

Heat Tolerance Screening Studies and Evaluating Salicylic Acid Efficacy against High Temperature in Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT

High temperature retards plant growth and development. Major objective of this study was to assess heat tolerant potencies of forty rice cultivars at seedling stage and check the effectiveness of salicylic acid (SA) in improving thermo-tolerance. Rice seeds were sown in plastic bowls containing sand and heat stress (45°C) was applied on 15-days old seedlings for 12 and 24 h in growth incubators. Growth related stress tolerance indices were used as screening tools. Among all, two heat sensitive (Rambir and DM 15-1) and heat tolerant (RP2 and DM1-30-15-04) cultivars were selected for SA studies. SA spray (100 mg L⁻¹) was used on 15-days old seedlings and heat stress (45°C) was imposed on 19-days old seedlings for 12 h and 24 h. Growth (root and shoot lengths and fresh and dry biomasses) and biochemical attributes (nitrate and nitrite reductase, total soluble sugars, proteins, nitrogen, potassium, phosphorous and magnesium) were measured. Heat shock reduced seedling growth, enzymatic activities and mineral contents. However, SA enhanced fresh and dry biomass, concentration of organic and inorganic solutes in all genotypes under heat stress. SA improved thermo-tolerance in RP2 and DM1-30-15-04 genotypes. These heat tolerant genotypes have capability to give better seedling growth and stand establishment.

Keywords: *Oryza sativa* L.; Seedlings; Growth; Stress tolerance indices; Soluble sugars and Protein; Mineral ions

INTRODUCTION

Rice is cultivated globally as a staple food and almost 40% of world's population depends on rice. Annual grain yield of rice must increase by 0.6 to 0.9% until to meet the growing demands by consumers [1]. According to survey 2003, total area for rice cultivation of rice is 153.8 M ha⁻¹ in the world and out of it, 132 M ha⁻¹ is present in Asia. Hence, 90% rice of world is cultivated in Asia because its environment is more suitable for rice cultivation than other parts of world. Production of rice has to increase up to 50% in order to meet food demands of increasing world population. In Pakistan, lower Punjab plains, flooded rivers and canal areas of Sindh are considered as most suitable for rice cultivation. Pakistan has become the 5th largest rice exporter country of world [2].

High temperature causes multiple changes in major plant processes which lead to reduction in crop yield. Heat stress causes reduction in yield by changing the phenomena of plant

growth and development [3]. High night temperatures commonly increase respiration rates which decline in yield by affecting its metabolisms [4-7]. Different rice growth stages have different sensitivity to increased day and night temperatures [8]. Photosynthetic rates decreased under heat stress which results decreased leaf chlorophyll and nitrogen content in rice.

A number of efforts have been made to decrease deleterious effects of high temperature. Abiotic stress tolerance capabilities can be enhanced by exogenous application of salicylic acid. Salicylic acid (SA) increases heat tolerance capacity of plants under environmental extremes [9]. Salicylic acid enhances root growth by increasing cell division in apical meristem in wheat seedlings which helped in better growth of plants under stress condition [10]. During last decade, exogenous application of plant growth regulators and antioxidant compounds to plants has been identified a solution to cope with the adverse effects of different stresses on plants [11,12]. Therefore, it is hypothesized that foliar application of salicylic acid could ameliorate the

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adverse effects of heat shock in rice varieties. Salicylic acid (SA) was very important plant growth regulator and helped to resist the abiotic stress. That is why a great interest was taken in SA because it had a protective ability under stress condition.

Objectives of this study were to screen out heat tolerant and sensitive varieties of rice at early seedling stage on the basis of growth-related stress tolerance indices. Effectiveness of salicylic acid spray in inducing thermo-tolerance was also evaluated in this study.

