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Evaluation of rice germplasm and introgression lines for heat tolerance

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ABSTRACT

Forty rice genotypes including 23 introgression lines (IL) derived from BC_2F_6 of Swarna × O nivara and KMR3 × O rufipogon and 3 mutants of N22 were evaluated for heat tolerance at germination, seedling and early vegetative stage of development under different temperature treatments. Swarna × O. nivara ILs 166-2, 175-2, 3-1K, KMR3 × O. rufipogon ILs 377-13, 50, 117, 13-7 and three IET hybrids 21528, 20907 and 20114 showed higher percent germination, mean shoot and root length, dry weight, chlorophyll, carotenoids and leaf senescence index compared to the tolerant check Nagina 22 at high temperature. At 40°C, two deep water rice varieties, Jalmagna and Madhukar and IL117 showed highest values for the first four traits. The two N22 mutants NH686 and NH787 had the least shoot and root length at 40°C. The effect of heat on carotenoid content was highly significant ($p<0.05$). Highly significant variations were observed between the genotypes for all other traits

Keywords: *Oryza rufipogon*, *Oryza nivara*, germination, leaf susceptibility index, heat tolerance index, chlorophyll stability index

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INTRODUCTION

Rice is one of the top five major cereals, and it is a major staple food which supports more than three billion people, and represents 50 to 80% of their daily calorie intake [1]. Even though rice is grown in tropical areas, rice is reported to be sensitive to high temperature [2-5].

High temperature is a stress factor that has a strong impact on the survival, growth, reproduction and distribution of plants [6]. Plants are characterized by a certain range of tolerance to high temperature, which reduces metabolic activity. The heat effect is manifested by physiological perturbations [2-5]. For example, any constraint in photosynthesis can limit plant growth at high temperatures. Photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of injury at high temperature. High temperature also caused significant decline in shoot dry mass, relative growth rate, net assimilation rate and thus final yield in many cereal crops [7]. Increased nighttime temperature has been reported to cause yield decline in rice [3].

There are fewer reports on effect of high temperature on germination, and vegetative stage of rice seedlings [8] than at reproductive stage of development [9, 10]. Keeping this in view, we analyzed twenty three rice introgression lines (ILs) and 3 parents of these ILs and 14 other rice genotypes for heat tolerance at germination, seedling and vegetative stage, in laboratory at different temperatures.

MATERIALS AND METHODS

2.1. Plant Materials & Sterilization

Forty accessions used in this study included 6 germplasm lines, 23 introgression lines (ILs) with parents, 3 mutants of Nagina 22 and 8 advanced breeding lines (Rice lines which are in Initial Evaluation Trials of All India Coordinated Rice Improvement Program at DRR). Of the 23 introgression lines, 11 lines are Swarna × *O. nivara* introgression lines and 12 lines are KMR3 × *O. rufipogon* (ILs) (Table-1). Seeds collected from the farm of Directorate of Rice Research were washed under running tap water and treated with 0.01% sodium hypochlorite (NaOCl) for two minutes and washed thrice in sterile distilled water.

2.2. Experimental Design

Ten seeds from each accession were placed in petri dishes containing germination paper moistened with sterile distilled water and incubated at four different temperatures (25°C, 30°C, 35°C, 40°C) at dark and each was replicated thrice.

Data on percentage of germination was tested initially at five days after incubation. Shoot and root length was measured 5 days after germination and subsequent readings were taken at regular interval of two days till 11 days after germination. 7 days old seedlings were transferred to disposable glasses supplemented with sterilized full strength Hoagland's medium (pH 5.8), and maintained at the four temperatures with 3000lux of cool white fluorescent light for 16 h/day.

Fifteen days old seedlings from disposable glasses were then transplanted into pots containing sterile soil and shifted to the glasshouse, and maintained in ambient conditions (25 ± 2 ^{0}C). For chlorophyll estimation, each 30 days old plantlet grown under 25 ±2°C temperature was transferred into small pots and incubated at different temperature (25, 30, 35 and 40°C) with 3000lux of cool white fluorescent light for 16h/day. Three days after incubation 100 mg leaf tissue was collected from individual plantlets and cut into small pieces and soaked in 80% acetone to extract pigments. The absorption of the extracts at wavelengths of 440nm (D_{440}), 645nm (D_{645}) and 663nm (D_{663}) was measured using spectrophotometer. The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll and carotenoids (Car) was calculated using the equations as follow [11]. The chlorophyll stability index (CSI) was determined according to Aghaee et al., [6] and ratio of carotenoids was measured

Ratio XK = Carotenoids at 40°C/ Carotenoids at 30°C

Dark induced senescence was measured using second leaf of 45 days old plants in three replicates. Initial SPAD values were taken and cut leaves were kept in long test tubes containing water and incubated at 6 days in dark. Final SPAD values were taken at 30 and 40°C in dark. Final SPAD value was compared with initial SPAD value at two different temperatures (30 and 40°C) and expressed in terms of Leaf Senescence Index (LSI).

