Osmopriming and Hormonal Priming Combined Improve Germination and Seedling Growth of Rice Var. FARO44

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Abstract

Osmopriming and hormonal priming have been practiced by farmers to stimulate fast germination and seedling growth of various crop species. In this study, laboratory experiments were carried out to examine the effect of combined osmopriming with potassium nitrate and hormonal priming with salicylic acid to promote germination and seedling growth of FARO44 rice. In the laboratory, FARO44 rice seeds soaked in 5 % KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA were germinated in specimen bottles for two weeks. Each treatment was replicated five times. The results revealed that combined osmopriming and hormonal priming with 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had significantly improved germination index and decreased mean germination time of FARO44 rice compared to control. However, germination percentage had not been improved substantially compared to control. However, there were no marked differences in GI and MGT between 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmoprimed and hormonal primed rice. Moreover, the results indicated that combined osmo-hormonal priming with 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had increased seedling growth, seedling vigour and biomass of FARO44 rice seedlings. However, length of plumule of FARO44 rice seedlings was not affected by these processes compared to control.

Keywords: *Osmopriming, Hormonal priming, potassium nitrate, salicylic acid, germination*

Introduction

Osmopriming is the physiological process of presoaking seeds in low osmotic solutions. Compounds mostly used for osmopriming seeds comprise of glycerol, polyethylene glycol, mannitol, sorbitol as well as inorganic salts like $CaCl_2$, $MgSO_4$, KH_2PO_4, K_3PO_4, KNO_3, KCl, and NaCl. Seed priming with salts sometimes called halopriming (Lutts et al., 2016). Osmopriming of cereals are proven to promotes germination, seedling emergence and growth. Osmopriming activates important pre-germination metabolic processes prior to appearance of the radicle (Jisha et al., 2013; Jisha & Puthur, 2014). This technique enhances germination and quality of seedling establishment, improves growth as well as flowering in plants (Bhargava, 2013; Jisha et al., 2013). Osmopriming is important in minimizing the risks of poor seedling emergence under stressful conditions of salinity and water (Kaya et al., 2004; Bhargava, 2013). Osmopriming enhances the performance rice seeds by allowing the initiation of essential metabolic processes thereby giving them unparalleled advantages over un-osmoprimed seeds.

On the other hand, hormonal priming involves the physiological process of presoaking seeds in different hormones such as kinetin, gibberellic acid (GA_3), salicylic acid, ascorbate and so on to enhance growth as well as rapid seedlings development (Yadav et al., 2018). Controlled soaking of seeds in plants' hormones causes rapid multiplication and growth of plants particularly during unfavourable environmental conditions. Changes stimulated by hormonal priming have significant effects on increasing germination as well as uniform seedling appearance particularly of several cereals in stressed conditions (Farooq et al., 2009). Hormonal primed seeds are characterized with faster germination and more uniform seedling emergence compared to their non-hormonal primed counterparts.

Poor germination, seedling growth and establishment have been main issues ravaging rice production in drier ecosystems that lead to decline in yields. Despite the reliability of physiological processes of osmopriming and hormonal priming with chemicals and hormones in enhancement of germination as well as rice seedling growth, there is scanty of study of employing these processes in increasing germination and seedling growth of FARO44 rice. Hence, this paper was examined the effect of combined osmopriming with potassium nitrate and hormonal priming with salicylic acid to improve germination and seedling growth of FARO44 rice.

Materials and methods

Study Location

This study was performed in the Biology Laboratory of the Department of Science Laboratory Technology, School of Science, Mai Idris Alooma Polytechnic, Geidam, Yobe State, Nigeria.

Sample collection

FARO44 rice seeds were obtained from Badeggi Rice Research Institute in Niger State, Nigeria in West Africa. FARO44 rice is a new rice variety developed in Nigeria, it is cultivated in different parts of Nigeria in irrigation as well as rain-fed agriculture (Oluwaseyi & Nehemmiah, 2016).

Optimisation of osmopriming and hormonal priming chemicals and duration

Preliminary studies were carried out to optimised the osmopriming and hormonal priming chemicals (salicylic acid and potassium nitrate) and duration. FARO44 rice seeds were presoaked in different concentrations of potassium nitrate (KNO_3) and salicylic acid (SA) (Zheng et al., 2016). The effective concentrations of 2.5% and 5% KNO_3 and 200 mM and 350 mM SA and 8 hour soaking time were used on the bases of germination and seedling growth performances such as germination percentage, germination index, mean germination time, seedling length, shoot and root length and seedling vigour (Hussain et al., 2016; Zheng et al., 2016).

