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Stability Analysis of Flowering and Yield Traits to High Temperature Stress Adopting Different Planting Dates in Rice (*O. sativa* L.)

N. Sravan Raju, P. Senguttuvel, S.R. Voleti, A.S. Hari Prasad, V.P. Bhadana, P. Revathi, K.B. Kemparaju, S. Ravi Chandran, Arun Kumar Singh, P. Koteswara Rao, N. Shobha Rani and B.C. Viraktamath
Center of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, 500 030, India

Corresponding Author: P. Senguttuvel, Center of Rice Research, Rajendranagar, Hyderabad, Andhra Pradesh, 500 030, India Tel: +919052694198, +914024591297

ABSTRACT

Rice grows mostly in tropical and subtropical regions, but it is very sensitive to higher temperature during reproductive stage especially flowering and anthesis. It is necessary to identify genetic donors for heat stress from high temperature rice growing environments. Temperature stress effects at reproductive stage by adopting three different planting dates with 15 days interval each in Environment-1(E1), Environment-2(E2) and Environment-3(E3) with forty three rice genotypes was studied. The temperature regimes were 35.6°C (E1) to 39.2°C (E3) at reproductive stage. From the results of AMMI analysis, the environment (E2) was found to be ideal for better identification of genotypes for heat tolerance with desirable traits. The elevated temperature at the time of flowering and maturity determines the yield *per se* of the genotypes. The hybrids adapted better than parental lines, showing the buffering nature and heterosis for stress tolerance. Under high temperature stress, the response of genotypes depended on developmental stage, but highest sensitivity was recorded at reproductive stage. The time of sowing, days to flowering (duration group), heat escape (early morning flowering) and inbuilt tolerance were the crucial factors in determining the performance of genotypes to varying temperature. Hence, it is necessary to select genotypes by keeping in view the above factors for different temperature stress within and across the environment.

Key words: Pollen, spikelet fertility, heat tolerance, AMMI, rice

INTRODUCTION

Rice (O. s*ativa* L.) is one of the three most important crops in the world and staple food for nearly half of the world population and it provides 35-80% of total calorie intake and consumed by 3.0 billion people (Wassmann *et al.*, 2009). More than 90% of global rice is produced and consumed in Asia. Hence, rice is immense need of food security in Asia, where more than 90% of rice is grown and consumed. Studies conducted at International Rice Research Institute (IRRI, 1976), Manila, Philippines indicated that the minimum and maximum temperatures have increased by 1.13 and 0.35°C, respectively for the period 1979 to 2003. (Peng *et al.*, 2004). Furthermore, global climate change is likely to aggravate the current vulnerability of the crop to climate, with a projected global average surface temperature increase of 2-4°C at the end of this century (IPCC, 2007). Rice has been model plant for years but its response to high temperature is poorly understood (Nagai and

Makino, 2009). Rice grows optimally at 20-32°C, but it is very sensitive to higher temperature (≥35°C) during flowering and to a lesser extent at booting (Satake and Yoshida, 1978). Yields of rice have been estimated to be reduced by 41% by the end of the 21st Century (Ceccarelli *et al.*, 2010) due to projected high temperature. The reduced grain yields are due to spikelet sterility under increased temperature during peak flowering period. the spikelet sterility is consequence of heat induced pollen inviability, failure of pollen or anther dehiscence, reduced pollen dispersal ratio, failure in penetration of pollen tube growth, loss of receptivity in stigma etc. Earlier studies also indicated that spikelets that are exposed to temperature ≥35°C for about 5 days during anthesis are sterile and set no seed. The spikelet sterility is varying among the genotypes at high temperature stress in both the sub species of *O. sativa*, *Indica* and *Japonica* (Matsui *et al.*, 2001). Adoption of high temperature tolerant cultivars is one of the most effective alternatives to maintain high productivity of rice under the anticipated climate change scenario (Horie *et al.*, 1996). Changing environment also affects genotype and its yield (Kempton, 1984), so it needs additional studies about the genotype (G)×Environment (E) interaction on rice production. Hence evaluation of genotypes under erratic environmental conditions within a location in different seasons is essential to understand the G×E interaction and stability of genotypes. The additive main effects and multiplicative interaction (AMMI) model was found to be useful for this purpose, as it utilizes both additive main effects for G×E and multiplicative components for an integrated least square analysis (Yan *et al.*, 2000).