MATERIALS AND METHODS

Planting material and screening studies

Rice germplasm was acquired from Rice Group of Plant Breeding and Genetics Division, NIAB, Faisalabad and tested

for high temperature stress tolerance potential using agronomical and physiological indices as screening tool. For screening against high temperature, rice germplasm was sown in sand culture pots. Experiment was conducted in growth incubators and after 15 days of sowing, high temperature stress was applied by shifting the plants in another growth chamber running at $45 \pm 2^\circ\text{C}$. Heat shock was applied for 12 h and 24 h.

After that, the seedlings were harvested and root length, shoot length, fresh and dry weight was measured and fresh matter stress tolerances (FMSTI), dry matter stress tolerances (DMSTI), roots length stress tolerances (RLSTI) and shoots length stress tolerances (SLSTI) were calculated [13]. On the basis of stress tolerance indices, two of each heat sensitive (V₂₆ and V₃₇) and heat tolerant (V₃₃ and V₃₈) rice cultivars were selected for further evaluation (Figures 1 and 2).

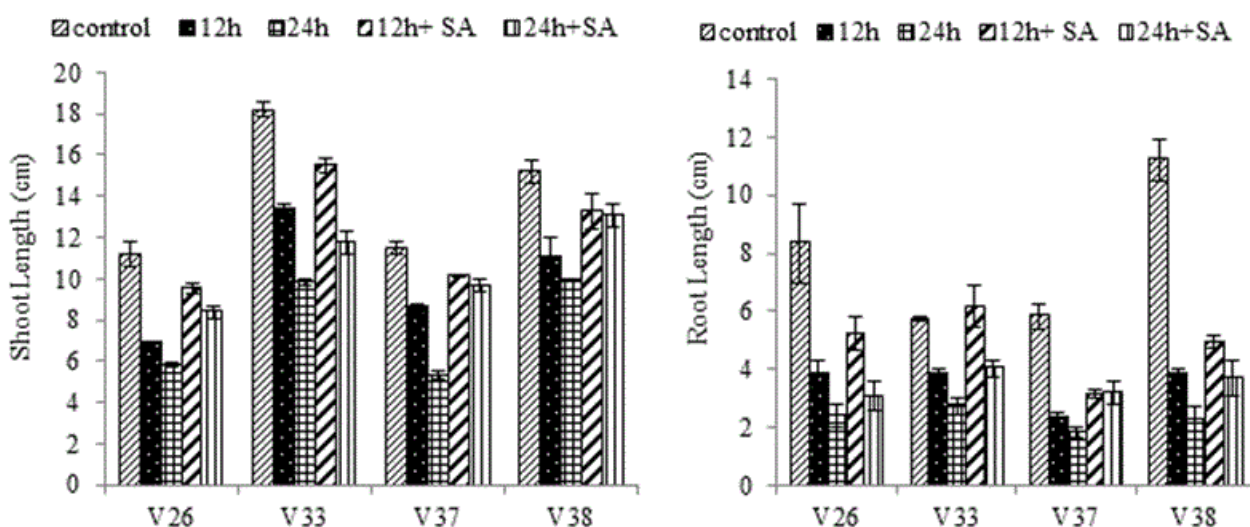


Figure 1: Effect of foliarly applied salicylic acid on shoot and root lengths of different rice cultivars under normal and high temperature stress conditions.

