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**Research Paper**



# **Influence of Synergistic Priming on Stimulating Germination and Seedling Growth of Rice VAR. FARO44**

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## *ABSTRACT*

*Priming of seed is an approach that stimulates fast germination and seedling growth of rice under normal and stressful conditions. Poor germination and seedling establishment cause colossal crop failure in direct-seeded rice production systems. We evaluated the effects of synergistic priming with KNO3, SiO<sup>2</sup> and SA on germination and seedling growth of 3-weeks old FARO44 rice seedlings under well-watered conditions. Germination experiments were laid in a completely randomised design with each treatment replicated five times. The results indicated that 2.5% KNO<sub>3</sub>+3.5% SiO<sub>2</sub></sub>, 5% KNO<sub>3</sub>+3% SiO<sub>2</sub><sup><i>, 1mM SA+5% KNO*<sub>3</sub>*, 2.5mM*</sup> *SA+2.5% KNO3, 3% SiO2+2.5mM SA and 3.5% SiO2+1mM SA synergistic priming significantly enhanced rice germination, seedling growth, seedling fresh and dry weights and seedling vigour differently. However, 2.5% KNO3+3.5% SiO<sup>2</sup> and 5% KNO3+3% SiO<sup>2</sup> synergistic priming showed more effects on rice germination and seedling growth improvement. Improved germination and seedling growth of synergistic primed rice are correlated to activated and increased pre-germination metabolic processes, cell division and elongation. Therefore, synergistic pre-soaking with KNO3, SiO<sup>2</sup> and SA are recommended for stimulating faster germination and seedling growth of direct-seeded rice production systems in arid ecosystems. KEYWORDS: Germination percentage, seedling vigour, KNO3, SiO2, SA*

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# **I. INTRODUCTION**

Rice (*Oryza sativa* L.) is an essential cereal crop cultivated on the earth surface. It is a major staple being consumed by more than 50% of the world's population, it is produced on both irrigation fields and rainfed agriculture (Dien et al., 2019; Esmaeili & Heidarzade, 2012). The world's production of rice has been estimated at around 650 million tonnes while the land under cultivation of rice has been estimated at around 156 million hectares (FAO, 2016).

Nutrient constituents of rice differ from one species to another and these are related to soil types and conditions where the cultivation has taken place; however rice contributes to the essential dietary energy requirement of the body (Rohman et al., 2014). Rice consists of about 80% carbohydrates, 7% protein, 2% water and 3.5% fat (Oko et al., 2012). Rice also consists of essential vitamins such as vitamin B1 (thiamine), vitamin B2 (riboflavin), tocopherol, B3 (niacin) some appreciable quantities of iron, phosphorus and calcium (Deepak & Shukla, 2011; Rohman et al., 2014)

The burgeoning world population necessitated the increased production of rice in Asian and African countries to meet the increasing demand of food and ensure its security. However, drought, declines of precipitation, salinization of water resources, frequent storms and extreme temperatures driven by a changing climate threatened rice production (Dien et al., 2019; Zheng et al., 2016). Erratic downfall patterns associated with the changing world's climate is the key factor responsible for the numerous drought episodes ravaging the agricultural crops around the globe. Drought severely impacts agricultural production, worldwide, it accounts for around 70% of crop yield declines (Huang et al., 2013). Sufficient production of food is one of the main challenges confronting many developing nations. These challenges are due to climate change, population growth and many other ecological pressures. Larger quantity of rice is being produced in Nigeria because the country has an estimated 4.6-4.9 million hectares of fertile land for rice production. However, insufficient precipitation and extreme temperature under a changing climate are putting more pressure on rice production. Ensuring food production and security in many countries that are extremely susceptible to climate variability have been ever more complex (Kim et al., 2017).

In many parts of rice-growing northern Nigeria, rice is generally produced by the system of direct seeding under rain-fed agriculture because the system has less input costs, enhances resource use effectiveness as well as increased yield. Nigeria's north is an arid ecosystem with very limited downfall annually posing serious problems to rice production in the events of poor germination and irregular seedling establishment leading to low productivity. Yousof, (2013) reported that poor germination and seedling establishment are key issues in direct seeded rice production occasioned by drought, salinity and harsh temperature. Drought threatens rice production and it is more serious especially under rain-fed conditions (Goswami et al., 2013). Drought at the onset of rainy season affects germination of seeds as well as establishment of seedlings as a result of decline in taking up water during imbibition stage of germination coupled with energy supply reduction to germinating seeds (Hussain et al., 2017; Liu et al., 2016).