Osmopriming and hormonal priming treatment and seed germination

Prior to presoaking of seeds, all beakers and specimen bottles were kept in an oven for 15 hours at 75 ºC; while the different soaking solutions of SA and KNO_3 were prepared and kept in a deep freezer (-20ºC).

Sterilized FARO44 rice seeds were presoaked separately in 10 ml each of 2.5% as well as 5% (w/v) of KNO_3 and 200 mM and 350 mM solutions (w/v) of SA for 8 hours as described by Chunthaburee et al. (2014), Yan (2015) & Abdel Latef & Tran (2016). The systems were placed in laboratory at 25 ºC as well as and photoperiod of 12h light/12 h dark. The ratio of 1:4 (w/v) seeds to volume of solution was retained throughout the osmopriming and hormonal priming processes. The seeds were then thoroughly rinsed with distilled water and air-dried for 48 hours to their initial mass of 11.1% at 25 ºC (Anosheh et al., 2011; Khan et al., 2019)

Combined osmoprimed and hormonal primed rice seeds in KNO_3 and SA were germinated in specimen bottles lined with Whatman filter (No.1 90mm) and 7 ml distilled water applied for two weeks in laboratory growth room with temperature of 25 ºC as well as a photoperiod of 12h light/12 h dark (Anwar et al., 2013; Khan et al., 2019). Each treatment had five replications. The first record of germinations was taken on the second day while the last was taken on the fourteenth day. Seed was considered germinated if the emerged radicle measures at least 2 mm long (Chunthaburee et al., 2014; Ruttanaruangboworn et al., 2017). All the measurements were based on randomly picked six normal rice seedlings in every replication. At the end of the experiment, final germination percentages were computed (Yan, 2015).

Statistical analyses

The osmopriming and hormonal priming experiments were laid down in a completely randomized design with five replicates per each treatments (Azeem et al., 2015). Data normality was tested with Shapiro Wilk test prior to analyses. One-way ANOVA) was carried out for comparing the effects of osmopriming and hormonal priming on germination and growth of seedling attributes studied (Khatami et al., 2015). Significant means differences were separated with Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$) (Yan, 2015).

RESULTS

Effect of hormonal and osmopriming on germination of rice

The analyses of variance showed that osmopriming and hormonal priming KNO_3 and SA had no significant effect ($P < 0.05$) on percentage of germination (GP) of FARO44 rice in comparison to control (non-osmoprimed and non-hormonal primed seeds) as detailed in Table 1. Osmoprimed and hormonal primed seeds with 5% KNO_3+200 mM SA as well as 2.5% KNO_3+350 mM SA significantly recorded maximum germination indices (GI) of 3.5 and 3.6 compared to control with lower GI of 2.3. However, there were no significant differences between 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmo-hormonal primed rice seeds. It was evident that there were marked significant differences in the mean germination time (MGT) of 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmoprimed and hormonal primed rice seeds compared to control. The 5% KNO_3+200 mM primed rice seeds completed germination within two days and 4 hours while 2.5% KNO_3+350 mM SA primed rice seeds took 2 days and 3 hours to complete germination compared to 3 days and 5 hours for control rice seeds as presented in Table 1 below.

Table 1: Osmo-hormonal priming influence on germination characteristics of FARO44 rice

Concentrations of osmo-hormonal priming agents	Germination Percentage (%)	Germination Index	Mean Germination Time (Days)
Control	100 ± 0.0^{a}	2.3 ± 0.0^{b}	3.5 ± 0.0^{a}
5% KNO_3+200 mM SA	100 ± 0.0^{a}	3.5 ± 0.1^{a}	2.4 ± 0.0^{b}
2.5% KNO_3+350 mM SA	98.9 ± 0.1^{a}	3.6 ± 0.1^{a}	2.4 ± 0.1^{b}
Levels of Significance	0.397	0.000	0.000

Means ± SE values in the columns having the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$), while figures with different letters were statistically different.

