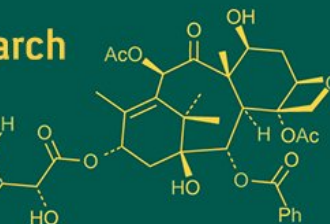
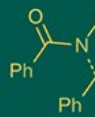


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2026): 5.29
 IJABR 2026; SP-10(9): 847-853
www.biochemjournal.com
 Received: 01-08-2026
 Accepted: 31-08-2026

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Improvement of biomolecules and antioxidants in rice leaves through imino acid

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9Sk.9920>

Abstract

Rice, second important staple food in world and it is a valuable source of complex carbohydrates with essential fibers and minerals. Drought is one of the major environmental constraints for productivity of rice. The physiological and biochemical traits of rice are adversely impacted by drought stress, severely affecting its production. Priming is presowing hydration treatment improves the morphological and biochemical characters. Proline as imino acid influence major reactive oxygen species scavenging and enhance the antioxidant enzyme activities during drought. Thus, this study aims combination of adaptive traits under drought stress between two rice genotypes viz., R 2307-73-1-38-1 and R-RF-212 at the vegetative stage associated with drought tolerance. Total five treatments (T1-control, T2-hydropriming, T3-proline priming (40 ppm), T4-drought stress and T5-T3+T4) were given to both rice genotypes under controlled condition. The results showed that genotype R 2307-73-1-38-1 is registered with higher moisture (70.23 %), relative water content (71.51 %), chlorophyll (1.73 mg/g), proline (49.45 mg/g), total soluble sugars (2.27 %), phenol (670.56 mg/100g) and protein (40.55 mg/g), while decreased membrane injury (15.53 %) and malondialdehyde (0.46 μ mol/g). Drought stress showed adverse effect on moisture content, relative water content and protein content by decreased amount while increased membrane injury, malondialdehyde content and peroxidase activity. The various biomolecules such as total soluble sugars, phenol, protein, proline as well as an antioxidant enzymes were improved by proline priming under drought conditions. Thus, present study showed that proline priming (40 ppm) effectively alleviated the adverse effects of drought stress in rice.

Keywords: Rice, proline, drought stress, priming, biochemical characters, antioxidant enzymes

1. Introduction

Rice (*Oryza sativa* L.) is the cereal crop belonging from *Poaceae* family. Rice is one of the most important food crops of India. Major share of rice is cultivated during Kharif season. Rice is also a valuable source of complex carbohydrates, which is the best source of energy. The major carbohydrates of rice are starch (72-75 %). Rice contains protein (6.8 %), moisture (13.7 %), fat (0.5 %), fiber (0.2 %) and mineral (0.2 %) which can be provide energy to human body (Umadevi *et al.*, 2012) [27]. Drought is one of the major environmental constraints on plant growth and crop productivity (Chaves and Oliveira, 2004) [5]. The availability of moisture during the period of cropping is the key factor influencing growth and yield (Dhruve and Vakharia, 2008) [7]. Drought can harmful to quality and quantity of field crops. Rice is a crop that is known to be sensitive to drought because of its short root system, quick stomatal closure, and low water stress tolerance (Hirasawa, 1999) [13]. Seed priming is basically a presowing controlled seed hydration treatment in which seeds are soaked in an osmotic solution or solid carrier with low matrix potential that allows protrusion through the seed coat. The seed priming techniques improve the germination rate, uniformity of germination and sometimes greater total germination percentage in crops (Hussain *et al.*, 2006) [15]. Proline is non-essential amino acid contains a secondary amine group, an imine, so called as imino acid. It plays a significant role in plant enhancements, flowering, pollen development, embryo development, and leaf expansion. With stress, an increase in proline quantity typically occurs within the cytosol, resulting in an adjustment in osmotic balance (Mushtaq *et al.*, 2025) [22]. Exogenous application of proline can influence the atmospheric nitrogen fixation, ROS scavenging as well as enhance the antioxidant enzyme activities.

Proline priming predominantly leads to the suppression of the adverse effects of abiotic stresses on plants, while it may also contribute to the activation of mechanisms leading to the induction of stress memory. Exogenous application of proline is a potential technique to prevent the harmful effects of drought on plant growth and other physiological traits (Pecka *et al.*, 2025) [23].