Developing varieties or hybrids with better yield stability under varied environments in changing climate is need of the hour. It is a established fact that hybrids are having higher yield advantage than inbreds i.e., 10-30% higher grain yield compared to inbred lines in irrigated ecosystems (Virmani *et al.*, 1982) and when they were evaluated under drought environments, some hybrids showed high yield heterosis as well (Xie, 2010). Breeding rice hybrids with stable performance in diverse environments is essential to stabilize the yields (Serraj *et al.*, 2009). Keeping earlier studies in view, the present investigation was undertaken to understand adaptability of rice genotypes and hybrids under different regimes of temperature at reproductive stage based on their *per se* performance.

MATERIALS AND METHODS

Site description and experimental design: The field experiment was carried out at experimental farm of Directorate of Rice Research (DRR), Hyderabad, India (Latitute-17°10'N, Longitude 78°39'E and Altitude 524 MSL) during *Rabi* 2011 in clay loam soil. The experiment was laid out in Randomized Block Design (RBD) with three replications. Forty-three genotypes including parental lines, hybrids as listed in Table 3 along with sensitive and tolerant checks were evaluated under field conditions and recommended packages of practices were followed.

Field preparation and crop management: The nursery was raised with three dates of sowing (E1, E2 and E3) at 15 days interval and 28 days old seedlings were transplanted with a spacing of 20×15 cm transplanted. The mean temperature during the month of January-February was 27.5/15.2°C (day/night) during seeding, whereas it was 39.2/25.4°C (day/night) at the time of flowering to grain filling (April-May).

Observations recorded: Plant height, productive tillers, panicle length, grain yield, test weight, spikelet fertility and pollen fertility pertaining to high temperature are measured and interpreted.

Statistical analysis: Statistical constants of mean for all the characters were estimated by Eberhart and Russell (1966) model. The interpretation and analysis was done using SAS ver. 2000 package and AMMI analysis and biplots were obtained from yield interaction and PCA (Kempthorne, 1957).

RESULTS

The forty three hybrid rice parental lines including hybrids were evaluated in all the three different dates of sowing at 15 days interval (each set is treated as one environment, hereafter sets will be mentioned as environment E1, E2 and E3) to ensure that every genotype gets exposed to high temperature stress at reproductive stage in any of the set as all may not flower at the same time. The maximum temperature during sowing for first environment(E1) was 29.5°C and flowering coincided in fourth week of March with max temperature attaining 35.6°C, similarly second environment(E2) flowering coincides in April with maximum temperature of 36.7°C and third set(E3) flowering commenced and coincided with max temperature of 39.2°C. The relative humidity was lowest at the time of flowering in E3 group (Table 1, Fig. 1).

The analysis of variance for yield and yield components over three environments showed that genotypes, environments and G×E interactions were significant (Table 2). The genotype×environment (linear) interaction component showed significance for tiller numbers, pollen fertility and single plant yield. The days to flowering ranged from 90-121 days in E1, 88-118 days in E2 and 85-119 days in E3. AMMI analysis for biplot and interaction effect for days to flowering, spikelet fertility and grain yield is depicted in Fig. 2-7.

Table 1: Month wise temperature, Rainfall, sunshine, wind speed and evaporation details throughout the experiment

Month	Max. temperature (°C)	Min. temperature (°C)	Mean temperature (°C)	Rainfall (mm)/days	Sunshine (h)	Wind speed	Evaporation
January	29.5	10.4	19.9	-	8.9	2.4	2.6
February	31.2	15.0	23.1	6.0	8.7	3.4	2.7
March	35.6	17.7	26.7	-	8.8	3.5	2.9
April	36.7	21.9	29.3	3.0	8.2	4.3	5.0

