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POLYETHYLENE GLYCOL MEDIATED DROUGHT STRESS IMPACTS ON GERMINATION, GROWTH AND ACCUMULATION OF PROLINE IN RICE (*Oryza sativa* L.)

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ABSTRACT

Drought is one of the major stress factors affecting the growth and development of plants, and is responsible for considerable crop yield losses worldwide. An experiment was conducted to compare three rice (*Oryza sativa* L.) varieties (BRRI dhan49, BRRI dhan71 and BRRI dhan75) for drought tolerance based on some growth parameters and physiological status in germination stage. Drought stress was imposed by five levels of polyethylene glycol (PEG) (0%, 10%, 15%, 20% and 25%). Rice varieties showed good germination with PEG concentration up to 15%. At 20% PEG concentration, a sharp reduction in germination percentage was observed and the highest germination percentage (55.53%) was found in the variety BRRI dhan71. Seeds of all the varieties treated with 25% PEG did not germinate. Seedling growth in terms of plumule and radicle length, fresh weight and dry weight decreased with increasing drought stress in all the varieties. Among the varieties, BRRI dhan71 was found to be the best at 20% PEG for seedling growth. Drought stress treatments decreased seedling length and weight vigor index in rice, whereas the highest seedling length and weight vigor index were obtained from BRRI dhan71. Accumulation of proline increased along with the increase of PEG concentration. The highest proline accumulation (3.08 μmol g^{-1} leaf) was obtained from the BRRI dhan71 at the higher treatment (20% PEG). This result suggests that BRRI dhan71 showed best performance under drought stress because of its own nature of tolerance.

Keywords: Drought stress, PEG, Proline, Rice

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INTRODUCTION

Plant growth and productivity is adversely affected by nature's wrath in the form of various biotic and abiotic stress factors. Drought is one of the important abiotic factors that impair the plant growth and development, especially in warm and dry areas (Fathi et al., 2016). Rice (*Oryza sativa* L.), is the staple food for half of the world’s population, especially people in developing countries (Seck et al., 2012) and supplies the main energy resource for almost 50% of the world’s population (Zhu et al., 2018). Worldwide, drought affects approximately 23 million hectares land of rain fed rice (Serraj et al., 2011). In Bangladesh, rice is an important economic crop and every year about 0.34 million ha of land are affected by severe drought in north-western region (Habiba et al., 2015).

Drought stress is primarily manifested as osmotic stress resulting in the disruption of homeostasis and ion distribution in the cell (Wang et al., 2003). It causes plant dehydration, stomatal closure, and limited gas exchanges, followed by inhibition of metabolism and photosynthetic rate (Jaleel et al., 2008), decreases chlorophyll content and inhibits enzymes of chlorophyll biosynthesis (Jain et al., 2013) and increases the activity of antioxidative enzymes (Pourtaghi et al., 2011). However, ability of plants to survive under stressed condition depends on plant species, growth stage, duration, and intensity of water deficit (Jaleel et al., 2008).

Seed germination and early seedling growth are the most critical stages of drought stress in many species including rice (Islam et al., 2018; Yousefi et al., 2020). Water availability and movement into the seeds are very important to promote germination, initial root growth, shoot elongation and therefore at the establishment of a uniform stand. The highly negative osmotic potential may affect the seeds water uptake, making germination not possible (Meneses et al., 2011).

Proline accumulation in leaves of plants during drought stress is well documented (Hare and Cress, 1997). Proline accords in stabilizing sub-cellular structure including membranes and proteins (Singh et al., 2015), scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007), thereby maintain turgor and stimulates the continued growth under stress (Mullet and Whitstitt, 1996). Furthermore, it also acts as a signaling molecule to regulate metabolite pools and the redox balance, to control gene expression, and ultimately to control drought tolerance (Szabados and Savourde, 2010).

Polyethylene glycol (PEG) can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in relatively controlled manner (Money, 1989; Zhu et al., 1997). PEG molecules are inert, non-ionic, and virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage (Lu and Neumann, 1998; Kulkarni and Deshpande, 2007). This approach has been used to selection of tolerant genotypes in different crops (Badiane et al., 2004) and it was reported to be an effective strategy for selection at the early growth stages of rice (Jing and Chang, 2003). The objective of this study was to know the impacts of PEG mediated drought stress on the germination, growth and accumulation of proline in rice.

