Indian Journal of Biochemistry & Biophysics
Vol. 57, February 2020, pp. 101-108

Effect of fungal biocontrol agents on enhancement of drought stress tolerance in rice (*Oryza sativa* L.)

Bishnu Maya Bashyal[1]*, Najam Waris Zaidi[2], Uma Shankar Singh[2] & Rashmi Aggarwal[1]

[1]Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi-110 012, India

[2]International Rice Research Institute, New Delhi-110 008, India

Received 15 June 2019; *revised 14 February 2020*

Rice is the staple food crop for about half of the population of the world. Drought is a major stress limiting factor of this crop. In the recent years, biocontrol agents like *Trichoderma* spp. have become popular as plant growth promoter and shown to enhance drought tolerance in plants. Therefore, present investigation investigation was undertaken to evaluate the different biocontrol agents *i.e. Trichoderma harzinum* 1, *Trichoderma harzianum* 2, *Chaetomium globosum* and *Talaromyces flavus* against the drought in resistant (DRR 42 and Sahbhagi Dhan) and susceptible (IR 64) varieties of rice. Prior to sowing seeds were bioprimed separately with each isolates of *Trichoderma harzianum* @ 10g/kg and were sown in pots. Drought treatment of 4 days, 7 days, 10 days and 13 days were given as per the standard protocol. Biocontrol agent *Trichoderma harzianum* (T2) was observed most effective for drought tolerance followed by *Chaetomium globosum*. After 13 days of drought treatment minimum wilting (20%) was observed in Sahbhagi Dhan treated with *Trichoderma harzianum* 2. Four and 10 days drought stressed plants were subjected to different biochemical analysis. Significantly positive correlation (r = 0.91) was observed between wilting and Malondialdehyde (MDA) content. While negative correlation (r = −0.67) was observed between wilting and average plant weight. Study suggested that *Trichoderma harzianum* 2 treatment during drought stress in rice plants can delay the drought upto 3-5 days.

Keywords: Biocontrol, *Cheatomium globosum*, Drought, Malondialdehyde (MDA), *Trichoderma*

Rice is the one of the main staple food for one-third of people worldwide[1], provides up to 80% of the individuals daily calories[2]. However, rice is considered one of the most susceptible plants for biotic and abiotic stress and drought is recognized as the primary constraint to rainfed rice production[3]. In rice, extent of drought tolerance varies between species to species and within species also. Drought susceptibility in rice is mainly due to its small root system, swift stomatal closure and thin cuticular wax[4]. Water deficit at early vegetative growth intensely affects the establishment of rice crops.

Drought affects plant–water potential and turgor, which interferes the normal functions of the plant[5] changing its physiological as well as morphological traits[6]. Most common affected growth parameters affected by drought stress are fresh weight and plant growth[7] and also influences the transport and availability of soil nutrients. The reaction of crop plants to water stress differs significantly at various organizational levels depending upon intensity, duration of stress, plant species and its growth stages. Further, drought as a multidimensional stress, affects at various sub cellular compartment, cell organs and whole plant level[8-9]. Thus, drought negatively affects quantity and quality of growth in plants. Worldwide extensive research is being carried out to develop strategies to cope with drought stress through development of drought tolerant varieties, shifting the crop calendars, resource management practices[10] *etc*. and most of these technologies are cost-intensive. Recent studies indicated that microorganisms can also help plants to cope with drought stress in rice[11-12]. Use of bio-agents such as *Trichoderma* is an effective and easily adaptive strategy in various plants[13].

Plants adopt different mechanisms to improve crop productivity under drought stress conditions[14]. Bio-agents such as drought tolerant *Trichoderma* are effective measures to control water stress and can be easily applied in field conditions[15-16]. The role of *Trichoderma harzianum* in plant growth, nutrient management and biocontrol activity is very well established. These beneficial microorganisms colonize the rhizosphere/endo-rhizosphere of plants

*Correspondence:
E-mail: bishnumayabashyal@gmail.com

and promote growth of the plants through various direct and indirect mechanisms[17]. Its colonization modulates the endogenous plant hormones, plant enzymes, antioxidants compatible solutes and compounds like phytoalexins and phenols level to confer drought tolerance[13]. It also improves the root growth and water-holding capacity of plants[19-20].

Overall, drought tolerance is a multifunctional output of numerous molecular, morphological, and biochemical characters. Further, to improve the efficiency of identified biocontrol agents and develop newer options, a better understanding of the drought tolerance mechanism is of utmost important. Although, the mechanisms by which rice adapts to drought stress have been studied extensively, limited information is available on the biocontrol induced drought tolerance. In this study, efforts were made to identify potential biocontrol agent for the drought tolerance and to elucidate the biochemical mechanism involved in drought stress tolerance in rice.