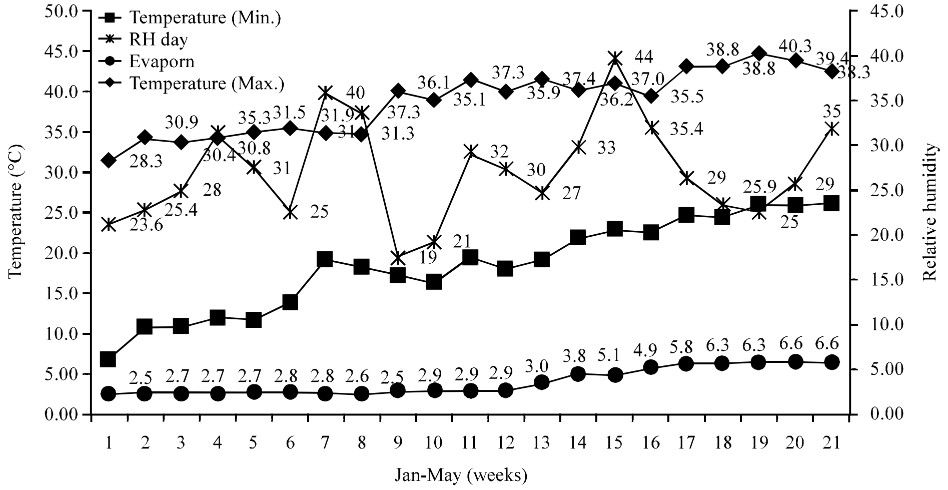

Fig. 1: Weekly environmental changes viz., Daily Temperature, RH and Evaporation (Jan-May) during 2011

Table 2: ANOVA for yield and yield components for stability of forty three rice parental lines and hybrids

	Mean sum of square									
Source	DFF	Plant height	Tiller numbers	No. of panicles	Panicle length	Pollen fertility (%)	Spikelet fertility (%)	Single plant yield	Harvest index	Plot yield
Rep within Env.	6.3	22.2	6.455**	2.300	2.4	22.401*	23.1	2.800	11.1	2714.14
Varieties	17.3	46.9	2.900	3.000	2.4	15.100	36.0	12.900	7.4	14013.40
Env+ (Var* Env.)	46.3	30.5	2.800*	2.400	3.5	9.700	34.1**	12.300*	9.4*	12720.10*
Var.* Env	35.1	45.0	11.054**	0.800	5.1	26.693**	19.0*	16.500**	20.0**	15651.20*
Environments (Lin.)	47.5	29.1	1.900	2.500	3.3	8.000	35.6	11.900	8.3	12427.00
Var.* Env.(Lin.)	70.2	89.9	22.108**	1.600*	10.2*	53.385*	38.0*	32.996*	40.1**	31302.30**
Pooled deviation	46.5	14.4	2.500	1.200	3.8	9.700	21.1	16.600	5.3	17613.40
Pooled error	44.01	39.8	1.200	3.498	2.6	5.700	45.5	6.500	10.4	6582.30
Total	10.0	37.4	3.100	1.000	2.6	12.500	28.0	4.200	9.6	4477.61

*Significant at 5% level, **Significant at 1% level

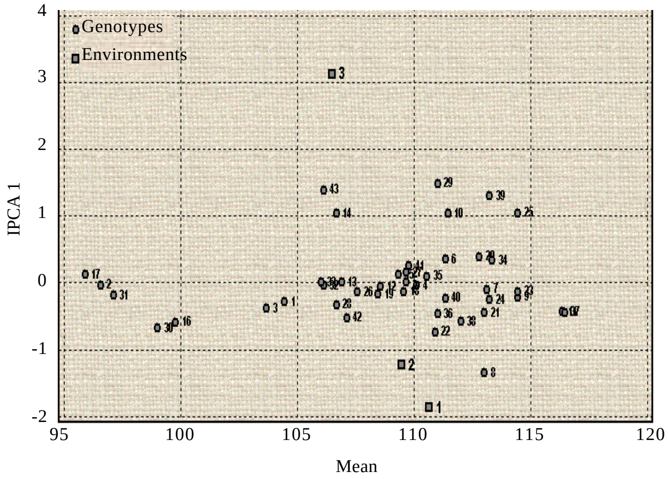

Fig. 2: AMMI 1 Biplot of 43 rice genotypes for days to flowering using genotype environment scores