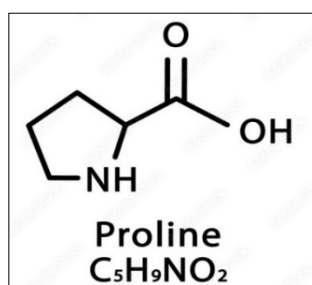


Fig 1: Proline structure

2. Materials and Methods

The experiment was carried out at Department of Biochemistry and Physiology, Anand Agricultural University, Anand. Two rice genotypes R 2307-73-1-38-1 (G1) and R-RF-212 (G2) used as an experimental material. The seed of resistant genotype (R 2307-73-1-38-1) and susceptible genotype (R-RF-212) were soaked in proline (40 ppm) for 12 hrs. Then these seeds were sowed in pots. Total five treatments (T1-control, T2-hydropriming, T3-proline priming (40 ppm), T4-drought stress and T5-T3+T4) were given to both rice genotypes under controlled condition. We used factorial completely randomized design (FCRD) as an experimental design. At 30 DAS the drought stress initiated in natural condition. At 45 DAS, plant showed visible symptoms of stress. Leaf was analyzed at 45 DAS for its various biochemical characters viz., moisture, relative water content, membrane injury, chlorophyll, total soluble sugars, protein, proline, antioxidant enzyme activity (catalase, superoxide dismutase and peroxidase activity) and total phenol content.

Moisture

Moisture content was estimated as per procedure developed by AOAC (1970) [1].

Relative water content

The method described by Weatherley (1950) [30] was used for the determination of relative water content of rice genotypes under control as well as water deficit stress conditions.

Membrane injury (%)

Membrane injury (MI) was determined by using the method of Suvillan (1971) [26] by measuring electrical conductivity at 40 °C and 100 °C.

Chlorophyll content

Chlorophyll content of rice leaf at vegetative stage was estimated as per DMSO method described by Hiscox and Israelstam (1979) [14].

Total soluble sugars

Total soluble sugars were determined using the phenol-sulphuric acid method as described by Dubois *et al.* (1956) [9].

Protein (mg/g)

Protein content was determined by the method developed by Lowry *et al.* (1951) [19].

Proline

The proline content in the rice leaves samples was analyzed at vegetative stage by the sulfosalicylic acid method suggested by Bates *et al.* (1973) [4].

Malondialdehyde

Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid (TBA) reaction (Heath and Packer, 1968) [12].

Enzymatic antioxidants

Catalase, Superoxide dismutase and Peroxidase

Enzyme assay of catalase, superoxide dismutase and peroxidase done as suggested method by Dhruv *et al.* (2021) [6].

Total Phenol

Total phenols were estimated from rice leaf by Folin-Ciocalteu reagent method as described by Dhruv *et al.* (2021) [6].

3. Result and discussion

3.1 Moisture (%)

Moisture refers to the amount of water present in a substance. It is fundamental for structure, metabolism, nutrient movement and survival and this character is most important factor for growth and development under drought stress conditions.

The significant difference of moisture content was recorded in leaf of rice genotypes (Table 1). The significant maximum and minimum moisture content was found for genotypes R 2307-73-1-38-1 (70.23 %) and R-RF-212 (62.87 %), respectively. Among the treatments significant difference was observed. Significantly higher moisture content was recorded for treatment T3 (77.38 %) which was at par with G₁T₅ (73.04 %) and G₂T₃ (76.35 %). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 1). Interaction effect indicated that maximum moisture content was observed in G₁T₃ (78.40 %) which was at par with G₁T₅ (73.04 %). Minimum moisture content was observed in G₂T₄ (51.60 %) which was at par with G₂T₅ (57.00 %). Proline priming treatment having more ability under drought stress and it may be a useful indicator of the drought-tolerance potential of rice.

Dien *et al.* (2019) [8] studied proline and carbohydrate metabolism in rice varieties under various drought and recovery conditions and found that under drought stress condition moisture content decreased as compared to control. From the above findings it was concluded that the priming effect of proline enhanced the moisture content significantly as compared with no proline treatment in rice.

3.2 Relative water content (%)

Relative water content (RWC) reflects the plant's internal water status and its ability to retain hydration under different treatments.

