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Effect of temperature regimes on biochemical traits of rice (*Oryza sativa* L.) genotypes for high temperature tolerance

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Abstract
High temperature stress is the one of the most important environmental factors that influencing growth, development, and yield of rice crop. The present paper studies the effects of temperature regimes on various biochemical parameters at different stages of growth for high temperature tolerance using the 50 IRRI and Indian rice genotypes with five check (Local, national and international) varieties were evaluated during normal sown (December-2014) and late sown conditions (January 2015). Result of the experiment reveals that late sowing of genotypes particularly in the month of January has shown the decreased various biochemical responses and yield attributes than the normal sown genotypes. Grain yield was lowest under late sown condition (11.92% reduction) as compared to normal sown condition, which was expected that plant experienced the high temperature stress mainly during flowering and grain filling stages. The results of mean sum of squares due to the genotypes and their interaction were significant for different biochemical parameters. Finally, among the screened genotypes EC792239, EC792185, EC792179, EC792240 and EC792316 are identified as heat stress tolerant genotypes. Hence, they are shown better performance for different biochemical parameters like chlorophyll a (3.49 mg/g Fw), chlorophyll b (2.24 mg/g Fw) and chlorophyll a/b ratio (6.93 mg/g Fw), pollen viability (97.90%) and protein and amylase content of grains. They could be used as potential donors for development of heat stress tolerant variety.

Keywords: Rice, High stress, heat tolerance, sowing dates, morpho-physiological, and yield

1. Introduction

Rice (*Oryza sativa* L.) is a "Global grain" cultivated widely across the world and feeds millions of mankind, is the staple food for more than half of the human population. Asia is considered as "Rice Basket" of the world, as 90 per cent of world's rice is grown and consumed with 60 per cent of population and where, about two-thirds of world's poor live (Khush and Virk, 2005) [16]. In India, rice is the second most produced commodity cultivated on an area of 43.95 m ha, with a production and a productivity of 106.54 m t and 2424 kg ha^{-1}, respectively (Anon., 2015) [3].

Global warming is a serious peril to the rice production. The optimum temperature for the normal development of rice ranges from 27 °C to 32 °C. High temperature stress is the one of the most important environmental factors that influencing growth, development, and yield of crop. High temperature (HT) affects almost all the growth stages of rice, *i.e.* from emergence to ripening. The increase in temperature has been striking and can cause irreversible damage to plant growth and development (Wahid *et al.*, 2007) [26]. It has been shown a 7-8% rice yield reduction for each 1 °C increase in daytime temperature from 28 °C to 34 °C (Baker *et al.*, 1992) [5]. However, flowering (anthesis and fertilization) and booting (microsporogenesis) are considered to be the stages of development most susceptible to temperature in rice. High temperatures ($\geq$ 35-40 °C) during anthesis stage of flowering induce spikelet sterility which in turn decreases the rice yield (Bhadana *et al.*, 2014) [6]. Plants possess a number of adaptive, avoidance or acclimation mechanisms to cope with HT situations.

Rice is being cultivated both in *kharif* and summer in Tungabhadra Project (TBP) command area of Karnataka state. Rice is being cultivated in an area of 4.92 lakh ha with a production of 13.6 lakh tonnes and a productivity of 2772 kg ha^{-1}. Due to late sowing of *kharif* crop because of late onset of monsoon harvest will be delayed and summer sowing is also delayed. Late sowing of rice in summer in the month of February lead to reduction the rice yields. The yield reduction is mainly due to late sowing coincide with high temperature during flowering and anthesis period. It is important to screen the rice germplasm for high temperature tolerance. Hence, with this background the present investigation was follow up.

Materials and methods

A field experiment was conducted at Agriculture research station Gangavati, university of agriculture sciences, Raichur. The paper studies the effects of temperature regimes on various physiological parameters at different stages of growth using 50 IRRI and Indian rice genotypes with five (Local, national and international) check varieties were evaluated during *early summer*/Normal sown (07-Dec.-2014) and *late summer/* late sown (20-Jan-2015). The details of genotypes and check verities are given in (Table 1). In our experiments we tried to expose our genotypes to high temperature at different crop growth stages especially during reproductive stage, which is a serious problem of this region. To coincide with the high temperature during reproductive stage of crop two (Normal & Late) different dates of sowing was done. The seedlings were planted in Randomized Block Design (RBD) at Agricultural Research Station, Gangavati. Adopting a spacing of 20 cm × 15 cm, in a plot size of 0.8 m × 3.5 m width and length respectively.