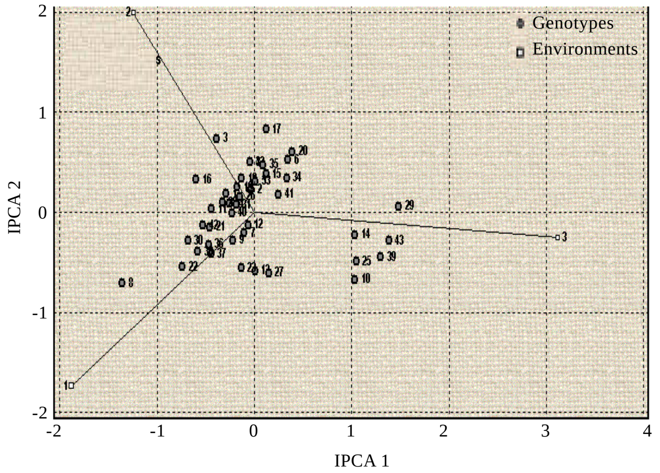

Fig. 3: Biplot of PCA 2 against PCA 1 scores for days to 50% flowering of 43 rice genotypes in three environments

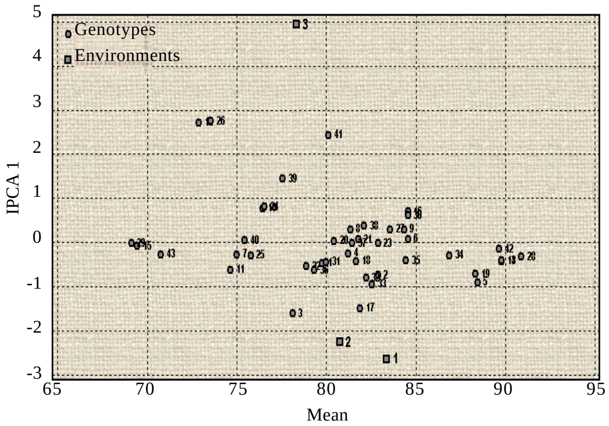

Fig. 4: AMMI 1 Biplot of 43 rice genotypes for spikelet fertility using genotype environment scores

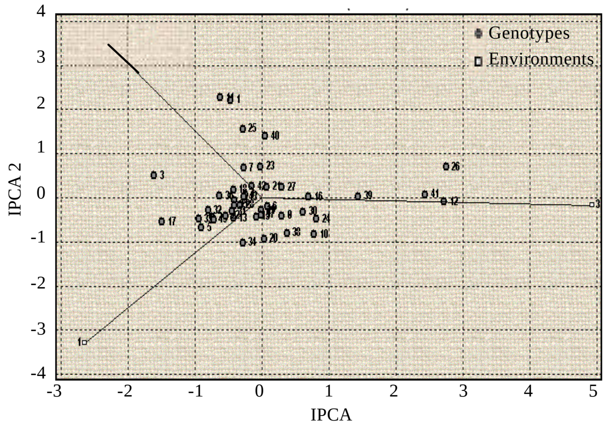

Fig. 5: Biplot of PCA 2 against PCA 2 scores for spikelet fertility of 43 rice genotypes in three environments

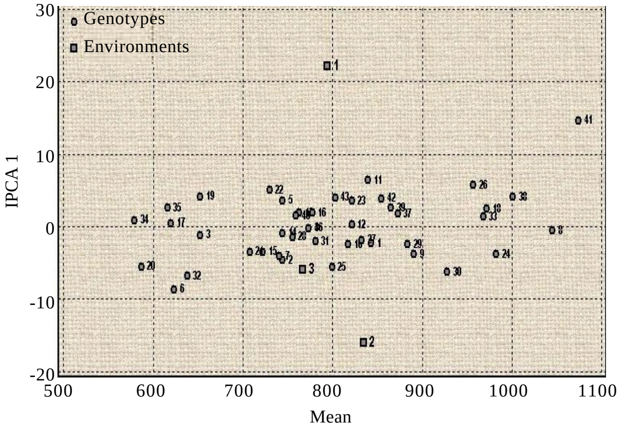

Fig. 6: AMMI 1 Biplot of 43 rice genotypes for grain yield using genotype environment scores

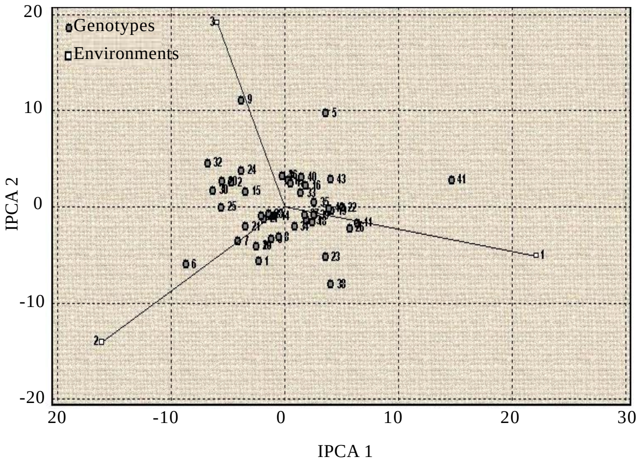

Fig. 7: Biplot of PCA 2 against PCA 2 scores for days to grain yield of 43 rice genotypes in three environments