Although, developing fast germinating and vigorous growing crop varieties remain a tedious task, however, significant breakthroughs were made in this direction. Priming of seeds, a regulated hydration approach has emerged as a reliable, pragmatic and an effective technique for improving germination, vigour of seedlings, seedling emergence, growth and drought resistance of crops such as rice, wheat and maize (Farooq et al., 2009; Farooq et al., 2010; Jisha et al., 2013; Hussain et al., 2015). Priming of seeds is an effective technique that increases germination, seed vigour, seedling establishment and eventually leads to higher yields under varying environmental pressures including drought. It is a regulated hydration process that stimulates pregermination metabolic processes within the seeds without the actual germination (Farooq et al., 2009; Hussain et al., 2015). Pre-germination metabolic processes in primed seeds include repair and synthesis of nucleic acids, protein, activation of reserve mobilising enzymes like acid phosphatase, dehydrogenase, α-amylase, β-amylase and antioxidants (Ashraf & Foolad, 2005; Marthandan et al., 2020). Different methods of seed priming including hydropriming, hormonal priming, osmopriming, nutrient priming, chemical priming, redox priming, halopriming and magnetopriming are used on rice in addressing several environmental stresses (Paparella et al., 2015; Jisha et al., 2013). Esmaeili and Heidarzade (2012) reported that priming 12 different rice cultivars with 0.75 and 1.5% concentrations of  $KNO_3$  and 3 and 6dS/m NaCl for 24h at 25°C enhanced germination percent, seedling growth, seedling vigour and biomass compared with control. Wheat seeds primed with 40 mM sodium silicate for 8 h exposed to water deficit conditions recorded improved germination, decreased emergence period, vigour index, energy of germination, germination index and seedling growth (Hameed et al., 2013). Wang et al. (2016) reported that two rice cultivars primed with 100 mgL salicylic acid (SA) for 24 hours at 25°C under low temperature stress recorded significantly improved germination, seedling growth, seedling emergence and seedling fresh and dry biomass compared to control.

Potassium nitrate, silicon dioxide and salicylic acid priming have been proven to enhance germination and seedling growth of diverse cereals such as rice, wheat, maize and sorghum (Abdel Latef & Tran, 2016; Ahmed et al., 2016; Anosheh et al., 2012; Wang et al., 2016). However, synergistic priming with KNO<sub>3</sub>, SiO<sub>2</sub> and SA to improve germination and seedling growth of rice has been poorly explored. Therefore, this study was undertaken to assess the synergistic effects of  $KNO<sub>3</sub>$ ,  $SiO<sub>2</sub>$  and SA priming on improving germination and seedling growth of rice var. FARO44 under well-watered conditions.

# **II. MATERIAL AND METHODS**

## **Source of Seed**

The seeds of FARO44 rice was gotten from Badeggi Rice Research Institute located in Niger State, Nigeria. It has a long grain and a hybrid between Taiwan Indica rice and African local rice (Oluwaseyi et al., 2016). It has a high yield and can mature in about three months. It can be cultivated under irrigation and rain-fed agriculture (Akinwale et al., 2012; Oluwaseyi et al., 2016). The initial seed moisture level was 10.9% while the dried seed moisture was 8.97% on the basis of dry weight.

## **Optimization of Priming Chemical concentration and Duration**

To obtain reliable results, preliminary priming studies were performed by soaking rice seeds in varying concentrations of  $KNO_3$ ,  $SiO_2$  and SA with different durations prior to obtaining the effective priming duration (8 hours) and priming chemical concentrations (Hussain et al., 2015). The effective concentrations used are: KNO<sub>3</sub> (2.5% & 5% w/v), SiO<sub>2</sub> (3% & 3.5%) and SA (1mM & 2.5mM) which were used in synergistic priming. These selections were on the bases of germination attributes and seedling growth performances such as germination, seedling length, biomass and vigour.