The data presented in Table 1 indicated that relative water content was varied among genotypes, treatments and their

interactions. Significantly maximum relative water content was observed in genotype R 2307-73-1-38-1 (71.51 %) and minimum relative water content was observed in genotype R-RF-212 (38.41 %). Among treatment, higher relative water content was recorded in treatment T3 (68.05), while lower relative water content was recorded in treatment T4 (37.18 %). While, treatment T1 (58.82 %) and T2 (60.23 %) found at par each other. The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 1). Maximum relative water content was found in G_1T_3 (81.49 %), while minimum relative water content was found in G_2T_4 (18.58 %). Interaction effect of G_1T_1 and G_1T_2 are found at par each other. Proline priming with highest relative water content among all treatments indicated that the proline accumulation plays a key role in acts as an osmolyte, helping cells retain water, stabilizes proteins and membranes and improves osmotic adjustment under mild stress. This leads to superior water retention and higher RWC. This result was accordance with Gaballah *et al.* (2020) [10]. They investigated genetic diversity of selected rice genotypes under water stress conditions and found maximum relative water content in control and minimum relative water content in drought condition. Thus, our results suggested that the genotype R 2307-73-1-38-1 and treatment T3 gave better performance for relative water content.

3.3 Membrane injury (%)

Membrane injury (%) reflects the extent of cellular damage, mainly caused by oxidative stress and loss of membrane stability.

Membrane injury was significantly varied among genotypes. Maximum and minimum membrane injury found in genotype R-RF-212 (25.27 %) and genotype R 2307-73-1-38-1 (15.53 %), respectively. Significant difference shows in membrane injury among different treatments. Maximum membrane injury showed in treatment T4 (37.50 %), while minimum membrane injury showed in treatment T3 (11.17 %). The maximum membrane injury was recorded in treatment T4 (37.50 %), which was followed by T5 (24.50 %) and T1 (15.50 %). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 1). In interaction higher membrane injury was recorded in G_2T_4 (46.00 %) while lower membrane injury was recorded in G_1T_3 (8.33 %). The interaction effect shows that genotype and treatment together influence the membrane injury. The highest membrane injury was found in drought stress treatment due to increased ROS production, lipid peroxidation damages membranes, loss of membrane permeability control. Thus, genotype R-RF-212 is much more affected than R 2307-73-1-38-1 due to poor defense mechanisms. Proline reduces damage by enhancing osmotic

balance, protecting membrane lipids and reducing oxidative stress.

This result was accordance with Abid *et al.* (2018) [12]. They have concluded from their experiment that due to drought stress membrane injury increased in wheat.

3.4 Chlorophyll (mg/g)

Chlorophyll plays a very important role in helping plants survive and continue producing energy.

The significant difference of total chlorophyll content was recorded between rice genotypes, treatments and its interactions (Table 1). The significant maximum and minimum total chlorophyll content was found in genotypes R 2307-73-1-38-1 (1.73 mg/g) and R-RF-212 (1.16 mg/g), respectively. Among treatments higher total chlorophyll content was recorded in treatment T3 (2.12 mg/g) while lower total chlorophyll content was recorded in treatment T4 (1.14 mg/g). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 1). Maximum chlorophyll content was found in G_1T_3 (2.25 mg/g), while minimum chlorophyll content was found in G_2T_1 (0.66 mg/g) which was at par with G_2T_4 (0.70 mg/g). Proline may protect chlorophyll from degradation, maintain photosynthetic apparatus under stress.

Similar finding observed by Hanif *et al.* (2022) [11]. They found out that exogenous application of proline (30 mM) improves the chlorophyll content in rice. Our results were also accordance with Khan *et al.* (2017) [17].

3.5 Total soluble sugars (%)

Total soluble sugars are key osmolytes that help plants cope with stress by maintaining osmotic balance, protecting cellular structures and serving as energy reserves.

Significant different of total soluble sugars was observed in genotypes (Table 1). Significantly maximum and minimum total soluble sugars found in R 2307-73-1-38-1 (2.27 %) and R-RF-212 (1.94 %), respectively. Among the treatments (Table 1), higher total soluble sugar content was recorded in T5 (2.49 %). Significantly the lowest sugar content was recorded in treatment T1 (1.65 %). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 1). Maximum total soluble sugars content was recorded in G_1T_5 (2.56 %) which was at par with G_2T_5 (2.42 %). Minimum total soluble sugars content was recorded in G_2T_1 (1.34 %). Proline priming treatment enhances metabolic activity and promotes synthesis of sugars.

Similar finding was recorded by Kumar *et al.* (2014) [18]. They found under drought condition sugars content increased compared with control. They found range of total soluble sugars content from 0.14 % to 2.78 %. Our results also accordance with Barik *et al.* (2025) [3] and Xu *et al.* (2015) [31].