Materials and Methods

Rice seed and biocontrol agents for the study

Seeds of rice varieties DRR-42 (drought tolerant), Sahbhagi Dhan (drought tolerant) IR-64 (drought susceptible check) and drought tolerant *Trichoderma harzianum* 1 and 2 (IRRI1, IRRI2)[21] were provided by International Rice Research Institute. Other effective biocontrol agents *Cheatomium globosum* (Cg2) and *Talaromyces flavus* (Tf2) were taken from Fungal Molecular Laboratory, Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi-110012[22-23].

Seed treatment

Prior to their use, rice seeds were surface sterilized with 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and dried. Seeds were bioprimed separately with each isolates of *Trichoderma harzianum* 1 (T1) *Trichoderma harzianum* 2 (T2) *Cheatomium globosum* (Cg2) and *Talaromyces flavus* (Tf2) @ 10 g/kg of seeds. After pre-soaking of seeds in sterile distilled water, seeds were coated with powder formulation of biocontrol agents and mixed thoroughly to provide uniform coating.

Maintaining drought stress in plants

Bulk surface soil (0-15 cm) was collected from the research farm of the Indian Agricultural Research Institute, New Delhi. The soil was air dried, mixed thoroughly and passed through 2 mm sieve. Seeds were sown in plastic pots (2.5 kg capacity) filled with 2.0 kg soil and saturated with water holding calibrated tensiometer. Ten plants per pot were maintained for each treatment combinations including control. Here, V1 is IR-64, V2 is DRR42 and V3 is Sahbhagi Dhan. T1 *Trichoderma harzianum* 1 (IRRI1), T2: *Trichoderma harzianum* 2 (IRRI 2); T3: *Cheatomium globosum* (Cg2) and T4: *Talaromyces flavus* (Tf2). Moisture was maintained by applying 100 mL of water per pot in every alternate day until plants attained the age of five weeks and at this point drought treatments was given by altering the water cycle. Watering was stopped for subsequent days for each drought treatment which included 4, 7, 10 and 13 days drought stress (DDS), while control seedlings were continuously watered every alternate day. Drought treatments were given for 4, 7 10 and 13 days and after each day drought stress (DDS) plants were uprooted carefully and washed with distilled water. The root length and shoot length of seedlings were observed and measured manually after each treatment. Subsequent to drought treatment application, observations were recorded on wilting and biochemical and physiological responses of rice plants. Leaf rolling and leaf drying ratings with a score of 0 for non-stress and 9 for stress near permanent wilting point, as described in Standard Evaluation System (SES)[24] of International Rice Research Institute (IRRI).

Hydrogen peroxide content estimation

Hydrogen peroxide content of plant leaves was measured by the method of Alexivea *et al*[25]. Hydrogen peroxide reacts with potassium iodide and absorbance measured by uv spectrophotometer at 390 nm. The reaction mixture contained 0·5 mL trichloroacetic acid (0.1 %) leaf extract supernatant, 0·5 mL potassium phosphate buffer (100 mM, pH- 7.8) and 2 mL potassium iodide solution (1 M *w/v* in double-distilled water) and left for 1 h in darkness for reaction. The blank probe consisted of 0·1 % trichloroacetic acid. The serial dilution of 0.1 % trichloroacetic acid was used for making standard curve which was used for calculating the concentration of hydrogen peroxide.

Proline content estimation

Proline content of plant tissue was determined spectrophotometrically[26]. The 0.5 g plant tissue was homogenized in 10 mL of 3 % (*w/v*) sulfosalicyclic

acid and supernatant collected after centrifugation (12000 g for 10 min). One mL of supernatant was added to the 1 mL acid-ninhdrin (prepared by adding 1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid) and 1 mL glacial acetic acid in the tube and incubated at 100°C for 1 h. The reaction mixture was placed at room temperature. After cooling, 2 mL of toluene was mixed with reaction mixture by pipetting and left for 30 min at room temperature. The reaction mixture was separated into two phases. The absorbance was measured at 520 nm and toluene was used as a blank. Standard curve was made by using proline to know the concentration of proline in samples.

Malondialdehyde (MDA) content

Lipid peroxidation can be measured by the amount of malondialdehyde (MDA), a product of unsaturated fatty acid peroxidation. MDA concentration (mg g^{-1} fresh weight) was estimated by the method of Li *et al*[27].