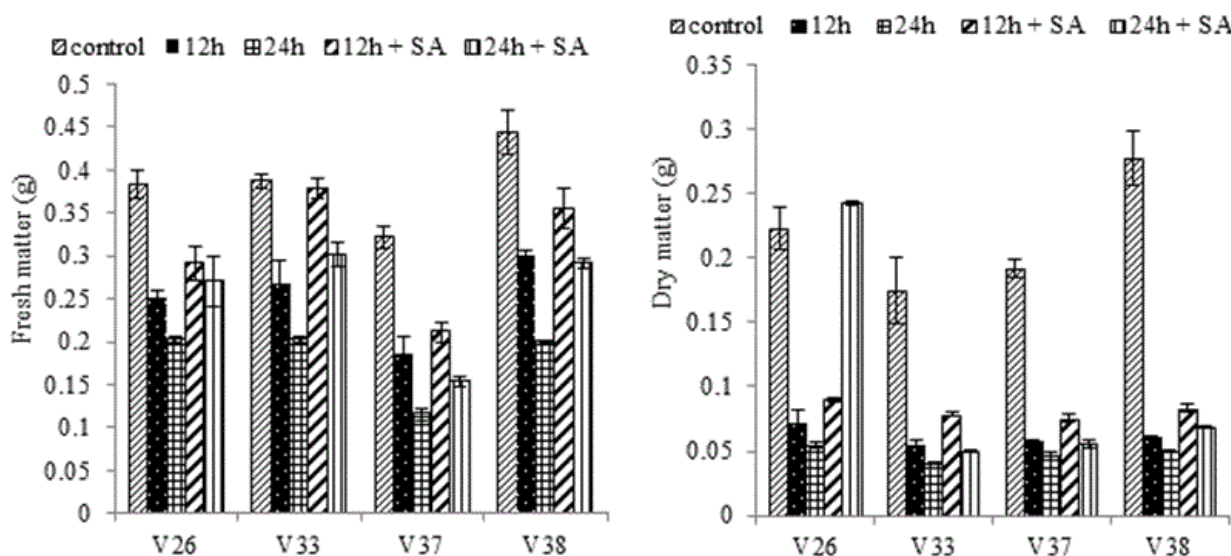


Figure 2: Effect of foliarly applied salicylic acid on fresh and dry matter of different rice cultivars under normal and high temperature stress conditions.

SA foliar spray studies on rice cultivars

To study the effects of SA in inducing heat tolerances, an experiment was conducted in pots filled with fine river sand in a complete randomized design (CRD) with three replicates and one SA treatment. SA solution of 100 mg L⁻¹ was used for foliar spray. Sprays were applied at 28 °C to reduce evaporation losses. Salicylic acid was applied on 15-days old seedlings and 19-days old seedling were subjected to heat shock treatments, i.e., 45± 2 °C for 12 h and 24 h. After that, various stress tolerance indices (STI) were measured [13]. Various biochemical traits such as total soluble sugars [14], total soluble proteins [15], nitrate reductase activity [16] nitrite reductase activity [17] and uptake of nutrients like K⁺, Mg²⁺, P and N were determined by the recommended procedures.

Statistical analysis

Both experiments were conducted in complete randomized design with three replicas. Data was collected was analyzed statistically using analysis of variance technique (ANOVA) and the LSD at 0.05% and 0.1% probability level was used to assess the differences among significant.

RESULTS

Screening of rice germplasm for heat stress tolerance Stress tolerance indices (STI)

Growth of rice seedlings were reduced under high temperatures. All genotypes behaved statistically differently under heat stress. Root and shoot lengths as well as fresh and dry biomasses of rice seedling were more decreased under the exposure of heat stress for longer time duration (24 h) than 12 h heat stress duration. Various stress tolerance indices such as root length stress tolerance indices (RLSTI), shoot length stress tolerance indices (SLSTI), fresh matter stress tolerance indices (FMSTI) and dry matter stress tolerance indices (DMSTI) showed reduction under both periods of heat stress. More the tolerance indices indicated their higher heat tolerance potentials. Heat tolerant genotypes showed greater stress tolerance indices as compared to other genotypes. RP 2 genotype showed highest fresh matter and root length stress tolerance indices under both periods of heat stress followed by DM 1-30-15-04 genotype (Table 1).

Table 1: Heat stress tolerance indices of forty rice genotypes after imposition of high temperature for 12 h and 24 h.

