PHYSIOLOGICAL AND BIOCHEMICAL MANIFESTATIONS OF SALICYLIC ACID IN RICE UNDER WATER STRESS CONDITION

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Abstract: Salicylic acid (SA) is a naturally occurring plant hormone of phenolic nature that has diverse effects on tolerance to abiotic stresses. It may act as endogenous signal molecule responsible for inducing abiotic stress tolerance in plants, especially water stress. An experiment was therefore, conducted with an aim to assess the role of exogenously applied SA in water stress tolerance of four different rice varieties. The pot culture was laid out in a completely randomized design (CRD) with three replications. Varieties were subjected to water stress at vegetative stage by withholding water application. The study revealed that moisture stress at vegetative stage is highly detrimental to most of the physiological and biochemical traits investigated in the current research. Drought caused a massive reduction in the basic physiological processes measured in terms of photosynthetic rate, stomatal conductance, transpiration rate, and chlorophyll stability index, but contrastingly, caused noticeable increase in proline accumulation. Foliar application of 100 ppm SA improved the plant growth by increasing the above stated parameters which were reduced due to moisture stress and helped the plants to overcome the adverse effects of water stress. The present finding envisaged that SA improved the drought tolerance of all the four rice cultivars particularly the sensitive ones. Therefore, it may be used as an ameliorant to alleviate the negative effect of drought injury in rice.

Key words: Rice, vegetative stage, water stress, physiological and biochemical traits

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than two-third of the world's population (Dowling *et al.,* 1998). Though the constraints of rice production are many, the most predominant is drought, as more than 70 percent of rice grown in India is rainfed. Productivity of rice in rainfed conditions is around 60-75 percent lower than that of in irrigated conditions (Rao and Venkateswarlu, 1998). It is a world wide spread problem seriously influencing grain production and quality and with increasing population and global climate change making the situation more serious. Water stress frequently occurs, either at one or more phenological stages of the upland rice crop raised under rainfed condition due to the erratic and unevenly distributed rains during the growing season. Water stress at vegetative stage causes irreparable loss of canopy, which leads to a series of morphological, physiological and molecular changes and that adversely affect the plant growth. Salicylic acid (SA) is an important signaling molecule with ubiquitous distribution in plants, and participates in plant physiological processes. It plays an important role in abiotic stress tolerance (Raskin, 1992). It has diverse effect on tolerance to abiotic stress. SA has been demonstrated to be messenger involved in signal transduction in response to biotic and abiotic stresses (Clarke *et al*., 2000). Exogenous application of SA may participate in the regulation of physiological processes in plants, such as stomatal closure, ion uptake and transport (Gunes *et al*., 2007). Therefore, objectives of the current study were to study the effect of induced water stress on morpho-physiological and biochemical traits vis à-vis variability among the rice varieties and to study the effect of salicylic acid in mitigation of drought injury. Analyzing these responses can be useful in understanding the physiological and biochemical mechanisms of this compound by which it cope up to drought stress in rice.

MATERIAL AND METHOD

A pot culture experiment was conducted during summer 2012-13 at the wire netting house of Department of Plant Physiology, OUAT, Bhubaneswar, Odisha, India. Four rice varieties namely; Subhadra (DR-92), Mandakini (OR-20774/IET 17847), Kalinga III (CR-237-1) and Khandagiri (IET 10396) were subjected to three treatments viz., control (normal irrigation), drought, drought + salicylic acid in the present experiment. Earthen pots of 10 inch diameter were used for raising the crop. The soil used for filling the pots was sandy loam type having a pH of 6.8 and the native available N, P and K contents were 100, 17.8 and 110.3 kg ha^{-1}, respectively. Seeds were first sown in the nursery and then 25 days old seedlings were transplanted in the earthen pots using two seedlings per hill and three hills per pot. N, P and K were applied @ 80:40:40 kg ha^{-1}. Half of the N and full dose of P and K were mixed in the soil before filling the pots. Remaining half of the nitrogen was added in two equal split doses, one at tillering and other at the time of panicle emergence. The experiment was replicated thrice with completely randomized design

(CRD). Moisture stress treatment was imposed at the vegetative stage (40 days crop age) by withholding irrigation till temporary wilting appeared. 100 ppm salicylic acid was applied as foliar spray at the beginning of the water stress. A water stress treatment without salicylic acid and a control set (irrigated normally) was also maintained for the study. The plants were irrigated at the end of water stress treatment. The different physiological and biochemical parameters were recorded by using methods as follows.