MATERIALS AND METHODS

A laboratory experiment was carried out at the School of Agriculture and Rural Development, Bangladesh Open University (90°38′N, 23°95′E), Gazipur, Bangladesh during November, 2018 to February, 2019. Three rice varieties, BRRI dhan49, BRRI dhan71 and BRRI dhan75 were chosen for this study. Seeds were obtained from Bangladesh Rice Research Institute (BRRI), Gazipur. The form of experiment was factorial, using a completely randomized design (CRD) with three replications. Seeds of three cultivars were subjected to five stress level of PEG6000 (0%, 10%, 15%, 20% and 25%). Distilled water was used as control. Ten grams (g) of PEG was dissolved in 100 ml of water to prepare 10% solution of PEG. Similarly, 15 g, 20 g, 25 g PEG was dissolved in 100 ml of water to make 15%, 20%, 25% solution of PEG, respectively. Rice seeds were disinfected with 1% sodium hypochlorite solution for 30 seconds. After the treatment the seeds were washed two times with distilled water. Twenty-five seeds of each variety were germinated on two layers of filter paper in 12 cm Petri dishes with respective treatment from PEG. The Petri dishes were covered to prevent the loss of moisture by evaporation under laboratory condition (25 ±1°C), a 12-h-light and 12-h-dark (100 mol $m^{-2} s^{-1}$) and a relative humidity of 60% in an incubator for 8 days. Every 24 h, 2ml of each solution was added to Petri dish. Germination percentage (%) was counted after 7 days following seeds placement in Petri dishes for germination, using radicle extrusion as germination indicator. Plumule and radicle length (cm) were measured on the eighth day after germination (end of experiment). The dry weight of plumule and radicle were examined after drying at room temperature. Length of plumule and radicle were measured by millimeter ruler and for measuring fresh weight of plumule and radicle an electric balance (Shimadzu Corporation, Japan) was used by accuracy of 0.1 mg. Germination percentage (%) was calculated using the following formula (Scott et al., 1984):

$$\text{Germination (\%)} = \frac{\text{Total numbers of germinated seeds}}{\text{Total seed placed for germination}} \times 100$$

Vigor index was calculated using following formula (Abdul-Baki and Anderson, 1973): Seedling length vigor index = Germination (%) × total seedling length (cm), and seedling weight vigor index = Germination (%) × seedling dry weight (g). Proline accumulation of leaves was determined according to Bates et al. (1973). The collected data were analyzed using statistical program cropstat7.2 software (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Effect of PEG mediated drought stress on seed germination

The effect of PEG mediated drought stress on the germination percentage of BRRI dhan49, BRRI dhan71 and BRRI dhan75 varieties is presented in Figure 1. The highest germination percentage was recorded at control (absence of stress) while the lowest at 20% PEG. There was no remarkable difference in germination percentage between control and drought stress up to 15% PEG concentration. At 20% PEG concentration, a sharp reduction in germination percentage was observed and the highest germination percentage (55.53%) was found in the variety BRRI dhan71, whereas the values for BRRI dhan49 and BRRI dhan75 in the same treatment were 31.06% and 26.66%, respectively. These results suggest that BRRI dhan71 is the most drought tolerant among the varieties. These results are consistent with those of other studies that have reported that high concentration of PEG reduce the final germination percentages of rice (Islam et al., 2018; Purbajanti et al., 2019). Seeds of all the varieties treated with 25% PEG did not germinate, which indicated that 25% PEG was the lowest osmotic potential for rice seed germination (Fig.1 and 2). Dodd and Donavon (1999) stated that PEG induced reduction in germination percentage was because of reduction in the water potential gradient between seeds and their surroundings. This could be also explained by metabolic disorders such as slower hydrolysis of storage compounds in endosperm or cotyledons and/or slower transportation of hydrolyzed material to developing embryo axis, under drought conditions (Ayaz et al., 2000).

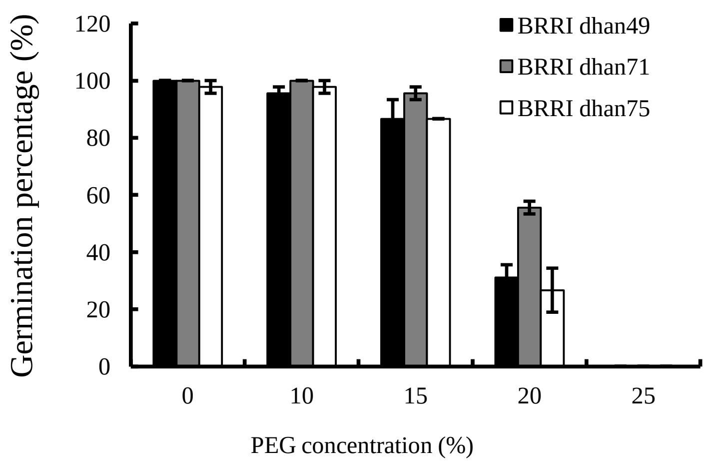


Figure 1. Effect of PEG mediated drought stress on germination percentage (%) of rice varieties.

