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POTENTIAL OF OSMOPRIMING FOR IMPROVED GERMINATION AND SEEDLING GROWTH OF RICE VAR. FARO44 UNDER DROUGHT STRESS

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Abstract

Osmopriming is a physiological approach that improves germination, seedling growth and imparts drought stress tolerance to germinating seeds and seedlings. Rice is an essential cereal produced and consumed worldwide. However, poor germination and irregular seedling emergence in direct-seeded rice production caused by drought in a changing climate are the major causes of low yield in hot and dry northeastern Nigeria. In this study, rice seeds var. FARO44 was subjected to different osmopriming treatments to improve germination, seedling growth and drought tolerance. The aims of this study include: (i) to determine the potential of osmopriming with potassium nitrate (KNO_3) and silicon dioxide (SiO_2) on germination and seedling growth of FARO44 rice (ii) to determine the potential of osmopriming with KNO_3 and SiO_2 on biochemical attributes and protective enzymes of FARO44 rice. Seeds were separately osmoprimed in KNO_3 (2.5% & 5% w/v); SiO_2 (3% & 3.5% w/v) and their combinations (2.5% KNO_3+3.5% SiO_2; 5% KNO_3+3% SiO_2). Osmoprimed seeds were then germinated in specimen bottles for three weeks with the third week drought stress being imposed. The experiments were arranged in a completely randomized design with each treatment replicated five times. Thereafter, germination percentage, mean germination time,

Keywords: Osmopriming, germination percentage, catalase, superoxide dismutase, ascorbate peroxidase

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germination index, seedling growth were evaluated. Likewise, total soluble protein, proline, malondialdehyde and activities of protective enzymes superoxide dismutase, catalase and ascorbate peroxidase were determined. The results found that osmopriming with KNO_3, SiO_2 and their combinations significantly ($P \leq 0.05$) improved germination percentage, germination index, shortened germination time and improved seedling growth, seedling and seedling vigour of rice seedlings. Osmopriming separately with KNO_3 and SiO_2 had more effects in improving these germination, growth and biochemical attributes and activities of protective enzymes than combined osmopriming.

Introduction

Rice (*Oryza sativa* L.) is an important cereal crop consumed by majority of people around the world which accounts for more than 50% of staple food need of the global population. Considering the essential roles rice plays there is the need for stable and adequate supply for absolute food security, poverty reduction and advancement of financial status of developing nations both in Africa and Asia. The old agricultural practices are inadequate to meet the increasing food demands of the world's growing population (Jisha & Puthur, 2016). Abiotic stresses such as drought, salinity and extreme temperatures affect germination and seedling establishments accounting for total rice yield loss of 50% and as such impede global rice production.

Poor germination and irregular establishment of seedlings associated with direct-seeded system of rice production limit achieving better crop growth and yields particularly under drought stress conditions during emergence (Liu et al., 2015). Sometimes, severe drought stress may lead to total cessation of seedling emergence (Kaya et al., 2006).

Most of dry rice-growing are in northern Nigeria are ravaged by severe drought cause by changing climate, this led to poor germination and establishment of rice seedlings. This lead to decline in the productivity of rice especially FARO44 variety. There are several approaches available that enhance germination, seedling growth and tolerance capability of cereals against abiotic stresses such as plant breeding, selection and production of transgenics (Jisha et al., 2013). However, these approaches are expensive compared to osmopriming. Osmopriming is a simple physiological process of soaking seeds in inorganic solutions that improves germination, seedling growth and imparts drought resistance to cereals such as rice, maize, wheat and so on. Previously, it was reported that silicon, potassium nitrate osmopriming enhanced germination, seedling growth and drought resistance of rice,

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barley, wheat, maize and sorghum seedlings (Biju et al., 2017; Fayez & Bazaid, 2014; Tabatabaei, 2013). However, there was scanty of studies that applied osmopriming on FARO44 rice variety. Thus, this study was carried out with the aim of (i) to determine the potential of osmopriming with potassium nitrate (KNO_3) and silicon dioxide (SiO_2) on germination and seedling growth of FARO44 rice (ii) to determine the potential of osmopriming with KNO_3 and SiO_2 on biochemical attributes and protective enzymes of FARO44 rice.

MATERIAL AND METHODS

FARO44 rice (*Oryza sativa* L.) was obtained from Badeggi Research Institute in the central State of Niger State, Nigeria. It is a hybrid rice having a long grain produced from hybridisation of African local rice and Taiwan's Indica rice. It is a high yielding variety that matures in three month and can be produced under irrigation and rain fed conditions (Akinwale et al., 2012; Oluwaseyi et al., 2016).