Table 1: List of rice genotypes used for present study

S. No.	Genotypes	S. No.	Genotypes	S. No.	Genotypes	S. No.	GGenotypes	S. No.	Genotypes
1	EC792216	12	EC792237	23	EC792215	34	EC792233	45	EC792270
2	EC792231	13	EC792267	24	EC792193	35	EC792179	46	EC792286
3	EC792227	14	EC792206	25	EC792185	36	EC792208	47	EC792289
4	EC792177	15	EC792234	26	EC792222	37	EC792219	48	EC792326
5	EC792226	16	EC792201	27	EC792240	38	EC792309	49	EC792217
6	EC792200	17	EC792257	28	EC792195	39	EC792203	50	EC792192
7	EC792224	18	EC792310	29	EC792235	40	EC792214	C1	Gangavati sona
8	EC792239	19	EC792199	30	EC792225	41	EC792176	C2	IR- 64
9	EC792194	20	EC792288	31	EC792316	42	EC792284	C3	MTU-1010
10	EC792236	21	EC792187	32	EC792204	43	EC792218	C4	N-22
11	EC792210	22	EC792205	33	EC792238	44	EC792186	C5	ES-18

Note: C1, C2, C3, C4 and C5 are check (Local, national and international) varieties

The genotypes were replicated twice. In each replication, each genotype was planted in four rows with 20 seedlings or hills per row. During crop growth period (December to May) summer 2014-2015, the maximum and minimum temperature was 41 °C and 26 °C, respectively and maximum and minimum relative humidity recorded was 99.5 and 29.7 per cent, respectively it is presented in (Fig. 1).

