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Biochemical Assimilates Accumulation and Remobilization at Reproduction Stage Drought Stress in Upland Rice (*Oryza sativa* L.)

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Abstract A very week correlation obtained between leaf rolling score and yield, shoot soluble sugar and RWC, respectively. Leaf depth ; score in well correlated with leaf soluble sugar. Plant height is often considered as a factor in plant response to drought stress. Rice accumulates significant amount of carbohydrate before and wide variability exists among genotypes. Improved variety like NDR-97, Vandana and DH line have higher content of total soluble sugar at flowering than do low yielding traditional cultivar like Azucena and Saita correlation study revealed strong positive relationship between grain yield in stress and shoot soluble sugar. Assimilate accumulated prior to flowering are of paramount importance when plant experience drought at later stage. We hypothesized that grain yields of rice crops drought stressed at flowering, directly related to reserve food materials and the ability of the plant to translocate those assimilate. Translocated-CHO for grain growth is supported by apparent contribution rate (ACR) and value of ACR was higher in stressed plant. This result indicated that under several water deficits at flowering stage, grain growth depend of previously accumulated assimilates. Correlation study showed strong positive regression coefficient ($R^2 = 0.54$) with grain yield, justified the ability of the plant to translocated assimilate to minimized the sterility due to water deficit. Among the cultivar tested here IR-64, NDR-97, Vandana, Azucena, Moroborokan and DHLs had at par ATR value in irrigated control but generally had more CHO coupled with high ATR and ACR under drought. In present investigation genotypic variability's were observed regarding other stress adoptive plant traits. Higher Nitrate reductase was observed in all genotypes at the end of drought stress and higher correlation coefficient was obtained with grain yield. It seems that higher NR active at the end of drought delayed the leaf senescence, which helps to sustain photosynthesis during stress. Furthermore, linear increase in SOD (antioxidant) was observed after re-watering and stressed plants. The increased in antioxidant activity is believed to be associated with recovery growth.

Keywords Rice, Reproductive stage drought stress, Biochemical assimilates, Soluble sugar, Enzymes.

Introduction

The plant breeding process for drought adaptation can be made more efficient when traits other than yield are added to the selection process. In rice, various researchers have identified putative traits for drought resistance. There has been a large effort to

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evaluate their effectiveness under a range of drought conditions. Limited work has been conducted on evaluating the contribution of putative drought resistance traits to grain yield in rice [1]. This needs to be rectified, particularly considering their importance relative to upland conditions in Asian countries. Multidisciplinary approach involving genetics, biochemistry, biotechnology, physiology, plant breeding and crop science will be appropriate to assess the complicated and integrated response of plants to drought and to evolve superior drought resistant genotypes [2]. Rice varieties differ greatly in their ability to tolerant aerobic soil and moisture deficit. The greatest ability to grow and produce some grain with chronic moderate water deficit is found in japonica varieties from uplant ecosystems such as those found in hilly Southeast Asia [3]. Notable levels of drought tolerance are also observed in the early-maturing aus and indica varieties traditionally grown in the plateau region of Eastern India, such as N22 and Dehula [4]. While these cultivars usually escape drought through early maturity, they can also produce some grain when rains around flowering, indicating that they avoid or tolerate drought. Enormous amount of variability is exhibited by traditional cultivars grown under fragile environments indicating that native landraces embody unique tolerance strategies appropriate to specific growing condition. Therefore, present investigation was carried out with to determine constitutive / adaptive traits associated with flowering stage drought tolerance in tolerant and susceptible rice varieties / genotypes and to find out the biochemical traits associated with drought tolerance.

Materials and Methods

Experimental sites, genotypes and years of screen

The present investigation was carried out in wet season at the Instructional Farm of Department of Crop Physiology, ND University of Agriculture and Technology Kumarganj (Faizabad), UP, India. The genotypes of upland rice (*indica* and *japonica* type) from different geographical regions were screened for drought tolerance. These genotypes responded well under severe drought conditions and displayed good drought score, recovery and early vegetative vigor, simultaneously, substantial yield also.

Management of water stress

Irrigated control (E_1) : The experimental field was left uncovered to receive natural rainfall. In addition to this, experimental plots were irrigated using well laid channels for supplying tube well water, as and when required, to maintain appropriate moisture levels as recommended for irrigated rice.