Leaf membrane stability index

Leaf membrane stability index of plant leaves was measured following the method of Premachandra *et al*[28]. One hundred mg of fresh leaf was added to 10 mL water in test tube and incubated in water bath at 40°C for 30 min. Parallel; one more set of leaves in water was boiled in water bath at 100°C for 30 min. The electric conductivity of leaf solution was recorded by using conductivity bridge meter. Electric conductivity was determined by using the formula:

$$MSI = [1 - (C1/C2)]*100$$

where, C1= conductivity of leaf sample incubated at 40°C; C2= conductivity of leaf sample incubated at 100°C.

β-1,3-glucanase activity assay

β-1,3-glucanase activity was determined by measuring the produced sugar[29]. Laminarin was reacted with β-1,3-glucanase enzyme and produced sugar by using dinitrosalicylic reagent. One mL of enzyme extract was mixed with 1 mL of 2 % laminarin solution and left at 50°C for 60 min in water bath. Three mL of the dinitrosalicylic reagent were added to the reaction mixture and left for heating at 100°C for 5 min. dinitrosalicylic reagent (prepared by adding 1 g of NaOH, of 0.2 g phenol, 0.05 g of sodium sulphite, 18.2 g of sodium potassium tartarate (Rochelle salt) and 1 g of 3-5, dinitrosalicyclic acid and raised the volume 100 mL with distilled water). The tubes were cooled at room temperature and the reaction mixture was diluted 1:10 with water. Absorbance was recorded at 500 nm. β-1,3-glucanase activity was expressed as Δ500 min^{-1} mg $protein^{-1}$.

Results

Evaluation of rice genotypes after different days of drought treatment

Observations were taken for root length, shoot length, average plant weight after each drought treatment. Genotypes were significantly different for the root length. Further, biocontrol agents and interaction of varieties and biocontrol agent was also significant for the root length (Table 1). After 4 days of drought treatment minimum root length was observed for the treatment V1T4 (2.77 cm) followed by V3T1 (6.83 cm). Significant differences were observed for the shoot length in different varieties and biocontrol agents. Maximum shoot length was observed in treatment V2T1 (60.67 cm) followed by V3T4 (56.0 cm). Minimum shoot length was observed for the treatment V1T2 (29.33 cm) followed by V1T3 (33.83 cm). Maximum shoot length was observed for the variety Sahbhagi Dhan. Shoot length was maximum in the *Trichoderma harzianum* (T1) treatments of varieties IR64 and DRR42, whereas, it was maximum for the *Talaromyces flavus* treated plants of Sahbhagi Dhan (Fig. 1). Significant differences were observed in varieties and biocontrol agent for plant weight. Maximum plant weight (average) was observed in the treatment V3T2 (14.42 g) followed by V3T3 (13.45 g), V3T1 (11.71 g) and V2T1 (10.92 g). Minimum average plant weight was observed for the treatment V1T4 (0.70 g) followed by V1T2 (1.0 g). Average plant weight was maximum in the variety Sahbhagi Dhan and it was minimum in the variety IR64.

Similar to the observations taken for 4 days drought stress, observations were taken after 7 days drought stress for root length, shoot length, average plant weight and spectral values (Table 1). Significant differences were observed for different treatments along with varieties. Maximum root length was observed in treatment V1T2 (9.67 cm) followed by V3T3 (9.17 cm) and minimum in V1T4 and V2T2 (6.11 cm). Shoot length varied significantly from being highest in treatment V3T4 (56.3 cm), variety Sahbhagi Dhan, to lowest 30.33 cm in treatment V1T2. Average plant weight was highest in V3T2 (27.06 g) and lowest in V2T4 (1.20 g).

After 10 days of drought stress maximum root length was observed in treatment V2T2 (variety

Table 1 — Effect of biocontrol agents on different rice varieties after different days of drought stress