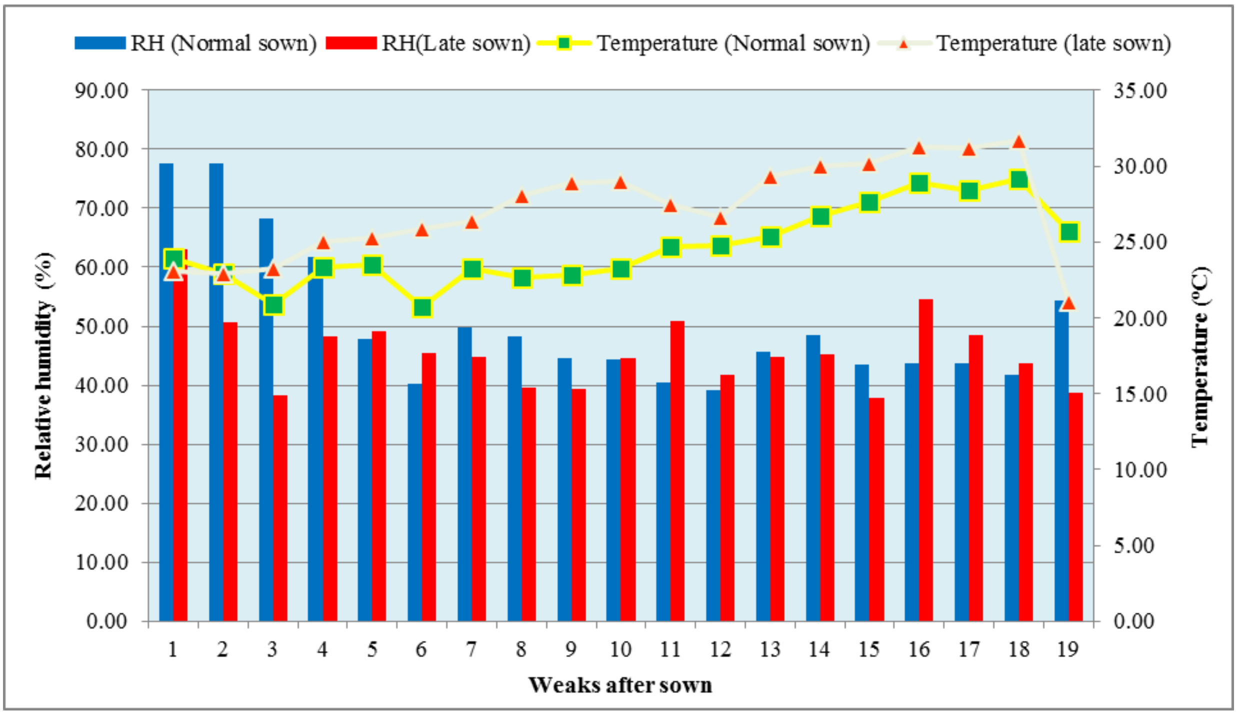

Fig 1: Standard meteorological week's data during the crop growth period (summer 2014-2015)

Five competitive plants were randomly selected from each entry, each replication and from each environment for the recording of the observations. The physiological parameters like leaf temperature (°C), chlorophyll content (mg/g FW), normalized difference vegetation index values, transpiration rate (mmol (H_2O) $m^{-2}s^{-1}$), stomatal conductance (mol (H_2O) $m^{-2}s^{-1}$), photosynthetic rate (μmol CO_2 $m^{-2}s^{-1}$), light interception (%), leaf area index and grain yield (kg/ha). The genotypes from normal and late sown condition were planted in randomised block design (RBD) with two replications and field observations were recorded during crop growth season. The data collected from experiments were analysed statistically by following the procedure prescribed by Sundararaj *et al.* (1972). Whenever, 'F' test was found significant the critical difference (CD) values were calculated and the treatment mean were compared at five per cent.

Plant height was recorded at flowering and harvest time from base of the plant up to the last sheath of main shoot. Five plants were randomly selected, tagged and mean values of height was expressed in centimeter. The canopy temperature was recorded with the help of infrared thermometer at 65, 75, 85, 95, 105 days after sowing (DAS) and at harvest. The canopy temperature was recorded in degree Celsius (ºC). It was focused on the canopy targeted by holding gun pistol grip at an angle of 45° and at a distance of 0.5 to 1 m from canopy for taking observation.

Chlorophyll was estimated following the standard procedure (Hiscox and Israelstam, 1979) [13] at 75 and 85 DAS. The third fresh leaves from top of the plants were collected. A known fresh weight of leaf sample were cut in to small pieces and suspended in test tubes containing seven ml of dimethyl sulphoxide (DMSO). Test tubes were incubated at 60 °C for 20 min in a water bath. The supernatant was decanted and another three ml of DMSO was added to the residue and incubated at 60° C for 20 min. The supernatants were pooled and the volume was made up to 10 ml by adding DMSO. The chlorophyll extract was transferred to a cuvette and the absorbance was read in a systronic spectrophotometer-169 at 645 and 663 nm against DMSO blank. Chlorophyll a, b, and chlorophyll a/b ratio were calculated by the following formulae (Arnon, 1949) [4]. The data on the chlorophyll content was recorded in mg/g fresh weight.

Chl. a (mg/g) = [12.7(OD663)-2.69(OD645)] x V/1000 x W x a

Chl. b (mg/g) = [22.9(OD645) - 4.68(OD663)] x V/1000 x W x a

Total Chl. (mg/g) = [20.2(OD645) + 8.02 (OD663)] x V/1000 x W x a

Where,

V = Volume of the acetone used in extract (ml)

W = Weight of fresh leaf tissue (g)

A = Light path length (cm)

A645 = Absorbance of the extract at 645 nm

A663 = Absorbance of the extract at 663 nm

The amylose and protein content in each rice sample was determined by using Spectrophotometerical techniques according to modified method of Juliano (1971).