LSI = Initial SPAD value – 6 days SPAD value / Initial SPAD value × 100
LSID = LSI 40°C - LSI 30°C

All the experiments were carried out in a complete randomized block design with three replications.

4.3. Statistical Analysis

ANOVA and correlation coefficients were determined following Snedecor [12] and using SAS software.

RESULTS

The percentage germination varied at each temperature and maximum response was observed at 30°C. Similarly other four characters, shoot and root length, pigment content (Chl a, b, total chlorophyll and carotenoids) and leaf senescence showed higher response at 30°C treatment compared to other temperature treatments. Hence 30°C treatment was considered as optimum for further comparative analysis.

2.1. Germination percentage:

Seeds incubated at different temperatures germinated 24h after incubation and all seeds germinated by 5th day at 25°C and 30°C but germination was delayed up to 9 days at 35°C and 40°C in few genotypes. Six ILs, 248, 50, 230, 3-1K, 463 and 7 showed more than 95 % germination in five days of incubation at 40°C. Three ILs, 478, 458 and 463 showed 100 percent germination in five days of incubation at 30°C, 35°C and 40°C (Table 1).

Percentage of germination at 30°C ranged from 47% in 14-3 to 100 % in eight lines but at 40°C it ranged from 27% in 20935 to 100% in four ILs 248, 463, 458 and 478 (Table-1). Among the forty genotypes, only IL 175-2, 248, and S-50 showed 4-8% increase in germination at 40°C compared to 30°C. Eleven other genotypes including seven ILs 7, 166-2, 463, 90-5, 13-5, 458, 478, Swarna, IET- 20907, 20893 and 20926 showed the same germination at 40°C as at 30°C. All other genotypes showed significant decrease in germination percentage with increasing temperature. IET20935 showed maximum (50%) reduction in germination at 40°C compared to 30°C (Table 2).

2.2. Shoot Length

Highly significant differences in shoot length at 11 days after germination were observed between the genotypes and temperature treatments based on two way ANOVA (Table 3). At 11 days after germination, mean shoot length ranged from 6.7 in IL175-2 to 11.1 in IL90-5 at 30°C and from 1.7 in NH 787 to 8.7 in Madhukar at 40°C. There was considerable reduction in mean shoot length at 35°C and 40°C compared to 30°C. The highest shoot length (8.7 cm) was observed in Madhukar at 40°C. Jalmagna, a deep water rice and N22, a heat tolerant variety had shoot length of 8.3cm and 7.9 cm respectively at 40°C (Table 1).

In terms of shoot length four ILs 50, 463, 166-2, and 175-2 and Madhukar, Jalmagana and IET20907 showed more heat tolerance compared to the well known heat tolerant line N22. IET20907 showed the lowest reduction of only 10% in shoot length from 30°C to 40°C. On the other hand, Jalmagna and Madhukar showed 2 to 6 % increase in shoot length from 30°C to 40°C (Table 2). The minimum shoot length of 1.7 and 1.9 cm was observed in NH787 and NH686 respectively. These lines also showed the maximum reduction of 83% and 81% respectively in shoot length at 40°C compared to 30°C (Table 2).

2.3. Root Length

ANOVA among the genotypes and effect of four temperatures on root length showed highly significant differences between the genotypes and temperature treatments (Table 3).The mean root length at 11 days after germination ranged from 6.3cm in NH219 to 11.4cm in IL-230 at 30°C. It was more than 10cm in 3 lines IL230, IL198 and IET 20915 at 30°C. The minimum root length was 0.8 cm in NH686 followed by 1.4 cm in NH787 at 40°C (Table 1). The highest root length of 8.2 cm was observed in Jalmagna followed by 8.0 cm in 198 at 40°C.

Ten lines including eight ILs 3-1K, 230, 50, 166-2, 463, 117, 13-7, 198, and Jalmagna and IET20907 showed higher root length at 40°C compared to the heat tolerant line N22. Madhukar and Swarna IL-7 showed equal root length of 6.5cm as in N22. N22 showed 29 percent reduction in root length at 40°C compared to 30°C. Fourteen out of forty lines including ten ILs, 117, 3-1K, 463, 166-2, 377-13,175-2, 198, 3-1S, 248 and 13-7 and Jalmagna, Madhukar, IET20907 and IET20893 showed less than 29% reduction in root length at 40°C compared to 30°C. Jalmagna and ILs 117 and 3-1K showed less than 10% reduction in mean root length from 30°C to 40°C. The highest reduction of 87% and 83% was observed in N22 mutants NH 686 and NH 787 respectively (Table 2).

