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To Study the Response Terminal Water Stress on Physio- Biochemical Changes in Drought Tolerant and Drought Sensitive Rice Genotypes

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ABSTRACT

A pot experiment was conducted during *kharif* season 2022 with two rice genotypes IR 64 (drought sensitive) and Nagina 22 (drought tolerant) to evaluate physio-biochemical changes under terminal water stress condition in department of Molecular biology and Biotechnology, Acharya Narendra deva University of agriculture and Technology, Kumarganj, Ayodhya 224229, Uttar Pradesh, India. The water treatment of 14 days was given at initiation of flowering under pot culture treatments. Leaf rolling pattern, Relative water Content (RWC), Chlorophyll content, Proline content, catalase and Peroxidase activity were recorded at the end of water stress against control condition. The Nagina 22 had less leaf rolling, high RWC, high proline content, catalase and peroxidase activity under water stress condition and showed less reduction in days to 50% flowering, effective tillers number, grains panicle^{-1}, test weight and grain yield plant-1 under water stress condition comparatively IR 64 rice genotype. The Transient leaf rolling, high RWC, stay green, accumulation of high proline content, high catalase activity at terminal stage water stress responsible for less reduction yield and yield contributing traits can be taken as physio-biochemical indices for screening water stress tolerant in rice genotypes.

Keywords Water stress, Catalase, Proline, Tolerance, Yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most consumed staple food crops worldwide. In developing countries, people often rely on rice as their sole of nutrition (Yang *et al.* 2019). It is important cereals crops be grown in any part of the world. Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Ray *et al.* 2019). Rice is grown in more than a hundred

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countries, with a total harvested area of approximately 158 million hectares, contributes about 40 to 43% of total food grain production, and continues to play wide role in the national food and livelihood security system. The total production of rice in 2020-21is 121.46 mt and the over 44 mha area of the country (Anonymous 2022).

Biotic and abiotic factors limit the productivity of the rice-growing areas of the world. It has been estimated that approximately more than 200 million tons of rice are lost every year due to environmental stresses, diseases, and insect pests (Chen *et al.* 2013). Drought, a period of no rainfall or irrigation that affects plant growth, is a major constraint for about 50% of the world production area of rice (Singh *et al.* 2017). Drought effects in lowland rice can occur when soil water contents drop below saturation and mild-drought stress reduced grain yield by 31%–64%, while severe stress reduced 65%–85% yield compared with normal conditions in rice crop (Kumar *et al.* 2008). Early and vegetative stages water stress reduce the leaf number, size and area, effective tillers number, plant height, days to 50% flowering, and ultimately grain yield of rice plant (Swain *et al.* 2017). The stresses that occur at early and vegetative stage can be recovered with minimum losses by rewatering of the crop. If water stress occurs at terminal or reproductive stage causes heavy losses up to 40-90 % depending upon the rice genotypes and cannot be recovered even after irrigation as it affects panicle initiation, grains panicle^{-1}, grain filling duration, thousand grain weight and yield of the rice crop (Mishra *et al.* 2019). The terminal water stress also altered the stay grenn duration, photosynthetic activity, source sink relation and induced force maturity of the rice genotypes (Ovenden *et al.* 2017).

The stress tolerant genotypes are less affected in drought stress condition and try to maintain growth and yield with minimum effects comparatively drought sensitive genotypes (Davatgar *et al.* 2009). The tolerant genotypes adopt various morpho-physiological, bio-chemical and molecular mechanism during water stress regimes and it reduces its leaf area by transient leaf rolling, rate of transpiration, stomatal, activity and minimize the loss of water (Hossain *et al.* 2020). It maintains the leaf water potential by osmotic adjustment and increasing the production of osmolytes like proline, sugar, polyols, sugar betaine and other Osmo protectants (Kumari *et al.* 2019). Under stress condition, plant produces excessive amount of super oxide radicals that damages cell and causes death of the plants (Gill and Tuteja 2010b). Therefore, the efficient way to enhance drought tolerance in rice is to reduce ROS over-production or enhance antioxidant activity in rice organs. Tolerant plant increases the activity of reactive oxygen species scavenging enzymes like super oxide dismutase, catalase, peroxidase to protect the cell and its membrane damage from super oxide radicals (Panda *et al.* 2021). In this paper, try to understand the effect of terminal water stress and mechanism adopted by tolerant and susceptible of rice genotypes to ameliorate the stress losses on yield and attributing traits.

