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The Effective Use of Phytohormones for Seed Priming: A Successful Method for Improving Germination and Vigour in Rice

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ABSTRACT: More than sixty percent of the world's population eats rice as staple food, making it both the most significant food in terms of human nutrition and calorie consumption. Poor field germination capacity, imminent water constraints, rising labour expenses and the continuously growing world population force us to make a better decision in order to ease the food crisis and increase rice output. For increasing seed vigour, synchronising germination, fostering the growth of seedlings, and increasing yield in a variety of crops, seed priming is an effective and useful technique. Pre-soaking seeds before planting encourages quick germination and growth, especially under difficult environmental circumstances. Abiotic stresses like drought, salinity, heat, cold, and heavy metals are commonplace for plants and cause complex reactions that lower crop yield and growth. It is commonly known that phytohormones regulate the growth and development of plants. They are also crucial chemical messengers that enable plants to function when they are under a variety of stressors. Through seed priming, plants can develop a greater ability to quickly and efficiently withstand a variety of stresses. So, phytohormones as seed priming have become a crucial strategy for reducing the negative effects of abiotic stress. Keeping these points in view, the present study was carried out with phytohormone priming materials. The research programme was formulated with seven priming materials on laboratory conditions with the objective of optimizing the appropriate concentration of GA_3 and Kinetin for better performance. Distinct findings were noted like germination parameters, different vigour and seedling parameters. Highest germination percentage (98.33), Vigour index-I (2599.933), and Vigour index-II (1445.767) was observed in 50 ppm GA_3. Seed priming with 50 ppm GA_3 was found very effective for seed germination, vigour, and seedling parameters. So, from this experiment, 50 ppm GA_3 was superior executant hormonal priming as it enhanced seed yield and most of the prime yield attributes.

Keywords: GA_3, germination, hormonal priming, kinetin, vigour.

INTRODUCTION

Rice is the staple food of more than sixty percent of world's population and has been cultivated in Asia since ancient times generation after generation. In Asia and Africa**,** about 90% of world's rice is grown as well as produced and consumed. As germination is a crucial stage in the plant's life cycle and is influenced by a number of environmental factors and the viability of the seed structure to determine the potential growth of the embryo, rice is typically grown by direct seeding or seedling transplanting.

The rice farmers who use the conventional transplanting procedure must contend with impending water shortages and rising labour costs. Poor germination capability under field conditions and the steadily increasing global population, we must choose a better choice to alleviate the food crisis and improve rice production. The issue of inadequate seed germination in crop plants is being addressed using a variety of methods, seed priming is an efficient and practical method for boosting seed vigour, synchronising germination, promoting the growth of seedlings and yield in various crops (Bajehbaj *et al.*, 2010). This pre-sowing method involves partially hydrating seeds to the point where germination-related metabolic processes start but imbibit development does happen (Chen *et al.*, 2001). In many field crops grown in challenging environmental conditions, seed priming is a strategy that has been proven to be effective and simple to use to increase yield, uniformity, and speed of seed emergence. The physiological activities at the early stage of seed imbibitions are activated by seed priming, including the elicitation of DNA repair pathways and antioxidant mechanisms to preserve genome integrity, ensuring enhanced germination and seedling establishment (Paparella *et al.*, 2015). Numerous recent research set up a strong emphasis on standardising seed priming methods in order to increase germination and shorten emergence times and achieve a consistent crop stand. Such beneficial effects of seed priming may result from the de novo synthesis of some germination-promoting compounds, early DNA replication, increased ATP availability, osmotic adjustments, membrane

reorganisation by restoring their original structures, and decreased metabolite leakage.

Priming techniques have shown some promising effects in better crop yield and increasing micronutrient concentrations in cereals. As it is expected to be species and dose- dependent (Wang *et al*., 2017), several seed priming techniques may be applied to enhance germination and stand establishment. Organic substances known as plant growth regulators (PGRs) are produced in trace amounts in crops. The growth, development, and yield of plants are significantly influenced by plant hormones, despite their modest concentration (Cothren and Oosterhuis 2010). According to certain studies, priming with indole acetic acid (IAA), gibberellic acid (GA_3), and ascorbic acid boosts germination rate, plant growth, early growth, grain quantity and quality, and grain quality (Anosheh *et al*., 2011). Recent research has focused on the utilisation of hormone priming, notably GA_3, in rice germination under unfavourable circumstances (Aymen *et al*., 2018). The germination performance and yield of many crops have been significantly enhanced by seed priming with plant growth hormones like auxin (IAA), gibberellins (GA), abscisic acid, and ethylene in both normal and stress situations. To improve the uniformity of seed germination, seedling establishment, and yield of different field crops like rice, corn, safflower, wheat, beetroot, and sunflower, plant growth regulators like gibberellins (GA), abscisic acid (ABA), and salicylic acid (SA) have been frequently used through hormonal priming (Anosheh *et al*., 2011).