Table 1: Influence of priming and drought stress on moisture, relative water content, membrane injury, chlorophyll, total soluble sugars and protein in leaves of rice

Treatments	Moisture (%)			Relative water content (%)			Membrane injury (%)		
	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T
T1	66.26	63.91	65.09	75.32	42.32	58.82	12.00	19.00	15.50
T2	69.21	65.49	67.35	77.29	43.17	60.23	10.33	16.33	13.33
T3	78.40	76.35	77.38	81.49	54.61	68.05	8.33	14.00	11.17
T4	64.27	51.60	57.94	55.78	18.58	37.18	29.00	46.00	37.50
T5	73.04	57.00	65.02	67.66	33.36	50.51	18.00	31.00	24.50
Mean G	70.23	62.87		71.51	38.41		15.53	25.27	
	G	T	G X T	G	T	G X T	G	T	G X T
S. Em ±	0.96	1.52	2.15	0.61	0.96	1.36	0.23	0.37	0.52
CD at 5 %	2.83	4.48	6.34	1.79	2.83	4.00	0.68	1.08	1.52
CV %	5.59			4.27			4.38		
Treatments	Chlorophyll (mg/g)			Total soluble sugars (%)			Protein (mg/g)		
	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T
T1	1.12	0.66	0.89	1.96	1.34	1.65	35.27	34.04	34.66
T2	1.87	1.43	1.65	2.17	1.78	1.97	40.69	38.00	39.35
T3	2.25	1.99	2.12	2.26	2.03	2.15	42.97	41.22	42.10
T4	1.57	0.70	1.14	2.40	2.15	2.28	33.82	30.32	32.07
T5	1.85	1.03	1.45	2.56	2.42	2.49	50.00	42.93	46.47
Mean G	1.73	1.16		2.27	1.94		40.55	37.30	
	G	T	G X T	G	T	G X T	G	T	G X T
S. Em ±	0.03	0.05	0.07	0.02	0.03	0.04	0.30	0.48	0.67
CD at 5 %	0.09	0.14	0.20	0.05	0.08	0.12	0.89	1.41	1.99
CV %	8.19			3.25			3.00		

3.6 Protein (mg/g)

True protein content reflects the balance between protein synthesis and degradation.

True protein found varied among genotypes (Table 1). The significant maximum and minimum true protein content were found in genotypes R 2307-73-1-38-1 (40.55 mg/g) and R-RF-212 (37.30 mg/g), respectively. Significant difference observed in true protein content among various treatments. Higher true protein content recorded in T5 (46.47 mg/g) while lower true protein content recorded in T4 (32.07 mg/g). The interaction effect was found to be significant which indicated that the genotype and treatment factor are dependent on each other (Table 1). Significantly the highest protein content was found in G₁T₅ (50.00 mg/g) while the lowest protein content was found in G₂T₄ (30.32 mg/g). Protein content is negatively affected by stress but improved by priming.

3.7 Proline (mg/g)

Proline content is a key biochemical indicator of plant response to stress, especially drought. It mainly functions in osmotic adjustment, stress protection and stabilization of cellular structures.

Among the two genotypes, maximum and minimum proline content was observed in genotypes R 2307-73-1-38-1 (49.45 mg/g) and R-RF-212 (39.91 mg/g), respectively (Table 2). Among the treatments (Table 4.8), the highest proline was observed in T5 (58.64 mg/g) followed by T4 (48.48 mg/g) and T3 (43.05 mg/g). Significantly the lowest proline was recorded in T1 (35.30 mg/g) which was at par with T2 (37.92 mg/g). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 2). Maximum and minimum proline was observed in G₁T₅ (67.99 mg g⁻¹) and

R-RF-212 under treatment G₂T₁ (32.19 mg g⁻¹), respectively. Proline priming directly increases internal proline levels which enhances plant's biochemical readiness for stress. Drought induces osmotic stress which triggers proline synthesis, protection against dehydration and oxidative damage.

Lum *et al.* (2014) [20] examined effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice and collected data on proline content and reported that increase of proline content in drought-tolerant variety Pulot Wangi, while lesser increased of proline content in drought sensitive variety Kusam. Overall range of proline content was 19.34 mg/g to 581.78 mg/g.

3.8 Malondialdehyde (μ mol/g)

Malondialdehyde (MDA) content is a widely used indicator of lipid peroxidation and membrane damage. Higher MDA means greater oxidative stress and cell membrane injury. Lower MDA content is an indication of strong antioxidant defence system, better scavenging of reactive oxygen species and reduced lipid peroxidation.