MATERIALS AND METHODS

The present investigation was conducted in pot culture at department of Molecular Biology and Biotechnology, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (U.P.), India during Kharif season of 2021-22. The rice genotypes IR 64 (drought susceptible) and Nagina 22 were exposed to 40% field capacity for 14 days water stress at flower initiation stage to know the impact of water stress on physio-biochemical changes and its effect on yield and yield contributing traits. Relative water content (RWC) was recorded as per method of Barrs and Weatherly (1962). Cut leaves were weighed (fresh weight, FW), then left saturated in water for three hours and their turgid weights (TW) were calculated. The samples were then dried in an oven at 80°C for 24 hours and weighed (DW). The RWC is determined as follows: RWC (%) =[(FW-DW)/(TW-DW)] ×100. Leaf rolling was recorded as method of IRRI, Scale (IRRI, 2013). Chlorophyll content (SPAD) was measured using chlorophyll analytical apparatus (chlorophyll meter SPAD-502. Konica Minolta sensing Inc. Ltd., Japan). Five flag leaves were measured from the widest part of the leaf of the main culm of each genotype in each replication.

Free proline content in leaves was estimated spectrophotometrically according to the methods of Bates *et al.* (1973). Catalase activity was assayed calorimetrically according to method given in Analytical Biochemistry (Sinha 1972). Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction. Peroxidase activity was measured according to methods of Curne and Galson (1959). The panicle bearing tillers plant^{-1} was counted at maturity. The number of grains panicle^{-1} of five randomly selected plants were counted from main panicles at maturity and averaged out to one as per panicle basis. Test weight was recorded by counting 1000 seeds from each genotype at 12% moisture and weighed for assessing test weight of each genotype. The randomly 5 plants were selected and grain weight were taken at 12% moisture level of each plant separately after threshing and average out to one as grain yield per plant.

RESULTS AND DISCUSSION

Rice genotypes showed differential response for water stress at terminal stage condition (Table 1). Leaf rolling of IR 64 reached at 7 (rolled completely) and unable to come at normal condition. The leaf rolling pattern of Nagina 22 was temporary type. It rolled during day time and regain it shape in morning hours. Nagina 22 score was 3-5 at the end of 14 days stress. IR 64 poorly recovered after releasing of water stress while Nagina 22 recovered without losing. Leaf rolling of plant is an indicator of relative water content of plants. The transient leaf rolling is an adoptive mechanism of plant to save the water for judicial use under water stress condition. Water stress reduce the leaf growth and total surface area of the plant (Zhu *et al,* 2020). Tolerant plant like Nagina 22 adopted transient leaf rolling and survive in water stress regimes.

Relative water content RWC) of IR 64 and Nagina 22 showed differential pattern under water stress condition while it was nearly same in normal condition (Table 1). The water stress reduced RWC irrespective of rice genotypes. Nagina 22 retained it leaf water showed less reduction comparatively IR 64. The RWC of Nagina 22 and IR 64 was 75.86 and 68.65 respectively at the end of continuous 14 days water stress. The recovery percentage of Nagina 22 was 85.25 while IR 64 was 40.25 after release of water stress. The relative water content of leaf is available water that remain in plants against a fully turgid leaf in environmental condition. The tolerant plant tries to maintain its RWC by mechanism of deep root system, reduced the leaf area and osmotic adjustment mechanism under water stress regimes (Mishra and Panda 2017).

Rice genotypes had variability in chlorophyll content under and water stress condition (Table1). The content in IR 64 and Nagina22 was 68.86 and 61.36 under normal condition at terminal stage. The waters tress reduced the chlorophyll content in rice Nagina 22 and IR 64 genotypes. The IR 64 showed high reduction (47.34) while Nagina 22 had less reduction (55.62) over control. The terminal water stress increases the senescence processes by decreasing the chlorophyll a, b and total chlorophyll content (Pirzad *et al.* 2011). Reactive oxygen species damages the chloroplast membrane, the activity of chlorophyll synthetic enzymes reduces decreases the stay green duration and photosynthetic activity of plant (Saha *et al*. 2020).

Proline content was nearly same in rice genotypes under normal condition but highly increased under water stress condition (Table 2). The water stress induced to increase the proline content in both IR 64 and Nagina 22 rice genotypes. The high proline content was recorded in Nagina 22(275.78) while comparatively less in IR 64 (175.10). The proline reduced to normal as control condition after rewatering

Table 1. Effect of terminal stage water stress on leaf rolling, RWC and total chlorophyll content (SPAD)of rice genotypes.

Rice genotypes	Leaf rolling (IRRI scale)			RWC (%)			Chlorophyll content (SPAD value)		
	C	S	PR	C	S	PR	C	S	PR
IR 64	1.25	7.15	472.00	91.24	68.65	24.76	68.86	47.34	31.25
Nagina 22	1.27	3.23	154.33	88.37	75.86	14.16	61.36	51.62	15.87
CD at 5%	0.23	2.12		2.84	3.18		2.13	2.86	

Where, C=Control, S= Stress, PR= Percent reduction.