It is generally known that gibberellic acid breaks down the starch contained in seeds so that growing embryos can use it during germination (Taiz *et al*., 2006). Therefore, when gibberellic was used as a priming solution for rice cultivars, seedling emergence and dry matter production significantly rose (Chen *et al*., 2005). Exogenous handling of plant hormones, notably GA_3, counteracts the effects of ABA and encourages the relaxation of dormancy, which results in seed germination (Chen *et al*., 2005).

MATERIALS AND METHODS

The present investigation was conducted with rice genotype (IET4786) with two different hormonal priming (GA_3 and Kinetin) having three different concentration in the seed testing laboratory, BCKV, Mohanpur, Nadia, WB, India during 2022 using a completely randomised design with three replications. Seeds priming materials were distilled water for soaking duration 12 hours (T_1 as control), GA_3 @ 30 ppm for soaking time 12 hours (T_2), GA_3 @ 50 ppm for soaking duration 12 hours (T_3), GA_3 @ 70 ppm for soaking time 12 hours (T_4), kinetin @ 2 ppm for soaking duration 18 hours (T_5), kinetin @ 4 ppm for soaking time 18 hours (T_6), and kinetin @ 6 ppm for soaking duration 18 hours (T_7). The seeds were collected from AICRP on seed BCKV. The collected seed were evaluated both in the Seed Testing Laboratory.

Germination percentage (%): The petri dish was cleaned with methylated spirit and filled with cotton and the blotting paper was placed on it. Distilled water was used to wet it. The treated seeds were placed on the blotting paper and the lid was covered. Four petri dishes were kept in the germinator for each treated seed, each petri dish having 100 seeds. After fourteen days, we took out the petri dishes from the seed germinator and counted the number of germinated and ungerminated seeds.

Calculation:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Root length (cm): After fourteen days of germination in the laboratory (glass plate method), the root lengths were measured and recorded in cm by measuring the length between the tip of the primary root to the collar region of ten randomly selected normal seedlings.

Shoot length (cm): The shoot lengths were measured after fourteen days of germination in the laboratory, and the mean value was recorded in cm for the length between the tip of the shoot and the collar region. The 10 normal seedlings which were taken for root length determination, also used for shoot length measurement.

Average seedling length (cm): Sum of root and shoot length of ten normal seedlings was recorded to calculate total seedling length.

Vigour index I: Computed the vigour index by multiplying the average seedling length with the germination percentage.

Vigour index I = Germination % × Average seedling length (cm) (Abdul-Baki and Anderson 1973)

Dry weight of ten seedlings (mg): 10 fresh seedlings were selected from each replication; care was taken to ensure that the least amount of water adhered to the seedlings. The unit of fresh weight was recorded in milligram (mg).

Dry weight of ten seedlings (mg): Ten seedlings, which were selected for measuring fresh weight, packed in an aluminium container without lid and dried at 80°C for 2 hours. The seedlings' dry weight was then measured.

Vigour index II: Vigour Index II computed the vigour index by multiplying the average seedling dry weight with the germination percentage (Abdul-Baki and Anderson 1973).

Vigour index II = Germination % × Average seedling dry weight (g)

Germination index: Coolbear *et al.* (1984) computed this parameter by using the following formula.

$$GI = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \text{----} + \frac{\text{Number of germinated seeds}}{\text{Day of last count}}$$

50% Germination time (days): We recorded the number of germinated seeds that germinated everyday according to the AOSA approach. By using the following formulas from Coolbear *et al.* (1984), to determine the time to achieve 50% germination (T50):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{(n_j - n_i)}$$

Where N is the total number of germinated seed and ni, nj are the progressive numbers of seeds that have germinated by adjoining calculate at period of times ti and tj, respectively, but ni > N/2 >nj.

Germination energy: Germination energy (GE) was measured on the fourth day of germination. It is the percentage of seeds that germinated 4 days after planting, in relation to the total number of seeds tested (Ruan *et al*., 2002).

Mean Germination Time (MGT) (days):

$MGT = \frac{\sum Dn}{\sum n}$ In this experiment, we followed the Ellis and Roberts (1980) equation to compute the MGT.

Where, 'n' denotes the no. of seeds that germinated on day D, and D denotes the number of days since the start of germination.

RESULTS AND DISCUSSION

Germination percentage: Significantly highest germination percentage was recorded in T_3 (98.33) followed by T_6 and T_2. But T_3, T_6 and T_6 priming materials showed non-significant difference. While lowest germination percentage (92.667) observed in control seed (T_1) preceded by T_4, T_7 and T_5. Among T_4, T_7 and T_5 treatments were non-significantly varied (Table 1). An analogous result was found in tomatoes by Ray and Bordolui (2022b).

Root length (cm): Significantly tallest root was recorded for T_7 (15.24 cm) and it was followed by T_5, T_6 and T_3, whereas, shortest roots were recorded for control (T_1) (12.24 cm) that was preceded by T_4 and T_2, though non-significant difference was noticed among all these three treatments. T_3 and T_6 were not able to produce significant length difference among them. Using potassium nitrate, Choudhury and Bordolui (2022b) saw a similar outcome in Bengal gram.