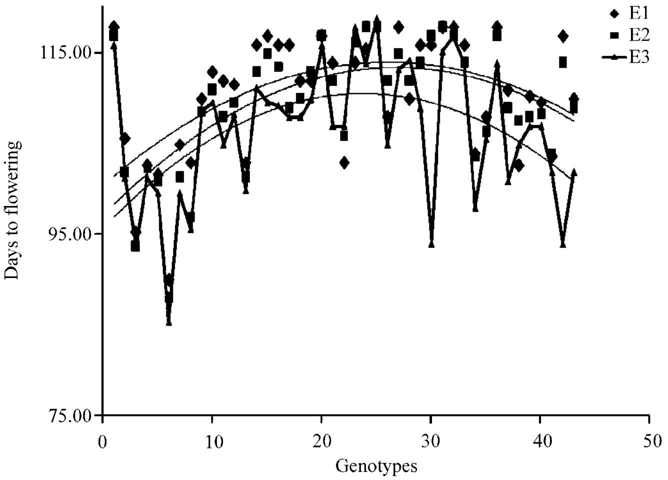

Fig. 8: Comparison of genotypes to days to 50% flowering in three environments

Days to flower among all the three environments varied significantly and higher variation was noticed in the third environment (Fig. 8). The genotypes viz., SG-27-175, DRRH 2, KRH 2 and CORH 3 showed early morning flowering behaviour and reached peak anthesis (1-1.5 h after dawn) as compared to other test entries which flowered normally 2.5 h after dawn (9.30 a.m.). Better seed set was observed in early morning flowered lines compared to lines flowered late and coincided with high temperature.

The pollen viability varied from 68% in RPHR 619-2 to 92% in IR 40750R (E1); 57% in IR 40750R to 90% in Rajalaxmi hybrid (E2) and 60% in RPHR 611-1 to 90% in IR 68897B (E3). Azucena a heat sensitive genotype showed 65% pollen viability in E3, 77% in E2 and 85% in E1, whereas heat tolerant genotype N22 recorded 88, 80 and 63% viability in E1, E2 and E3 environments. The mean pollen viability among the E1 is 83% and remains same in E2 and E3 as 80% (Fig. 9).

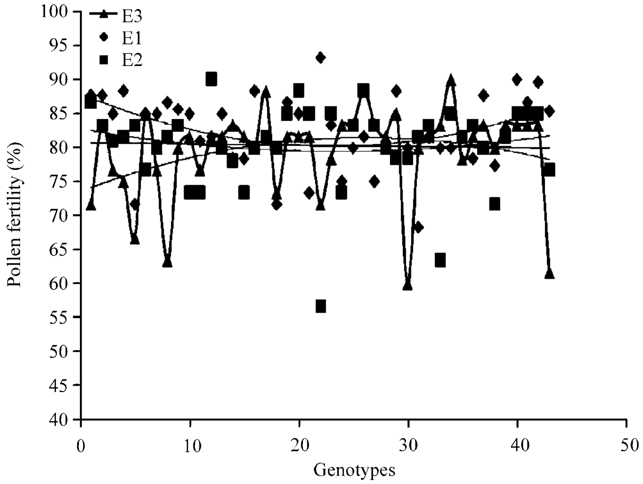

Fig. 9: Response of pollen fertility for forty three genotypes in three environments