Treatments*	Drought stress given (days)											
	4 days drought stress			7 days drought stress			10 days drought stress			13 days drought stress		
	Root length (cm)	Shoot length (cm)	Average plant weight (g)	Root length (cm)	Shoot length (cm)	Average plant weight (g)	Root length (cm)	Shoot length (cm)	Average plant weight (g)	Root length (cm)	Shoot length (cm)	Average plant weight (g)
V1T1	7.0	38.2	1.3	7.8	39.0	2.1	10.2	40.0	2.1	11.3	40.3	1.5
V1T2	8.0	29.3	1.0	9.6	30.3	1.5	9.7	35.3	2.0	12.3	35.3	2.0
V1T3	8.8	33.8	1.3	8.8	36.8	1.5	8.5	36.9	4.5	13.0	36.7	2.1
V1T4	2.7	33.9	0.7	6.1	38.5	1.9	6.2	49.7	9.5	6.7	46.0	4.6
V2T1	5.5	60.7	10.9	6.1	45.5	10.2	14.2	56.0	16.2	16.3	57.0	10.0
V2T2	11.8	40.2	10.1	12.3	54.7	10.2	14.3	49.7	10.3	8.7	46.7	8.4
V2T3	8.6	34.2	1.6	9.0	56.0	6.2	9.3	40.7	1.6	14.3	35.7	10.5
V2T4	7.6	43.2	1.1	8.0	38.3	1.2	12.3	40.0	1.0	8.3	36.0	0.6
V3T1	6.8	37.8	11.7	6.6	38.1	12.7	8.3	57.3	17.5	10.7	54. 3	0.6
V3T2	7.6	51.7	14.4	8.5	40.2	27.1	8.5	55.3	19.7	6.3	39.3	10.2
V3T3	8.3	44.5	13.4	9.2	41.3	13.8	10.0	58.3	12.7	10.3	59.0	10.3
V3T4	5.6	56.0	8.6	7.7	56.3	15.5	7.7	52.0	12.0	12.0	49.0	9.7
V1T0	8.0	34.5	1.2	9.2	34.7	2.5	11.3	34.7	9.5	12.3	34.5	11.0
V2T0	10.2	32.8	7.0	10.1	33.5	8.0	11.5	34.0	9.0	12.3	35.3	9.5
V3T0	11.9	38.7	10.5	10.8	39.0	22.6	12. 7	36.3	21.0	13.0	36.67	10.0
C.D.												
Factor (V)	2.3	4.5	0.3	2.4	5.6	0.5	1.1	2.4	0.5	N/A	5.1	1.0
Factor (T)	2.7	5.2	0.3	4.8	6.5	0.6	1.3	2.8	0.6	N/A	5.9	1.2
Factor (V XT)	4.7	9.0	0.5	2.4	11.5	0.9	2.3	4. 9	1.2	6.2	10.1	2.0

*V1 is IR-64, V2 is DRR42 and V3 is Sahbhagi Dhan. T1 *Trichoderma harzianum* 1 (IRRI1), T2: *Trichoderma harzianum* 2 (IRRI 2); T3: *Cheatomium globosum* (Cg2) and T4: *Talaromyces flavus* (Tf2).

Fig. 1 — Rice variety Sahbhagi Dhan showing wilting after 13 days of drought stress

DRR42, 14.33 cm) and minimum in treatment V1T4 (6.17 cm) (Fig. 2). Highest shoot length (58.33 cm) was observed in treatment V3T3 (variety Sahbhagi dhan). The lowest shoot length and average plant weight was observed in variety IR64 in treatment V1T2 (35.33 cm and 2.0 g, respectively).

After 13 days of drought stress, very few treatments could survive and most of the others dried or withered with no recovery afterward (Fig. 1). This highly influenced the plant weight and root-shoot length which decreased compared to other treatments. Root length was lowest (6.67 cm) in treatment V1T4 (variety IR64), and highest (16.33 cm) in treatment V2T1 (variety DRR42). Shoot length varied from highest being 59.0 cm in treatment V3T3 (variety Sahbhagi Dhan) and lowest 35.33 cm in treatment V1T2 (variety IR64). Average plant weight went as low as 0.63 g in treatment V2T4 and highest upto 10.28 g only in treatment V3T3 (variety Sahbhagi Dhan). Overall response showed that Sahbhagi dhan had the highest root length, shoot length, average plant weight compared to all the other varieties.

Evaluation of wilting in rice plants after drought treatment

Significant differences were observed in plants after 10 and 13 days of drought stress, therefore, plants were evaluated for the wilting 10 and 13 days of drought stress. After 10 days of drought stress more than 100% wilting was observed in control (negative control), while it was 10%, 20%, 30% and 50%, respectively, for the treatments T1, T2, Cg and Tf in the variety Sahbhagi dhan. While it was 40, 20,