Seed osmopriming and germination

Prior to osmopriming, all beakers and Petri dishes were kept in an oven for 24 hours at 110 ºC; while the different osmopriming solutions of SiO_2 and KNO_3 were prepared and kept in a fridge freezer (4 ºC). Viable selected quality rice seeds were surface sterilized with 0.5% sodium hypochlorite (v/v) for 10 minutes to suppress microbial growth. They were then rinsed thoroughly with distilled water. Seeds were separately soaked in solutions of KNO_3 (2.5% & 5% w/v); SiO_2 (3% & 3.5% w/v) and their combinations (2.5% KNO_3+3.5% SiO_2; 5% KNO_3+3% SiO_2) for 8 hours and the systems were kept in the laboratory growth room of temperature 25±2ºC and relative humidity of 50-70% (Khan et al., 2019). The solutions were thereafter drained out and the seeds were dried at room temperature of 25 ºC for 48 hours (Anosheh et al., 2011; Azeem et al., 2015).

Seed germination and drought stress imposition

Ten KNO_3 and SiO_2 osmoprimed and un-osmoprimed rice seeds (control) were separately germinated in specimen bottles lined with Whatman filter paper (90mm size) wetted with 6 ml distilled water for 14 days. Seed is considered to have germinated if the radicle measures about 2 mm (Chunthaburee et al., 2014). Prior to drought stress induction, Kaufmann (Kaufmann, 1973) formula was used for calculating drought stress solution of -0.3 Mpa using polyethylene glycol (PEG6000). After 14 days of germination, drought stress was induced to the young rice seedlings by watering with 6 ml of -0.3 Mpa PEG6000 for one week (Yan, 2015). The specimen

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bottles were kept in the laboratory growth room of temperature 25±2ºC and relative humidity of 50-70%, seed germination was recorded for 3 weeks (Khan et al., 2019). From the data of germination collected, germination percentage (GP), germination index (GI) and mean germination time (MGT) were evaluated (Ghodrat et al., 2013; Aloui et al., 2014; Ruttanaruangboworn et al., 2017). Seedling length, root length and shoot length were measured with a ruler while seedling vigour was evaluated (Abdul-baki & Anderson, 1970).

Determination of biochemical attributes and protective enzymes of rice seedlings

Preparation of protein extract

The slightly modified method of Muchate et al. (2019) was used. Leaves (0.25g) of FARO44 rice seedlings osmoprimed with KNO_3 (2.5% & 5% w/v) and SiO_2 (3% & 3.5% w/v) and their combinations (2.5% KNO_3+3.5% SiO_2 and 5% KNO_3+3% SiO_2) were first frozen in liquid nitrogen to prevent proteolytic activity. Thereafter, the samples were separately ground in ice-chilled mortar in 2 ml of 62.5 mM Tris-HCl (pH 6.7). The samples were then centrifuged at 13,000 g for 10 min. at 4 ºC. The supernatant was collected and used for determination of total soluble protein and assays of catalase and ascorbate peroxidase activities.

Determination of protein Content

Total soluble protein was determined according to the slightly modified method of Bradford, (1976) using Bovine serum albumin (BSA) as a standard. The 1 ml reaction mixture comprised of 100µl protein extract and 900µl Bradford reagent. The reaction mixture was read with a spectrophotometer (Model-Hitachi U-1900, Tokyo, Japan) at wavelength 620nm. The constituent of protein was computed using the linear equation derived from BSA protein standard curve ($y=0.77x$, $R^2=0.97$).

Determination of proline Content

Proline content of KNO_3 and SiO_2 osmoprimed FARO44 rice seedlings grown under drought stress were determined according to the slightly modified method by Bates et al. (1973). A 250 mg of FARO44 rice seedling was ground in ice chilled mortar in 3 ml aqueous sulfosalicylic acid (pH 7.8). The mixture was centrifuged for 13,000 g for 6 minutes at 4 ºC. The 2 ml of the supernatant was mixed with 2 ml ninhydrin reagent (30 ml of glacial acetic acid, 1.25 g of ninhydrin and 20 ml 6 Molar H_3PO_4). The reaction was incubated in water bath at 100 ºC for 1 hour and then cooled at room temperature, 4 ml of toluene was added while the mixture was vortexed for 4 minutes at 40 rpm. Following phase separation, the upper layer was used in measuring the proline content and read at absorbance of 520nm with a spectrophotometer (Model-Hitachi

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U-1900, Tokyo, Japan). The content of proline was determined from a linear equation derived from pure L-proline (Sigma-Aldrich)) standard curve (y=2.78x, R2=0.99).