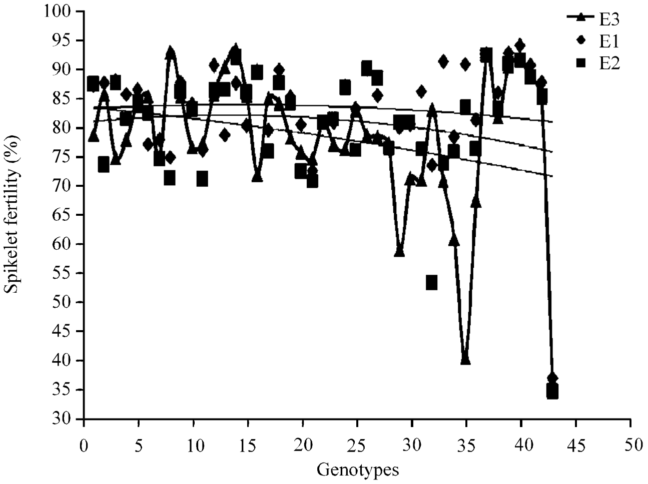

Fig. 10: Response of spikelet fertility for forty three genotypes in environments

The spikelet fertility varied from 37, 35 and 35% (E1, E2 and E3) in Azucena (sensitive) to 93% (in all three environments) in N22 (tolerant); Suruchi, PHB 71(hybrids), RPHR 1005 and SG-27-77 were also observed to be moderately tolerant to high temperature stress (Table 3, Fig. 10, 11). Other genotypes viz., BPT 5204, IR 58025B, DRR6B were affected adversely when flowering coincided with high temperature. The pollen viability did not correlate with spikelet fertility as in case of N22 which is relatively tolerant to heat, the pollen viability was low (62%) but it had high percentage (83%) of seed set (spikelet fertility). Azucena which is sensitive genotype recorded high pollen viability (65%) but spikelet fertility (filled grains) was comparatively very low (16%).

The grain yield is the sum of all effects and the response of the genotype in terms of economic yield ranged from 473-1144 g m^{-2} in E1, 583-1210 g m^{-2} in E2 and 396-1144 g m^{-2} in E3 (Table 3). Among the environments only the E2 had recorded highest mean yield 834 g m^{-2} over the other two environments of 794 kg m^{-2} (E1) and 767 kg m^{-2} (E3). The cultivar N22 was the highly tolerant genotype and Azucena was most sensitive genotype in terms of spikelet fertility and grain yield.

Table 3: Comparative performance of forty three rice genotypes under three environments (E1, E2 and E3) for key traits to heat tolerance (DFF-days to fifty per cent flowering, PF-Pollen fertility, SF-Spikelet fertility, grain yield)

	Environment I				Environment II				Environment III			
Entry name	DFF	PF (%)	SF (%)	Yield (kg m^{-2})	DFF	PF (%)	SF (%)	Yield (kg m^{-2})	DFF	PF (%)	SF (%)	Yield (kg m^{-2})
Ajaya	118	88	87	825	117	87	88	935	116	72	79	924
CORH 3	106	88	88	748	102	83	74	924	101	83	86	858
DRRH 2	95	85	88	803	94	81	88	913	94	77	75	792
DRRH 3	103	88	86	825	102	82	82	979	101	75	78	847
JKRH 401	102	72	87	638	101	83	85	803	100	67	84	726
JRH 8	90	85	77	660	88	77	83	847	85	85	86	605
KRH 2	105	85	78	550	101	80	75	726	100	77	75	594
NK 5251	103	87	75	638	97	82	71	616	96	63	93	528
PA 6129	110	86	88	737	109	83	86	781	109	80	86	748
PHB 71	113	85	84	803	111	73	83	946	110	82	77	858
PRH 10	112	81	76	1012	108	73	71	1155	105	77	78	935
Rajalaxmi	112	82	91	913	110	90	87	781	108	82	86	704
Sahyadri 4	103	85	79	737	101	80	87	616	100	82	91	649
Suruchi	116	78	88	671	113	78	92	693	111	83	94	396
US-312	117	78	81	748	115	73	86	803	110	82	86	649
BCW-56	116	88	90	495	114	80	90	583	109	80	72	517
DR714-1-2R	116	82	80	473	109	82	76	682	108	88	85	726
EPLT-104	112	72	90	473	110	80	88	715	108	73	84	638
EPLT-109	112	87	86	638	113	85	85	792	110	82	79	836
GQ-25	117	85	81	693	117	88	73	770	116	82	76	594
IBL-57	114	73	73	770	112	85	71	913	107	82	75	792
IR40750R	103	93	81	803	106	57	81	847	107	72	81	704
KMR 3	114	83	81	1089	116	85	82	1023	118	78	77	737
RPHR-1096	116	75	87	814	118	73	87	770	114	83	77	1144
SC$_5$-2-2-1	121	80	83	891	118	83	76	748	119	83	83	847
SG-27-77	108	82	90	1012	112	88	90	1056	105	88	79	924
SG-27-175	118	75	86	803	115	83	89	1210	113	83	79	825
RPHR-111-3	110	80	77	990	112	80	77	737	114	82	77	935
RPHR-517	116	88	80	836	114	78	81	704	109	85	59	781
RPHR611-1	116	80	81	770	117	78	81	990	94	60	72	825
RPHR619-2	118	68	86	682	118	82	77	957	115	80	71	836
APMS6B	118	82	74	957	117	83	53	693	117	82	83	781
IR58025B	116	80	92	781	114	63	74	880	114	83	71	748
IR68897B	104	80	79	1012	104	85	76	891	98	90	61	880
IR79156B	108	82	91	1144	106	82	84	858	106	78	41	770
PUSA 5B	118	78	82	869	117	83	77	913	114	82	68	1001
N22 (Check)	111	88	93	1012	109	80	93	803	101	63	93	814
BPT-5204	103	77	86	957	108	72	83	814	105	80	82	880
IR-64	110	83	93	880	108	82	91	781	107	83	91	715
Jaya	110	90	94	803	108	85	92	814	107	83	92	704
Rasi	104	87	91	627	104	85	89	781	102	83	89	737
Apo	117	90	88	704	114	85	86	781	94	83	85	693
Azucena	110	85	37	891	109	77	35	869	102	65	16	803
Mean	111	82	83	795	109	80	81	835	106	80	78	767
CD (0.05)	1	7	5	560.1	0.8	10.7	8.5	544	1.8	8.3	4.0	480.5
Range	90-121	68-93	37-94	473-1144	88-118	57-90	35-93	583-1210	85-119	60-90	35-94	396-1144
CV	1	5	4	43.4	0.5	8.21	6.5	40.3	1.0	6.4	3.17	38.56