Table 2. Effect of terminal stage water stress on proline content, catalase and total peroxidase activity of rice genotypes.

Rice genotypes	Proline content (mg g^{-1}ft)			Catalase activity			Peroxidase activity		
	C	S	R	C	S	R	C	S	R
IR 64	144.18	175.10	96.35	134.09	167.23	140.27	34.23	68.53	37.21
Nagina 22	150.06	275.78	151.35	120.31	290.61	125.65	39.64	84.26	40.24
CD at 5%	3.65	5.36	4.78	3.76	5.64	3.26	2.14	3.63	2.43

Where, C=Control, S= Stress, R= Data at recovery.

Table 3. Effect of terminal stage water stress on days to 50% flowering, panicle bearing tillers, grain $panicle^{-1}$, test weight and grain yield $plant^{-1}$ of rice genotypes.

Rice genotypes	Days to 50% flowering			Panicle bearing tillers			Grains $panicle^{-1}$			Test weight (g)			Grain yield $plant^{-1}$(g)		
	C	S	PR	C	S	PR	C	S	PR	C	S	PR	C	S	PR
IR 64	86.24	70.52	18.23	7.10	2.510	64.49	198.54	108.24	45.48	26.37	18.48	29.92	63.14	20.18	68.04
Nagina 22	68.41	62.16	9.14	6.40	4.780	23.49	156.33	135.45	13.36	24.25	20.34	16.12	52.57	42.51	19.14
CD at 5%	4.63	5.32		0.75	1.24		7.24	6.83		1.37	1.21		3.21	4.28	

Where, C=Control, S=Stress, PR= Percent reduction over control.

and Nagina 22 recovered. The IR 64 did not recovered after releasing the water stress and later on failed to survive. Higher accumulation of proline is usually associated with drought tolerance and it helps for maintenance of leaf turgor and progress in stomatal conductance (Kumar *et al.* 2016). Thus, proline content can act as a biochemical marker under drought screening of plants (Pandey and Shukla 2015).

The catalase and peroxidase activity suddenly increased significantly IR 64 and Nagina 22 in terminal water stress condition but it showed less variation under control condition (Table 2). The catalase activity was 134.09 and 120.31 in IR 64 and Nagina 22 under normal condition. Water increased the catalase activity in both the genotypes. High catalase activity was recorded in Nagina22 (290.61) comparatively IR 64 (167.23) at the end of water stress regimes. Peroxidase activity was also highly increased under stress condition and less fluctuated under normal condition. High peroxidase activity was noted in Nagina 22 and less increased in IR 64 under water stress condition. Exposure of plants to drought stresses initially causes oxidative damage by the formation of ROS. These ROS pose serious threat to the cell functioning by damaging lipids and proteins. The ROS are mainly produced in the chloroplast (Mishra *et al.* 2017) however, reaction of oxygen with the components of electron transport chain in mitochondria also results in the generation of ROS (Gill and Tuteja 2010b). Beside these enzymes, certain carotenoids and glutathione can also play part in the antioxidant system as

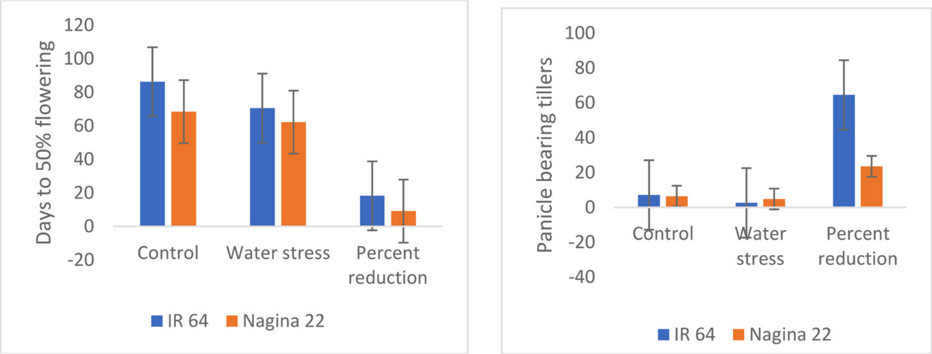

Fig. 1. Water stress on days to 50 % and panicle bearing tillers in rice genotypes.

