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Morphological and Biochemical Studies of Root Cells of Rice (*Oryza sativa* L.) under Drought Stress Condition at Seedling Stage through PEG

Md. Haroon Rashid[1], Guatam Kumar[1], Anil Kumar[2]*, Sumit Saurabh[1] and Aditi Singh[1]

[1]*Department of Life Science, Central University of South Bihar, Patna, Bihar*

[2]*Department of Plant Breeding and Genetics, BAU, Sabour, Bhagalpur, Bihar*

**Corresponding Author Email : dranilbau@gmail.com*

Abstract

Rice (*Oryza sativa* L.) is one of the major staple food crops for about 65% of the world's population and it requires large amount of water to grow as compared to other plants. It is one of the important source of dietary energy, proteins and minerals. High yielding seed were selected of threedifferent genotypes of rice (IR64, SahbhagiDhan, Sarnashreyi) and germinated in the dark for 3-4 days under normal temperature and pressure. These seedlings were transferred to hydroponic media (Yoshida media) for the culture and treated with PEG for 48 hours.Present study aimed to identify the potential drought tolerant rice genotype for eastern region of India. This study morphological, biochemical and yield attributes of rice has been used to identify the most tolerated rice variety. The study included the comparative analyses of morpho-physiological and biochemical alterations with special emphasis on ROS generation and detoxification through antioxidant enzyme activity.In future, studies are needed with longer stress duration to achieve severe drought in plant tissue in several varieties with contrasting tolerance to drought stress.

Key words : *Yield and climate variability root cells,drought stress, osmotic stress, seedling, rice.*

Introduction

Rice (*Oryza sativa* L.) is one of the major staple food crops for about 65 % of the world's population and it requires large amount of water to grow as compared to other plants. It is one of the important source of dietary energy, proteins and minerals. The sheer diversity of plant species grown across climatic regions that include extreme dry conditions suggests that in nature, plants have evolved to endure drought stress with an array of morphological, physiological and biochemical adaptations (1). The overall rice yield variability due to climate variability over the last three decades has been estimated and it was concluded that approximately 53% of rice harvesting regions experiences the influence of climate variability on yield and approximately 32% of rice yield variability is explained by year-to-year global climate variability (2).

Red areas indicate regions with declined yield and the green areas indicate regions with high yield.

Major cause of decrease in rice yields in rain-fed rice is drought. It is a major production constraint in rain-fed rice. In the eastern Indian states of Jharkhand, Orissa, and Chhattisgarh alone, total rice production losses in severe droughts (about 1 year in 5) average 40 %, valued at $ 650 million. Most of the traditional as well as high yielding varieties cultivated in the eastern region are highly susceptible to drought, particularly reproductive stage drought. The higher frequency and intensity of drought spells necessitates development of rice cultivars, which are able to survive under water deficit stress at reproductive stage and quickly recover after the drought spells, by rapid growth upon improved availability of soil moisture (2, 3).

Tolerance in rice varies with genotypes and developmental stages, with early vegetative stage being relatively more susceptible. Present study aimed to identify the potential drought tolerant rice genotype for eastern region of India. This study morphological, biochemical and yield attributes of rice has been used to identify the most tolerated rice variety. The study included the comparative analyses of morpho-physiological and biochemical alterations with special emphasis on ROS generation and detoxification through antioxidant enzyme activity.

Effect of osmotic stress on rice : The first and foremost effect of drought is impaired germination and poor stand establishment (5). In rice, osmotic stress during the vegetative stage greatly reduced the plant growth and development. Decreasing water availability under drought condition usually results in limited total nutrient uptake and their decrease in tissue concentrations in crop plants. Reactive oxygen species (ROS), such as $^1O_2$, H_2O_2, O_2^- and HO^-, are toxic molecules capable of causing oxidative damage to proteins, DNA and lipids (6).