2.4. Dry weight

Dry weight of 11 days old seedlings at 30°C ranged from 0.3mg in 3-1S and IET20935 to a maximum of 1.2mg in Madhukar and at 40°C, it ranged from 0.2mg in 3-1S to 1.9mg in 20893 followed by 1.5 mg in Madhukar (Table 1). Ten lines IET20893, IET20114 IL248, IET21510, IL478, IET 21528, IL175-2, Jalmagna, 166-2 and Madhukar showed more than 25% increase in dry weight at 40°C (Table 2). Only seven lines IL13-7, 458, 7, 3-1S, Swarna, NH 787 and 20935 showed reduction in dry weight at 40°C. The dry weight of seven lines 16-3, 230, NH219, KMR3, NH686, 117 and 75 was the same at 30°C and 40°C. . The other lines showed increase at 40°C compared 30°C. Percent increase in dry weight at 40°C varied from 6% in 13-5 to 160% in 20114.

2.5. Chlorophyll and Carotenoids

The chlorophyll content of the leaves was significantly influenced by high temperature stress and percent reduction of chlorophyll and carotene content at 40°C in each line varied significantly depending on genotype which is also supported by ANOVA (Table 3).

Five ILs 117, 142, 14, 3-1K, 458 and two IET lines 20935 and 21528 showed highest total chlorophyll (50µg/g fresh weight) at 30°C. Of these, IL 117 showed maximum 56.2µg/g total chlorophyll and 20.1µg/g chlorophyll b. 142 showed 54µg/g total chlorophyll and 39.3µg/g Chl a. The quantity of total chlorophyll, Chl a, Chl b reduced with increase in temperature. Total chlorophyll content at 40°C ranged from 20µg/g in Swarna to 50µg/g in stay green line IL142 and IET 21528 (Table 1).

At 30°C lowest total chlorophyll 28.9µg/g was observed in N22, whereas at 40°C lowest values were from 20µg/g to 25µg/g in Swarna, Krishnahamsa, NH787, Madhukar and KMR3 in increasing order. At 40°C known heat tolerant variety N22 showed 26µg/g total chlorophyll which is lower than that of all 23 ILs studied and also of N22

mutant NH686. NH686 showed 6% more chlorophyll compared to N22 at 40°C. Fifteen out of forty lines including eleven introgression lines 142, 166-2, 14, 3-1K, 117, 458, 377-13, 175-2, 50, 7, 13-7 and four IET lines 21528, 20114, 20907 and 21510 showed ≥40µg/g total chlorophyll and 10µg/g carotenoids at 40°C.

At 30°C maximum leaf carotenoids (12µg/g) were observed in Krishnahamsa but were reduced to 9.3µg/g at 40°C. All other lines showed less than 1µg/g carotenoids at 30°C but it increased almost ten times at 40°C in leaf of all accessions. Maximum quantity of carotenoids was observed in 21528 followed by 142 (Table 1).

The ratio of carotenoids at 40°C to 30°C was highest in 3-1K (65) and lowest ratio was observed in Krishnahamsa (0.8). 25-57 fold increase was observed at 40°C in 11 lines, 3-1S, 21528, 20114, 13-7, 458, 175-2, 14, 142S, 20907, 377-13 and 20935 compared to 30°C. Seven other lines 7, 75S, 463, Madhukar, 50, 230 and 166-2 showed 20-25 fold increase in carotenoids at 40°C. and it was less than 10 fold in 8 lines NH686, Swarna, N22, 467, 90-5, KMR3, 13-5 and Krishnahamsa.

Table 1. Response of rice genotypes at germination, seedling and early vegetative stage at 30˚C and 40˚C