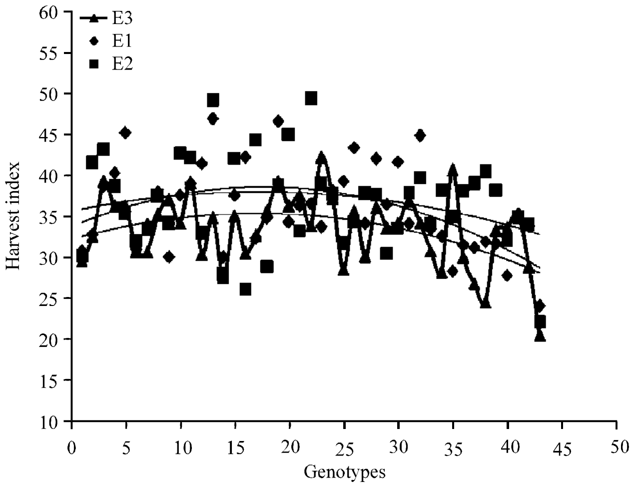

Fig. 11: Effect of Temperature on harvest index of forty three genotypes in three environments

DISCUSSION

The experiment was planned in different sowing date in same location keeping in mind that the parameters pertaining to soil and edaphic factors will remain constant and only ensuring variation in temperature regime. The wind speed and evaporation loss increased as the temperature went up during peak flowering period; it has also had triggering effects on pollen and spikelet fertility. Matsui *et al.* (1997) reported that a wind velocity above 0.85 m sec^{-1} drastically increased spikelet sterility at 37.5°C and relative humidity of 60% in a chamber experiment. The optimum temperature for the normal growth of rice plants to produce maximum biological yield should range from 27°C to 32°C (Yin *et al.*, 1996). Even though the temperature roses to higher level of 40°C, the pollen fertility decreased tremendously in sensitive and very less in case of tolerant genotypes (N22, PHB 71 and RPHR 1005) which means these genotypes are able to cope up with the changing climate and also prevailing microclimate inside the floret and field. The high Vapor Pressure Deficit (VPD) helped the plants to avoid heat stress and to maintain their microclimate below critical levels by efficient transpiration cooling, rather than by temperature *per se* (Weerakoon *et al.*, 2008).