Pollen viability of genotypes was tested for pollen fertility status by using the acetocarmine staining method (Qureshi *et al.*, 2009). The acetocarmine method is suitable test for determining viability of pollens in rice crop. Flower buds, flowers or inflorescences of the genotypes were collected from field during early in the morning (9:00 to 11:00 am) and where pollen was analyzed for viability within one of collection or kept in the fridge at 4 ºC for analysis the next day. Using dissecting forceps, scalpel and a needle, anthers of genotypes were opened to allow extraction and subsequent transfer of pollen dust on to a microscopic glass slide in a drop of acetocarmine stain. Mature anthers were crushed and pollen grains mixed thoroughly with the acetocarmine stain. Cover slips were gently placed on to different slides for each species. The slides were than observed under a light microscope. For each genotype flowers were collected from at least three different plants. For each plant, three slides were prepared. For each slide ten randomly selected fields were observed under the 10X objective (100X magnification). To determine pollen fertility, darkly stained pollen grains were recorded as fertile and viable, and unstained or very lightly stained ones were considered as sterile or non viable. Pollen fertility was calculated by dividing the number of viable pollen grains by the total number of grains counted in the field of view and averaging them for all plants in that genotype. Pollen viability was expressed as percentage pollen fertility in each genotype.

Results and discussion

Rice is one of the important staple food crop grown in the areas where temperature is optimum for rice production and heat stress may limit sustainable rice production in these areas Tian *et al.* (2009). The effect of high temperature on rice yield depends on several complex factors individually and in combination including genotype, the growth stage at which it encounters heat stress, duration of stress, time of the day/night, the prevalent conditions of water vapour deficit, wind velocity, radiation, and ambient recovery conditions. Morita *et al.* (2005), Prasad *et al.* (2006) in sorghum, Jagadish *et al*. (2010), and (Zhou *et al.*, 2012) in rice. However, flowering stage is the most heat sensitive stage as heat stress during this stage results in loss of yield due to low pollen fertility and low seed set.

High temperature (heat) stress is considered to be one of the major environmental factors limiting crop growth and yield. Heat stress induces many biochemical, molecular, and physiological changes and also influence various cellular and whole plant processes that affect crop yield and quality. Heat stress (increase in above-optimum air temperature) causes different effects on various physiological growths, development, and yield processes. The rise in atmospheric temperature causes detrimental effects on growth, yield, and quality of the rice crop by affecting its phenology, physiology, and yield components (Singh 2001, Sheehy *et al.* 2005 and Peng *et al.* 2004). The sensitivity of rice to high temperature varies with growth phase, an increase in day/night temperature and genotype (Yoshida 1981, Singh 2001 and Peng *et al*. 2004). The impact of increased temperature has an accumulative effect on the later phases of plant development and alter the grain filling phase and affect the grain quality of the rice.

In general, there was 12.95 per cent decrease in pollen viability due to late sown compared to normal sown. The reduction of pollen viability under late condition is significantly more due high temperature coincidence during flowering stage of crop growth period. The results on pollen viability were significantly influenced by temperature regimes. Among the genotypes, EC792195 (97.90%) and EC792218 (68.10%) recorded significantly highest and lowest pollen viability percent recorded under normal and late sown conditions, respectively. Similar results were agreed with the Roy *et al.* (1995), Tsutomu *et al.* (2002) [27], Prasad *et al.*, (2006), Cao *et al.* (2008), Zhou *et al*. (2012), Jeffrey *et al.* (2013) and Yugandhar *et al.* (2013) [29].

In the present investigation, chlorophyll a, chlorophyll b content and chlorophyll a/b ratio of leaf increased from 75 DAS to 85 DAS but later decreased. Among the genotypes EC792227 and EC792179 recorded highest chlorophyll a (3.49 mg/g FW) and chlorophyll b (2.24 mg/g FW), respectively and the chlorophyll a/b ratio recorded highest in Gangavati sona (8.96 mg/g FW). The increase in chlorophyll content has been reported by several authors Burker (1990) [8], Hae-Ran *et al.* (2010) [12], Panigrahy *et al.* (2011) [19], Yalcaum *et al.* (2011) [28], Aghaee *et al.* (2011) [1], Prasanth *et al.* (2012) [20, 21], Yugandhar *et al.* (2013) [29] and Gerson *et al.* (2015) [11], This result is in conformity with the result of Rupinder Kaur and Saxena (2011) [22] and Zhou *et al*. (2012).