Determination of lipid peroxidation

Lipid peroxidation of KNO_3 and SiO_2 osmoprimed FARO44 rice seedlings grown under drought stress was determined in terms of the content of malondialdehyde (MDA) and the slightly modified method of Heath & Packer, (1968) was used. First, fresh leaf sample (250 mg) was frozen in liquid nitrogen. The frozen leaves were ground in ice-chilled mortar in 3 ml of trichloroacetic acid (TCA). Then, the homogenate was centrifuged at 13,000 g for 6 minutes at 4 ºC. From the supernatant, 2 ml was taken and mixed with 2 ml of 0.67% thiobarbituric acid. The mixture was then heated in water-bath set at 100 ºC for 30 minutes and the reaction was quickly terminated by cooling in ice bath. The mixture was thereafter centrifuged for 10 minutes at 10,000 g for suspended impurities to settle. The absorbance of the collected supernatant was finally read at 532 and 600 nm. The mixture of 0.025% thiobarbituric acid in 10% trichloroacetic acid was used as a blank sample. The content of MDA (mM) was calculated using the following formula:

MDA (mM) = (A532-A600)/ε, ε is the extinction coefficient (=155 $mM^{-1}cm^{-1}$).

Determination of protective enzymes' activities of rice seedlings

Superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase activities from the leaves of KNO_3 and SiO_2 osmoprimed FARO44 rice seedlings were determined according to the slightly modified methods of Esfandiari et al. (2007) Nakano & Asada, (1981).

Experimental Design and Statistical Analyses

The osmopriming experiments were laid down in a completely randomized design with 5 replicates per each treatments (Azeem et al., 2015). Data normality was checked with Shapiro-Wilk test before statistical analyses. One-way analysis of variance was performed to compare osmopriming effect on growth parameters and biochemical components studied and two-way analyses of variance (ANOVA) were carried out to compare osmopriming effect on drought stress both with SPSS (window version 23), and significant differences of means were separated using Duncan's Multiple Range test ($p < 0.05$) (Khatami et al., 2015).

RESULTS AND DISCUSSION

Effect of osmopriming on germination attributes of FARO44 rice under drought stress

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Osmopriming of rice with KNO_3, SiO_2 and their combinations, drought stress and interaction of osmopriming and drought stress had significant effect ($P \leq 0.05$) on germination percentage (GP), germination index (GI) and mean germination time (MGT) of FARO44 rice under drought stress as shown in Table 1 below.

Under drought stress, germination percentage of FARO44 rice was significantly increased by 2.5% and 5% KNO_3 and 3% and 3.5% SiO_2 osmopriming compared with control. Likewise, under drought stress, GP of FARO44 rice was increased by combined osmopriming with 2.5% KNO_3+3.5% SiO_2 and 5% KNO_3+3% SiO_2 compared with control.

Furthermore, under drought stress, GI of FARO44 rice was significantly increased by KNO_3, and SiO_2 osmopriming compared with control. However, combined osmopriming with 2.5% KNO_3+3.5% SiO_2 and 5% KNO_3+3% SiO_2 showed no significant effect on enhancing GI of FARO44 rice compared with control. Osmopriming with 2.5% and 5% KNO_3 and 3% SiO_2 showed more effect in enhancing GI of FARO44 rice.

Under drought stress, MGT of FARO44 rice was significantly decreased by osmopriming compared with control. Osmopriming with 2.5% KNO_3 followed by 3.5% SiO_2, 5% KNO_3 and 3% SiO_2 showed more effect in decreasing the MGT of FARO44 rice.

Table 1: Germination attributes of FARO44 rice osmoprimed with KNO_3, SiO_2 and their combinations under drought stress

Osmopriming Treatments	GP (%)	GI	MGT (Days)
Control	96 ± 2.44^{ab}	2.88 ± 0.07^{cd}	4.6 ± 0.24^{f}
2.5% KNO_3	100 ± 0.00^{a}	3.68 ± 0.08^{a}	2.8 ± 0.20^{a}
5% KNO_3	100 ± 0.00^{a}	3.60 ± 0.08^{a}	3.2 ± 0.20^{c}
3% SiO_2	100 ± 0.00^{a}	3.70 ± 0.05^{a}	3.2 ± 0.20^{c}
3.5% SiO_2	100 ± 0.00^{a}	3.48 ± 0.13^{ab}	3.0 ± 0.00^{b}
2.5% KNO_3+3.5% SiO_2	100 ± 0.00^{a}	2.82 ± 0.09^{cde}	4.4 ± 0.24^{e}
5% KNO_3+3% SiO_2	100 ± 0.00^{a}	2.86 ± 0.05^{cd}	4.0 ± 0.00^{d}
Osmopriming TRTs	**	**	**
Drought stress	**	**	**
Osmopriming× Drought	Ns	**	**