The significant difference of MDA content was observed among genotypes (Table 2). Maximum and minimum MDA content was recorded for genotypes R-RF-212 (0.81 μ mol/g) and R 2307-73-1-38-1 (0.46 μ mol/g), respectively. Treatments significantly influenced MDA levels (Table 2). Significantly higher MDA content was observed in T4 (0.82 μ mol/g) while lower MDA content was observed in T2 (0.51 μ mol/g). The interaction effect was found significant which indicated that genotype and treatment factor is dependent from each other (Table 4.9). Maximum MDA content was recorded in G₂T₄ (0.95 μ mol/g) which was at par with G₂T₅ (0.93 μ mol/g). Minimum MDA content was recorded in G₁T₃ (0.32 μ mol/g). Proline priming protects

membranes, reduces ROS and lipid peroxidation.

Our results are accordance with Mahatthanaphacharakun and Taratima (2025) ^[21]. They studied comparative effect of drought stress on growth and physiological performance of three different rice cultivars and found malondialdehyde content was increased in drought stress condition and decreased in control condition.

3.9 Catalase ($\mu\text{M H}_2\text{O}_2/\text{min/g}$)

Catalase is an antioxidant enzyme that breaks down hydrogen peroxide (H_2O_2), so its activity increases when plants experience stress that generates reactive oxygen species. Generally, with increasing stress activity of catalase enzyme increased for H_2O_2 breakdown.

The significant difference of catalase activity found between genotypes (Table 2). Maximum ($36.71 \mu\text{M H}_2\text{O}_2/\text{min/g}$) and minimum ($33.67 \mu\text{M H}_2\text{O}_2/\text{min/g}$) catalase activity recorded for R-RF-212 and R 2307-73-1-38-1, respectively. Among treatments varied catalase activity observed. Significant maximum catalase activity observed in treatment T5 ($56.43 \mu\text{M H}_2\text{O}_2/\text{min/g}$) and minimum in treatment T1 ($21.07 \mu\text{M H}_2\text{O}_2/\text{min/g}$). The interaction effect was found to be significant which indicated that the genotype and treatment factor are dependent on each other (Table 2). Maximum and minimum catalase activity was recorded in interaction G_2T_5 ($60.30 \mu\text{M H}_2\text{O}_2/\text{min/g}$) and G_1T_1 ($20.17 \mu\text{M H}_2\text{O}_2/\text{min/g}$), respectively. The interaction effect indicates that catalase activity is affected by genotype and treatment together. Similar result observed by Kamarudin *et al.* (2018) ^[16] and Wang *et al.* (2019) ^[29]. They state that catalase activity found higher in drought stress condition in rice.

3.10 Superoxide dismutase (% scavenge activity)

Superoxide dismutase (SOD) is the first line of defence against oxidative stress, converting superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2).

Significant SOD activity difference recorded between the genotypes (Table 2). Superoxide dismutase activity was observed to be maximum (55.76 % scavenge activity) and minimum (50.55 % scavenge activity) for R 2307-73-1-38-1 and R-RF-212, respectively. Among treatments varied SOD activity observed. Significant maximum SOD activity observed in treatment T5 (64.72 % scavenge activity) and minimum in treatment T1 (42.68 % scavenge activity). The non-significant interaction effect suggested that the treatment component and genotype are unrelated to one another (Table 2).

Saha *et al.* (2020) ^[25] examined on effects of drought stress on pigment and protein contents and antioxidant enzyme

activities in rice. They state that SOD activity found minimum in control condition while higher in drought stress condition.

3.11 Peroxidase ($\Delta\text{OD}/\text{min/g}$)

Peroxidase (POX) is another key antioxidant enzyme that helps detoxify hydrogen peroxide (H_2O_2) by converting it into water using various substrates.

Influence of proline on peroxidase activity displayed in Table 2. The significant maximum and minimum peroxidase activities were found in genotypes R 2307-73-1-38-1 ($1.92 \Delta\text{OD}/\text{min/g}$) and R-RF-212 ($1.55 \Delta\text{OD}/\text{min/g}$), respectively. Among treatments varied POX activity observed (Table 4.12). Significant maximum POD activity observed in treatment T4 ($2.25 \Delta\text{OD}/\text{min/g}$) and minimum in treatment T1 ($1.35 \Delta\text{OD}/\text{min/g}$) which was at par with T2 ($1.49 \Delta\text{OD}/\text{min/g}$). Treatment T3 recorded $1.79 \Delta\text{OD}/\text{min/g}$ POX activity which was at par with T5 ($1.78 \Delta\text{OD}/\text{min/g}$). The significant interaction effect suggested that the treatment component and genotype are dependent to each other (Table 2). Maximum POX activity was found in G_1T_4 ($2.45 \Delta\text{OD}/\text{min/g}$) while minimum POX activity was found in G_2T_1 ($1.10 \Delta\text{OD}/\text{min/g}$), which was at par with G_2T_2 ($1.28 \Delta\text{OD}/\text{min/g}$). The interaction effect demonstrates that POX activity was influenced by both genotype and treatment.