Among rice genotypes grain filling percentage was significantly influenced by normal sown and late sown conditions. There was a significant reduction in grain filling percentage due to temperature stress under late sown condition in the genotype EC792238 (6.07%). However lowest grain filling percentage was recorded in the genotype ES-18 (68.71%). Similar finding with different date sown conditions on filled grain percentage were reported by Stone (2001) and Shi *et al.* (2008). However, interaction effect revealed that ES-18 recorded significantly higher grain filled percentage and lowest were rerecorded in EC792238 genotype compared to all other interaction values. These results are in conformity with the findings of Yoshida *et al*. (1981), Prasad *et al.* (2006), Lur *et al.* (2009) [17], Liao *et al.* (2011), Prerna *et al. (*2012) and Tenorio *et al*. (2013).

In the present investigation, the grain yield in rice was significantly influenced by temperature regimes and significantly higher grain yields recorded under normal sown condition in the genotype EC792239 (6,334 kg ha^{-1}) compared to all other genotypes. However, late sown condition recorded significantly lower grain yield in the local check ES-18 (1,553 kg ha^{-1}). The normal sown conditions favour maximum production of dry matter accumulation due to non stressful condition as compared to late sown condition. There was significant reduction in grain weight $plant^{-1}$, Test weight of seeds (1000 seeds) and panicle length due to high temperature stress under late sown condition. Since, there is coincidence of high temperature during peak anthesis stages compared to normal sown condition. Similar results were reported by various researchers like Daubenmire (1974) [9], Muchow *et al.* (1990) [18], Wilhelm *et al.* (1999), Wardlaw *et al.*, 2002 [27], Mian *et al.*, 2007, Prerna *et al.* (2012), Tenorio *et al.* (2013) and Pratap and Dwivedi (2015) [10]. Performance of rice genotypes for yield character under normal and late sown conditions during 2014-2015 presented in the (Figure 3).

Among the genotypes, the maximum amylose content was recorded in genotype having ascension EC792218 (120.71%) compared to all other genotypes under normal sown condition and the lowest amylose content was recorded in EC792257 (43.75%). Under late sown condition the maximum amylose content in seed was recorded in ascension EC792218 (125.31%) and the lowest amylose content in grain was recorded in ascension EC792310 (55.11%).

Mechanisms involved in during high temperature tolerance in rice genotypes

Plants respond to high temperature tolerance by various physiological and biochemical adaptations like proline synthesis, wax synthesis and synthesis of heat shock proteins (HSP_S) *etc...* And also leaf and panicle transpiration plays an important role in reducing canopy temperature. In present investigation the high yielding genotypes *viz.*, EC792239, EC792185, EC792179, EC792240 and EC792316 recorded significantly higher transpiration, lower canopy temperature and higher photosynthetic rate indicated clearly the leaf transpiration play an important role tolerating high temperature stress. Similar results were agreed with the findings of Tenorio *et al*. (2013) and Santosh and Rawson. 2013 [23].

Table 2: Performance of rice genotypes under normal and late sown conditions and percent decrease of late sown over the normal sown condition for different physiological parameters during summer 2014-2015

Characters	Condition	Genotypes showing highest value		Genotypes showing lowest value		Overall mean	Per cent decrease
		Genotypes	Values	Genotype	Values		
Plant height (cm)	Normal	N-22	112.0	EC792240	76.9	92.7	6.26
	Late	EC792227	105.7	EC792205	67.9	86.9	
Chlorophyll- a (mg/g Fw)	Normal	EC792227	3.49	EC792201	2.71	3.23	18.75
	Late	EC792316	3.25	EC792234	1.81	2.72	
Chlorophyll- b (mg/g Fw)	Normal	EC792179	2.24	EC792224	0.40	1.27	37.80
	Late	EC792240	2.22	ES-18	0.20	0.79	
Chlorophyll- a/b ratio (mg/g Fw)	Normal	EC792224	6.93	EC792179	1.55	2.95	-49.83
	Late	Gangavati sona	9.05	EC792179	1.46	4.42	
Pollen viability (%)	Normal	EC792195	97.90	EC792218	78.90	89.20	12.95
	Late	EC792195	88.20	EC792218	68.10	79.30	
Number of spikelet's per panicle	Normal	EC792215	183.95	EC792214	61.40	110.50	122.55
	Late	EC792215	135.08	EC792186	55.30	87.40	79.78
Chaffynessb (%)	Normal	ES-18	68.71	EC792238	6.96	19.52	61.75
	Late	MTU-1010	47.08	EC792238	6.07	31.26	41.01
Grain yield (kg/ha)	Normal	EC792239	6,334	MTU-1010	2,557	3,671	14.94
	Late	EC792239	5,713	ES-18	1,553	3,123	

Table 3: Performance of Rice genotypes for important seed quality traits like Protein, Amylose and Moisture content under normal and late sown condition during *summer* season 2014-2015.

