميه هذه التراكيب الوراثيه لنقل صفات تحمل الاجهاد الحرارى فى الأرز فى برامج التربيه.أظهرتحليل المعلمات الوراثيه أن **12** منها كانت قادره على تحديد الاصناف المتحمله عن الآصناف الحساسه من خلال الشجره الوراثيه وبناءا عليه سوف يتم استخدام هذه المعلمات الوراثيه فى التربيه بالانتخاب المعتمد على المعلمات الجزيئيه **(RM6100,RM160,RM310,RM3471,RM225,RM592,RM148,RM153,RM5687,RM471,RM43 RM٣٥٨٦, 0,RM440)**