Our result accordance with Wang *et al.* (2019) ^[29]. They state that highest POX activity observed in drought stress as compared to control. As days of stress increased POX activity increased in rice genotypes.

3.12 Total Phenol (mg/100g)

Total phenols are important secondary metabolites involved in plant defense. They act as antioxidants, helping plants tolerate stress by scavenging harmful molecules and protecting cellular structures.

Among genotypes, the significant maximum and minimum total phenol content was recorded for genotypes R 2307-73-1-38-1 ($670.56 \text{ mg}/100\text{g}$) and R-RF-212 ($506.52 \text{ mg}/100\text{g}$), respectively. Among treatments, significantly highest phenol content (Table 4.13) observed in treatment T5 ($798.30 \text{ mg}/100\text{g}$). While, lower phenol content observed in treatment T1 ($311.93 \text{ mg}/100\text{g}$). The non-significant interaction effect suggested that the treatment component and genotype are unrelated to one another (Table 2).

Quan *et al.* (2016) ^[24] studied on involvement of secondary metabolites in response to drought stress of rice and found that phenol content was higher in drought stress condition as compared to control.

Table 2: Influence of priming and drought stress on moisture, relative water content, membrane injury, chlorophyll, total soluble sugars and protein in leaves of rice

	Proline (mg/g)			Malondialdehyde (μ mol/g)			Catalase activity (μ M H ₂ O ₂ /min/g)		
Treatments	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T
T1	38.42	32.19	35.30	0.45	0.71	0.58	20.17	21.97	21.07
T2	41.24	34.60	37.92	0.43	0.59	0.51	25.00	23.60	24.30
T3	47.07	39.03	43.05	0.32	0.85	0.59	30.10	27.17	28.63
T4	52.50	44.46	48.48	0.68	0.95	0.82	40.50	50.50	45.50
T5	67.99	49.29	58.64	0.42	0.93	0.67	52.57	60.30	56.43
Mean G	49.45	39.91		0.46	0.81		33.67	36.71	
	G	T	G X T	G	T	G X T	G	T	G X T
S. Em $\pm$	0.59	0.94	1.33	0.01	0.02	0.03	0.14	0.21	0.30
CD at 5 %	1.75	2.76	3.91	0.04	0.06	0.09	0.39	0.63	0.89
CV %	5.14			8.16			1.49		
	Superoxide dismutase (% scavenge activity)			Peroxidase (Δ OD/min/g)			Total phenol (mg/100g)		
Treatments	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T	R 2307-73-1-38-1	R-RF-212	Mean T
T1	47.57	37.80	42.68	1.61	1.10	1.35	387.03	236.83	311.93
T2	49.57	44.53	47.05	1.70	1.28	1.49	535.34	338.53	436.93
T3	54.97	51.57	53.27	1.81	1.78	1.79	743.92	586.19	665.05
T4	60.00	56.13	58.07	2.45	2.05	2.25	798.53	662.46	730.50
T5	66.70	62.73	64.72	2.01	1.54	1.78	887.99	708.60	798.30
Mean G	55.76	50.55		1.92	1.55		670.56	506.52	
	G	T	G X T	G	T	G X T	G	T	G X T
S. Em $\pm$	0.70	1.10	1.56	0.03	0.05	0.07	12.54	19.38	28.04
CD at 5 %	2.06	3.26	NS	0.10	0.15	0.21	36.99	58.50	NS
CV %	5.09			7.16			8.25		

Conclusion

Overall, the results clearly demonstrated that drought stress adversely affected in leaf of rice genotypes. However, seed priming with 40 ppm proline effectively mitigated drought-induced damage by improving water status, chlorophyll content, osmolyte accumulation, antioxidant enzyme activities and membrane injury. The drought-resistant genotype R 2307-73-1-38-1 consistently outperformed the susceptible genotype R-RF-212, indicating superior drought adaptation mechanisms.

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