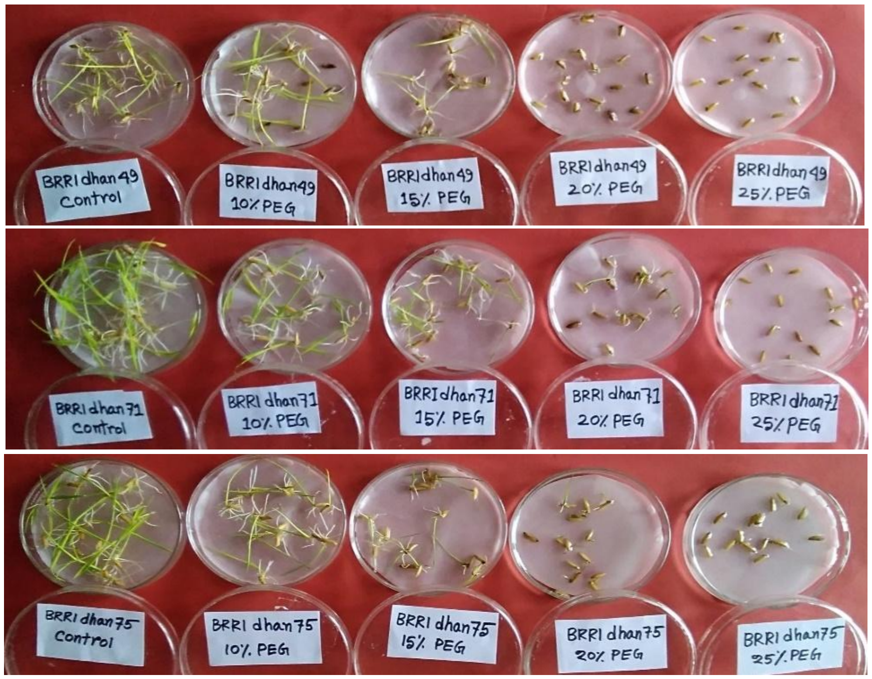


Figure 2. Performance of BRRI dhan49, BRRI dhan71 and BRRI dhan75 in petri plate technique on test solution subjected to control (0% PEG), 10% PEG, 15% PEG, 20% PEG and 25% PEG.

Effect of PEG mediated drought stress on plumule length

The plumule length decreased with the increase in PEG concentration and the difference was remarkable at high concentration of PEG (20%) when compared with control, 10% and 15% PEG concentration (Fig. 3A). Rice variety BRRI dhan71 performed better with the longest plumule length (1.74 cm) than BRRI dhan49 (1.22 cm) and BRRI dhan75 (1.29 cm) at high concentration of PEG (20%). No plumule length was recorded for all varieties at 25% PEG since there was no seed germination. The radicle length of rice varieties studied is presented in figure 3B. The longest radicle length was observed in BRRI dhan71 at control (3.1 cm) and all other concentration of PEG (2.22 cm for 10%, 1.7 cm for 15% and 1.43 cm for 20%) than BRRI dhan49 (1.8 cm for control, 1.55 cm for 10%, 1.24 cm for 15% and 1.15 cm for 20%) and BRRI dhan75 (1.42 cm for control, 1.2 cm for 10%, 0.99 cm for 15% and 0.866 cm for 20%). This result suggested that BRRI dhan71 developed the longest radicle compared to the other varieties, indicating its higher tolerance to drought stress during early seedling growth stage. Decrease in growth rate with increasing osmatic stress was reported in many studies (Kulkarni and Deshpande, 2007; Hamayun et al., 2010). Under drought stress, it has been shown that the inhibition of radicle emergence is mainly because of decrease in water potential gradient between the external environment and the seed and consequently impairs seedling height (Sokoto and Muhammad, 2014). Reduced root and shoot lengths under PEG environment might be due to decreased cell division and elongation rates under water stress condition (Choi et al., 2000).