Reproductive stage drought stress (E_2) : The experiment field was covered by constructing temporary rainout shelter with proper drainage channel. Care was taken to check the inflow or seepage of water from the adjoining areas by making adequate bunds around the experiment and covered with polythene in drought condition. The heading stage drought was created by with holding the irrigation for 15 days up to 80 K Pa at 0—15 cm soil profile and 60 K Pa at 30 cm soil depth. Plants were exposed for two weeks ((60—80 K Pa). Soil moisture content during stress period was monitored. Recovery was measured at 10th days after released of drought. Genotypes were scored for leaf rolling and leaf drying at the peak stress period using the IRRI Standard Evaluation System [5].

Experimental design

The genotypes were seeded and transplanting was done 21 days after seeding. Each genotype was transplanted in randomized block design with three replications. Recommended agronomic practices were followed. All other crop management practices were at the optimum level.

Observation and evaluation

Observations were recorded on five competitive plants of the middle row of each plot for yield and 18 biochemical traits. The biochemical traits estimated viz., chlorophyll according to [6], and protein content [7], soluble sugar [8], starch estimation [9], proline content [10], superoxide dismutase activity (SOD) [11], nitrate reductage (NR) [12], α–amylase activity [13]. The data of biochemical and grain yield were

Table 1. Performance of rice genotype for biometrical traits under irrigated and drought (60—80 kpa) conditions over the years.

Genotypes	Chlorophyll a		Chlorophyll b		Total chlorophyll		Protein content		Proline content	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
NDR-359	0.76	0.60	0.29	0.21	0.97	0.75	4.21	3.43	18.96	31.60
NDR-97	0.75	0.62	0.35	0.27	0.97	0.69	3.90	3.42	31.84	35.76
Moroberekan	0.75	0.60	0.24	0.20	0.98	0.75	3.75	2.98	33.94	37.83
Vandana	0.77	0.62	0.45	0.35	0.95	0.77	4.10	3.84	30.50	34.69
Azucena (DT Check)	0.86	0.66	0.31	0.23	0.98	0.77	3.95	3.34	19.49	30.21
P-0326	0.72	0.55	0.27	0.21	0.95	0.73	3.90	3.03	19.72	3.80
TN-1	0.84	0.64	0.26	0.18	0.98	0.65	4.07	2.60	18.13	32.87
DSU-18-6	0.76	0.43	0.26	0.21	0.94	0.65	4.06	3.35	21.72	33.92
DGI-138	0.77	0.51	0.21	0.16	0.99	0.69	4.14	3.14	22.29	29.15
DGI-21	0.75	0.56	0.23	0.18	0.93	0.75	3.98	2.90	21.36	35.99
P-0397	0.79	0.49	0.29	0.13	0.81	0.67	3.90	2.89	16.96	31.77
P-0090	0.81	0.48	0.27	0.16	0.97	0.64	3.54	3.10	30.26	36.73
DGI-379	0.87	0.54	0.37	0.27	0.97	0.63	3.36	3.31	28.07	34.73
DGI-75	0.77	0.44	0.26	0.19	0.97	0.67	3.95	3.02	19.89	32.73
P-0088	0.74	0.43	0.29	0.20	0.97	0.63	3.75	2.44	22.61	24.13
DGI-152	0.79	0.53	0.34	0.22	0.99	0.65	3.81	3.25	17.05	27.27
Saita	0.74	0.25	0.38	0.24	0.95	0.52	4.00	2.70	20.69	37.86
IR-64 (DS Check)	0.86	0.41	0.27	0.13	0.99	0.63	3.32	3.38	21.00	38.22
SEm±	0.025	0.024	0.023	0.010	0.017	0.021	0.015	0.04	2.11	1.62
CD at 0.5%	0.051	0.069	0.067	0.028	0.049	0.061	0.042	0.13	6.00	4.67

Table 1. Continued.