Photosynthesis and ancillary parameters

Photosynthetic rate and other gas exchange parameters viz. transpiration rate and stomatal conductance were measured on the second fully expanded leaf of three representative plants per genotype with a portable photosynthesis system (CIRAS-2 of version 2.02, USA).

Root : shoot ratio

After destructive sampling of plants, the root and stem were separated and kept in an oven for 48 hours at 80^0C. After 48 hours, dry weight was taken. Root:shoot ratio was calculated as the ratio between root dry weight and shoot dry weight.

Chlorophyll stability index (CSI)

Chlorophyll stability index (CSI) was calculated by following methodology outlined by Kar *et al*. (2005) as follows: CSI = (Total chlorophyll content in stressed plant/ Total chlorophyll content in control plants) x 100. Total chlorophyll content in leaf sample was estimated according to method of Arnon (1949) and expressed as mg g^{-1} fresh weight of leaves. In this method, chlorophyll was extracted in the 80% acetone. 100 mg of fresh leaves were taken from the middle portion of the leaves and were cut into small pieces. The leaf discs were then put in 80% v/v acetone solution and kept in dark for 24 hours. Then they were filtered by Whatman No. 1 filter paper and the filtrate was used to record the absorbance (OD) at 645 nm and 663 nm. The amount of chlorophyll was calculated as, total Chl = $(20.2 \times OD_{645} + 8.02 \times OD_{663}) \times V/ (1000 \times W_F)$; Where, OD = Optical density of the chlorophyll extract at a specific wave length and W_F is Fresh weight of leaf in gram.

Proline content

Proline estimation was done as per the protocol described by Gilmour *et al.* (2005) and Sadasivam & Manickam (1996). Fresh leaves (0.5 g) were ground in mortar and pestle with 10 ml of 3% sulpho-salicylic acid and the homogenate was centrifuged at 18000 rpm. The homogenate was filtered. 2 ml of filtrate was added to 2 ml of glacial acetic acid and 2 ml of acid ninhydrin and test tubes were kept for 1 hour at 100^0C in water bath, followed by ice bath. The reaction mixture was vortexed with 4 ml of toluene. Toluene layer was separated & OD was read at 520 nm. A standard curve of proline was used for calibration & expressed as mg g^{-1} FW.

Statistical analysis

Statistical analyses were done in analysis of variance (ANOVA) technique following the methodologies outlined by Panse and Sukhatme (1985).

RESULT AND DISCUSSION

Photosynthesis and ancillary parameters

Results showed that due to imposition of water stress at vegetative stage, there was significant decrease in photosynthetic rate in all the varieties tested in the current experiment, whereas treatment with 100 ppm salicylic acid reduced the impact of stress and increased the photosynthesis. Photosynthetic rate, irrespective of varieties, decreased significantly by 35% under water stress over control. SA application improved photosynthetic rate in stressed plants and the decrement was to the tune of 14% in stressed plants compared to control. The reduction in photosynthesis under stress and stress plus salicylic acid relative to control was minimum in Subhadra (26 and 4%, respectively) and maximum in Kalinga-III (56% and 38%, respectively). The mean effect of stress, variety and their interaction effect were found to be significant. This enhancement in photosynthetic rate due to exogenously applied SA under drought condition was in agreement with A. R. Mohammed (2011) in rice plants. It has been reported that SA protects the chloroplast and the photosynthetic enzymes from the stress injury which in turn maintains the photosynthesis and its related process much higher than that of lone stressed plants (Marschner, 1995). The decrease in net photosynthetic rate under drought stress observed in many studies is often explained by the lower internal CO_2 concentration, which result in a limitation of photosynthesis at the acceptor site of Rubisco (Ribulose-1,5-biphosphate carboxylase /oxygenase) (Cornic *et. al.*, 1992) or by the direct inhibition of photosynthetic enzymes like Rubisco (Haupt-Herting and Fock, 2000) or ATP synthase (Tezara *et. al.*, 1999; Nogues and Baker, 2000). Drought injury is manifested both at zone of cell turgor and zone of cell flaccidity. This is mainly attributed to stomatal closure, increased mesophyll resistance, decreased diffusion and metabolic shift, which concomitantly inhibit growth and development of plant leading to its productivity (Levitt, 1980).