Plant material and experimental design : Seeds of selected high yielding three rice genotypes (IR64, Sahbhagi Dhan, Swarna Shreya) were germinated in the dark for 3-4 days under normal temperature and

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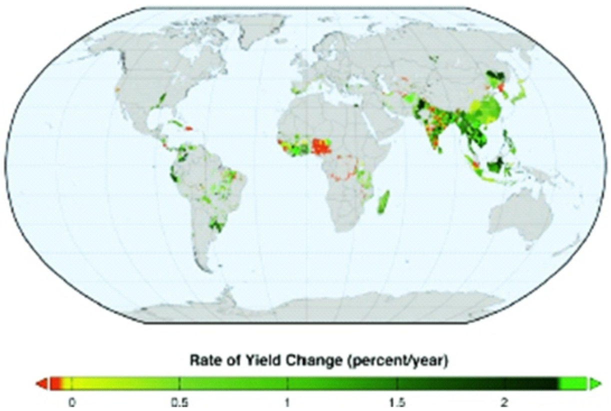

Fig.-1 : Global map showing percentage rate of change in rice yield (Ray et al., 2015).

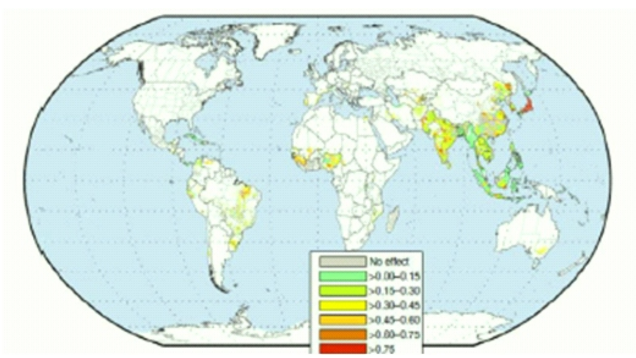

Fig.-2 : Global rice yield variability due to climate variability over the last three decades (Ray *et al.*, 2015).

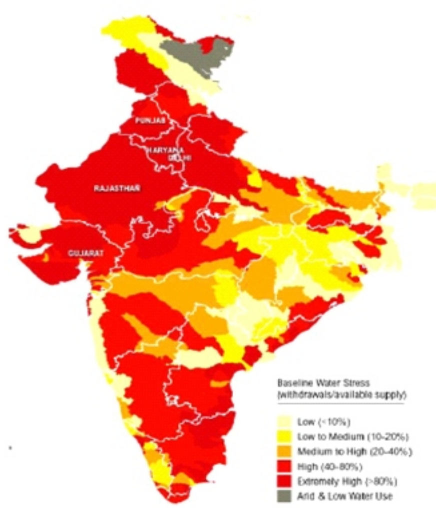

Fig.-3 : Drought affected regions in India.

pressure.These seedlings were transferred to hydroponic media(Yoshida media) for the culture. The seedlings were allowed to grow for 15 days (days after germination) with continuous media aeration at 25°C illuminated at 400-500 mol $m^{-2}s^{-1}$ using fluorescent lighting (Philips, Kolkata, India) in a day and night cycle of 48 hours each. The nutrient solution was replaced at 5 days interval.

Stress protocol : 15 days old plants grown seedling in Yoshidamedia, were divided into two groups. The first group was kept untreated (control) and the second group was treated with 20 % PEG-6000 (drought) for 48 hours. Morphological and biochemical studies were performed at the seedling stage.

Morphological studies : Root length of plant was studied for all three genotypes under control and stress conditions. Relative change of root length of plant for each genotype was calculated using the formula of (7).

Protein extraction : 15 days seedlings after stress treatment for 48 hours, roots are harvested. Harvested roots were washed 2-3 times with distilled water, crushed in liquid nitrogen properly and diluted it in potassium phosphate buffer for 2ml, centrifuged the crushed sample at 1g at 4^{o}C.

Quantification of total protein : Total protein was quantified according to (8). The absorbance of Coomassie Brilliant Blue after binding with the protein was recorded at 595 nm. Total protein was estimated from the BSA standard curve.

Assay of antioxidant enzyme activities : Lyophilized plant tissue was homogenized with ice-cold phosphate buffer (pH 7.0). The plant extract was used to detect different antioxidant enzyme activities.

Superoxide dismutase (SOD) activity : Superoxide dismutase activity was determined at 560 nm by its ability to reduce the formation of blue coloured formazone by nitro blue tetrazolium and O_2^- radical (9).