#### **Seed Priming Treatments**

Before priming of rice seeds, priming concentrations of  $KNO<sub>3</sub>$ ,  $SiO<sub>2</sub>$  and SA were prepared and kept in a fridge. Viable quality rice seeds were sterilized in 0.5% sodium hypochlorite for 5 minutes in order to inhibit microbial growth and rinsed thrice in distilled water. The rice seeds were soaked separately in 2.5% KNO<sub>3</sub>+3.5% SiO<sub>2</sub> (w/v), 5% KNO<sub>3</sub>+3% SiO<sub>2</sub>, 1mM SA+5% KNO<sub>3</sub>, 2.5mM SA+2.5% KNO<sub>3</sub>, 3% SiO<sub>2</sub>+2.5mM SA and  $3.5\%$  SiO<sub>2</sub>+1mM SA for a period of 8 hours and the systems were kept in dark laboratory growth room of temperature  $25 \pm 2^0$ C and relative humidity of 50-70%. Ratio of 1:5 (w/v) seed weight to solution volume was maintained (Khan et al., 2019). The seeds were dried back to their near-original weight of 10.1% under the laboratory temperature of 25<sup>o</sup>C for 48 hours before germination (Anosheh et al., 2011).

#### **Germination of Synergistic Primed Seeds**

In order to effect germination of synergistic primed rice seeds, ten  $(n = 10)$  seeds were placed separately in Petri dishes with filter paper (diameter-90 mm) wetted with 7 ml of distilled water (Yan, 2015). Petri dishes were place in the laboratory growth room of temperature  $25 \pm 2^0C$  and relative humidity of 50-70% with a photoperiod of 12h dark/12 h light duration (Khan et al., 2019). Each treatment was replicated five times. Records of germination was taken on a daily until all seeds germinated, a seed was considered to be germinated if the emerged radicle was about 2 mm long (Yan, 2015). Seedlings were harvested after 3 weeks and the following seedling growth parameters from six randomly selected seedlings were measured:

Germination percentage was evaluated with the formula  $GP = \frac{No \text{ of seeds normally germinated}}{T_{total}} \times 100$  (Abdul-baki Total no.of seeds germinated

& Anderson, 1970).

Germination index-total number of seeds which germinated daily was calculated using<br>  $\frac{GI}{dV}$  are  $\frac{GI}{dV}$  and  $\frac{GI}{dV$  $GI=\frac{No\ of\ germinated\ seeds}{\text{days\ of\ first\ count---No.\ of\ seeds\ germinated/day\ of\ final\ count}}$  (Aloui et al., 2014).

Length of rice seedling, shoot and root were measured using a plastic ruler (Abdul-baki & Anderson, 1970). Fresh and dry seedling biomass was weighed with an electronic balance (Yan, 2015).

Mean germination time was determined using MGT =  $\frac{2Dt}{\Sigma n}$ , where n represents the number of seeds which germinated on day D and D represents the number of days counted from the beginning of germination (Ruttanaruangboworn et al., 2017).

Seedling vigor index was computed using SVI I = Seedling length  $\times$  germination percentage (Abdul-baki & Anderson, 1970) while seedling vigor II was computed using SVI II = Seedling dry weight  $\times$  germination percentage (Abdul-baki & Anderson, 1970).

#### **III. RESULTS**

#### **Influence of synergistic priming on germination attributes of FARO44 rice**

Synergistic priming with  $KNO<sub>3</sub>$ , SiO<sub>2</sub> and SA has significantly affected germination percentage (GP), germination index (GI) and mean germination time (MGT) of FARO44 rice as shown in figures 1, 2 and 3. Synergistic priming has no significant effect in improving GP of rice. However, priming with 2.5%  $KNO<sub>3</sub>+3.5%$  SiO<sub>2</sub> and 5%  $KNO<sub>3</sub>+3%$  SiO<sub>2</sub> significantly improved GI of rice compared to control. However, 1mM SA+5% KNO<sub>3</sub>, 2.5mM SA+2.5% KNO<sub>3</sub>, 3% SiO<sub>2</sub>+2.5mM SA and 3.5% SiO<sub>2</sub>+1mM SA showed no significant effects in improving GI of rice compared to control. Similarly, except  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub>, 5%  $KNO<sub>3</sub>+3\%$  SiO<sub>2</sub>, synergic priming with  $KNO<sub>3</sub>$ , SiO<sub>2</sub> and SA significantly decreased MGT of rice compared to control.