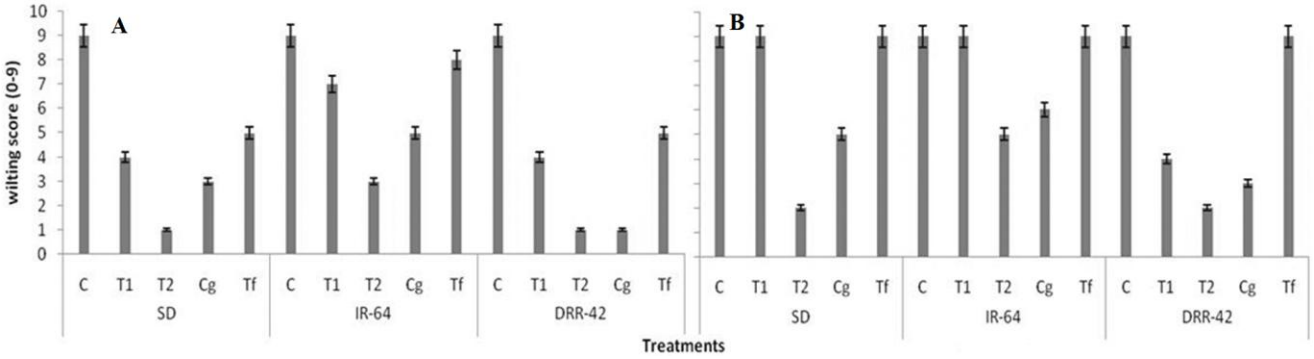

Fig. 2 — Wilt score in rice varieties Sahbhagi Dhan, IR 64 and DRR 42. (A) After 10 days; and (B) After 13 days of drought stress

10, 10, 5% for the DRR42. Wilting was maximum for the genotype IR-64 which was 70%, 40%, 50% and 80% for the treatments T1, T2, Cg and Tf, respectively. After 13 days of drought stress only few plants could survive in treatments T2 and Cg. Wilting was 20% for Sahbhagi Dhan, 50% for IR-64 and 20% for DRR-42 treated with *Trichoderma harzianum* 2. While, it was 50%, 60% and 30%, respectively, for the *Chaetomium globosum* treated plants. For other treatments wilt score was more than 90% (Fig. 2).

H_2O_2 assay

Variable results were observed for H_2O_2 in all the genotypes tested. H_2O_2 was observed minimum in genotype Sahbhagi dhan compared to the genotypes DRR42 and IR64. Slightly increased concentration of H_2O_2 was observed for the treatment T1, Cg and Tf. Whereas, it was less for the treatment T2 in variety DRR 42 after 4 and 10 days of drought stress. For the variety Sahbhagi dhan H_2O_2 concentration was less in the treatment Cg and Tf after 4 and 10 days of drought stress. For the variety IR 64 H_2O_2 concentration was highest for the TF after 4 days of drought stress (Fig. 3A-C).

Proline estimation

Proline content was maximum in all the genotypes after 10 days of drought treatment. For all the genotypes proline was minimum in T2 treatment after 4 days of drought whereas it was observed maximum in same treatment of DRR42 after 10 days of drought treatment. Proline content was observed minimum for the *Talaromyces flavus* inoculated treatments in all the genotypes (Fig. 3D-F).

Electrical conductivity

Rice varieties have shown variable results for the electrical conductivity (Fig. 3G-I). Electrical conductivity was observed maximum in 4 days drought stress compared to 10 days stress in all treatments and varieties. For the genotype DRR 42 increased conductivity was observed after 4 days of drought stress in all the treatments compared to control (positive control). Minimum electrical conductivity was observed in the treatment T2 after 4 and 10 days of drought stress in Sahbhagi dhan and IR64 varieties after 4 and 10 days of drought treatment (Fig. 3G-I).

Malondialdehyde (MDA) content

Malondialdehyde content was observed increased in all the drought stresses treatments compared to positive control. Minimum concentration of MDA was observed for the treatment T2 in varieties DRR42 and Sahbhagi dhan (60, 70 µM g^{-1}, respectively). For the variety IR64, MDA content was minimum for the treatment T2 (40, 50 µM g^{-1}, respectively) and Cg (35, 50 µM g^{-1}, respectively) after 4 and 10 days of drought stress. Significantly positive correlation ($r = 0.91$) was observed between wilting and MDA content (Fig. 3J-L).

β-1,3-glucanase assay

For the variety DRR42, β-1,3-glucanase was minimum (9.22 mg/protein) in control after 4 days of drought stress (Fig. 3M-O). β-1,3-glucanase was maximum in T1 (13.73 mg/protein) followed by T2 (12.30 mg/protein). After 10 days of drought treatment, it was maximum in T2 (14.81) followed by T1 (13.7).

For genotype Sahbhagi dhan, maximum quantity of β-1,3-glucanase was observed in treatment T2 (13.16) followed by control (10.23). For the genotype IR64, maximum expression of β-1,3-glucanase was observed after 4 days of drought stress compared to 10 days treatment. It was maximum in treatment T2 (14.01) followed by control (10.25) after 10 days of drought stress (Fig. 3M-O).