Genotypes	Leaf soluble sugar		Upper root soluble sugar		Total shoot soluble sugar		
	Control	Drought	Control	Drought	Before drought	Control	Drought
NDR-359	66.50	56.33	0.12	0.10	177.33	99.33	86.17
NDR-97	84.67	69.67	0.13	0.13	186.17	153.83	140.00
Moroberekan	69.00	56.17	0.14	0.10	237.33	158.67	98.50
Vandana	73.50	66.00	0.22	0.11	205.00	185.00	169.27
Azucena (DT Check)	81.83	71.83	0.19	0.17	214.83	247.17	126.50
P-0326	45.68	39.00	0.13	0.11	165.83	129.17	58.50
TN-1	66.83	55.00	0.24	0.13	244.50	143.50	122.99
DSU-18-6	52.50	39.00	0.19	0.10	183.50	126.50	111.00
DGI-138	60.67	47.83	0.14	0.12	208.17	143.17	107.00
DGI-21	75.33	53.83	0.15	0.14	183.50	144.83	109.17
P-0397	73.83	41.67	0.24	0.17	206.33	298.50	32.83
P-0090	67.50	39.67	0.21	0.12	143.83	123.33	98.64
DGI-379	67.50	36.67	0.14	0.11	178.33	118.83	67.60
DGI-75	77.67	42.50	0.20	0.11	205.50	124.17	44.00
P-0088	56.50	36.33	0.17	0.11	187.33	213.67	50.00
DGI-152	68.67	35.50	0.24	0.14	202.17	124.50	56.83
Saita	84.17	37.83	0.12	0.09	195.33	228.17	48.37
IR-64 (DS Check)	63.33	39.33	0.21	0.12	191.00	125.17	59.67
SEm±	2.66	1.53	0.018	0.016	11.30	6.48	3.77
CD at 5%	7.67	4.39	0.023	0.028	32.53	15.65	10.87

Table 1. Continued.

	Shoot starch			Leaf starch		Upper root starch		Lower root soluble sugar		Lower root starch	
Genotypes	Before drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
NDR-359	158.83	105.33	92.00	46.17	34.50	0.19	0.05	0.09	0.07	0.08	0.07
NDR-97	143.50	194.67	180.17	44.33	33.83	0.14	0.11	0.13	0.07	0.11	0.11
Moroberekan	177.17	158.50	143.00	32.00	21.83	0.22	0.13	0.13	0.09	0.09	0.04
Vandana	145.37	96.17	84.00	54.67	46.17	0.21	0.13	0.09	0.07	0.10	0.09
Azucena (DT Check)	176.17	125.33	107.50	55.00	47.17	0.21	0.13	0.21	0.11	0.13	0.09
P-0326	134.17	164.17	69.33	33.33	25.00	0.21	0.12	0.18	0.11	0.09	0.06
TN-1	190.33	172.83	97.83	59.67	39.83	0.21	0.12	0.18	0.09	0.12	0.08
DSU-18-6	119.83	169.33	132.00	53.83	40.00	0.23	0.13	0.13	0.11	0.09	0.07
DGI-138	134.00	103.50	77.00	33.00	29.17	0.17	0.12	0.12	0.09	0.16	0.09
DGI-21	112.83	124.00	71.17	53.33	34.00	0.21	0.12	0.13	0.07	0.09	0.08
P-0397	161.33	173.83	130.17	57.33	29.50	0.22	0.11	0.12	0.06	0.09	0.04
P-0090	191.50	96.83	53.67	60.33	53.00	0.32	0.21	0.12	0.09	0.09	0.04
DGI-379	162.33	83.00	33.17	33.35	23.67	0.20	0.09	0.11	0.07	0.07	0.04
DGI-75	167.00	112.67	79.00	73.17	44.67	0.20	0.13	0.06	0.03	0.11	0.04
P-0088	144.50	181.50	132.17	34.83	27.33	0.19	0.13	0.11	0.10	0.11	0.08
DGI-152	127.67	106.17	55.83	55.00	35.17	0.26	0.13	0.06	0.04	0.12	0.07
Saita	139.67	90.33	38.33	36.83	27.83	0.26	0.12	0.14	0.08	0.09	0.04
IR-64 (DS Check)	142.67	104.17	33.50	39.00	22.37	0.19	0.13	0.21	0.10	0.13	0.10
SEm±	0.09	3.12	2.09	3.54	2.25	0.007	0.004	0.008	0.006	0.009	0.005
CD at 0.5%	11.77	8.99	6.02	10.20	8.75	0.019	0.011	0.013	0.011	0.013	0.009

Table 1. Continued.