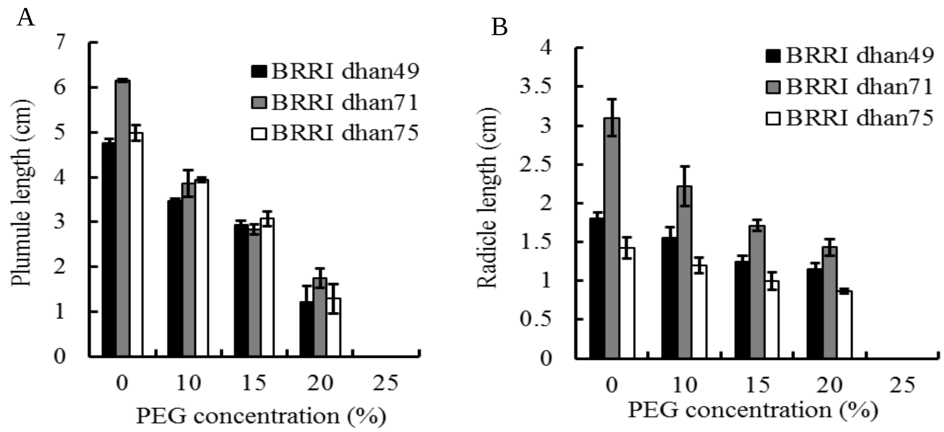
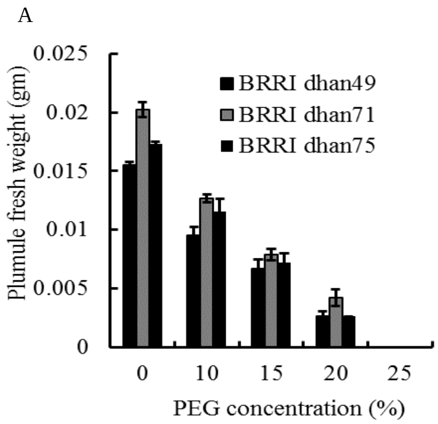


Figure 3. Effect of PEG mediated drought stress on plumule length (A) and radicle1ength (B) of rice varieties.

Effect of PEG mediated drought stress on fresh weight of plumule and radicle

Fresh weight of plumule and radicle are shown in Figure 4A and Figure 4B. Increasing PEG concentration caused a remarkable reduction in fresh weight of plumule (Fig.4A). The highest plumule fresh weight was recorded at control and the lowest was recorded at 20% PEG concentration in all the tested varieties. At 20% PEG, reduction of plumule fresh weight over control was higher in BRRI dhan49 (81.51%) and BRRI dhan75 (84.77%), whereas reduction was less in BRRI dhan71 (79.45%). Among the varieties, lower percentage reduction of radicle fresh weight was observed in BRRI dhan71 (80.40%) and higher in BRRI dhan49 (71.83%) and BRRI dhan75 (66.62%) (Fig.4B). Lower percent reduction of shoot and root growth in tolerant cultivar over control were recorded in soybean as reported by Ange et al. (2016). Demir and Kazim (2008) reported that the higher the osmotic stress concentration the lower was the seedling fresh weight of pepper. Similarly, Hellal et al. (2018) reported that root and shoot fresh weights of barley cultivars declined when osmotic potential was decreased.



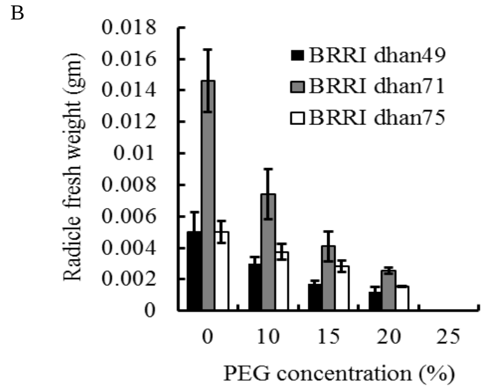


Figure 4. Effect of PEG mediated drought stress on plumule fresh weight (A) and radicle fresh weight (B) of rice varieties.