Mean values ± SE in the same column followed with similar letters are not significantly different according to DMRT $P \leq 0.05$; SE = standard error of the mean; GP = Germination percentage; GI = Germination index; MGT = Mean germination time; * = Significant at 5% level of probability; ** = Significant at 1% level of probability; ns = Not significant

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Drought is one of the key causes of yield decline in rice worldwide. Germination of seed is a critical stage in rice production especially in rain-fed agricultural systems. Even a moderate drought stress could affect seed germination and seedling emergence and consequently causes yield decline of rice.

Drought stress decreased germination percentage (GP), germination index (GI) and prolonged the mean germination time (MGT) of FARO44 rice. However, osmopriming with KNO_3, SiO_2 their combinations improved these germination attributes of FARO44 rice. Osmopriming with combinations of KNO_3+SiO_2 showed less effect in improving germination attributes of FARO44 rice. This might be attributed to the low osmotic potential of the osmopriming solutions thereby slowed down water imbibition into seeds hence affected germination processes.

Enhanced GP, GI and shorter MGT of KNO_3 and SiO_2 osmoprimed FARO44 rice under drought stress were as a result of completion of phases I, II and III of seed germination with their associated physiological and metabolic processes; which include increased water imbibition, biosynthesis of protein and starch within seeds for embryonic growth and development, activation of seed reserve mobilising enzymes that hydrolysed reserve for embryonic growth and faster emergence.

Improved GP, GI and shorter MGT of KNO_3 and SiO_2 osmoprimed FARO44 rice under drought stress conditions indicated increased resistance to drought. Drought suppressed cell division and enlargement, activation of reserve mobilising enzymes, increase pH, causes nutritional and metabolic impairment and ion disorders in rice seedlings (Dien et al., 2019).

Effect of osmopriming with KNO_3, SiO_2 and their combinations on seedling growth of FARO44 rice under drought stress

Osmopriming with KNO_3, SiO_2, their combinations, drought stress and their interactions had significant effect ($P \leq 0.05$) on seedling length, shoot and root length of FARO44 rice as shown in Table 2. Under drought stress, 2.5% and 5% KNO_3 and 3.5% SiO_2 increased seedling length of FARO44 rice by about 2-folds compared with control. Likewise, 3% SiO_2 and combinations of KNO_3 and SiO_2 osmopriming increased seedling length of FARO44 rice compared with control. Osmopriming with 2.5% and 5% KNO_3 showed more effect in increasing seedling length of FARO44 rice followed by 3.5% SiO_2 osmopriming.

Likewise, under drought stress, 2.5% and 5% KNO_3 and 3% and 3.5% SiO_2 osmopriming increased shoot length of FARO44 rice by about 2-folds compared with control. Also, osmopriming with combinations of KNO_3 and SiO_2 increased shoot length of FARO44 rice compared with control.

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Furthermore, under drought stress, root length of FARO44 rice was increased by osmopriming with KNO_3 and SiO_2 by more than 2-folds compared with control. Also, combination of 2.5% KNO_3+3.5% SiO_2 and 5% KNO_3+3% SiO_2 osmopriming significantly increased root length of FARO44 rice seedlings by about 2-folds compared with control.

Table 2: Growth performance of FARO44 rice seedlings osmoprimed with KNO_3, SiO_2 and their combinations under drought stress

Osmopriming Treatments	SL (cm)	ShL (cm)	RL (cm)
Control	7.80 ± 0.02^{f}	3.84 ± 0.10^{e}	3.91 ± 0.08^{ij}
2.5% KNO_3	15.50 ± 0.04^{ab}	6.56 ± 0.13^{b}	8.92 ± 0.16^{a}
5% KNO_3	16.05 ± 0.01^{a}	7.40 ± 0.03^{a}	8.67 ± 0.03^{ab}
3% SiO_2	13.57 ± 0.09^{c}	5.99 ± 0.23^{c}	7.58 ± 0.22^{def}
3.5% SiO_2	15.31 ± 0.05^{b}	6.22 ± 0.13^{bc}	9.08 ± 0.27^{a}
2.5% KNO_3+3.5% SiO_2	12.41 ± 0.31^{d}	4.68 ± 0.30^{d}	7.74 ± 0.17^{cde}
5% KNO_3+3% SiO_2	11.64 ± 0.21^{e}	5.25 ± 0.27^{cd}	6.38 ± 0.18^{gh}
Osmopriming TRT	**	**	**
Drought stress	**	**	**
Osmopriming × Drought	**	**	**

Mean values ± SE in the same column followed with similar letters are not significantly different according to DMRT P ≤ 0.05; SE = Standard error of the mean; SL = Seedling length; ShL = Shoot length; RL = Root length; * = Significant at 5% level of probability; ** = Significant at 1% level of probability; ns = Not significant

Drought is one of the environmental pressures that influences rice farming in rain-fed and irrigation systems and has been a serious impediment for crop yield in drought ravaged ecosystems of the globe. Decreased in the growth of rice seedlings is the most distinctive sign of deficit water stress. Seedling growth is a precursor to productive agriculture.