Peroxidase (POX) activity : Peroxidase activity was determined at 436 nm by its ability to convert guaiacol to tetraguaiacol (10). The increase in absorbance was recorded by the addition of H_2O_2 at 436 nm for 1 min.

Results and Discussion

Rice genotypes showed increase in root length in response to drought as compared to their normal condition (Fig-4). Sahbhagi Dhan showed increase in root length by 15.66 % after 20% PEG induced drought for 48 hours, Swarna Shreya also exhibited higher increase in root length 15.5% after 48 hours drought; whereas, IR64 also showed slight increase in length by 12% after 48 hours of drought.

Drought stress resulted in decreased total protein content in considered rice genotypes. Minimum decrease in total protein content was observed in Swarna Shreya (6.84%) after 48 hours; whereas, Sahbhagi Dhan exhibited the maximum decrease in protein content about

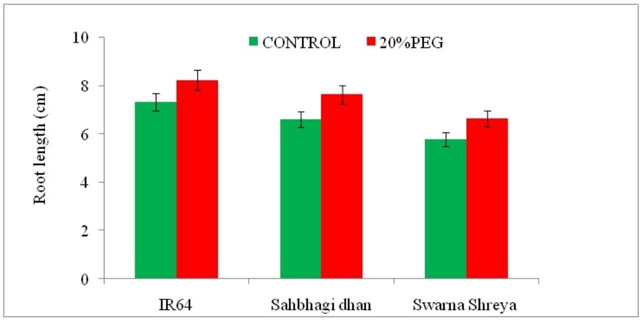

Fig.-4 : 20% PEG induced drought stress disturbed growth of rice seedlings.

11 % after 48 hours of stress and IR64 showed decrease in protein content about 9.79% after 48 hours of stress

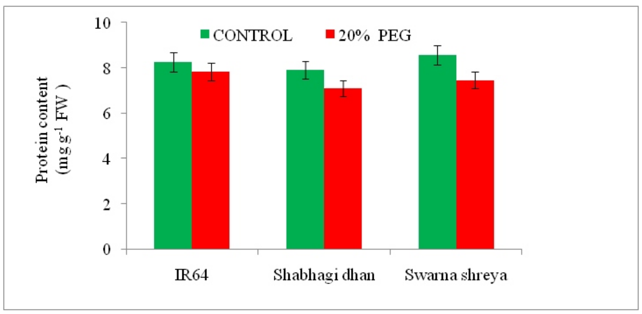

Fig-5 : 20% PEG induced drought stress resulted in decreased total protein.

(Fig.-5).

Osmotic stress increased the antioxident enzyme activities in all rice genotypes. Successive increase in duration of drought stress increased the antioxidant enzyme activities in all rice genotypes. Superoxide dismutase activity was found to be increased in response

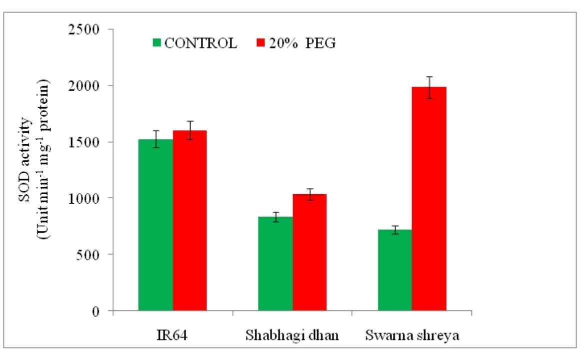

Fig.-6 : Effect of osmotic stress on superoxide dismutase activity of root in different rice genotypes at seedling stage under 20% PEG-induced drought stress.

to drought as compared to that of the normal condition. IR64 showed the lowest increase in SOD activity (5.25% increase after 48 hours stress), whereas, Swarna Shreya showed the highest increase in SOD activity (58.89%

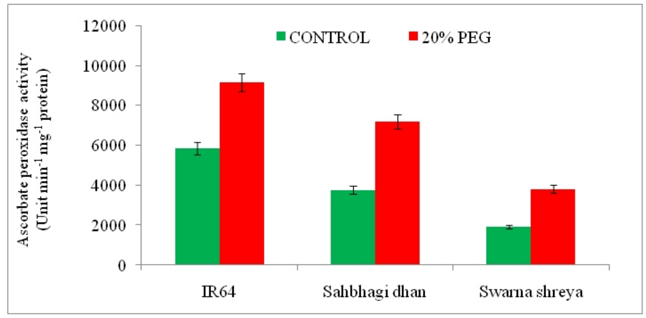

Fig.-7 : Effect of osmotic stress on ascorbateperoxidase activity of root in different rice genotypes at seedling stage under 20% PEG-induced drought stress for 48 hours.