Rice genotypes	FMSTI		RLSTI		SLSTI		DMSTI	
	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
RP 73	48.29	5.79	91.6	43.7	87.7	31	64.1	14
EF 1-30-79	38.26	43.61	85.9	90.6	63.7	57.1	43	54.8
C 49-1-80	7.78	4.58	42.73	27.3	16.4	17.7	8.9	5.9
EF 1-20-59-04	32.22	22.26	69.3	102.1	67.1	53.8	64.8	99
EF 1-30-54-02	18.94	9.56	69.7	106.1	68.4	56.8	61.3	77.6
DM 1-20-38	24.3	30.04	93.7	90.1	52.1	39	40.9	34.1
RP 69	71.35	84.4	96.7	104.1	59.3	46.8	65.7	67.9
C 75-8-79	58.18	64.87	80.4	49.7	76.3	76.6	49.2	53.4
C 75-7-79	82.06	59.26	70.5	94.6	80.6	74.8	21.4	69.4
EF1-20-119-02	99.23	81.7	151.5	114.9	73.9	60.7	68.3	57.9
EF 1-20-52-04	83.06	44.48	96.6	84.1	52.9	56.7	67.4	67.5
Dhera Dhun	32.3	43.72	100	62.7	64.8	64.1	56.8	57.5
RP 144	45.26	61.82	111	75.4	46.6	64.6	39.7	52.3
EF 11	52.46	38.5	87.6	100	64.8	66.6	55.7	61.8
EF 4	62.46	80.01	134.9	117.4	38.8	73.7	67.3	69.5
Kashmir Basmati	56.21	71.71	82.4	64	64	72.2	50.7	21.5

EF 1-25-16	98.46	18.13	95.3	88.2	55.9	52.6	58.2	55.5
EF 1-20-5-99	77	48.3	106.5	111.4	65.5	66.2	62	62.8
EF 1-25-19	7.99	68.74	77.5	111.4	80.4	72.6	51.3	62.2
Basmati 6141	43.27	97.56	140.4	118.4	68.4	92.9	55.3	80.4
EF 1-25-3-02	86.3	88.47	65.7	83.1	73.9	61.7	44.5	33.6
EF 25-4-2	67.2	60.95	55.8	121.4	64.1	61.8	30.3	26
Kashmir Basmati(F.R.)	68.25	42.33	90.2	162.7	60.1	71.3	12.8	28
EF 6	62.83	28.52	105.7	144.6	59.3	65.4	16.5	22.7
Desi Basmati	75.11	99.67	78.3	96.1	43.5	73.8	25	33.3
Rambir (EF)	30.65	16.71	227.7	73.9	53.9	54.3	14.9	20
DM 1-20-2	77.64	15	163.3	130	59.3	68.2	25	23
DM 1-15-1	72.58	79.01	98.6	238.9	61.3	76.8	26.5	52.1
DM 1-20-3,	23.12	69.87	85.2	144.5	86.7	66	80.6	99.5
DM 107-4	97.13	71	82.9	80.41	69.1	66.7	15.7	19.2
DM 15-13-1	7.24	33.28	156	183.5	82	78.8	32.4	38.5
DM 1-20-29-4-1	91.72	77.35	117.3	93.4	71.7	71.7	24	37.4
DM 1-30-15-04	85.61	87.32	140.6	91.2	86.1	88.9	70.7	68.9
EF 76-1	57.78	24.13	95.2	108.5	70	68.6	16.7	16.2
RP 1	27.46	41.64	101.6	115.4	86.6	91.5	23.7	34.2
DM 1-20-7-99	82.69	33.28	146.8	138.3	65.8	61.2	16.8	16.3
DM 15-1	24.21	16.26	117.3	102.4	58.5	55.2	20.4	22
RP 2	89.55	99.78	83.7	69	76.3	82.3	80.7	79.3
DM 1-30-3-99	88.47	6.32	115.2	71.7	63.4	60.7	8.3	15.2
EF 1-25-32-1	37.92	21.83	127.7	115.8	61.1	61.3	12.8	20

FMSTI= Fresh matter stress tolerance index; RLSTI= Root length stress tolerance index, SLSTI= Shoot length stress tolerance index, DMSTI= Dry matter stress tolerance index

Effect of SA foliar spray in inducing heat tolerance

SA foliar spray had improved the growth of both heat tolerant and sensitive rice genotypes. The stress tolerance indices for root

and shoot length, fresh and dry matter of rice seedlings were increased under the heat stress for 12 h and 24 h durations (Table 2).