S. No	Genotypes	Ger %		Shoot Length		Root Length		Dry Weight		Xa		Xb		Xt		CSI	XK		Ratio of XK	LSI		LSID
		30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C	30°C	40°C		30°C	40°C		30°C	40°C	
Swarna -*O nivara* ILs																						
1	Swarna	93	93	9.6	6.0	9.4	5.2	0.9	0.7	17	14	15	6	32	20	61	0.7	5	7.9	18.4	41.0	22.7
2	248	93	100	8.6	5.9	8.5	6.2	0.4	0.7	22	21	13	9	33	30	91	0.4	8	19.4	16.1	56.3	40.2
3	3-1K	97	93	8.6	5.4	8.2	7.4	0.9	1.1	37	34	13	13	50	48	95	0.2	13	64.5	14.2	14.3	0.1
4	3-1S	77	63	7.2	4.4	6.7	4.9	0.3	0.2	23	19	10	9	33	28	84	0.1	7	56.5	12.7	40.1	27.4
5	230	100	93	10.0	6.1	11.4	6.7	0.7	0.7	35	26	10	10	46	37	81	0.4	9	21.9	9.0	13.9	4.9
6	142	93	83	8.5	5.6	8.5	4.8	0.6	0.7	39	36	15	14	54	50	92	0.5	13	28.4	4.4	22.8	18.4
7	14	93	80	8.4	5.0	8.5	5.5	0.7	0.8	36	34	14	14	50	48	95	0.5	13	28.6	4.9	11.2	6.3
8	14-3	47	40	8.4	4.4	8.9	4.7	0.4	0.5	27	22	13	9	39	31	79	0.5	9	17.0	8.4	10.4	2.0
9	7	93	93	7.7	5.2	9.6	6.5	0.7	0.6	32	31	13	13	45	44	96	0.5	12	24.3	14.5	42.5	28.0
10	166-2	97	97	7.5	5.5	8.6	7.2	0.6	0.7	34	31	12	10	46	42	91	0.5	10	21.5	5.9	2.6	-3.2
11	175-2	87	93	6.7	4.9	7.8	6.2	0.5	0.6	38	31	7	12	44	44	98	0.3	12	38.0	10.6	17.9	7.3
12	75	93	87	8.5	4.3	8.2	5.3	0.7	0.7	28	25	9	10	38	35	92	0.4	9	23.2	9.5	68.5	58.9
N22 - Mutants																						
13	N22	97	93	10.9	7.9	9.1	6.5	0.8	0.9	25	18	4	7	29	26	89	0.9	7	7.8	13.9	75.1	61.2
14	NH 787	100	87	10.1	1.7	8.5	1.4	0.4	0.3	30	17	12	7	42	24	57	0.5	7	14.7	21.8	62.3	40.5
15	NH 686	97	80	9.6	1.9	6.4	0.8	0.7	0.7	27	22	11	10	38	32	85	0.9	8	8.9	15.2	51.1	35.9
16	NH 219	100	80	9.8	3.0	6.3	2.4	0.7	0.7	24	23	10	9	35	32	93	0.5	9	18.4	28.4	56.3	27.9
KMR3 -*O rufipogon* ILs																						
17	KMR3	100	97	10.7	6.8	8.9	5.9	0.9	0.9	25	18	11	7	36	25	69	0.9	7	7.5	4.0	53.6	49.5
18	463	100	100	8.8	6.7	8.2	6.8	0.8	0.9	27	26	11	10	38	36	95	0.4	9	23.1	4.9	80.8	75.9
19	50	87	90	8.5	6.8	8.8	6.2	0.7	0.8	30	28	14	13	43	41	81	0.5	12	22.2	18.5	25.4	6.9
20	117	100	83	9.9	6.1	7.3	6.8	0.6	0.6	36	34	20	13	56	47	84	1.0	12	12.4	14.1	43.6	29.4
21	377-13	80	67	10.2	5.7	6.9	5.6	0.9	0.9	38	33	11	13	49	46	94	0.5	12	26.2	5.2	6.9	1.7
22	13-7	97	93	10.8	5.9	9.7	6.9	1.0	0.9	30	28	13	12	43	40	90	0.2	10	42.7	5.5	8.0	2.6
23	16-3	63	37	9.3	5.1	8.3	5.5	0.4	0.4	32	27	14	10	46	37	80	0.8	10	13.2	2.2	70.3	68.1
24	90-5	97	97	11.1	5.3	8.7	5.3	0.9	1.1	37	26	12	9	49	35	87	1.5	11	7.5	4.5	51.8	47.3
25	198	90	93	10.8	6.8	10.8	8.0	0.9	0.9	-	-	-	-	-	-	-	-	-		-	-	-
26	13-5	93	93	9.4	5.9	8.9	5.7	0.9	1.0	27	26	12	11	39	37	99	1.4	10	7.2	3.3	16.9	13.6
27	458	100	100	10.8	2.8	9.5	2.6	0.9	0.8	38	34	13	13	50	47	93	0.3	12	39.7	3.2	34.5	31.3
28	467	90	87	9.0	3.1	7.9	2.3	0.8	1.0	26	25	11	10	37	35	95	1.2	9	7.8	18.9	71.2	52.3
29	478	100	100	9.3	6.7	7.4	3.4	0.5	0.7	28	24	8	8	37	33	90	0.5	9	16.8	11.6	50.5	38.9
Germplasm lines																						
30	Jalmagna	100	97	8.2	8.3	8.7	8.2	0.7	0.9	25	19	11	8	36	27	75	0.7	7	10.3	12.2	68.5	56.4
31	Krishnahamsa	87	70	10.7	5.9	8.7	5.5	0.8	0.9	30	14	13	8	42	22	40	11.7	9	0.8	12.4	59.7	47.3
32	Madhukar	97	93	8.2	8.7	8.0	6.5	1.2	1.5	21	16	9	8	30	24	82	0.3	6	22.2	42.5	50.2	7.7
IET lines																						
33	20114	93	73	8.1	5.7	9.0	4.4	0.5	1.3	37	33	13	13	49	47	95	0.3	13	45.4	6.0	5.2	-0.8
34	20935	53	27	8.9	4.0	8.4	3.8	0.3	0.3	36	20	15	9	50	29	58	0.3	9	25.3	5.3	13.4	8.0
35	21528	97	73	7.3	5.2	7.9	5.0	0.8	1.1	36	35	14	14	50	50	98	0.2	13	56.3	7.6	13.3	5.8
36	20893	67	67	8.5	4.9	7.3	5.3	0.8	1.9	27	20	11	10	38	30	90	0.7	12	17.3	20.7	34.6	13.9
37	21510	83	57	9.6	2.8	9.0	2.5	0.9	1.3	36	31	13	14	48	45	93	1.0	12	12.1	14.1	20.2	6.1
38	20907	87	87	7.6	6.9	9.3	7.2	0.7	0.8	35	30	14	10	49	40	87	0.4	12	27.5	39.0	40.6	1.5
39	20915	77	50	10.0	6.3	10.1	4.0	1.1	1.3	26	20	10	9	36	29	81	0.5	8	16.2	25.3	83.5	58.1
40	20926	97	97	9.8	6.5	8.0	3.0	0.5	0.6	25	21	11	9	36	30	83	0.6	8	12.6	35.7	52.0	16.3