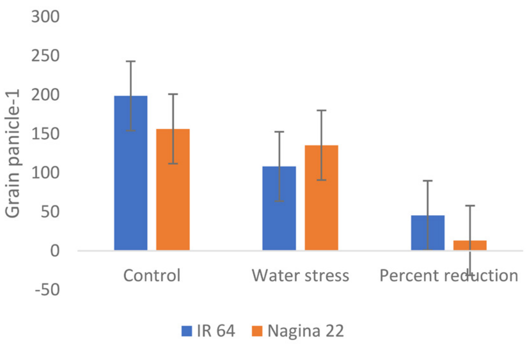

Fig. 2. Effect of water stress on grains panicle^{-1} in rice genotypes.

non-enzymatic components. The enzymes such SOD, POD and CAT either directly scavenge the ROS or protect plants indirectly by managing non-enzymatic defence (Anjum *et al*. 2017). Therefore, maintenance of the higher levels of the anti-oxidants can be a good strategy by the plants to counter the negative effects of ROS (Bhattacharjee and Dey 2018).

The water stress reduced the days to 50% flowering in rice genotypes (Table 3). High reduction in days to 50% flowering was recorded in IR 64 (18.23) and less in Nagina 22 (9.14) during terminal stage drought treatment. The days to 50% flowering under control condition in IR 64 and Nagina 22 was 86.24 and 68.41 respectively (Fig.1). The panicle bearing tillers was affected under terminal water stress condition. The panicle bearing tillers under control condition was 7.11 in IR 64 and 6.11 in Nagina 22 (Fig.1). Under water stress, panicle bearing tillers highly reduced in IR 64 (64.49%) and less in Nagina 22(23.49). The rice genotypes was varied in grains panicle^{-1} (Fig.2). In control condition, high grain number was recorded in IR 64 (198.54) and comparatively less in Nagina 22(156.33). the grain number panicle^{-1} was reduced under water stress. High reduction was recorded in IR 64 (45.48%) while less in Nagina 22 (13. 36). Rice genotypes had variation in test weight under normal and control condition. High test weight was recorded in IR64 (26.37) and low in Nagina 22 (24.25) under control condition. The test weight of both genotypes was reduced under water stress condition. High reduction was noted in IR 64 (29.92 %) while less in Nagina 22 (16.12 %) in water stress regimes (Fig. 3.) High grain yield plant^{-1} was recorded in IR 64 (63.13 g) and less in Nagina 22 (52.57 g) in control environment (Fig. 3.). Water stress affected the grain yield of rice genotypes. High percent reduction was recorded in IR 64 (68.04 %) and less in Nagina 22 (19.14 %) under stress treatment.

Drought stress during different growth stages might decrease translocation of assimilates to the grains, which lowered grain weight and increased the empty grains. Gupta *et al,* 2020 have reported reduced rice yield because of drought stress at critical growth stages. Yield contributing and yield traits such as effective tillers hill^{-1}, total spikelets panicle^{-1}, filled grains panicle^{-1}, 1000-grain weight, % sterility and grain yield hill^{-1} are the most popular parameters used to identify drought tolerance in rice breeding programs (Yang *et al*. 2019). Our findings have also harmony with those of Zhu *et al.* (2020) who have studied of different agronomic parameters during screening of rice varieties for drought prone areas.

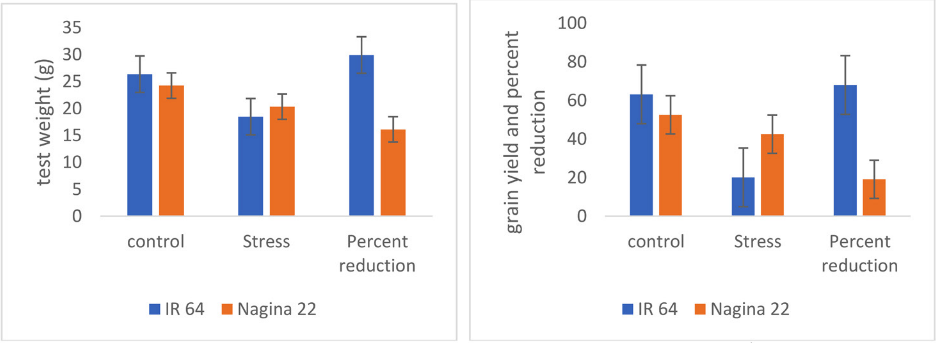

Fig. 3. Effect of water stress on grains test weight and grain yield plant^{-1} in rice genotypes.

CONCLUSION

The terminal stage water stress affected the yield and yield contributing traits in rice genotypes. The loss occurred at terminal stage was not recoverable after releasing the stress and causing more damage comparatively early stage drought stress. The tolerant rice genotypes Nagina 22 was less affected by maintaining transient leaf rolling, high RWC, proline content, stay green, catalase and peroxidase activity and consequently less reduction in yield contributing traits and yield over water stress sensitive genotypes IR 64.

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