In the present study, drought decreased seedling length, shoot length, root length and growth of FARO44 rice seedlings. Osmopriming of FARO44 rice with KNO_3, SiO_2 and their combinations countered the grim effect of drought and increased seedling growth of FARO44 rice. However, osmopriming with combinations of KNO_3 and SiO_2 showed less effect in increasing shoot length and root length of FARO44 rice seedlings. This was because of the toxicity of combinations of KNO_3 and SiO_2, or low osmotic potential of the osmopriming solutions that slowed water entry into the seeds to cause breakdown of seed reserve by enzymes such as dehydrogenase, acid phosphatase and amylases for embryonic growth and development.

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Improved seedling growth of KNO_3 and SiO_2 osmoprimed FARO44 rice might be due to prolific cell division, enlargement and elongation, increased activation of superoxide dismutase, ascorbate peroxidase and catalase activities and low lipid peroxidation in osmoprimed rice seedlings. Increased seedling growth of FARO44 rice under drought conditions were associated with the effect of KNO_3 and SiO_2 in triggering increased production of proline, soluble sugars, glycine betaine and anti-stress proteins that helped in osmotic adjustment and maintenance of seedlings' health under drought conditions.

Increased root length in osmoprimed FARO44 rice seedlings was because KNO_3 and SiO_2 osmopriming stimulated enzymes that weakened the cells encircling the radicle thereby sped up germination by stimulating elongation of seedlings. These results indicated an improved drought tolerance of FARO44 rice seedlings by exhibiting enhanced growth and vigour under deficit water conditions. Silicon, KNO_3 and SA impart drought resistance to rice, wheat, maize and sorghum (Anwar et al., 2013; Biju et al., 2017; Tayyab et al., 2020). Previously, it was reported that silicon, KNO_3 and SA application enhanced seedling growth and drought resistance of rice, barley, wheat, maize and sorghum seedlings (Biju et al., 2017; Fayez & Bazaid, 2014; Tabatabaei, 2013).

Effect of osmopriming on seedling vigour of FARO44 rice under drought stress

Osmopriming with KNO_3, SiO_2, their combinations, drought stress and their interactions had significant effect ($P \leq 0.05$) on seedling vigour indices of FARO44 rice as shown in Table 3.

Under drought stress, seedling vigour index I (SVI I) of FARO44 rice was increased by KNO_3 and SiO_2 osmopriming by more than 2-folds, and combined osmopriming also increased significantly SVI I of FARO44 rice compared with control. Osmopriming with 2.5% and 5% KNO_3 showed more effect in enhancing SVI I of rice seedlings.

Similarly, drought stress, seedling vigour index II (SVI II) of FARO44 rice was increased by KNO_3 and SiO_2 osmopriming by more than 2-folds, and combined osmopriming also increased significantly SVI II of FARO44 rice compared with control. Osmopriming with 2.5% and 5% KNO_3 showed more effect in enhancing SVI II of rice seedlings.

Table 3: Seedling vigour indices of FARO44 rice seedlings osmoprimed with KNO_3, SiO_2 and their combinations under drought stress

Osmopriming treatments	SVI I	SVI II
Control	774 ± 11.96^{gh}	2840.26 ± 12.07^{f}
2.5% KNO_3	1489.46 ± 11.64^{ab}	3619.33 ± 20.97^{b}
5% KNO_3	1533.06 ± 8.69^{a}	4403 ± 35.95^{a}
3% SiO_2	1346 ± 31.72^{bc}	3490.33 ± 51.77^{c}

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3.5% SiO_2	1481.4 ± 31.23^{bc}	3098.25 ± 19.02^{d}
2.5% KNO_3+3.5% SiO_2	1252.66 ± 32.03^{cde}	2735.66 ± 4.49^{g}
5% KNO_3+3% SiO_2	1160.66 ± 28.00^{ef}	3083.5 ± 27.56^{e}
Osmopriming TRT	**	**
Drought stress	**	**
Osmopriming × Drought stress	**	**