	α amylase		Nitrate reductase			Superoxide dismutage		
Genotypes	Control	Drought	Control	Drought	Control	1 day	2 days	3 days
NDR-359	525.33	647.00	170.83	128.17	450.83	966.50	996.00	1025.33
NDR-97	522.67	636.33	161.00	122.50	444.83	936.00	1003.00	1045.67
Moroberekan	557.67	670.67	166.83	112.17	443.83	963.00	1011.00	1043.83
Vandana	560.50	658.67	174.50	127.67	455.17	954.00	997.00	1031.00
Azucena (DT Check)	601.83	660.00	165.17	122.17	449.17	962.00	1008.00	1040.33
P-0326	560.67	622.33	153.00	91.83	448.83	969.00	985.00	1069.67
TN-1	680.67	673.67	176.17	88.00	440.83	959.50	974.00	1031.17
DSU-18-6	409.83	601.17	169.33	94.50	442.00	971.50	1028.00	1025.33
DGI-138	503.83	710.83	174.83	92.50	450.50	992.00	1008.00	1079.67
DGI-21	487.50	690.00	139.17	109.50	444.33	968.00	988.00	1042.00
P-0397	479.33	606.50	180.83	100.33	450.83	931.50	908.00	986.50
P-0090	489.17	620.33	175.33	54.17	451.83	918.50	953.00	974.00
DGI-379	505.67	414.00	153.67	106.83	448.83	943.00	962.00	854.00
DGI-75	467.83	631.50	154.67	102.83	450.17	969.50	1007.00	1070.00
P-0088	494.00	669.50	169.83	76.33	455.50	961.50	977.00	1018.83
DGI-152	453.00	568.50	175.00	93.50	454.33	947.00	972.00	1006.33
Saita	510.17	753.17	174.33	81.50	452.00	899.50	942.00	988.00
IR-64	480.50	648.50	180.50	110.33	443.50	937.00	1005.00	1001.17
SEm±	17.00	22.22	5.78	4.22	12.81	8.20	12.28	13.10
CD at 0.5%	30.21	45.60	10.29	2.25	20.25	23.60	22.74	37.70

analyzed by appropriate statistical analysis using Crop Stat 7.2 program [14].

Results and Discussion

Chlorophyll content (mg g^{-1} fresh weight)

Table 1 revealed that chl a, chl b and total chl content were significantly reduced in stressed rice plant. Maximum chl a content was observed in Azucena (0.66 mg g^{-1} fresh weight) followed by Vandana as well as NDR-97 and NDR-359, respectively. Extent of reduction was higher in Saita (66.20%) followed by IR-64 (52.32%) susceptible check, DGI-75, P-0088 and P-0090, respectively. Similarly, in E_2–significantly reduced the chl b content of leaf of all tested rice cultivars/genotypes. Minimum degradation in chl b content was observed in DGI-138 (2.38%) followed by Moroberekan (16%), DSU-18-6 as well as DGI-21 (21%), respectively. Moreover, maximum reduction was obtained in IR-64 (51%) followed by P-0397 (55%) and P-0090 (40%), respectively.

Protein content (mg g^{-1} fresh weight)

Significant genotypic variability was obtained in leaf protein content among tested rice genotypes/cultivars during drought stress (Table 1). Minimum reduction per cent in leaf protein contents (mg g^{-1} fresh weight) were observed in Vandana (6.34) followed by NDR-97 (12.30) and P-0090 (12.42), respectively. Extent of per cent reduction was higher in TN-1 (36 approx.) followed by Saita (32.50) susceptible cultivar.

Proline content (mg g^{-1} fresh weight)

Genotype moroberekan (33 mg g^{-1} fresh wt.), NDR-97 (31.84 mg g^{-1} fresh weight) and Vandana as well as P-0090 (30.50 mg g^{-1} fresh weight) showed relative higher proline content even in non stress condition. Maximum increase over non stressed plants were observed in P-0397(87%), Saita (83%), IR-64 (82%), TN-1 (81%). Likewise, Moroberekan showed minimum increase in proline content of leaf (11.46%) followed by NDR-97 (12%), Vandana (14%) and P-0090 (21%) respectively (Table 1). Higher genotypic variability in leaf proline content was obtained among rice genotypes.