Effect of PEG mediated drought stress on dry weight of plumule and radicle

Dry weight of plumule and radicle are shown in Figure 5A and Figure 5B. Both parameters showed a significant decrease with increasing PEG concentration. The highest plumule and radicle dry weight were recorded at control and the lowest was recorded at 15% PEG concentration in all the tested varieties. At 15% PEG, reduction of plumule dry weight was more in BRRI dhan49 (65.74%) and BRRI dhan75 (63.39%) than BRRI dhan71 (57.48%). The inhibitory degree on radicle dry weight by 15% PEG was the highest on BRRI dhan49 (58.82%) followed by BRRI dhan75 (53.57%) and the lowest was on BRRI dhan71 (61.53%). Despite fresh weight was recorded in all varieties at 20% PEG, they weigh was zero gram i.e., no detectable by the weighing balance (no dry weight). The result indicated that BRRI dhan71 had better growth under drought than BRRI dhan49 and BRRI dhan75 and as the drought level increased, seedling dry weight decreased. Qayyum et al. (2021) also noted that seedlings root and shoot dry weight of wheat cultivars was decreased by increasing PEG. These results are consistent with many reports which presented that drought tolerant genotypes could maintain higher growth than sensitive ones (Kumar et al., 2014; Mejri et al., 2016).

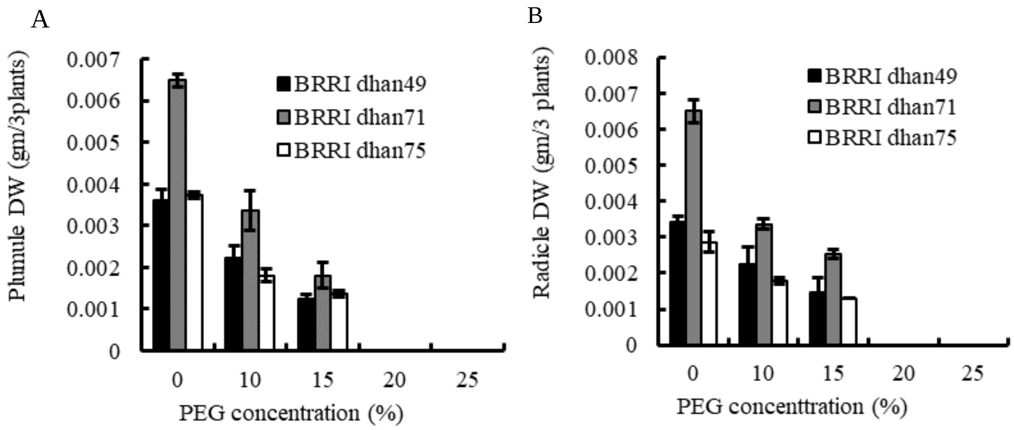


Figure 5. Effect of PEG mediated drought stress on plumule dry weight, DW (A) and radicle dry weight, DW (B) of rice varieties.

Effect of PEG mediated drought stress on vigor index

Tolerant rice cultivar recorded higher vigor index than sensitive cultivar under stress as indicated by Sadasivam et al. (2000). The seedling vigor index has been used as a tolerance index to evaluate the effect of drought stress on seedling growth (Liu et al., 2015). The length vigor index decreased with the increase in PEG concentration in all the varieties (Fig. 6A). These results evidenced the negative effect of drought on the elongation of the seedlings, as reported by the lower length of the plumule (Fig. 3A) and radicle (Fig. 3B). Among the varieties, higher seedling length vigor index was observed in BRRI dhan71 (889.3), while lower in BRRI dhan49 (694.4) and BRRI dhan75 (647.7) in control treatment. The highest seedling vigor index at 20% PEG were recorded in BRRI dhan71 (183.19) followed by BRRI dhan49 (74.01). The lowest was observed in BRRI dhan75 (57.74). The weight vigor index decreased with the increase in PEG concentration in all the tested varieties (Fig. 6B). Among the varieties, higher seedling weight vigor index was observed in BRRI dhan71 (1.3 for control, 0.673 for 10% PEG and 0.415 for 15% PEG), while lowest in BRRI dhan49 (0.703 for control, 0.424 for 10% PEG and 0.232 for 15% PEG) and BRRI dhan75 (0.643 for control, 0.352 for 10% PEG and 0.23 for 15% PEG). The lower seedling weight vigor index was obtained with decreasing osmotic potential level due to that of drought stress reduced germination rate of seeds and inhibited the dry weight of seedlings. The reduction in vigor index of seedlings under water restriction conditions was reported by other research (Zahedifar and Zohrabi, 2016). Copeland and McDonald (1995) reported that vigor of seedlings relates with their ability upon germination to grow rapidly and well.