It was observed that drought significantly decreased stomatal conductance (Gs) of all rice varieties over control, wherein SA application increased Gs in the water stress condition. Application of SA as compared to no application, increased stomatal conductance by 31% under drought condition. The highest Gs (across the treatments) was found in Subhadra (40.87 µmol CO_2 m^{-2} S^{-1}), while minimum

Gs was observed in Kalinga-III (29.34 µmol CO_2 m^{-2} S^{-1}) (table 1). According to Cornic (1994), plants react to water deficit with a rapid closure of stomata to avoid further water loss via transpiration. The varieties having higher Gs are supposed to maintain higher photosynthetic rate as compared to other varieties. In the present study, stomatal conductance and transpiration rate were increased with increase in photosynthetic rate due to exogenously applied SA through foliar application, which suggests that this increase in photosynthesis might have been due to stomatal factors (Athar & Ashraf, 2005).

Table 1. Effect of imposed moisture stress and salicylic acid application at vegetative stages on photosynthetic parameters like (a) Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), (b) Stomatal conductance (µmol CO_2 m^{-2} s^{-1}), (c) Transpiration rate (µmol CO_2 m^{-2} s^{-1}) in rice varieties.

Varieties		Control	Stress	Stress + SA	Mean
Subhadra	(a)	9.11	6.70	8.73	8.18
	(b)	47.50	31.95	43.16	40.87
	(c)	8.55	7.25	8.17	7.99
Mandakini	(a)	7.26	5.01	6.17	6.15
	(b)	39.81	24.97	32.06	32.28
	(c)	8.39	5.46	6.33	6.73
Kalinga III	(a)	6.49	2.86	4.01	4.45
	(b)	40.64	17.17	30.21	29.34
	(c)	7.53	3.78	5.22	5.51
Khandagiri	(a)	8.87	5.94	8.37	7.73
	(b)	46.20	34.57	36.59	39.12
	(c)	8.97	5.09	8.23	7.43
		V	T		V x T
SEm(±)	(a)	0.16	0.14		0.27
	(b)	1.04	0.90		1.80
	(c)	0.15	0.13		0.26
C.D. at 5%	(a)	0.46	0.40		0.80
	(b)	3.03	2.63		5.25
	(c)	0.43	0.37		0.75

Results pertaining to transpiration rate showed that transpiration rate of all the four varieties decreased under drought conditions (35% as compared to control). On the other hand SA treatment increased it and a reduction of 17% was found under stress treated with 100 ppm salicylic acid as compared to control (table 1). Irrespective of stress treatment, lowest transpiration was found in Kalinga III followed by Mandakini, Khandagiri and Subhadra. On exposure to water stress, plants close their stomata more rapidly to avoid drought injury. Early stomatal closure in response to water stress helps to reduce water loss. But it reduces the gas exchange between the plant and the ambient air also, which results in reduced CO_2 intake and reduced photosynthesis (Sharkey *et. al.*, 1989 and Chaves *et. al.*, 2002). Transpiration rate reduced markedly by water stress (Cabuslay *et. al.*, 1999; Wade *et. al.*, 2000; Ravindrakumar *et. al.*, 2003). Waseem *et. al.*, (2006) reported that with the application of 100 ppm SA in wheat under drought through foliar spray, transpiration rate (E) increased, which is in consonance with the present findings.

Root : shoot ratio

Moisture stress was found to significantly increase the root: shoot ratio in rice (Table 2). On an average, root: shoot ratio increased by 13.9% due to the moisture stress. Among the cultivars, root: shoot was highest in Subhadra followed by Khandagiri, Mandakini and lowest in Kalinga III. Application of SA had increased the ratio by 4.3% as compared to control. In this case, root : shoot ratios were found to increase compared to control in almost all the varieties (except Kalinga III), when salicylic acid was applied in stress condition. This means root dry weights increased more over shoot dry weight due to SA application. It indicates that rice varieties diverted more of their dry matter to the root than the shoot, when SA was applied at stress condition. Acceleration of root growth in the plants cause rapid establishment of seedlings and helps to avoid moisture stress. The above result corroborates with the results of Chang *et al.* (1972) in rice and Levitt (1980).

