Potassium nitrate, Silicon dioxide and Salicylic Acid Mediation Increase Seedling Growth, Biochemical Characteristics and Antioxidant Enzymes Activities in Rice (*Oryza sativa*) FARO44 Under Water Deficit Conditions

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Abstract

Application of potassium nitrate, silicon dioxide and salicylic acid to seed are approaches that impart stress tolerance to germinating seeds and seedlings under stressful conditions. This study evaluated the effects of synergistic osmopriming and hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA on seed germination, seedling growth, antioxidant enzymes, protein, proline and malondialdehyde contents in 3-weeks old FARO44 rice seedlings under drought conditions induced by polyethylene glycol (PEG6000). Germination experiments were laid in a completely randomised design (CRD) with each treatment replicated five times. The results revealed that synergistic priming significantly seedling growth, antioxidant enzymes catalase, ascorbate peroxidase and superoxide dismutase activities in rice seedlings by 2 folds compared to unprimed seedlings under drought. Synergistic effect of priming methods improved protein and proline contents in rice seedlings while decreased the content of malondialdehyde. Osmopriming and hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA as well as 2.5% KNO_3+1 mM SA showed different responses, however, they were found to be efficient in increasing germination, growth of seedling, antioxidant enzymes' activities as well as biochemical characteristics in rice seedlings grown under water deficit conditions. Enhanced activities of antioxidants, proline content as well as low malondialdehyde content in rice seedlings correlate with improved drought tolerance. Therefore, pre-soaking of FARO44 in 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA or 2.5% KNO_3+1 mM SA is recommended for fast germination, seedling growth and drought tolerance in arid regions.

Keywords: *Germination; water deficit; antioxidants; biochemical attributes; proline*

Introduction

Rice is an important crop consumed by many societies across the world. It serves as an essential daily food for more than half of the world's population (Dien *et al*., 2019). Considering the significance of rice in both developed and developing nations, there is the need for its adequate supply, poverty reduction as well as improvement of financial status of people and nations (Zheng *et al*., 2016).

Environmental constraints such as drought, salinity and extreme temperatures affect germination and seedling growth, accounting for 50% yield loss and as such impede the global rice production (Dien *et al.*, 2019; Pandey & Shukla, 2015; Quan *et al.*, 2016). Water is a key resource for successful crop production and is a highly limited resource. Drought causes colossal loss to crop production worldwide and is therefore a severe threat to sustainable agriculture. Globally, 23 million hectares of rice produced using rainfall as source of water are affected by water deficit situation (Pandey & Shukla, 2015). Moreover, changing climate that affect the world caused by the anthropogenic release of greenhouse gases is aggravating the water deficit conditions upon crop production (Intergovernmental Panel on Climate Change, 2007). Providing food to teeming population under depleting water resources needs crop varieties which can adapt and thrive under dry environments (Pandey & Shukla, 2015).

Poor germination as well as establishment of seedlings in rice under broadcasting systems of rice production caused by water deficit results in enormous yield loss to rice growers (Liu *et al*., 2016). Water shortage at early stage of crop production is responsible for delayed germination as well as irregular establishment of seedling (Kaya *et al.*, 2006). Shortage of water coupled with other environmental stresses are responsible for rapid production of reactive oxygen species (ROS) that ruin RNA, lipids, DNA, carbohydrates and proteins in crops (Gill & Tuteja, 2010). Reactive oxygen species impairment known as oxidative stress is a critical harm for plants facing abiotic stresses such as water shortage (Price *et al*., 1989). While responding to stresses, plants deploy antioxidant systems consisting of catalase, glutathione, superoxide dismutase, peroxidase, superoxide dismutase and ascorbic acid to convert the toxic ROS to harmless substances (Gill & Tuteja, 2010; Hussain *et al*., 2016).