Genotypes	Protein			Amylose		
	Normal	Late	Mean	Normal	Late	Mean
EC792216	8.33	8.38	8.36	89.70	96.50	93.10
EC792231	7.91	8.33	8.12	88.35	112.45	100.40
EC792227	7.33	8.21	7.77	92.81	96.31	94.56
EC792177	7.81	7.99	7.90	94.96	98.06	96.51
EC792226	7.39	7.48	7.44	81.97	93.87	87.92
EC792200	7.19	7.73	7.46	89.40	122.30	105.85
EC792224	7.48	7.91	7.70	84.55	81.65	83.10
EC792239	7.81	7.49	7.65	102.61	98.61	100.61
EC792194	7.71	8.08	7.90	86.36	90.86	88.61

EC792236	7.56	11.23	9.40	85.57	75.47	80.52
EC792210	8.11	8.01	8.06	102.00	94.60	98.30
EC792237	7.72	7.69	7.71	87.15	100.25	93.70
EC792267	7.90	7.88	7.89	92.91	91.91	92.41
EC792206	8.81	8.73	8.77	99.26	114.66	106.96
EC792234	7.21	7.31	7.26	87.07	99.27	93.17
EC792201	7.29	7.19	7.24	86.50	92.60	89.55
EC792257	7.79	7.28	7.54	43.75	92.55	68.15
EC792310	8.68	8.63	8.66	91.51	55.11	73.31
EC792199	8.19	8.31	8.25	85.96	99.96	92.96
EC792288	7.61	7.19	7.40	74.07	82.10	78.09
EC792187	7.60	7.38	7.49	88.50	95.10	91.80
EC792205	9.29	9.03	9.16	98.95	111.55	105.25
EC792215	7.99	7.81	7.90	89.91	98.91	94.41
EC792193	7.50	8.19	7.85	82.46	96.26	89.36
EC792185	7.90	7.38	7.64	78.87	110.67	94.77
EC792222	8.00	7.33	7.67	98.90	92.30	95.60
EC792240	9.02	8.21	8.62	100.05	113.55	106.80
EC792195	7.59	7.99	7.79	90.41	97.21	93.81
EC792235	8.01	7.68	7.85	65.76	103.06	84.41
EC792225	7.29	6.93	7.11	82.57	92.57	87.57
EC792316	7.90	8.01	7.96	69.40	78.90	74.15
EC792204	7.71	7.69	7.70	103.05	88.45	95.75
EC792238	8.89	9.68	9.29	105.01	95.11	100.06
EC792233	7.88	8.33	8.11	86.66	88.86	87.76
EC792179	7.70	7.61	7.66	93.87	83.67	88.77
EC792208	8.41	7.99	8.20	77.00	82.50	79.75
EC792219	7.50	7.58	7.54	90.95	85.85	88.40
EC792309	8.30	7.93	8.12	87.91	95.81	91.86
EC792203	8.00	8.01	8.01	88.56	99.56	94.06
EC792214	7.31	7.89	7.60	85.57	90.57	88.07
EC792176	7.31	7.48	7.40	97.60	108.90	103.25
EC792284	7.41	7.63	7.52	86.65	96.25	91.45
EC792218	7.69	7.61	7.65	120.71	125.31	123.01
EC792186	7.91	7.49	7.70	75.26	96.46	85.86
EC792270	8.91	8.08	8.50	101.67	103.67	102.67
EC792286	7.01	7.53	7.27	84.30	94.30	89.30
EC792289	7.50	7.61	7.56	85.65	78.25	81.95
EC792326	7.59	7.79	7.69	86.51	89.01	87.76
EC792217	8.49	8.08	8.29	92.76	103.46	98.11
EC792192	7.80	7.73	7.77	89.77	87.27	88.52
Gangavati sona	7.60	8.51	8.06	89.30	117.90	103.60
MTU-1010	7.70	7.79	7.75	96.05	88.45	92.25
IR-64	7.59	7.78	7.69	99.41	95.21	97.31
N-22	7.39	7.83	7.61	88.66	96.06	92.36
ES-18	8.31	7.61	7.96	101.37	56.87	79.12
Mean	7.85	7.93		89.21	95.03	
	S Em±	C.D. (5%)		S Em±	C.D. (5%)	
Dates	0.022	0.061		0.004	0.011	
Genotypes	0.011	0.030		0.021	0.058	
Date × Genotype	0.016	0.044		0.030	0.083	