Fig. 1: Germination percentage of KNO<sub>3</sub>, SiO<sub>2</sub> and SA synergistic primed FARO44 rice



**Fig. 2**: Germination indices of KNO<sub>3</sub>, SiO<sub>2</sub> and SA synergistic primed FARO44 rice



**Fig. 3**: Mean germination time of KNO<sub>3</sub>, SiO<sub>2</sub> and SA synergistic primed FARO44 rice

**Effects of Synergistic Seed Priming on Seedling Growth Performance**

Synergistic priming with  $KNO_3$ ,  $SiO_2$  and SA has significantly affected seedling length, shoot length and root length of FARO44 rice as shown in figures 4, 5 and 6. Synergic priming with  $2.5\%$  KNO<sub>3</sub>+3.5%  $SiO_2$ , 5% KNO<sub>3</sub>+3%  $SiO_2$ , 1mM SA+5% KNO<sub>3</sub>, 2.5mM SA+2.5% KNO<sub>3</sub>, 3%  $SiO_2$ +2.5mM SA and 3.5%  $SiO<sub>2</sub>+1mM SA$  significantly enhanced rice seedling length compared to control. However, synergistic priming with 2.5% KNO<sub>3</sub>+3.5% SiO<sub>2</sub> and 5% KNO<sub>3</sub>+3% SiO<sub>2</sub> showed more effects on seedling length enhancement compared to other priming treatments. Similarly, all  $KNO<sub>3</sub>$ , SiO<sub>2</sub> and SA synergistic priming significantly enhanced shoot length compared to control. However,  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub> priming showed more effect on shoot length increase compared to other priming treatments. Likewise, all  $KNO<sub>3</sub>$ ,  $SiO<sub>2</sub>$  and SA synergistic priming significantly enhanced root length of rice compared to control. However,  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub> and  $5\%$  KNO<sub>3</sub>+3% SiO<sub>2</sub> synergistic priming showed more effects on root length enhancement compared to other priming treatments.



## **Effects of Synergistic Seed Priming on Seedling Biomass and Vigour**

Synergistic priming with  $KNO<sub>3</sub>$ ,  $SiO<sub>2</sub>$  and SA has significantly affected seedling biomass and seedling vigour indices of FARO44 rice as shown in figures 7 to 9. Synergistic priming with  $KNO_3$ ,  $SiO_2$  and SA showed no significant effect in improving seedling fresh weight of rice compared to control. Except  $2.5\%$  KNO<sub>3</sub>+3.5%  $\text{SiO}_2$ , 5% KNO<sub>3</sub>+3%  $\text{SiO}_2$  priming, other synergic priming significantly improved seedling dry biomass of rice compared to control. Similarly, all  $KNO<sub>3</sub>$ ,  $SiO<sub>2</sub>$  and SA synergistic priming significantly enhanced seedling vigour index I (SVI I) of rice compared to control. However,  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub> and 5% KNO<sub>3</sub>+3% SiO<sub>2</sub> synergistic priming showed more effects on improving SVI I compared to other priming treatments. Likewise, except 2.5% KNO<sub>3</sub>+3.5% SiO<sub>2</sub> priming, other synergic priming significantly improved seedling vigour index II (SVI II) of rice compared to control. However, 5%  $KNO<sub>3</sub>+3%$  SiO<sub>2</sub> synergistic priming showed more effect in enhancing SVI II of rice compared to other priming treatments.







**Fig. 8**: Seedling dry biomass of  $KNO_3$ ,  $SiO_2$  & SA synergistic primed FARO44 rice



**Fig. 10**: Seedling vigour index II of KNO<sub>3</sub>, SiO<sub>2</sub> and SA synergistic primed FARO44 rice

# **IV. DISCUSSION**

Poor germination and seedling emergence of rice are key agricultural deterrence that gravely decrease crop yield in dry and semi-dry ecosystems of the world (Liu et al., 2016). Synergistic priming with 2.5% KNO<sub>3</sub>+3.5% SiO<sub>2</sub>, 5% KNO<sub>3</sub>+3% SiO<sub>2</sub>, 1mM SA+5% KNO<sub>3</sub>, 2.5mM SA+2.5% KNO<sub>3</sub>, 3% SiO<sub>2</sub>+2.5mM SA and 3.5% SiO<sub>2</sub>+1mM SA improved germination, seedling growth performances and seedling vigour of FARO44 rice differently. The results of this study indicated that synergistic priming improved germination index (GI) and decreased mean germination time (MGT) of FARO44 rice. However, synergistic priming showed no effects on improving germination percentage of FARO44 rice. Enhancement of GI and decrease of MGT of rice was associated with increased cell division, activation of important pre-germination processes in seeds, activation of activities of catalase, superoxide dismutase, ascorbate peroxidase and increased soluble sugar, proline content, and increased water imbibition of primed seeds (Jisha & Puthur, 2016). Agreeing the findings of this study, Ghobadi et al. (2012) reported that gibberellic acid and polyethylene priming of two wheat cultivars increased germination percentage and decrease germination. Calcium chloride priming treatment of rice reduced emergence time and time to attains a 50% emergence (Rehman et al., 2011).