Numerous approaches were employed aimed at induction of pressure tolerance in different crops (Hussain *et al*., 2016). Priming of seed is a practical, easy, efficient and affordable method of inducing quicker as well as even seedling development. The technique also heightened vigor of seedling and yield of agricultural facing pressures of water shortage and salt stress (Jisha *et al*., 2013; Paparella *et al*., 2015). It is a controlled soaking of seeds in solutions of salts, water, and hormones or exposing to magnetic conditions, heat aimed at stimulating crucial processes of germination before the radicle emerge and sowing the seeds (Jisha *et al*., 2013; Thomas *et al.*, 2013). Rapid and quality germination of soaked seeds are due to stimulation of enzymes, reduction of imbibition period, accelerated repair and production of germination metabolites as well as osmotic modification (Ashraf & Foolad, 2005; Farooq et al., 2006; Hussain *et al*., 2015; Hussain *et al.*, 2016). The common approaches of rice priming used to deal with many stresses include osmopriming, hormonal priming, hydropriming, nutrient priming, redox priming, chemical priming as well as magnetopriming (Jisha *et al.*, 2013; Paparella *et al*., 2015).

Northern Nigeria grow rice commonly using rainfall for germinating seeds, this system faces dry spell problems. Drought gravely upsets rice crop since rice have small roots, a slight water deficit cause hasty stomatal closing resulting in cessation of photosynthesis, change in metabolism of carbohydrate, osmolyte accumulation and other organic acids (Ji *et al.*, 2012; Kamoshita et al., 2004). Thus, drought stress is an impediment for rice production, it is more unsettling in areas that depend on rainfall. Thus, this paper studied the synergistic or combined potentials of seed priming using potassium nitrate, silicon dioxide and salicylic acid priming on seedling growth, antioxidant enzymes, and biochemical attributes in FARO44 rice under drought stress.

Results

Synergistic effects of osmopriming and hormonal priming on FARO44 rice seedling under drought condition

Osmopriming and hormonal priming had significant ($P \leq 0.05$) effects on seedling length, seedling biomass and root length in FARO44 rice under drought stress (Table 1). Synergistic priming using 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA or 2.5% KNO_3+1 mM SA significantly improved seedling length in FARO44 rice under drought stress compared with control. However, priming with 2.5% KNO_3+3.5% SiO_2 produced significantly higher seedling under drought stress compared to other priming treatments. Similarly, priming using 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA or 2.5% KNO_3+1 mM SA significantly enhanced rice seedling biomass and root length compared with control under drought stress. Priming with 2.5% KNO_3+3.5% SiO_2 significantly improved root length of rice subjected to drought in comparison to other priming treatments evaluated in this study.

Table 1: Seedling growth in FARO44 rice primed with combinations of KNO_3, SiO_2 and SA under PEG-induced drought stress

Priming Treatments	SL (cm)	SB (Mg)	RL (cm)
Control	2.00 ± 0.03^{d}	51.91 ± 1.04^{b}	0.88 ± 0.04^{d}
2.5% KNO_3+3.5% SiO_2	5.76 ± 0.09^{a}	52.48 ± 0.95^{ab}	4.79 ± 0.10^{a}
3.5% SiO_2+1 mM SA	4.53 ± 0.10^{b}	52.43 ± 0.46^{ab}	3.61 ± 0.10^{b}
2.5% KNO_3+1 mM SA	3.12 ± 0.02^{c}	54.34 ± 0.41^{a}	2.19 ± 0.03^{c}
Levels of Significance	0.000	0.000	0.000

Means and standard error (SE) in same column having similar superscripts are statistically the same as shown by Duncan Multiple Range Test at $P \leq 0.05$; SL stands for Seedling length; SB = Seedling biomass; RL = root length.

Synergistic potentials of osmopriming and hormonal priming on superoxide dismutase, catalase and ascorbate peroxidase activities in FARO44 rice seedlings under drought stress

Osmo-hormonal priming had significant ($P \leq 0.05$) effects on superoxide dismutase and catalase activity in FARO44 seedlings subjected to drought stress (Table 2). However, osmo-hormonal priming showed no substantial effect on ascorbate peroxidase activities in rice seedlings under drought stress (Table 3). Synergistic priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA significantly improved superoxide dismutase and catalase activity of rice seedlings under drought stress compared to control. However, osmo-hormonal priming treatments had no effects in improving activities of ascorbate peroxidase in rice seedlings under drought compared with control. However, osmo-hormonal priming with 3.5% SiO_2+1 mM SA produced significantly higher catalase activity in rice seedlings under drought stress in comparison to other priming treatments.