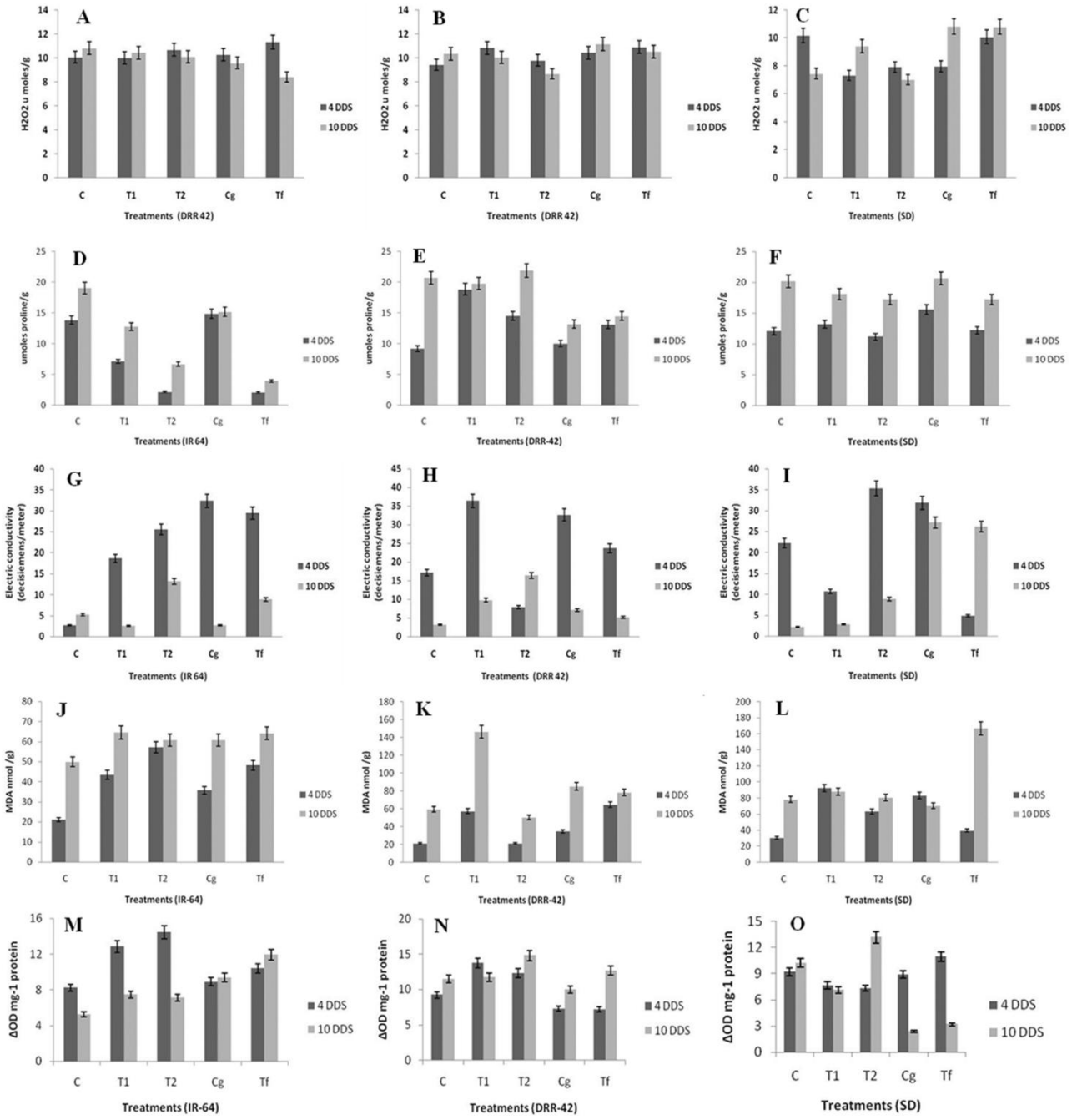

Fig. 3 — Biochemical changes in different rice varieties treated with different biocontrol agents after 4 and 10 days of drought treatment. (A-C) H_2O_2 content; (D-F) Proline content; (G-I) Electrical conductivity; (J-L) Melonaldihyde (MDA) content; and (M-O) β-1-3-glucanase activity

Discussion

Drought is regarded as a major abiotic factor reducing rice grain yield. Biocontrol agent treated rice plants have shown higher root length, shoot length and higher plant weight compared to uninoculated control. Based on drought tolerance and plant weight *Trichoderma harzianum* isolate 2 and *Chetomium globosum* isolate (Cg2) were able to delay the drought even after 13 days of stress in rice variety Sahbhagi dhan. *Trichoderma* helps to avoid drought through morphological adaptations[30]. It mediates drought tolerance via physiological and biochemical adaptations[4] and enhances drought recovery[31].