The findings of this study revealed that synergistic priming improved seedling growth of FARO44 rice. Enhanced rice seedling growth and emergence might be related to the useful roles of synergistic priming in cell division & elongation, repair of damaged nucleic acid, synthesis and activation of growth proteins. The previous findings of Farooq et al. (2013) revealed that two wheat cultivars hydroprimed and primed with ascorbic acid recorded increased seedling length, shoot length, root length as well as biomass weight owing to enhancement of proline and phenolic contents. Super Basmati rice primed with CaCl2, KCl and ascorbate substantially has enhanced seedling length, shoot length, root length as well as seedling biomass (Farooq et al., 2010).

This study found that synergistic priming with  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub>, 5% KNO<sub>3</sub>+3% SiO<sub>2</sub>, 1mM  $SA+5\%$  KNO<sub>3</sub>, 2.5mM  $SA+2.5\%$  KNO<sub>3</sub>, 3%  $SiO<sub>2</sub>+2.5$ mM SA and 3.5%  $SiO<sub>2</sub>+1$ mM SA improved FARO44 rice seedling biomass and vigour. Improvement of rice seedling biomass and vigour by priming might be related to fast completion of germination-linked metabolic processes such as activation reserve hydrolysing cellulase, amylase and xylanase that are stimulated by priming. At the start of germination, hydrolases are modulated that essential roles in building germination metabolites that stimulate uniform seedling emergence (Farooq et al., 2006). Consistent with findings of this study, the previous study of Farooq et al. (2013) found that wheat cultivars primed with ascorbic acid exhibited improved seedling dry weight and seedling vigour. Similarly, Rehman et al.  $(2011)$  reported that rice primed with CaCl<sub>2</sub> showed increased emergence index, kernel weight and seedling vigour. Gibberellic acid and PEG priming enhanced shoot and root dry weights and seedling vigour of wheat (Ghobadi et al., 2012). Consistent with these results, Zhang et al. (2015) found that priming sorghum with PEG substantially improved seedling dry weight and vigor under water scarcity stress.

#### **V. CONCLUSIONS**

Poor germination and seedling emergence are main deterrence of direct seeded rice production that cause decline of seedling growth and yield around the world. Synergistic priming with  $2.5\%$  KNO<sub>3</sub>+3.5% SiO<sub>2</sub>, 5% KNO<sub>3</sub>+3% SiO<sub>2</sub>, 1mM SA+5% KNO<sub>3</sub>, 2.5mM SA+2.5% KNO<sub>3</sub>, 3% SiO<sub>2</sub>+2.5mM SA and 3.5% SiO2+1mM SA were effective on enhancing germination, seedling growth, seedling biomass and seedling vigour of FARO44 rice seedlings. However, 2.5%  $KNO<sub>3</sub>+3.5%$  SiO<sub>2</sub> and 5%  $KNO<sub>3</sub>+3%$  SiO<sub>2</sub> synergistic priming showed more effects on improving germination and seedling growth of rice compared to other priming treatments. Improved germination and seedling growth in primed rice seedlings are associated with activated pre-germinative metabolites, antioxidants and osmolytes. Synergistic priming with  $KNO<sub>3</sub>$ , SiO<sub>2</sub> and SA showed different responses, however, they were all found to be effective in enhancing germination and seedling growth. The findings of this study substantiated the reliability of priming, a simple and cheap method which can be adopted by farmers in dry regions of the world for improving germination, seedling establishment and growth of rice under normal and water deficit conditions. Thus, FARO44 rice synergistic priming with  $KNO_3$ ,  $SiO_2$  and SA are recommended for faster germination and growth of seedlings in dry ecosystems.

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