Table 2: Superoxide dismutase, catalase and ascorbate peroxidase activities of FARO44 rice seedlings primed with combinations of KNO_3, SiO_2 and SA under PEG-induced drought

Priming Treatments	SOD (U/mgFW)	CAT ($U/mg^{-1}min^{-1}FW$)	APX ($U/mg^{-1}min^{-1}FW$)
Control	0.06 ± 0.02^{c}	0.00 ± 0.00^{c}	0.08 ± 0.01^{a}
2.5% KNO_3+3.5% SiO_2	0.16 ± 0.03^{bc}	0.04 ± 0.01^{b}	0.14 ± 0.02^{a}
3.5% SiO_2+1 mM SA	0.46 ± 0.02^{a}	0.09 ± 0.01^{a}	0.43 ± 0.22^{a}
2.5% KNO_3+1 mM SA	0.25 ± 0.06^{b}	0.01 ± 0.00^{bc}	0.23 ± 0.06^{a}
Levels of Significance	0.000	0.000	0.197

Means and standard error (SE) in same column having similar superscripts are statistically the same as shown by Duncan Multiple Range Test at $P \leq 0.05$; SL stands for Seedling length; SB = Seedling biomass; RL = root length

Synergistic effects of osmopriming and hormonal priming on protein, proline and malondialdehyde contents in FARO44 rice seedlings under drought

Synergistic osmo-hormonal priming had significant ($P \leq 0.05$) effects on protein, proline and malondialdehyde content of FARO44 rice seedlings as shown in Table 3. Osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2 and 2.5% KNO_3+1 mM SA produced significantly higher content of protein in rice seedlings subjected to drought stress in comparison with 3.5% SiO_2+1 mM SA.

Synergistic osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA significantly improved proline content and reduced the conent of malondialdehyde in rice seedlings subjected to drought stress in comparison with control. However, osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2 and 2.5% KNO_3+1 mM SA significantly enhanced proline content in rice seedlings compared with 3.5% SiO_2+1 mM SA. Equally, osmo-hormonal priming manifestly reduced content of MDA in rice seedlings compared with control.

Table 3: Protein, proline and malondialdehyde contents in FARO44 rice seedlings primed with combinations of KNO_3, SiO_2 and SA under PEG-induced drought

Priming Treatments	Protein (mg/ml)	Proline (mg/ml)	MDA (mM/mg^{-1}FW)
Control	0.35 ± 0.03^{c}	3.22 ± 1.27^{c}	0.070 ± 0.00^{a}
2.5% KNO_3+3.5% SiO_2	0.73 ± 0.01^{a}	15.51 ± 1.63^{a}	0.001 ± 0.00^{b}
3.5% SiO_2+1 mM SA	0.63 ± 0.03^{b}	9.79 ± 2.26^{b}	0.001 ± 0.00^{b}
2.5% KNO_3+1 mM SA	0.72 ± 0.02^{a}	17.74 ± 2.09^{a}	0.001 ± 0.00^{b}
Levels of Significance	0.000	0.000	0.000

Means and standard error (SE) in same column having similar superscripts are statistically the same as shown by Duncan Multiple Range Test at $P \leq 0.05$; SL stands for Seedling length; SB = Seedling biomass; RL = root length; MDA stands for malondialdehyde