Ger% - Germination Percentage; Xa - Chlorophyll A; Xb – Chlorophyll B; Xt – Total chlorophyll; CSI-Chlorophyll Stability Index; LSI – Leaf Senescence Index (Initial SPAD value – 6 days SPAD value / Initial SPAD value × 100); LSID – (LSI 40°C - LSI 30°C); IET lines – Rice lines which are in Initial Evaluation Trials of All India Coordinated Rice Improvement Program at DRR

Table 2. Heat tolerance indices

S.No	Genoypes	Ger %	S. L.	R. L.	D. Wt.	Xa	Xb	Xt
Swarna -*O nivara* ILs								
1	Swarna	0.0	37.4	44.6	27.8	19.1	61.5	38.9
2	248	-7.1	31.3	27.1	-75.0	3.3	27.0	9.3
3	3-1K	3.4	37.2	9.3	-22.2	7.6	1.2	4.5
4	3-1S	17.4	39.2	26.2	20.0	17.2	11.9	15.6
5	230	6.7	38.6	41.4	0.0	25.3	2.8	18.6
6	142	10.7	33.5	43.9	-16.7	9.6	2.1	7.6
7	14	14.3	39.8	35.2	-15.4	5.5	2.2	4.6
8	14-3	14.3	47.7	47.4	-12.5	17.5	27.3	20.6
9	7	0.0	32.6	32.3	14.3	4.6	1.7	3.8
10	166-2	0.0	25.9	16.3	-27.3	7.3	15.2	9.3
11	175-2	-7.7	26.6	20.1	-33.3	17.8	-91.9	1.9
12	75	7.1	49.4	35.4	0.0	12.8	-6.5	7.9
N22 - Mutants								
13	N22	3.4	27.6	28.6	-13.3	27.2	-91.1	11.3
14	NH 787	13.3	83.2	83.0	28.6	44.3	41.0	43.3
15	NH 686	17.2	80.7	86.8	0.0	17.3	11.0	15.4
16	NH 219	20.0	68.8	61.5	0.0	4.7	11.8	6.8
KMR3 -*O rufipogon* ILs								
17	KMR3	3.3	36.8	34.0	0.0	29.7	33.8	30.9
18	463	0.0	23.7	17.1	-6.3	4.6	5.9	5.0
19	50	-3.8	19.9	28.9	-7.1	5.7	4.1	5.2
20	117	16.7	38.7	7.3	0.0	6.0	33.9	16.0
21	377-13	16.7	44.1	18.5	-5.9	13.8	-21.0	6.3
22	13-7	3.4	45.5	28.5	5.3	6.0	3.8	5.3
23	16-3	42.1	45.9	33.5	0.0	17.0	27.0	20.1
24	90-5	0.0	52.1	39.2	-22.2	29.6	27.1	29.0
25	198	-3.7	37.0	25.8	-5.9	-	-	-
26	13-5	0.0	37.2	36.4	-5.6	2.1	11.4	4.2
27	458	0.0	74.5	72.3	11.1	10.2	0.0	6.6
28	467	3.7	65.5	70.7	-18.8	2.1	11.4	4.9
29	478	0.0	28.5	53.5	-40.0	16.4	4.7	10.2
Germplasm lines								
30	Jalmagna	3.3	-1.5	5.9	-28.6	23.7	26.5	24.5
31	Krishnahamsa	19.2	45.3	36.8	-6.3	53.0	40.9	48.2
32	Madhukar	3.4	-5.6	18.8	-26.1	22.1	10.1	18.5
IET lines								
33	20114	21.4	30.1	51.2	-160.0	9.1	-5.4	5.4
34	20935	50.0	54.7	55.0	28.6	43.0	38.8	41.8
35	21528	24.1	28.2	36.9	-40.0	2.7	0.0	1.5
36	20893	0.0	42.2	27.1	-131.3	25.9	10.2	21.3
37	21510	32.0	70.8	72.2	-52.9	11.8	-8.3	6.5
38	20907	0.0	9.7	21.8	-14.3	14.3	27.0	18.0
39	20915	34.8	37.0	60.5	-18.2	22.6	10.4	19.2
40	20926	0.0	33.8	62.0	-20.0	15.9	20.4	17.3