Table 2: Analysis of Variance (ANOVA) for the effects of high temperature stress and foliar application of salicylic acid on shoot length, root length, fresh matter and dry matter of different rice (*Oryza sativa* L.) cultivars.

SOV	df	Mean sum of squares
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		Fresh matter	Root length	Shoot length	Dry matter
Treatment (T)	4	0.06934***	53.0376***	32.3994***	0.00936***
Variety (V)	3	0.004476***	9.7042***	72.7925***	0.05456 ^{ns}
TXV	12	0.00111 ^{ns}	4.4598***	4.0531 ^{ns}	0.00513 ^{ns}
Error	40	8.1543	0.7612	5.8987	0.00432
Total	59				

*Significance at $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ probability level; NS, non-significance.

Analysis of Variance (ANOVA) showed that the SA and heat treatments were highly significant ($p \leq 0.001$) in terms of all growth traits while interactive effect of treatment and genotypes was found highly significant in case of seedling root length (Table 3).

Table 3: Effects of high temperature stress and foliar spray of salicylic acid on nitrate reductase activity (NRA), nitrite reductase activity (NiRA), phosphorus (P), potassium (K), total soluble sugars (TSS), total soluble protein (TSP), nitrogen (N) and magnesium (Mg) of different rice cultivars of rice under normal and high temperature stress conditions. [C=control, 12 h and 24 h=Heat stress of $45 \pm 2^\circ\text{C}$ for 12 h and 24 h, 12 h+SA and 24 h+SA=Foliar application of Salicylic acid(100 ppm) at 12 h and 24 h heat stressed seedlings].

Varieties	Treatment	NRA	NiRA	TSS	TSP	K	N	P	Mg
		$\mu \text{ mol. g}^{-1} \text{ F.W. h}^{-1}$	$\mu \text{ mol. g}^{-1} \text{ F.W. h}^{-1}$	$\text{mg g}^{-1} \text{ F.W.}$	$\text{mg g}^{-1} \text{ F.W.}$	mg D.W.	$\text{g}^{-1} \%$	mg D.W.	$\text{g}^{-1} \text{ mg D.W.}$
Rambir (V ₂₆)	C	4	4.1	6.1	6.1	4	1.7	5.9	30
	12 h	3.8	4	4.6	4.7	1.7	0.5	3.5	10
	24 h	3.6	3.7	3.3	3.5	2.2	0.6	4.3	10
	12 h+SA	4.2	7.3	5.9	6	2.4	1.4	4.7	20
	24 h+SA	4.1	5.7	4.9	4.9	2.1	1.1	4.8	20
DM 15-1 (V ₃₇)	C	4.6	6	6.5	6.5	4.8	3.5	6.8	40
	12 h	4	3.7	3.8	3.4	2.6	1.7	4	30
	24 h	3	3.6	2.7	2	4	0.8	2.7	20
	12 h+SA	4.5	4.7	4.5	4.7	3.8	1	4.7	40
	24 h+SA	3.7	5.1	4.1	4.5	3.2	2.2	3.7	30
RP 2 (V ₃₈)	C	4.7	7	5.1	5.5	3.8	1.6	5.7	30
	12 h	3.7	5.6	3.7	3	2	0.6	4	20
	24 h	3.6	6.2	2.7	2.5	1.1	0.5	3	10
	12 h+SA	4.3	6.5	4.5	4.5	3.1	1.2	4.6	30
	24 h+SA	4	5.5	4.2	4.1	3	1.2	3.2	20
DM 1-30-15-04 (V ₃₃)	C	5.3	6.6	6.1	6	5.1	2.6	6.5	40