Increased amount of proline was observed in biocontrol inoculated plants compared to uninoculated control. Higher proline content was also reported

in *Pseudomonas fluorescens* inoculated maize plants[32]. Higher proline accumulation in inoculated plants generally indicates higher plant tolerance to water stress[14] which helps in maintaining cell water status, protects membranes and proteins from stress[33-34].

Exposure of plants to drought stress leads to the generation of reactive oxygen species (ROS), including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), ROS react with proteins, lipids and deoxyribonucleic acid causing oxidative damage and impairing the normal functions of plant cell. In order to over-come these effects, plants develop antioxidant defense systems comprising both enzymatic and non-enzymatic components that serve to prevent ROS accumulation and alleviate the oxidative damage occurring during drought stress[35-36]. Drought stress negatively affects the membrane integrity which reflects the extent of membrane injury. Less electric conductivity in *Trichoderma harzianum* 2 compared to other treatments suggests that *Trichoderma harzianum* 2 reduced the membrane damage in rice plants. Malondialdehyde (MDA) is a naturally occurring product of lipid peroxidation and the level of MDA in plant is often used as a parameter to evaluate the damage to plants' cells due to stress. Plant with lower amounts of MDA under drought conditions is generally considered as more tolerant to drought.

Conclusion

Trichoderma harzianum 2 treatment in drought stressed rice plants can delay the drought upto 3-5 days. Lower concentraion of MDA, reactive oxygen species, electrical conductivity and higher concentration of proline was observed in *Trichoderma harzianum* 2 treated rice plants under drought conditions.

Acknowledgement

The authors are grateful to Head, Division of Plant Pathology, ICAR-IARI for providing facilities and IRRI-EC IFAD project for providing financial support.

Conflict of interest

Authors do not have any conflict of interest for this manuscript.

References

1 Ashkani S, Yusop MR, Shabanimofrad M, Harun AR, Sahebi M & Latif MA, Genetic analysis of resistance to rice blast: a study on the inheritance of resistance to the blast disease pathogen in an F3 population of rice. *J Phytopathol*, 163 (2015) 300.

2 Khush GS, What it will take to feed 5.0 billion rice consumers in 2030. *Plant Mol Biol*, 59 (2005) 1.

3 Datta SK De, Chang TT & Yoshida S, Drought tolerance in upland rice. *Major Res Upland Rice* IRRI: (Los Banos, Philippines) 1994, 101.

4 Ji K, Wang Y, Sun W, Lou Q, Mei H, Shen S & Chen H, Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *J Plant Physiol*, 169 (2012) 336.

5 Hsiao A, Effect of water deficit on morphological and physiological characterizes in Rice (*Oryza sativa). J Agric*, 3 (2000) 93.

6 Rahdari P & Hoseini SM, Drought stress, a review. *Int J Agron Plant Prod*, 3 (2012) 443.

7 Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram R & Vam RP, Drought stress in plants: a review on morphological characteristics and pigments composition. *Int J Agric Biol*, 11 (2009) 100.

8 Choluj D, Karwowska R, Jasinska M & Haber G, Growth and dry matter partitioning in sugar beet plants (*Beta vulgaris* L.) under moderate drought. *J Plant Soil Environ*, 50 (2004) 265.

9 Rahdari P, Hoseini SM & Tavakoli S, The studying effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in Purslane (*Portulaca oleraceae* L.) leaves. *J Med Plant Res*, 6 (2012).

10 Venkateswarlu B & Shanker AK, Climate change and agriculture: adaptation and mitigation strategies. *Indian J Agron*, 54 (2009) 226.

11 Saakre M, Baburao TM, Salim AP, Ffancies RM, Achuthan VP, Thomas G & Sivarajan SR, Identification and characterization of genes responsible for drought tolerance in rice mediated by *Pseudomonas fluorescens*. *Rice Sci*, 24 (2017) 291.

12 Shukla N, Awasthi RP, Rawat L & Kumar J, Seed biopriming with drought tolerant isolates of *Trichoderma harzianum* promote growth and drought tolerance in *Triticum aestivum*. *Ann Appl Biol*, 166 (2015) 171.

13 Evans HC, Holmes KA & Thomas SE, Endophytes and mycoparasites associated with an indigenous forest tree *Theobroma gileri* in Equador and prelimnary assessment of their potential as biocontrol agents of cocoa diseases. *Mycol Prog*, 2 (2003)149.