Chlorophyll stability index

It was noticed that CSI significantly decreased with increase in moisture stress irrespective of varieties (Table 2). The decrease was 42% in stress and 18% in stress with SA treatment as compared to control. The mean CSI computed was in order of: 72.9% < 80.2% < 82.3% < 83.8% in Kalinga III, Mandakini, Khandagiri and Subhadra, respectively. Application of SA significantly increased the CSI over the stressed plants (table 2). Agarie *et al.*, (1995) reported about decrease in CSI in rice genotypes under drought stress condition. Yildirim *et al*., (2008) found that 100 ppm SA significantly increased chlorophyll stability index, which corroborate with our results.

Proline content

Higher proline accumulation in plants during stress is credited to maintenance of high turgor and continuous growth even under stress condition. A spurt in proline content was found in plants grown under stress-prone environment irrespective of cultivars. Application of SA showed further significant increase of proline accumulation (26%) over the stressed plants. Mean proline content (across treatments) was highest in Subhadra (302.66 µg g^{-1} FW leaf) followed by Mandakini (269.8 µg g^{-1} FW leaf), Khandagiri (243.4 µg g^{-1} FW leaf) and the lowest value was found in Kalinga III (150.17 µg g^{-1} FW leaf) (table 2). Under SA treatment, Mandakini registered the highest increase in proline accumulation (47%) followed by Subhadra (26%), Khandagiri (14%) and Kalinga III accumulated the least (10%) over their respective values under stress. The interaction effect on proline accumulation was significant between variety and the treatments. Increased trend of proline content with moisture stress suggest its protective and stabilized role. These results were in conformity with the earlier findings of Mafakeri *et. al.*, (2010); Maggio *et. al.* (2002), and Baruah *et. al.*, (1998). Azooz and Youssef (2010) and Demiralay *et. al.*, (2012) reported that proline content increased with application of SA under drought stress in wheat and roscoe respectively, which are in consonance with our present findings.

Table 2. Effect of imposed moisture stress and salicylic acid application at vegetative stages on (a) Root: Shoot ratio (b) Chlorophyll stability index (%), (c) Proline content (µg proline g^{-1} fresh weight leaf) in rice varieties.

Varieties		Control	Stress	Stress + SA	Mean
Subhadra	(a)	0.200	0.198	0.240	0.213
	(b)	100.00	66.28	84.96	83.75
	(c)	93.15	359.91	454.92	302.66
Mandakini	(a)	0.215	0.171	0.232	0.206
	(b)	100.00	59.31	81.37	80.23
	(c)	91.72	290.11	427.61	269.81
Kalinga III	(a)	0.226	0.160	0.153	0.180
	(b)	100.00	42.07	76.56	72.88
	(c)	66.93	182.02	201.55	150.17
Khandagiri	(a)	0.190	0.188	0.242	0.207
	(b)	100.00	63.15	83.76	82.30
	(c)	82.71	302.57	344.82	243.37
		V	T	V x T	
SEm(±)	(a)	0.006	0.005	0.011	
	(b)	1.51	1.31	2.62	
	(c)	12.28	10.63	21.27	

C.D. at 5%	(a)	0.018	0.015	0.031
	(b)	4.41	3.82	7.63
	(c)	35.84	31.03	62.07

CONCLUSION

Analyzing the findings of the present investigation, it was concluded drought caused a severe reduction in photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll stability index and root:shoot ratio, but contrastingly, caused noticeable increase in proline accumulation in rice. Foliar application of 100 ppm increased the drought tolerance of rice by overcoming the adverse effect of stress on stated parameters. The beneficial response of salicylic acid in ameliorating the adverse effect of water stress was more in sensitive genotype Kalinga III. Therefore, it may be used as an ameliorant to alleviate the negative effect of drought injury in rice.

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