Effects of Osmopriming and hormonal priming upon growth of rice seedling

Variance analyses showed that 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmoprimed and hormonal primed FARO44 rice seedlings had recorded significantly ($P < 0.05$) maximum seedling height in comparison to control seedlings as detailed in Table 2. The 5% KNO_3+200 mM SA osmoprimed and hormonal primed rice seedlings had seedling height of 12.6 cm while 2.5% KNO_3+350 mM SA osmo-hormonal primed seedlings had seedling height of 12.9 cm compared to 10.7 cm seedling height for control. There was no significant difference in seedling height between 5% KNO_3+200 mM SA and 2.5%
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KNO_3+350 mM SA primed seedlings. Osmoprimed and hormonal priming had not significantly affected the plumule length of FARO44 rice seedlings as seen in Table 2 below. The 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmo-hormonal priming had significant effect in promoting root length of FARO44 rice seedlings compared to control's root. The 5% KNO_3+200 mM SA osmo-hormonal primed seedlings had root length of 6.7 cm while 2.5% KNO_3+350 mM SA had root length of 7.1 cm and control with root length of 5.2 cm. There was no marked difference in root length of 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmo-hormonal primed seedlings

Table 2: Effect of osmo-hormonal priming on seedling growth FARO44 rice

Concentrations of osmo-hormonal priming agents	Seedling height (cm)	Plumule length (cm)	Root length (cm)
Control	10.7 ± 0.4^{b}	5.8 ± 0.1^{a}	5.2 ± 0.3^{b}
5% KNO_3+200 mM SA	12.6 ± 0.5^{a}	6.2 ± 0.2^{a}	6.7 ± 0.4^{a}
2.5% KNO_3+350 mM SA	12.9 ± 0.5^{a}	5.9 ± 0.2^{a}	7.1 ± 0.4^{a}
Levels of Significance	0.003	0.202	0.001

Means ± SE values in the columns having the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$), while figures with different letters are statistically different.

Effect of osmo-hormonal priming on biomass of FARO44 rice seedlings

It has been observed that 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmo-hormonal priming had significantly ($P < 0.05$) affected seedling biomass both fresh weight and dry weight of rice seedlings as detailed in Table 3 below. Seedlings osmo-hormonal primed with 5% KNO_3+200 mM SA had seedling fresh weight of 116.4 mg and seedling dry weight of 28.8 mg while seedlings osmo-hormonal primed 2.5% KNO_3+350 mM SA had seedling fresh weight of 116.1 mg and seedling dry weight of 29.7 mg. Control had lower seedling fresh weight of 110.6 mg and seedling dry weight of 27.9 mg. However, there was no marked difference in seedling fresh weight between 2.5% KNO_3+350 mM SA osmo-hormonal primed and 5% KNO_3+200 mM SA primed rice seedlings, although 2.5% KNO_3+350 mM SA osmo-hormonal primed rice seedlings had higher seedling dry weight than 5% KNO_3+200 mM SA osmo-hormonal primed rice seedlings.

Table 3: Effect of osmo-hormonal priming on biomass of FARO44 rice seedling

Concentrations of osmo-hormonal priming agents	Seedling fresh weight (mg)	Seedling dry weight (mg)
Control	110.6 ± 0.9^b	27.9 ± 0.2^c
5% KNO_3+200 mM SA	116.4 ± 1.2^a	28.8 ± 0.2^b
2.5% KNO_3+350 mM SA	116.1 ± 1.5^a	29.7 ± 0.3^a
Levels of Significance	0.002	0.000

Means ± SE values in the columns having the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$), while figures with different letters were statistically different.

Effect of osmo-hormonal priming on seedling vigour of rice

Osmo-hormonal priming of FARO44 rice with 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had significantly marked effects ($P < 0.05$) on seedling vigour indices I and II as detailed in Table 4 below. Rice seedling osmo-hormonal primed with 5% KNO_3+200 mM SA had seedling vigour index I (SVI I) of 1259.7 and seedling vigour index II (SVI II) of 2884.7 while rice seedlings osmo-hormonal primed with 2.5% KNO_3+350 mM SA had SVI I of 1239.9 and SVI II of 2889.3. Un-osmo-hormonal primed rice seedlings (control) had SVI I of 1069.7 and SVI II of 2798.7 lower than that of osmo-hormonal primed seedlings.

Table 4: Effect of osmo-hormonal priming on seedling vigour of FARO44 rice seedling

Concentrations of osmo-hormonal priming agents	SVI I	SVI II
Control	1069.7 ± 39^b	2798.7 ± 21.1^b
5% KNO_3+200mM SA	1259.7 ± 25.8^a	2884 ± 21.6^a
2.5% KNO_3+350mM SA	1239.9 ± 36.3^a	2889.3 ± 10.4^a
Levels of Significance	0.005	0.001

Means ± SE values in the columns having the same letter (s) are not significantly different according to Duncan's Multiple Range Test ($P < 0.05$), while figures with different letters were statistically different.