Mean values ± SE in the same column followed with similar letters are not significantly different according to DMRT at P ≤ 0.05; SE = Standard error of the mean; SVI I = Seedling vigour index I; SVI II = Seedling vigour index II; * = Significant at 5% level of probability; ** = Significant at 1% level of probability; ns = Not significant

Osmopriming with KNO_3 and SiO_2 improved seedling vigour of FARO44 rice under drought stress. However, combination of KNO_3 and SiO_2 showed less effect on enhancing seedling vigour of FARO44 rice. Improved seedling vigour might be due the roles of KNO_3 and SiO_2 in stimulating cell growth and cell expansion.
Earlier study reported that Si osmoprimed wheat under salinity stress recorded significantly increased seedling biomass and seedling growth (Azeem et al., 2015). Consistent with these results, the earlier study of Farooq et al. (2010) revealed that $CaCl_2$, KCl and ascorbate osmopriming increased seedling vigour index of rice as well as increased activities of alpha amylase. Spring maize primed with SA, extract of Moringa leaf and $CaCl_2$ showed improved seedling vigour and seedling growth (Rehman et al., 2015). Eskandari & Kazemi, (2011) reported that KNO_3 and NaCl osmopriming and hydropriming of cowpea substantially improved seedling vigour index, seedling establishment and seedling growth of cowpea. Urea and KNO_3 osmopriming were effective in imparting drought resistance, enhancing vigour index and growth of Chinese cabbage (Yan, 2015).
Agreeing the present results, Ahmad et al. (2012) reported that maize primed with SA under low temperature stress recorded improved root and shoot dry biomass, seedling vigour and increased activities of POD and CAT. It has been reported that osmopriming wheat with 1% KNO_3 and tomato with 0.75% KNO_3 enhanced seedling vigour index, shoot fresh and dry biomass, seedling fresh biomass, seedling length, plant height, harvest index, grain yield and phenolic and soluble sugar content (Sarlach et al., 2013; Ali et al., 2020).

Effect of osmopriming on soluble protein, proline and malondialdehyde content of FARO44 rice seedlings under drought stress

Osmopriming with KNO_3, SiO_2, their combinations, drought stress and their interaction had significant effect (P ≤ 0.05) on soluble protein, proline and malondialdehyde (MDA) content of FARO44 rice seedlings as shown in Table 4.
Under drought stress conditions, FARO44 rice seedlings osmoprimed with KNO_3, SiO_2 and combinations had higher total soluble protein content compared with control.

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FARO44 rice seedlings osmoprimed with 2.5% and 5% KNO_3 had the highest total soluble protein content followed by 3% and 3.5% SiO_2 osmoprimed rice seedlings.
Under drought stress, proline content of FARO44 rice seedlings was increased by 2.5% and 5% KNO_3 osmopriming by more than 3-folds, 3% and 3.5% SiO_2 osmopriming by increased about 3-folds, while combinations of KNO_3 and SiO_2 osmopriming significantly increased proline content of FARO44 rice seedlings compared with control.
Malondialdehyde content (MDA) of FARO44 rice seedlings was significantly decreased by KNO_3, SiO_2 osmopriming and their combinations under drought stress conditions compared with control. Malondialdehyde causes lipid peroxidation in plants.

Table 4: Soluble protein, proline and MDA content of FARO44 rice seedlings osmoprimed with KNO_3, SiO_2 and their combinations under drought stress

Osmopriming Treatments	Total Soluble Protein (mg/FW)	Proline (mg/L)	MDA (µmolmg^{-1}FW)
Control	0.86 ± 0.01^{jk}	7.05 ± 0.34^{rs}	0.068 ± 0.00^{ab}
2.5% KNO_3	1.35 ± 0.03^{a}	33.67 ± 1.17^{bc}	0.002 ± 0.00^{e}
5% KNO_3	1.25 ± 0.03^{abcd}	27.76 ± 1.03^{cd}	0.002 ± 0.00^{e}
3% SiO_2	1.21 ± 0.10^{cde}	22.43 ± 1.11^{e-h}	0.002 ± 0.00^{e}
3.5% SiO_2	1.30± 0.02^{abc}	23.64 ± 1.64^{def}	0.002 ± 0.00^{e}
2.5% KNO_3+3.5% SiO_2	1.09 ± 0.01^{fgh}	21.29 ± 1.25^{e-j}	0.004 ± 0.00^{e}
5% KNO_3+3% SiO_2	1.08 ± 0.02^{fghi}	21.63 ± 0.48^{e-k}	0.055 ± 0.01^{bcd}
Osmopriming TRT	**	**	**
Drought stress	**	**	**
Osmopriming × Drought	**	**	**