Discussions

Scarcity of water has been a crucial ecological burden that austerely reduces crop harvest in arid and semi-arid biomes across the globe (Yan, 2015). Synergistic osmopriming and hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA improved germination attributes, seedling growth, antioxidant enzymes' activities as well as biochemical characteristics of FARO44 rice seedlings grown under PEG-induced water deficit stress. The findings of this study showed that osmo-hormonal priming improved germination index and decreased time taken for completion of germination (mean germination time) of FARO44 rice under PEG-induced drought stress. However, osmo-hormonal priming had no substantial effect in improving germination percentage of rice under PEG-induced drought. Enhancement of GI and decrease of MGT of rice was associated with increased cell division, activation of important pre-germination processes in seeds, accelerated activities of antioxidants such as ascorbate peroxidase, superoxide dismutase and catalase as well as high content of proline, soluble sugar as well as improved imbibition of water in osmo-hormonal primed seeds. Heightened antioxidant activities in rice seedlings point out to resistance to water stress conditions. Osmotic potential regulation has been a strategy employed by plants to respond to prevalent water deficit circumstances (Farooq *et al*., 2009). This study found that synergistic osmo-hormonal priming improved seedling growth of FARO44 rice under drought. Improved seedling growth might be due to the pivotal roles of osmo-hormonal priming in triggering cell division and elongation, restoration of impaired nucleic acid, production and galvanization of development proteins as well as heightened drought coping factors. Plants growing in drought threatened settings typically amass osmolytes like proline and soluble sugar that aid in improving osmotic modification (Yan, 2015). Previously, Farooq *et al*. (2013) found that ascorbic acid osmoprimed and hydroprimed wheat had improved biomass, seedling length plus phenolic and proline content.

It had been found that synergistic osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA improved seedling biomass under PEG-induced drought. Enhancement of biomass of rice seedling by osmo-hormonal priming was closely correlated to speedy germination initiated by hastened metabolic processes. Modulated hydrolases are paramount for production of metabolites that stimulate accelerated germination and uniform establishment of seedlings (Farooq *et al.*, 2006). Supporting these results, Farooq *et al.* (2013) found that ascorbic acid osmoprimed and hydroprimed wheat had improved biomass, seedling length. Equally, Rehman *et al*. (2011) stated that $CaCl_2$ osmoprimed rice had higher kernel mass as well as emergence index. Agreeing these results, PEG osmoprimed sorghum grown under limiting water supply was reported by Zhang *et al*. (2015) to have had improved dry biomass and vigour.

Synergistic osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA further improved activities of APX, CAT and SOD in rice seedlings grown under water constraint. While responding to stresses, plants deploy antioxidant systems consisting of catalase, glutathione, superoxide dismutase, peroxidase, superoxide dismutase and ascorbic acid to convert the toxic ROS to harmless substances (Gill & Tuteja, 2010; Hussain *et al*., 2016). Improved activities of antioxidants in rice seedlings suggested increased resistance to drought. Corroborating these results, the study by Yan (2015) found that KNO_3 soaked *Brassica rapa* grown under limited water had high POD, SOD and CAT activities.

It had been shown in this study that synergistic osmo-hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5% KNO_3+1 mM SA enhanced proline content as well as lessened malondialdehyde content in rice seedlings subjected to limited supply of water. Proline is an essential osmolyte for osmotic adjustment in plants under stress, while malondialdehyde causes lipid peroxidation which is responsible for oxidative stress. Plants amass glycine betaine, proline, carbohydrate and soluble sugar as resistance tactics in limited water settings (Chowdhury *et al*., 2017). Corroborating these results, Abdul Latef & Tran, (2016) stated that Si mediated maize grown in alkaline conditions had substantially higher total chlorophyll and soluble sugar. Salicylic acid and selenium osmo-hormonal primed rice cultivars under cold stress had high proline, glutathione, SOD, POD and CAT activities; low malondialdehyde content (Hussain *et al*., 2016).

Conclusions

Drought is a crucial ecological constraint that affects seed germination, growth of seedling and yield of cereals particularly rice across the globe. Synergistic osmo hormonal priming with 2.5% KNO_3+3.5% SiO_2, 3.5% SiO_2+1 mM SA and 2.5%

KNO_3+1 mM SA proved effective in improving germination characteristics, growth of seedling as well as biochemical components of FARO44 rice subjected PEG-induced drought situations. Activities of radical destroying ascorbate peroxidase, superoxide dismutase and catalase were heightened in osmo-hormonal primed rice seedlings. Synergistic osmo-hormonal priming treatments also improved protein and proline contents in rice seedlings while decreased malondialdehyde content.

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