Shoot soluble sugar (mg g^{-1} dry weight)

At end of drought treatment TN-1, P-0090, Moroberekan, Azucena, Vandana, DGI-75, P-0397, DGI-138 and DGI-152 showed higher initial soluble sugar content. Moreover, drought significantly reduced the total shoot soluble sugar of tested rice genotypes/cultivars (Table 1). Minimum reductions were observed in Vandana (7%), P-0326 (8%), TN-1 (10%), Moroberekan and DGI-DGI-75 (13%) and Azucena, NDR-97 and NDR-359 (15%) over control. Maximum reduction in total shoot soluble carbohydrate were observed in DGI-138 (62%) and Saita (55%) followed by DGI-379 (50%), DGI-152 (49%), P-0090 (46%), P-0397 (40%), IR-64 (35%) and P-0088 (32%) respectively.

Leaf soluble sugar (mg g^{-1} dry weight)

The tested genotypes namely NDR-97, Azucena, Saita, DGI-21, DGI-75 and P-0397 showed higher leaf soluble sugar, ranged from 70 to 80 mg g^{-1} dry weight. Flowering stage drought significantly reduced the leaf soluble sugar in all rice genotypes. Extent of reduction were lesser in Vandana (10%) followed by Azucena (12%), P–0326 (15%), NDR-359 (15%), TN-1 and NDR-97 (18%), respectively (Table 1).

Soluble sugar in upper root (mg g^{-1} dry weight)

In general, higher soluble sugar observed in upper portion of the root of tested rice genotypes, except P-0326, Azucena and Saita showed higher soluble sugar in lower part of roots. Extent of reduction in soluble sugar of roots was found at par in all genotypes. Azucena (DT) showed higher reduction in soluble sugar of lower root (48%) whereas ; IR-64 (DS) showed higher reduction in both upper root and lower part of root. Higher reduction in soluble sugar in upper root of stressed rice plants were observed in DSU-18-6 (47%) followed by Vandana (50%).

Soluble sugar in lower root (mg g^{-1} dry weight)

Higher reduction in soluble sugar of lower roots were obtained in IR 64 (52%) followed by DGI-75, TN-1 and Vandana (50%) and DGI-21 and NDR-97 (46%), respectively. Comparative study clearly indicated that tolerant cultivar like Azucena (DT check) and NDR-97, DGI-21 and P-0397 showed lesser reduction in upper root soluble sugar and higher reduction in lower root soluble sugar. In contrast, DSU-18-6 and Vandana showed higher reduction in upper root soluble sugar and lesser reduction in lower root soluble sugar (Table 1).

Reserve carbohydrate

Total shoot starch content (*mg g^{-1} dry wt*)

Maximum reduction in shoot starch content were obtained in IR-64 (65%) followed by DGI-0379 (60%) Saita and P-0326 (58% approx.) and TN-1 (50% approx.), respectively over control. NDR-97, Moroberekan, NDR-359, Vandana, Azucena and DSU-18-6 showed only 7.44, 9.77, 12.65 and 22.6% reduction respectively, when plants were exposed to drought at flowering stage. Moreover, Higher starch content of shoot at the end of drought stress was obtained in NDR-97 (180.17 mg g^{-1} dry wt.) followed by Moroberekan (143 mg g^{-1} dry wt.).

Leaf starch content (*mg g^{-1} dry weight*)

Higher leaf starch content were obtained in P-0090 (53) followed by Azucena (47.17), Vandana (46.17) DGI-75 (44.67) and DSU-18-6 (40.0), However, maximum reduction in leaf starch in stressed plants were obtained in P-0397 (45.54) followed by IR-64 (42.74%) and DGI-75 (38.95%) respectively. Minimum reduction were obtained in DGI-138 (11.75%) and P-0090 (12.14%).

Upper root starch content (*mg g^{-1} dry wt*)

Large genotypic variability's were observed at flowering stage drought stress. Drought significantly reduced the starch content of upper root. NDR-359, DGI-379, Saita, P-0326, DGI-152 and P-0397 showed more than 50% reduction in upper root starch content in stressed condition. Drastic reduction in upper root starch content were obtained in NDR-359 (73%) followed by DGI-379 (55%), respectively.