Mean values ± SE in the same column followed with similar letters are not significantly different according to DMRT at P ≤ 0.05; SE = Standard error of the mean; MDA = Malondialdehyde; * = Significant at 5% level of probability; ** = Significant at 1% level of probability; ns = Not significant

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Osmopriming of FARO44 rice with KNO_3, SiO_2 and their combinations improved total soluble protein, proline and decreased malondialdehyde content in rice seedlings under drought stress. However, KNO_3 and SiO_2 osmopriming individually had more effect in improving these biochemicals more than combined osmopriming. These might be associated with toxicity with combined KNO_3 and SiO_2 osmopriming that hindered initiation of essential biochemical processes in the rice seeds. Improved protein, proline and decreased malondialdehyde content in osmoprimed rice seedlings increased water stress resistance of rice seedlings.

It has been reported that Si osmoprimed maize recorded about 2-folds higher soluble protein, soluble sugar, glycine betaine and proline contents and improved resistance to water deficit stress (Abdel Latef & Tran, 2016; Pandey & Shukla, 2015). Agreeing these results, earlier studies revealed that SA and Si osmopriming of rice and maize increased content of protein, proline, glycine betaine, decreased malondialdehyde content and improved drought and chilling resistance (Hussain et al., 2016; Parveen et al., 2019). Supporting these results, Jafar et al. (2012) found that two wheat varieties primed with SA under salinity constraint recorded significantly higher soluble protein content and amylase and protease activities.

Effect of osmopriming on superoxide dismutase, catalase and ascorbate peroxidase activities of FARO44 rice seedlings under drought stress

Osmopriming with 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, their combinations and drought had significant effects ($P \leq 0.05$) on superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities of FARO44 rice seedlings. Drought stress had significant effect ($P \leq 0.05$) on SOD and CAT activities of FARO44 rice seedlings but not on APX activities. However, interaction of osmopriming and drought had significant effect ($P \leq 0.05$) on CAT activities of FARO44 rice seedlings but not on SOD and APX activities of FARO44 rice seedlings as shown in Table 5.

Under drought stress, 2.5% and 5% KNO_3 osmopriming increased SOD activities of FARO44 rice seedlings by more than 2-folds, 3% and 3.5% SiO_2 priming increased SOD activities of FARO44 seedlings by more than 4-folds, and combinations of 2.5% KNO_3+3.5 SiO_2 increased SOD activities of FARO44 rice seedlings by more than 2-folds compared with control. However, 5% KNO_3+3% SiO_2, osmopriming slightly increased SOD activities of FARO44 rice seedlings compared with control.

Under drought stress, osmopriming with 2.5% KNO_3, 5% KNO_3, 3% SiO_2 and 3.5% SiO_2 similarly increased CAT activities of FARO44 rice seedlings by more than 7-folds; combinations of 5 % KNO_3+3% SiO_2 osmopriming increased CAT activities of FARO44 rice seedlings by more than 3-folds. However, osmopriming with combinations of 2.5% KNO_3+3.5% KNO_3 was not significantly different from control.

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Under drought stress, APX activities of FARO44 rice seedlings were increased by 3% and 3.5% SiO_2 by 2-folds and by more than 4-folds; 2.5% and 5% KNO_3 osmopriming enhanced APX activities of FARO44 rice seedlings compared with control. Combinations of 5% 2.5% KNO_3+3.5% SiO_2 and KNO_3+3% SiO_2 osmopriming slightly increased APX activities of FARO44 rice seedlings compared with control.

Table 5: SOD, CAT and APX activities of FARO44 rice seedlings osmoprimed with KNO_3, SiO_2 and their combinations under drought stress

Osmopriming TRTs	SOD Activity (U/mg^{-1}FW)	CAT Activity (U/mg^{-1}protein)	APX Activity (U/mg^{-1}protein)
Control	0.07 ± 0.01^{f}	0.01 ± 0.01^{e}	0.21 ± 0.02^{f}
2.5% KNO_3	0.18 ± 0.06^{d}	1.05 ± 0.00^{a}	0.27 ± 0.06^{e}
5% KNO_3	0.18 ± 0.02^{d}	0.90 ± 0.02^{abc}	0.34 ± 0.10^{c}
3% SiO_2	0.34 ± 0.07^{a}	0.96 ± 0.01^{ab}	0.40 ± 0.07^{b}
3.5% SiO_2	0.26 ± 0.05^{b}	0.85 ± 0.02^{abc}	0.87 ± 0.17^{a}
2.5% KNO_3+3.5% SiO_2	0.19 ± 0.06^{c}	0.02 ± 0.00^{e}	0.19 ± 0.05^{g}
5% KNO_3+3% SiO_2	0.09 ± 0.02^{e}	0.04 ± 0.01^{d}	0.28 ± 0.08^{d}
Osmopriming TRT	**	**	**
Drought stress	*	**	Ns
Osmopriming× Drought	ns	**	Ns