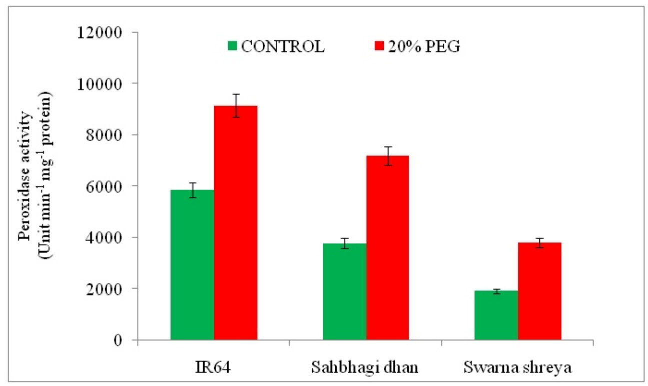

Fig.-8 : Effect of osmotic stress on Peroxidase activity of root in different rice genotypes at seedling stage under 20% PEG-induced drought stress for 48 hours.

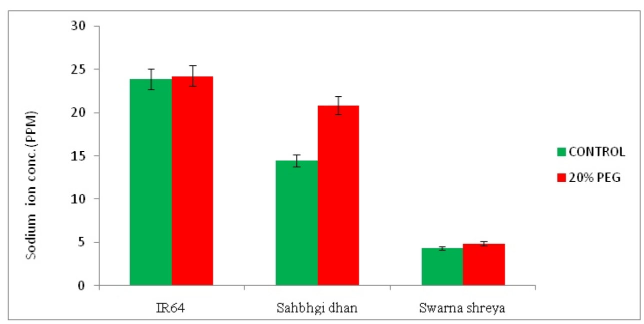

Fig.-10 : Sodium and Potassium ion accumulation inside root of different rice genotypes at seedling stage under 20% PEG-induced drought stress (B) for 48 hours.

increase after 48 hours stress), followed by Sahbhagi Dhan (24% increase after 48 hours stress). Peroxidise activity and ascorbate peroxidase activity showed the similar trend (Fig.-6).

Ion accumulation during PEG-induced drought stress : PEG-induced drought stress cause more accumulation sodium and potassium ion concentration than normal condition (Fig-10). Sahbhagi Dhan showedincrease in sodium ion concentration by 44.22% after 48 hours of drought stress.

Drought stress treatment inhibited overall plant growth, which might be due to restriction of cell division and impaired mitosis, cell elongation (11). IR64 showed maximum increase in root length under PEG-induced drought condition after 48 hours. Osmotic stress is also associated with higher accumulation of ROS (6). Deleterious effect of ROS on cell was determined by the equilibrium between the production and detoxification of ROS through antioxidant enzyme system (12). Reactive oxygen species-induced cell toxicity could be eliminated in Swarna Shreya. Through strong antioxidant defence mechanism against ROS built-up. Further more, increased activity of SOD, POX and APX in Swarna Shreya seedlings might result in enhanced ROS scavenging, providing better protection under drought stress. Drought stress interrupted seed germination of all rice genotype as described by. It can observe significant change in morphological and biological activity in plant tissues during drought induce condition treating PEG. During stress condition amount of Na^+ is increase which creates ion stress by partial uptake of Na ion by root.

Conclusions

Present work high light for the search rice genotype with better tolerance towards drought grounded on morphological, biochemical analyses. In summary, Swarna Shreya exhibited considerably enhanced drought tolerance at seedling. Biochemical studies provided the evidence that SwarnaShreya improved their tolerance by strengthening their antioxidant defensive mechanism. Thus SwarnaShreya may be considered as the potential osmotic tolerant rice genotype.

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