Discussions

Combined osmopriming and hormonal priming with 5% KNO_3+200 mM SA as well as 2.5% KNO_3+350 mM SA had improved germination index and decreased mean germination time of FARO44 rice compared control. However, germination percentage had not been improved considerably compared to control. However, there were no marked differences in GI and MGT between 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA osmoprimed and hormonal primed rice. Moreover, the results indicated that osmo-hormonal priming with 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had increased seedling growth, seedling vigour and biomass of FARO44 rice seedlings. Despite the effectiveness of these processes, plumule length of FARO44 rice seedlings was not affected. Similar findings were reported by Farooq et al. (2006) on hormonal priming of fine and course rice varieties with 10 and 20 ppm salicylic acid and ascorbic acid for 48 h at 27^{0}C found the treatments enhanced seed germination and seedling growth. The study of Rehman et al. (2015) revealed that maize seed hormonal priming with 50 mg/L salicylic acid for 18 h have significantly reduced the mean seedling emergence time (MET) and the time required by seedlings to reach 50% emergence. Likewise, salicylic acid hormonal priming improved seedling vigour, shoot length, root length, seedling fresh and dry weights equally increased compared to unprimed and hydroprimed seedlings. In the study conducted of Esmaeili and Heidarzade (2012) on osmopriming 12 different rice cultivars with 0.75 and 1.5% concentrations of KNO_3 and 3 and 6dS/m NaCl for 24 h at 25^{o}C affected positively both the germination percentage and germination rate of two cultivars (Tarom & Neda) compared to control.

Conclusions

Combined osmopriming and hormonal priming with 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had improved germination, seedling growth and seedling vigour of FARO44 rice. However, there were no marked differences between the two aforementioned combinations in terms of germination, seedling biomass and vigour increase as both indicated almost similar responses in improving these attributes. Thus, these combinations of 5% KNO_3+200 mM SA and 2.5% KNO_3+350 mM SA had been suggested for farmers to apply in order to improve faster rice germination as well as seedling growth of FARO44 rice grown in Nigeria under rain fed and irrigated systems.

References

Abdel Latef, A. A., & Tran, L.-S. P. (2016). Impacts of Priming with Silicon on the Growth and Tolerance of Maize Plants to Alkaline Stress. *Frontiers in Plant Science*, *7*, 1–10. https://doi.org/10.3389/fpls.2016.00243

Anosheh, H. P., Sadeghi, H., & Emam, Y. (2011). Chemical Priming with Urea and KNO3 Enhances Maize Hybrids (Zea may L.) Seed Viability under Abiotic Stress. *Journal of Crop Science and Biotechnology*, *14*(4), 289–295.

Anwar, S., Iqbal, M., Raza, S. H., & Iqbal, N. (2013). Efficacy of seed preconditioning with salicylic and ascorbic acid in increasing vigor of rice (Oyza sativa L) seedling. *Pakistan Journal of Botany*, *45*(1), 157–162.

Azeem, M., Iqbal, N., Kausar, S., Javed, M. T., Akram, M. S., & Sajid, M. A. (2015). Efficacy of silicon priming and fertigation to modulate seedling's vigor and ion homeostasis of wheat (Triticum aestivum L.) under saline environment. *Environmental Science and Pollution Research*, *22*(18), 14367–14371. https://doi.org/10.1007/s11356-015-4983-8

Bhargava, B. (2013). *EFFECT OF SEED PRIMING ON GROWTH AND FLOWERING OF Antirrhinum majus L.* Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, India.

Chunthaburee, S., Sanitchon, J., Pattanagul, W., & Theerakulpisut, P. (2014). Alleviation of salt stress in seedlings of black glutinous rice by seed priming with spermidine and gibberellic acid. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(2), 405–413. https://doi.org/10.1583/nbha4229688

Esmaeili, M. A., & Heidarzade, A. (2012). Investigation of different osmopriming techniques on seed and seedling properties of rice (Oryza sativa) genotypes. *International Research Journal of Applied and Basic Sciences*, *3*(2), 242–246.

Farooq, M, Basra, S. M. A., Wahid, A., Ahmad, N., & Saleem, B. A. (2009). Improving the Drought Tolerance in Rice (Oryza sativa L .) by Exogenous Application of Salicylic Acid. *Journal of Agronomy & Crop Science*, *195*, 237–246. https://doi.org/10.1111/j.1439-037X.2009.00365.x

Farooq, Muhammad, Barsa, S. M. A., & Wahid, A. (2006). Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regulation*, *49*(2–3), 285–294. https://doi.org/10.1007/s10725-006-9138-y

Hussain, S., Khan, F., Cao, W., Wu, L., & Geng, M. (2016). Seed Priming Alters the Production and Detoxification of Reactive Oxygen Intermediates in Rice Seedlings Grown under Sub-optimal Temperature and Nutrient Supply. *Frontiers in Plant Science*, *7*, 1–13. https://doi.org/10.3389/fpls.2016.00439