12 h	3.5	4.1	3.6	3.5	3.5	0.4	4.8	30
24 h	2.7	4.3	3.1	2.7	2.5	0.2	4.1	20
12 h+SA	4.8	5.2	5	4.9	3.3	1.9	5.7	40
24 h+SA	3.8	5.2	4	3.5	2.4	1.8	5	30

Biochemical analyses

All biochemical aspects such as nitrite and nitrate reductase enzyme activities, total soluble sugars, total soluble proteins and nutrient ions (phosphorous, potassium, nitrogen and magnesium) concentrations were reduced under the exposure of

heat stress for 12 h and 24 h durations. However, SA foliar spray had improved the enzymatic activities of NiR and NR enzymes, ameliorated TSS, TSP and mineral contents under heat stress (Table 4).

Table 4: Analysis of Variance (ANOVA) showing the effects of high temperature stress and foliar spray of salicylic acid (SA) on nitrate reductase activity; nitrite reductase activity; phosphorus; K, potassium, total soluble sugars, total soluble protein, nitrogen and magnesium.

SOV	df	Mean sum of squares for different parameters							
		NRA	NiRA	P	K	TSS	TSP	N	Mg
Treatment (T)	4	3.5952***	6.0244***	17.486***	14.178***	18.791***	15.911***	5.512***	772.513***
Variety (V)	3	0.0712 ^{NS}	3.6434***	2.3356***	6.9718***	0.6901***	3.9116***	1.3321***	76033***
TXV	12	0.4234***	3.3256***	0.4122***	0.223***	0.256***	0.1843***	0.454***	22.578***
Error	40	0.0358	0.0572	0.0987	0.0345	0.0283	0.0636	0.0356	4.254
Total	59								

***Significance at 0.001 probability levels; NS non-significance respectively; NRA= nitrate reductase activity; NiRA= nitrite reductase activity; P=phosphorus; K=potassium; TSS=total soluble sugars; TSP=total soluble protein; N=nitrogen; Mg=magnesium.

The maximum improvement in NR activity was seen in heat tolerant variety RP 2 and heat sensitive variety MD 15-1 after the SA spray on stress seedlings. Likewise, highest increment in NiRA via SA application was analyzed in Rambir (EF) genotype under heat stress.

TSS content of seedlings were decreased by 39% and 53% under 12 h and 24 h heat shock durations respectively as compared to control in Rambir (EF) genotype. However, foliar application of SA improved TSS contents by 37% and 59% relate increased respectively under 12 h and 24 h heat stress as compared to stressed seedlings. Similarly, there was a decrease in protein contents, i.e., 26% in 12 h and 43% in 24 h heat shock periods as compared to control in Rambir (EF) while SA spray improved protein contents i.e., 30% (in 12 h) and 41% (in 24 h) as compared to stressed seedlings. In high temperature tolerant

variety RP 2, the protein content increased up to 37% at 12 h + SA and 44% in 24 h + SA treatment. Concentration of inorganic components (K, N, P and Mg) was also affected by both heat shock and SA treatments. Highest reduction under 24 h heat shock duration and minimal increment *via sa* was observed in Rambir (EF) genotype in terms of all inorganic mineral contents (Table 4).

Treatment means clearly indicated that foliar application of SA significantly ($p < 0.001$) more enhanced all biochemical traits in tolerant rice varieties (RP2 and DM 1-130-15-04) as compared to non-tolerant ones (Rambir and DM 15-1). ANOVA indicated that mean sum of squares of all biochemical traits were found significant ($p \leq 0.001$) in terms of genotypes and treatments interaction (Table 5).

Table 5: Pearson's correlation analysis among organic and inorganic biochemical constituents.