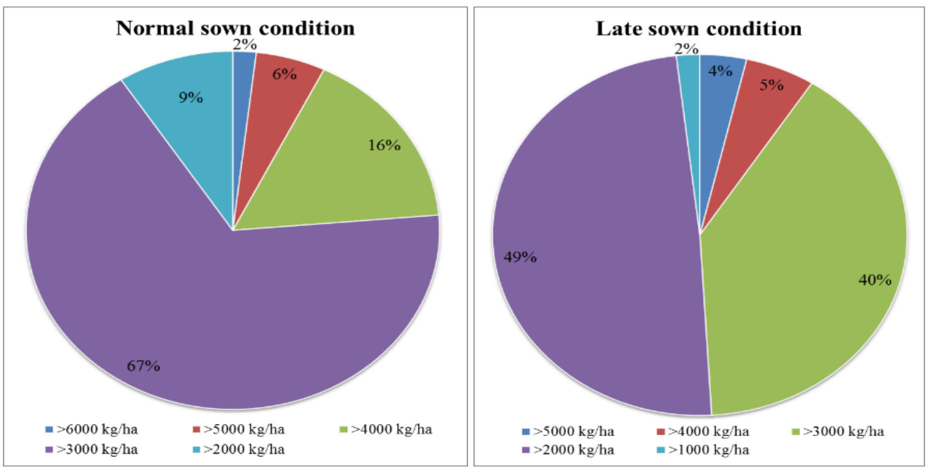

Fig 2: The per cent performance of rice genotypes for yield under normal and late sown condition during 2014-2015.

Conclusion

Rice is the one of the important crop cultivating both during *kharif* and summer in Tungabhadra project (TBP) command area. Due to late sowing of *kharif* crop and late onset of monsoon harvest will be delayed and summer sowing is also delayed. Late sowing of rice in summer (February) lead to reduction the rice yield. Due to variation in sowing period, the late sown crop was exposed to higher temperature by 2.88 ºC compared to normal sown conditions. There was not much difference in the other meteorological events, like rain fall received and the relative humidity between the two temperature regimes. In all the cases, differences between the sowing dates and genotypes were observed in the present investigation. Both yield and quality traits were shows significant differences for genotypes, sowing conditions and their interaction. Among the sowing conditions, late sown (heat stress) having elevated temperature shows the decreased values for all yield traits of rice genotypes as compared to the normal sown condition.

Genotypic variability was observed for biochemical traits and yield and its attributes for heat stress tolerance under normal and late sown conditions. Among the normal and late sown conditions, normal sown crops perform better for various morphological, physiological and yield attributes compared to late sown crops due to high temperature stress. Among the genotypes EC792239, EC792185, EC792179, EC792240 and EC792316 were responded better to heat stress in terms of morphological, physiological and yield parameters under both normal and late sown conditions. Thus the genotypes indicating the high temperature tolerance. The highlight of the results obtained and inferences drawn from the different aspects of the study have given rise to some directions for future line of work.

The identified heat stress tolerant genotypes *viz.*, EC792239, EC792185, EC792179, EC792240 and EC792316 could be used as potential donors for development of heat stress tolerant variety. The genetic nature of heat stress tolerance could be studied by utilizing the identified heat stress susceptible and heat stress tolerant genotypes. Further specific molecular markers associated with heat stress tolerance could be identified using identified variability for marker assisted selection. The identified heat stress tolerant genotypes *viz.*, EC792239, EC792185, EC792179, EC792240 and EC792316 could be used for further breeding purpose or directly used for release as a variety to formers for late sown cultivation. The identified suitable heat tolerant donors may be used for development high yielding heat tolerant varieties.

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