Mean values ± SE in the same column followed with similar letters are not significantly different according to DMRT P ≤ 0.05; SE = Standard error of the mean; CAT = Catalase; APX = Ascorbate peroxidase; SOD = Ascorbate peroxidase; * = Significant at 5% level of probability; ** = Significant at 1% level of probability; ns = Not significant.

Osmopriming with KNO_3, SiO_2 and their combinations increased the activities of superoxide dismutase (SOD) catalase (CAT) and ascorbate peroxidase (APX) of FARO44 rice seedlings. Osmopriming with SiO_2 had more effect in increasing SOD activities FARO44 rice seedlings while KNO_3 osmopriming had more effect in increasing CAT and APX activities in FARO44 rice seedlings under drought stress. This might be associated with effect of KNO_3 and SiO_2 in stimulating antioxidant activities and stress resistance in plants. However, osmopriming with combinations of KNO_3+SiO_2 showed slight effect in increasing SOD, CAT and APX activities in FARO44 rice seedlings under drought compared to osmopriming with these chemicals individually. This might be associated with either due to low osmotic potential of combined KNO_3 and SiO_2 solutions that might have slowed water entry into the rice seeds during the priming process to activate the radical-scavenging machinery.

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Increased SOD, CAT and APX activities in KNO_3 and SiO_2 osmoprimed FARO44 rice seedlings ensured vigorous scavenging of oxidative stress inducing-ROS hence conferred increased tolerance to stress. Catalase, ascorbate peroxidase, peroxidase and superoxide dismutase break down superoxide radicals and H_2O_2 into water and molecular oxygen (Khaliq et al., 2015; Refli and Purwestri, 2016).

Previous studies reported that Si osmoprimed maize recorded about 2-folds higher catalase, superoxide dismutase and ascorbate peroxidase activities, soluble protein, soluble sugar, glycine betaine and proline contents in seedlings, and improved resistance to alkaline stress (Abdel Latef & Tran, 2016; Pandey & Shukla, 2015). Consistent with these results, the previous study of Zheng et al. (2016) found that KNO_3 osmoprimed Indica rice varieties grown under drought had substantially enhanced CAT, POD and SOD activities in seedlings. Ahmed et al. (2015) reported that wheat osmopriming with Si increased seedling proline content and activities of nitrate reductase, SOD and APX.

CONCLUSIONS AND RECOMMENDATIONS

Osmopriming with potassium nitrate, silicon dioxide and their combinations improved germination, germination index and decreased mean germination time of FARO44 rice under drought stress. However, combined potassium nitrate and silicon dioxide showed slight effect in increasing germination attributes of FARO44 rice. Slight effect of combined potassium nitrate and silicon dioxide might be due lower osmotic potential that hindered activation of essential germination processes.

Osmopriming with potassium nitrate, silicon dioxide and their combinations improved seedling growth of FARO44 rice under drought stress. Improved rice seedling growth might be due to prolific cell division and cell expansion in osmoprimed rice seedlings under drought stress. Combined osmopriming with potassium nitrate and silicon dioxide had less effect in increasing rice seedling growth. Osmopriming with potassium nitrate and silicon dioxide similarly improved seedling vigour and biomass under drought stress. Osmopriming with potassium nitrate, silicon dioxide and their combinations improved biochemicals comprising of soluble protein, proline and decreased malondialdehyde content of FARO44 rice under drought stress. However, combined potassium nitrate and silicon dioxide showed slight effect in improving biochemicals of FARO44 rice. Furthermore, potassium nitrate and silicon dioxide osmopriming improved activities of superoxide dismutase, catalase and ascorbate peroxidase of FARO44 rice seedlings under drought. Increased activities of these protective enzymes were linked with heightened tolerance to drought of rice seedlings. As revealed in the results of this study, osmopriming of FARO44 rice with 2.5% and 5% KNO_3, 3% and 3.5% SiO_2, proved effective in increasing faster germination,

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seedling growth and drought tolerance of FARO44 rice. It is thus suggested for farmers involved in rain fed and irrigation agriculture to osmoprime (soak seeds prior to planting) their seeds with KNO_3 (2.5% and 5%) and SiO_2, (3% and 3.5%) prior to planting to better manage drought which is prevalent during early stages of crop production.

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