Inorganic constituents	Organic constituents			
	NRA	NiRA	TSS	TSP

P	0.925*	-0.756	-0.977*	0.956*
K	-0.768	0.572	0.798	-0.973*
N	0.844*	0.832	0.756	0.753
Mg	0.478	0.922*	0.974*	-0.956*

*Significance at $p \leq 0.001$; nitrate reductase activity (NRA), nitrite reductase activity (NiRA); total soluble sugars (TSS); total soluble protein (TSP); phosphorus (P); potassium (K); nitrogen (N); magnesium (Mg)

Pearson correlation analysis was performed among biochemical characters. According to this, NRA is positively and significantly ($p \leq 0.001$) correlated with potassium and nitrogen. Likewise, positive correlation exists among NiRA and magnesium. TSS and TPC were significantly correlated with potassium and magnesium.

DISCUSSION

Early seedling stage is considered as the most critical for vigorous growth stand. Heat stress drastically effected the early seedlings growth. The crop varieties which survive at seedling stage and produce high fresh and dry matter under high temperature stress conditions might be tolerant and may produce high yield. In present study, forty genotypes were tested for high temperature stress tolerance at an early seedling stage and genotypes which produced higher matter and maintained higher shoot and root lengths were categorized as high temperature stress tolerant. Rice germplasm showed significant variation in response to temperature stress. Results indicated that heat stress significantly reduce the seedling growth [18]. However, foliar application of SA was beneficial in enhancing the growth and biomass in all rice genotypes. Previous studies reported that foliar spray of SA was effective in alleviating adverse effects of high temperature [19,20].

High temperature stress adversely influences the physiological and biochemical processes in crops, which ultimately results in reduction of biomass and in extreme condition, plant death occurs. There are several endogenous and exogenous factors which affect the plant growth and development. Among endogenous factors, hormones play a vital role in the regulation of plant growth. Foliar SA application increased the production of fresh matter in Indian mustard (*Brassica juncea* L.) [21] and dry matter [18,22,23] under high temperature stress

Salicylic acid has significant influences on biochemical attributes of rice seedlings. Nitrogen is an important constituent of chlorophyll pigment and increase or decrease in N concentration directly influences the process of photosynthesis [24]. High temperature damages the photosynthetic machinery due to reduction in nitrogen concentration as well as activity of NR and NiR enzymes of N-cycle. NR activity decreased in plants which were subjected to heat stress because the activity of NR depends upon NO_3 concentration in substrate Previous studies reported that NR activity was improved by the exogenously applied SA [20,21].

Concentrations of N, P and K were decreased with increasing temperature stress [21]. The SA spray increased leaf nitrogen, inorganic phosphate, potassium and magnesium as compared to control. A positive response of SA on plant growth regulations may be associated with chemical changes in plants by preventing the intake of Na^+ and Cl^- and enhancing easy uptakes of ions like NO_3^- , Mg^{2+} , Fe^{2+} , Mn^{2+} and Cu^{2+} [25,26] documented that SA treatment increased the N and K contents of leaf under high temperature stressed condition in Indian mustard (*Brassica juncea* L.). SA spray increased N, P and K contents even under stressed condition [27-32].

Soluble sugars act as osmoprotectants under heat stress. Salicylic acid had amplified the TSS and TSP concentrations under heat stress. Increase level of total soluble sugar may be linked with increased invertase activity. In addition, the increased amount of sugar acted as adaptive mechanism to heat stress because sugar could act as signaling molecule during abiotic stresses. The treatment of heat and SA increased the level of total soluble sugars in mungbean seedlings.

CONCLUSION

Salicylic acid exerts positive effects in improving thermo-tolerance in rice cultivars at seedling stage. SA highly enhanced the heat tolerance potencies of tolerant cultivars (RP 2 and DM 1-30-15-04) while it's less effects were observed in sensitive ones (Rambir and DM 15-1). It facilitated in establishment of better seedling stands, growth and biochemical attributes of rice cultivars under heat stress. Hence, it can be confer that foliar spray of SA could be recommended for obtaining good rice seedling growth in the areas facing high temperature extremities.

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