Jisha, K. C., & Puthur, J. T. (2014). Seed halopriming outdo hydropriming in enhancing seedling vigor and osmotic stress tolerance potential of rice varieties. *Journal of Crop Science and Biotechnology*, *17*(4), 209–219. https://doi.org/10.1007/s12892-014-0077-2

Jisha K Vijayakumari, K. C., & Puthur, J. T. (2013). Seed priming for abiotic stress tolerance : an overview. *Acta Physiol Plant*, *35*, 1381–1396. https://doi.org/10.1007/s11738-012-1186-5

Kaya, M., Arif, Ş., & Tonguç, M. (2010).

Effect of sowing dates and seed treatments on yield , some yield parameters and protein content of chickpea (Cicer arietinum L .). *9*(25), 3833–3839. https://doi.org/10.5897/AJB10.268

Khatami, S. R., Sedghi, M., & Sharifi, R. S. (2015). Influence of Priming on the Physiological Traits of Corn Seed Germination Under Drought Stress. *Annals of West University of Timişoara, Ser. Biology*, *18*(1), 1–6.

Langeroodi, A. R. S., & Noora, R. (2017). SEED PRIMING IMPROVES THE GERMINATION AND FIELD PERFORMANCE OF SOYBEAN UNDER DROUGHT STRESS. *The Journal of Animal & Plant Sciences*, *27*(5), 1611–1620.

Lutts, S., Benincasa, P., Wojtyla, L., Lechowska, K., & Quinet, M. (n.d.). *" Seed priming : new comprehensive approaches for an old empirical technique " Référence bibliographique Seed Priming : New Comprehensive Approaches for an Old Empirical Technique*.

Mansour Heydariyan, Nasrallah Basirani, Majid Sharifi-Rad, Isa Khmmari, S. R. P. (2014). Effect of Seed Priming on Germination and Seedling Growth of the Caper (Capparis Spinosa) Under Drought Stress. *International Journal of Advanced Biological and Biomedical Research*, *2*(8), 2381–2389.

Mittova, V., Guy, M., Tal, M., & Volokita, M. (2004). Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species Lycopersicon pennellii. *Journal of Experimental Botany*, *55*(399), 1105–1113. https://doi.org/10.1093/jxb/erh113

Mohammad Nauman Khan, Jing Zhang, Tao Luo, Jiahuan Liu, Muhammad Rizwan, Shah Fahad, Zhenghua Xu, L. H. (2019). Seed priming with melatonin coping drought stress in rapeseed by regulating reactive oxygen species detoxi fi cation : Antioxidant defense system , osmotic adjustment , stomatal traits and chloroplast ultrastructure perseverati. *Industrial Crops & Products*, *140*, 111597. https://doi.org/10.1016/j.indcrop.2019.111597

Oluwaseyi, A. B., & Nehemmiah, D. (2016). Genetic Improvement of Rice in Nigeria for Enhanced Yeild and Grain Quality - A Review. *Asian Research Journal of Agriculture*, *1*(3), 1–18. https://doi.org/10.9734/ARJA/2016/28675

Rehman, H. U., Kamran, M., Basra, S. M. A., Afzal, I., & Farooq, M. (2015). Influence of Seed Priming on Performance and Water Productivity of Direct Seeded Rice in Alternating Wetting and Drying. *Rice Science*, *22*(4). https://doi.org/10.1016/j.rsci.2015.03.001

Ruttanaruangboworn, A., Chanprasert, W., Tobunluepop, P., & Onwimol, D. (2017). Effect of seed priming with different concentrations of potassium nitrate on the pattern of seed imbibition and germination of rice (Oryza sativa L.). *Journal of Integrative Agriculture*, *16*(3), 605–613. https://doi.org/10.1016/S2095-3119(16)61441-7

Thomas, S., Anand, A., & Chinnusamy, V. (2013). Magnetopriming circumvents the effect of salinity stress on germination in chickpea seeds. *Acta Physiol Plant*, *35*, 3401–3411. https://doi.org/10.1007/s11738-013-1375-x

Yan, M. (2015). Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. *South African Journal of Botany*, *99*, 88–92. https://doi.org/10.1016/j.sajb.2015.03.195

Zheng, M., Tao, Y., Hussain, S., Jiang, Q., & Peng, S. (2016). Seed priming in dry direct-seeded rice: consequences for emergence , seedling growth and associated metabolic events under drought stress. *Plant Growth Regulation*, *78*(2), 167–178. https://doi.org/10.1007/s10725-015-0083-5