Ger % - Germination Percentage; S.L. – Shoot Length; R.L. – Root Length; D. Wt. – Dry Weight; Xa – Chlorophyll A; Xb – Chlorophyll B; Xt – Total Chlorophyll

Table 3. Correlation coefficients between traits at 30°C and 40°C

	Ger% 30°C	Ger% 40°C	S.L. 30°C	S.L. 40°C	R.L.30°C	R.L. 40°C	D.Wt. 30°C	D.Wt. 40°C	Xa 30°C	Xa 40°C	Xb 30°C	Xb 40°C	Xt 30°C	Xt 40°C	CSI	XK 30°C	XK 40°C
Ger %3 0°C	1.00																
Ger % 40°C	0.87**	1.00															
S.L. 30°C	0.19	0.09	1.00														
S.L. 40°C	0.18	0.32*	-0.03	1.00													
R.L. 30°C	0.05	0.14	0.27	0.31*	1.00												
R.L. 40°C	0.09	0.29	-0.20	0.76**	0.36*	1.00											
D.Wt. 30°C	0.33*	0.31*	0.38*	0.31*	0.31*	0.21	1.00										
D.Wt. 40°C	0.11	0.11	0.06	0.28	0.11	0.15	0.71**	1.00									
Xa 30°C	-0.02	-0.17	-0.22	-0.25	-0.14	-0.10	-0.15	-0.02	1.00								
Xa 40°C	0.12	0.00	(-0.35)	-0.19	-0.17	-0.02	-0.05	0.05	0.87**	1.00							
Xb 30°C	-0.09	-0.24	-0.15	-0.21	-0.15	-0.11	-0.14	-0.14	0.58**	0.56**	1.00						
Xb 40°C	0.03	-0.10	(-0.37)	-0.23	-0.21	-0.07	-0.03	0.12	0.85**	0.95**	0.59**	1.00					
Xt 30°C	-0.05	-0.21	-0.22	-0.26	-0.16	-0.12	-0.16	-0.06	0.96**	0.86**	0.78**	0.85**	1.00				
Xt 40°C	0.10	-0.02	(-0.36)	-0.20	-0.18	-0.03	-0.05	0.07	0.88**	0.99**	0.57**	0.97**	0.86**	1.00			
CSI	0.15	0.11	(-0.39)	-0.12	(-0.35)	-0.12	0.01	0.18	0.62**	0.79**	0.33*	0.78**	0.58**	0.79**	1.00		
XK 30°C	-0.05	-0.09	0.25	0.06	0.03	0.02	0.11	0.05	0.03	-0.20	0.11	-0.12	0.06	-0.18	(-0.32)	1.00	
XK 40°C	-0.01	-0.10	-0.29	-0.21	-0.19	-0.05	-0.01	0.20	0.90**	0.92**	0.60**	0.93**	0.89**	0.94**	0.71**	0.03	1.00

Note: *=significant at 5%; **=Significant at 1% (Highly significant); ( ) = Significant negative correlation coefficients;

Ger % - Germination Percentage; S.L. – Shoot Length; R.L. – Root Length; D. Wt. – Dry Weight; Xa – Chlorophyll A; Xb – Chlorophyll B; Xt – Total Chlorophyll; CSI – Chlorophyll Stability Index; XK – Carotenoids

2.6. Dark Induced Leaf Senescence

All accessions, except 166-2,3-1K and IET20114 showed leaf senescence after 6 days of dark treatment. The reduction in SPAD from zero to 6 days varied from 2.2% in 16-3 to 42.5% in Madhukar at 30°C and it was 2.6% in 166-2 to a maximum 84% in 20915 at 40°C (Table 1).Thus 166-2 was least affected at 40°C in leaf senescence assay for 6 days.