The days to flowering varied with the environment and the effect was more pronounced in late planting (E3). However, E2 showed better variation and consistency for adaptability based on AMMI analysis. The genotypes in the E3 flowered earlier than other two environments indicating the severity of the heat stress leads to early flowering coupled with incomplete exsertion of the panicle. However, flowering (anthesis and fertilization) and to a lesser extent the preceding stage i.e. booting (microsporogenesis) are considered to be the most sensitive developmental stages to high temperature in rice (Satake and Yoshida, 1978; Farrell *et al.*, 2006). By studying, differences among the forty three parental lines of rice genotypes for linear response to sowing date (bi), the behaviour of the rice genotypes can be predicted over environments more precisely as G×E interaction is outcome of the linear function of environmental components. Hence, prediction of performance of genotypes based on stability parameters would be feasible and reliable.

The tolerant genotypes even though recorded moderate pollen viability resulted in higher degree of spikelet fertility indication tolerance to heat stress. Sensitive genotype Azucena have maximum variation in flowering duration, as reported by Nagarajan *et al.* (2010). The percentage of pollen viability reduction is much pronounced in E3 than the other two environments. It is reported that more than 10-20 pollen grains are enough to get germinated on the stigmata to ensure successful fertilization (Satake and Yoshida, 1978). Anthers of high temperature tolerant cultivars dehisce more easily than those of susceptible cultivars and contribute to pollination under high-temperature conditions (Satake and Yoshida, 1978; Mackill *et al.*, 1982; Matsui *et al.*, 2000, 2001).

Exposure to 41°C for 4 h at flowering caused irreversible damage and plants became completely sterile (IRRI, 1976), whereas this high temperature (41°C) had no effect on spikelet fertility at one day before or after flowering (Yoshida *et al.*, 1981). Sheehy *et al.* (2005) reported altered responses of rice genotypes in terms of spikelet fertility to different levels of temperature increases. It is clearly evident that when the genotypes (E3) coincides with high temperature during the anthesis period results in drastic reduction in fertility level and in turn decreased the grain yield. The effect of heat stress in many plant species is induced sterility particularly during anthesis (Wahid and Close, 2007; Nakagawa *et al.*, 2003). High temperature not only affected pollen viability but also inhibits pollen germination, pollen tube growth, fertilization and early stages of embryo development.

The Early-morning Flowering (EMF) trait is proposed to be one approach to mitigate heat-induced spikelet sterility (Satake and Yoshida, 1978; Imaki *et al.*, 1987). Identification of early morning flowering trait is a valuable phenotypic marker and it was reported that *O. officinalis* possess this trait. The genotypes namely SG-27-175, DRRH2, KRH2 and CORH3 flowered early in morning than other genotypes and could be used to introgress into elite cultivars. Identification of EMF trait in *O. sativa* accessions is an added advantage than having in *O. officinalis* or *glaberrima* species as trait can easily be introgressed in high yielding varieties.

From the study it is clearly evident that hybrids (known for heterosis) respond better than inbred lines under abiotic stress. Yield superiority of hybrid rice under unfavorable environments, including rainfed drought-prone lowlands and problem soils (Virmani, 2003). The phenomenon of internal buffering mechanism of hybrids under adverse climatic environments especially to high temperature stress is more pronouncing than inbred varieties. Earlier it is reported that hybrids tended to be superior in drought tolerance (Xu *et al.*, 1998; Serraj *et al.*, 2009; Xie, 2010). The tolerance level depicted in terms of grain yield is comparatively higher than inbred varieties and however this may not be broadly applicable to all set of hybrids, unless bred for specific stress ecology or having inbuilt mechanism of tolerance within the hybrid parental lines. The present findings confirms that the possibility of breeding of new varieties/hybrids possessing heat tolerance, growing and evaluating in dry season upto 40°C. It is evident that breeding hybrids for tolerance and early morning escape to temperature stress can pave the way for developing genotypes better adapted to future climates. So far, none of the hybrids were bred for abiotic stress, if breeding efforts are focused towards abiotic stresses, there is a possibility in development of better hybrids for unfavorable environments.

CONCLUSION

Effect of temperature stress in rice more pronounced during reproductive stage and pollen grains are more sensitive to heat stress which ultimately results in yield reduction. It is obvious that hybrids respond very well under adverse climatic conditions. So selection of parents should be focused on duration, EMF traits and higher pollen with spikelet fertility genotypes.

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