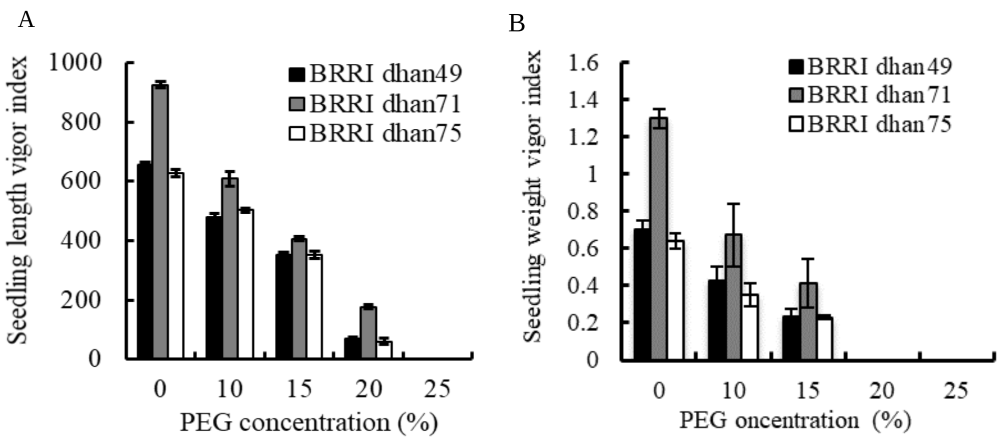


Figure 6. Effect of PEG mediated drought stress on seedling length vigor index (A) and seedling weight vigor index (B) of rice varieties.

Effect of PEG mediated drought stress on the accumulation of proline in the leaf

The accumulation of proline in PEG treated rice was investigated. The PEG concentration affected the accumulation of proline in rice varieties (Fig. 7). BRRI dhan71 showed higher accumulation of proline than BRRI dhan49 and BRRI dhan75 at 0% (control), 15% and 20% PEG concentration.

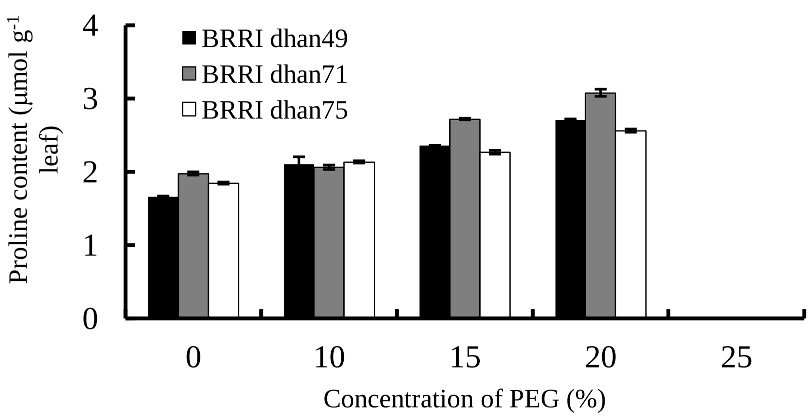


Figure 7. Effect of PEG mediated drought stress on the accumulation of proline in the leaf of rice varieties.

The highest proline accumulation was observed at the higher concentration of PEG (20%) in BRRI dhan71. The results indicated that higher accumulation of proline during drought stress might be a potential indicator of drought tolerance in BRRI dhan71. In parallel to the above results, other researchers have also approved the impact of PEG in the accumulation of proline in rice leaves (Lum et al., 2014; Kadhimi et al., 2016). These results were in accordance with the findings of Mohammadkhani and Heidari (2008), and Tatar and Gevrek (2008) who reported that proline content increased under drought stress conditions. Proline acts as an osmotic subsists water particulars, stores carbon and nitrogen after water stress recovery, and stabilizes macromolecule, proteins and cell membranes in plant tissues (Farooq et al., 2009) and is the main strategy of the plant to avoid the detrimental effects of drought.

CONCLUSION

The germination percentage, as well as seedling growth was considerably lowered, except for accumulation of proline, which increased with the increasing levels of stress using PEG. Promising rice variety BRRI dhan71 had the largest value of germination percentage; plumule and radicle length, fresh weight, seedling length vigor index, and proline accumulation in concentration of 20% PEG compared with the BRRI dhan49 and BRRI dhan75. It could be suggested that BRRI dhan71 might possess drought tolerance characteristics on the basis of their germination, growth and proline accumulation. Nevertheless, further research is required to investigate the impact of different drought mediating agents in more species in germinating stage.

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