Cut leaves of 15 lines, 166-2, IET20114, 3-1K, IET20907, 377-13, 14-3, 13-7, 230, IET20935, IET21528, IET21510, 14, 175-2, Madhukar and 50, showed almost similar SPAD values at 40°C as at 30°C. Only two lines, 166-2 and IET20114 showed higher SPAD values at 40°C compared to 30°C. Three lines, 3-1K, 20907 and 377-13 showed less than 2% reduction at 40°C. 10 lines showed 2-10% reduction from 30°C to 40°C. Seven lines 463, 16-3, 166, 467, Jalmagna, N22 and IET20915 showed more than 50% reduction in SPAD value at 40°C compared to 30°C (Table 2).

2.6. Correlation

Significant positive correlation was found between germination percent at 30°C and at 40°C, root length at 30°C and shoot length at 40°C. Root length at 40°C correlated significantly with root length at 30°C and shoot length at 40°C. Dry weight at 30°C was positively correlated with germination, shoot and root length at both temperatures and dry weight at 40°C. Chl a at 30°C correlated with Chl a at 40°C, Chl b at 30°C was correlated positively with Chl a at both temperatures. Chl b at 30°C was correlated with Chl b at 40°C. Total chlorophyll content at both temperatures was significantly correlated with both chlorophyll a and b. Carotenoids at 40°C were correlated significantly with Chl a, Chl b, and total chlorophyll at both 30°C and 40°C and with CSI (Chlorophyll stability index). Chlorophyll stability index (CSI) was significantly correlated with Chl a, Chl b, and total chlorophyll at both temperatures. (Table 3)

Significant negative correlation was observed between shoot length at 30°C and Chl a, and between Chl b and total chlorophyll at 40°C. Chlorophyll stability index (CSI) also showed significant negative correlation with shoot length and root length at 30°C (Table 3).

ANOVA revealed highly significant differences among the genotypes and temperature treatments (P<0.05). Though the differences among genotypes were not significant, the effect of heat on carotenoids content was highly significant (P<0.05; Table 4).

Table 4. Estimation of variance on genotype and temperature treatment at germination, seedling and early vegetative stages of rice development

Germination, seedling stages of rice						
	df	Ger%	Shoot length	Root length	*df*	Dry weight
Source		*MS*	*MS*	*MS*		MS
Genotypes	39	751.2**	3.6**	3.19**	39	0.12**
Temperature	3	384.1**	105.6**	85.6**	1	0.29**
Error	117	64.5	1.08	1.4	39	0.02
Early vegetative stage of rice						
	df	Xa	Xb	Xt	XK	LSI
Source		*MS*	*MS*	*MS*	*MS*	*MS*
Genotypes	39	104.04**	14.22**	183.18**	5.26	438.08*
Temperature	1	444.85**	45.23**	741.32**	1519.75**	1292.89**
Error	39	7.06	3.73	13.62	5.09	257.9

Note: *=P<0.05; **=P<0.01

Ger% - Germination Percentage; Xa – Chlorophyll A; Chlorophyll B; Xt; Total Chlorophyll; XK – Carotenoids; LSI – Leaf Senescence Index

DISCUSSION

The present study in rice germplasm revealed that seedling traits were significantly influenced by high temperature. In general, the high temperature treatment reduced germination, shoot and root length and photosynthetic pigments. The response of these lines to two temperatures 30° and 40°C was different and was genotype dependent (P<0.05). Seven lines 166-2, 175-2, 50, Jalmagna, Madhukar, IET20907 and IET 21528 showed higher germination, shoot and root length at 40°C compared to the known heat tolerant variety N22. It may be noted that the reported heat

tolerance of N22 is based on seed set and not seedling response [2]. The two N22 mutants NH686, NH787 showed the lowest response for all traits compared to N22 indicating they are loss of function mutants for heat tolerance in the stringent method of screening under continuous heat stress. It is possible that the response is different if a diurnal variation in temperature is used for screening as it will allow time for recovery from stress.

High temperature is one of the most important factors that limit photosynthetic activity [13]. In our study, chlorophyll a, b and total chlorophyll content were significantly reduced at 40°C. However, 15 lines 142, 166-2, 14, 3-1K, 117, 458, 377-13, 175-2, 50, 7 13-7, 21528, 20114, 20907 and 21510 showed consistent chlorophyll content and more than10µg/g carotenoids at 40°C. Among these, 10 lines 3-1K, 13-7, 458, 175-2, 14, 142, 377-13, 20114, 20907and 20935 showed more than 25 times increase in carotenoids at 40°C compared to 30°C. The relative high carotenoids content under high temperature is an indicator of plants adaptive response to heat stress, because carotenoids protect the light harvesting complexes (LHC) against the impact of ROS under stress conditions [14]. The increase in carotenoids content in some lines at 40°C indicates that these lines respond to heat stress by increasing the level of carotenoids thus protecting the LHC.