Lower root starch content (*mg g^{-1} dry wt*)

Similarly significant reduction in root starch content were obtained DGI-75 (63.63%) followed by Saita, P-0397 and P-0090 (55.55%) over irrigated control. Distinct variability was also observed among tested rice genotypes / cultivars. In general varieties i.e. NDR-97 (0.00%), Vandana (10.00%) and NDR-359 (12.50%) showed very less reduction in lower root starch content. However / cultivar like Moroberekan and TN-1 (38.33%) showed moderate reduction including IR-64 susceptible check.

Superoxide dismutase activity (*enzyme unit g^{-1} fresh weight*)

SOD activity was significantly increased when plants exposed to drought stress. Linear increased in SOD activity were obtained after re-watering in all tested rice genotypes / cultivars. Maximum SOD activity was observed at 3rd day re-watering in all rice genotypes. Extent of increase in SOD activity were higher in DGI-138 and DSU-18-6 (120%) followed by DGI-21 (118%). Minimum SOD activity in leaves were obtained in susceptible cultivars like Saita, P-0090, P-0397, DGI-152, DGI-397 showed only 2 to 3 fold higher SOD activity over control at 1st day re-watering, Furthermore, maximum SOD activity in leaves were observed on 3rd day re-watering in all tested genotypes, except DGI-379, Saita, P-0397 and P-0090.

α-amylase (*enzyme unit g^{-1} fresh weight*)

Two week exposure of drought at flowering stage significantly increased the α-amylase activity in all cultivars. Extent of increase were higher in DSU-18-6 (47%) followed by Saita (44%) over control. Least increase in α-amylase activity were obtained in Azucena (9.66%) followed by P-0326 (11%).

Nitrate reductase (μ*M* NO^-_2 *produce* g^{-1} *fresh wt* h^{-1})

Higher nitrate reductase activity were recorded in P-0397 and IR-64 (180.5), rest genotypes showed at par in E_1. Flowering stage drought significantly reduced / increased the leaf NRase activity among all tested rice genotypes / cultivars. Drastic reduction in NRase activity were obtained in P-0088 (55%) and Saita (53%) followed by P-0090 (51%) and TN-1 (50%). Genotypes NDR-97, NDR-359, Azucena still maintained higher NRase activity in leaf of stressed plant. Assimilate accumulated prior to flowering are of paramount importance when plant experience drought at later stage. We hypothesized that grain yields of rice crops drought stressed at flowering, directly related to reserve food materials [15] and the ability of the plant to translocate those assimilate. Translocated -CHO for grain growth is supported by apparent contribution rate (ACR) and value of ACR was higher in stressed plant. Grain yield was significantly correlated with ACR ($r = 0.78$), this result indicated that under several water deficits at flowering stage, grain growth depend of previously accumulated assimilates as was also observed by Chaturvedi et al. [16]. Furthermore, secondary traits like leaf water potential showed higher correlation values with leaf starch ($r = 0.47$), shoot soluble sugar ($r = 0.71$) and leaf soluble sugar ($r = 0.48$) which might be indirectly help to minimize the effect of water deficit on spikelet sterility [17]. The relative importance of translocation and supply CHO to grain increases when plant stress develops [18]. Among the cultivar tested here IR-64, NDR-97, Vandana, Azucena, Moroborokan and DHLs had at par ATR value in irrigated control but generally had more CHO coupled with high ATR and ACR under drought. Moreover, spikelet sterility at flowering stage water deficit is the great concern to limit the grain yield of rice in upland ecosystem. In present investigation variation in soluble sugar is considered to be major determinant of water potential. Strong negative regression coefficient ($R^2 = 0.51$) were obtained between leaf water potential and shoot soluble sugar. Accumulated sugar in plant water relation serve to sustained turgor and cellular dehydration. Ultimately these traits are important to minimize the spikelet sterility. In contrast we found week correlation between proline and LWP as well spikelet-sterility. Although, higher Nitrate reductase were observed in all genotypes at the end of drought stress and higher correlation coefficient (r=0.58) was obtained with grain yield. It seems that higher NR active at the end of drought delayed the leaf senescence, which helps to sustain photosynthesis during stress. Furthermore, linear increase in SOD (antioxidant) was observed after re-watering and stressed plants. The increased in antioxidant activity is believed to be associated with recovery growth.

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