14 Gusain YS, Singh US & Sharma AK, Enhance activity of stress related enzymes in rice (*Oryza sativa* L.) induced by plant growth promoting fungi under drought stress. *Afr J Agric Res*, 9 (2014) 1430.

15 Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Samuels GJ, Choi IY & Holmes KA, Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta*, 224 (2006) 1449.

16 Alwhibi MS, Hashem A, AbduAllah EF, Alqarawi AA, Soliman DWK, Wirth S & Egamberdieva D, Increased resistance of drought by *Trichoderma harzianum* fungal treatment correlates with increased secondary metabolites and proline content. *J Integr Agric*, 16 (2017) 1751.

17 Grover M, Ali SZ, Sandhya V, Rasul A & Venkateswarlu B, Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World J Microbiol Biotechnol*, 27 (2011) 1231.
18 Chen J, Hall DE, Murata J & De Luca V, Alanine induces programmed cell death in *V. labrusca* cell suspension cultures. *Plant Sci*, 171 (2006) 734.
19 Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL & Bailey BA, The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J Exp Bot*, 60 (2009) 3279.
20 Doni F, Isahak A, Zain CRCM, Ariffin SM, Mohamad WNW & Yusoff WMW, Formulation of *Trichoderma* sp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. *Springer plus*, 3 (2014) 532.
21 Singh R, Singh R, Singh US, Prasad D & Kumar A, Management of early drought in rice with most suitable strains of *Trichoderma harzianum*. *Int J Curr Microbiol App Sci*, 7 (2018) 1480.
22 Aggarwal R, Tiwari AK, Srivastava KD & Singh DV, Role of antibiosis in the biological control of spot blotch (*Cochliobolus sativus*) of wheat by *Chaetomium globosum*. *Mycopathologia*, 15 (2004) 369.
23 Bashyal BM, Etiology of an emerging disease: bakanae of rice. *Indian phytopath*, 71 (2018) 485.
24 IRRI, Standard evaluation system. International Rice Research Institute, Manila, Philippines, (2002) 29.
25 Alexieva V, Sergiev I, Mapelli S & Karanov E, The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ*, 24 (2001) 1337.
26 Bates LS, Waldren RP & Teare ID, Rapid determination of free proline for water stress studies. *Plant Soil*, 39 (2006) 205.
27 Li X, Yang YQ, Sun XD, Lin HM, Chen JH, Ren J, Hu XY & Yang YP, Comparative physiological and proteomic analyses of poplar (*Populus yunnanensis*) plantlets exposed to high temperature and drought. *PLoS One*, 9 (2014). 107605.
28 Premachandra GS, Saneoka H & Ogata S, Cell membrane stability, an indicator of drought tolerance as affected by applied nitrogen in soybean. *J Agric Sci*, 115 (1990) 63.
29 Abeles FB & Forrence LE, Temporal and Hormonal Control of β-1,3-Glucanase in *Phaseolus vulgaris* L. *Plant Physiol*, 45 (1970) 395.
30 Doni F, Anizan I, Che Radziah CMZ & Wan Mohtar WY, Physiological and growth response of rice (*Oryza sativa* L.) plants to *Trichoderma* spp. inoculants. *AMB Express*, 4 (2014) 45.
31 Rawat L, Bisht TS, Kukreti A & Prasad M, Bioprospecting drought tolerant *Trichoderma harzianum* isolates promote growth and delay the onset of drought responses in wheat (*Triticum aestivum* L.). *Mol Soil Biol*, 7 (2016) 1.
32 Ansary MH, Rahmani HA, Ardakani MR, Paknejad F, Habibi D & Mafakheri S, Effect of *Pseudomonas fluorescens* on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Ann Biol Res*, 3 (2012) 1054.
33 Sandhya V, Ali SKZ, Grover M, Reddy G & Venkateswaralu B, Effect of plant growth promoting *Pseudomona*s spp. on compatible solutes antioxidants status and plant growth of maize under drought stress. *Plant Growth Regul*, 62 (2010) 21.
34 Kaur G, Asthir B & Bains NS, Modulation of proline metabolism under drought and salt stress conditions in wheat seedlings. *Indian J Biochem Biophys*, 55 (2018) 114.
35 Miller G, Susuki N, Ciftci-Yilmaz S & Mittler R, Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ*, 33 (2010) 453.
36 Prajapat P, Singh D, Tripathi S, Patel K, Abbas H & Patel A, Effect of water stress on antioxidative enzymes and glycine betaine content in drought tolerant and drought susceptible cotton (*Gossypium hirsutum* L). *Indian J Biochem Biophys*, 55 (2018) 198.