Cut leaves of all accessions, except IL-166-2, IL3-1K and IET20114 showed complete senescence after 6 days dark treatment. The chlorophyll degradation during dark induced senescence was more at 40°C than at 30°C. However the extent of reduction at 40°C than 30°C was limited in sixteen lines indicating that chlorophyll degradation in these lines is minimal. It may be mentioned that levels of free and conjugated polyamines, as well as arginine decarboxylase and polyamine oxidase activities, were found higher in tolerant rice cultivar N22 than that in sensitive rice cultivar IR8 under non-stressed condition. Heat stress resulted in higher levels of free and bound polyamines in the heat tolerant callus than in the heat sensitive callus. Furthermore, uncommon polyamines, norspermidine and norspermine were detected in tolerant cultivar which increased under stress, while they were not detected in sensitive IR8 cultivar under normal or stressed condition. It was concluded that under heat stress, a tolerant rice cultivar such as N22 had the capacity to maintain or increase its total pools and to shift these pools to uncommon polyamines while the sensitive cultivars do not have this capacity [15]. Whether the data on callus can be extrapolated to whole plant level is debatable.

Lines tolerant to heat at one stage of development may or may not tolerate at another stage of development but in the present study five ILs 166-2, 175-2, 377-13, 3-1K and 50, three IET lines 21528, 20907 and 20114 showed heat tolerance at germination, seedling and early vegetative stages of development. Under high temperature treatment, two deep water varieties, Jalmagna and Madhukar showed tolerance in terms of shoot length and root length at germination and seedling stage of development but both chlorophylls and carotenoids were reduced in these lines at seedling stage. It appears that the mechanisms of tolerance operating in these 2 varieties do not confer protection to the photosynthesis related pigments.

Utilization of wild species to develop abiotic stress tolerance was suggested in barley [16].In the present study, introgression lines obtained from crosses of elite lines with wild progenitors of Asian cultivated rice *O. rupfipogon* and *O. nivara* were used to screen for heat tolerance. In all, 7 ILs derived from KMR3 × *O.rufipogon*, and 4 ILs from Swarna × *O. nivara* withstand 40°C at germination and seedling stage. These lines also retained higher amount of chlorophyll and carotenoids compared to their respective recurrent parents and also the heat tolerant check N22. The tolerance of ILs to high temperature can be attributed to the introgressions from the wild species.

In this experiment highly significant positive correlation was found between closely related traits as expected. . Among the traits CSI was significantly correlated with Chl a, Chl b and total chlorophyll at both temperatures and with carotenoids only at 40°C suggesting the role of Chl a and b and specifically carotenoids under heat stress. Earlier work shows the role of carotenoids to protect cellular structures in various plant species irrespective of the stress type [7].

Significant negative correlations were observed between shoot length and chl a, chl b, total chlorophyll and carotenoid content at both temperatures. CSI was also negatively correlated with shoot length and root length at normal conditions, indicating that dwarf lines in this study seem to produce high chl a, chl b, total chlorophyll and carotenoids content than tall lines [17]. The difference in carotenoids among the genotypes was not significant at 5% level but the effect of heat on carotenoids was highly significant indicating a clear role of carotenoids in responding to heat stress.

CONCLUSION

In conclusion, Swarna × *O.nivara* ILs 166-2, 175-2, 3-1, KMR3 × *O.rufipogon* ILs 50, 377-13, 50, 13-7 and three IET lines 21528, 20907 and 20114 showed highest heat tolerance response at germination, seedling and early

vegetative stages of development. Two deep water rice varieties, Jalmagna and Madhukar and IL117 showed highest response at germination, shoot length, root length and dry weight at seedling stage of development. These lines can be considered as more heat tolerant at early seedling stage compared to N22 a known heat tolerant variety at reproductive stage. It was interesting to note that all the elite lines identified for heat tolerance at germination and seedling and vegetative stage of development are also known for their drought, salinity or submergence tolerance indicating considerable overlap of pathways and mechanisms conferring abiotic stress tolerance. The two N22 mutants NH686 and NH787 were identified as loss of function mutants for early vegetative stage heat tolerance. Lines and varieties used in the present study provide suitable experimental system to undertake detailed analysis of genetic, genomic, biochemical and molecular processes underlying heat tolerance.

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