Shoot length (cm): Among the treatments, shoot length was varied significantly. T_4 showed the highest shoot length (13.44 cm) which was followed by T_3, T_2, and T_5. But T_1 showed the shortest shoot length (7.20 cm) which was preceded by T_7 and T_5. But non-significant difference was observed in between T_5 and T_7. Using sodium molybdate (Na_2MoO_4) nutri-priming to lengthen shoots.

Average length of seedling (cm): Average length of seedling was varied significantly. The minimum average length of seedling was observed in T_1 (19.44 cm) preceded by T_6, T_5 and T_7, while longest seedling was recorded for control (T_3) (26.44 cm) followed by T_4 and T_2. But non significant distinction could be noticed among only T_5 and T_6; T_2 and T_7.

Table 1: Effect of different hormonal priming on some physiological characters such as germination percentage, root length, shoot length, total seedling length

Sr. No.	Priming material	Germination (%) (Tr value)	Length of root (cm)	Length of Shoot (cm)	Average Length of Seedling (cm)
1	T_1	92.667 (74.266)	12.240	8.400	20.640
2	T_2	97.000(80.087)	12.680	11.720	24.400
3	T_3	98.333(82.816)	13.480	12.960	26.440
4	T_4	96.000(78.686)	12.480	13.440	25.920
5	T_5	97.000(80.087)	14.360	8.920	23.280
6	T_6	97.667(81.223)	13.520	9.720	23.240
7	T_7	96.000(78.686)	15.240	8.880	24.120
CD (0.01)		**3.762**	**0.833**	**0.437**	**0.728**
SEm (±)		**1.228**	**0.272**	**0.143**	**0.238**
SE(d)		**1.737**	**0.385**	**0.202**	**0.336**
C.V.		**2.679**	**3.510**	**2.373**	**1.727**

Note: T_1 (control), T_2 (GA_3 30ppm), T_3 (GA_3 50ppm), T_4 (GA_3 70ppm), T_5 (Kinetin 2 ppm), T_6 (Kinetin 4 ppm), T_7 (Kinetin 6 ppm)

Fresh weight of ten seedlings (mg): Significantly maximum seedling fresh weight was noticed for T_3 (58.333mg), followed by T_2, T_4, and T_5. However, the lowest magnitude was found in control seed (T_1; 37.667mg) preceded by T_7 and T_6. Though some treatments were not statistically significant varied like T_2, T_4, and T_5; T_6, T_4, and T_5 (Table 2). When Chakraborty and Bordolui (2021) compared Ag nano priming to other treatments, they discovered that it increased the fresh weight of green grams seedlings.

Dry weight of ten seedlings (mg): There was no statistically significant difference between primed and control seeds for dry weight of the seedlings (mg). Although T_4 (14.733 mg) treatment resulted in the highest dry weight, T_1 (13.867 mg) treatment showed the lowest dry weight. Osmo-priming with PEG solution increased dry weight following seed treatment in wheat, according to Ghiyasi *et al*. (2008).

Vigour index I: Vigour index I was seen significantly highest magnitude for T_3 (2599.933) which was followed by T_4 and T_2. Lowest value was noted in control (T_1) (1801.44) which was preceded by T_5, T_6, and T_7, albeit there was no significant difference between these three treatments. There was non- significant variation between T_2 and T_7. According to Masood *et al*. (2012), hormonal seed priming is a widely used technique to enhance seed germination, seedling growth, and vigour under challenging circumstances.

Vigour index II: Highest vigour index II, T_3 (1445.767) was the most notable finding for me, followed by T_4 and T_6, albeit there was no statistically significant difference between any of these three treatments. The lowest value was recorded for control (1284.933), preceded by T_5, T_2 and T_7. There was no noticeable Vigour index II change between T_2, T_5, and T_7. A popular method for improving vigour index-II in difficult situations is hormonal seed priming (Masood *et al*., 2012).

Table 2: Effect of different hormonal priming on fresh and dry weight of seedling, Vigour index I and II (VI-I and VI- II).

Sr. No.	Priming material	Fresh weight of ten seedlings (mg)	Dry weight of ten seedlings (mg)	VI- I	VI- II
1	T_1	37.667	13.867	1,801.440	1,284.933
2	T_2	47.333	14.133	2,366.893	1,370.800
3	T_3	58.333	14.700	2,599.933	1,445.767
4	T_4	45.667	14.733	2,488.400	1,415.200
5	T_5	45.667	14.000	2,257.873	1,357.733
6	T_6	44.333	14.433	2,269.594	1,409.433
7	T_7	41.000	14.467	2,315.960	1,388.933
CD (0.01)		**2.315**	**NS**	**94.090**	**92.486**
SEm (±)		**0.756**	**0.272**	**30.722**	**30.199**
SE(d)		**1.069**	**0.385**	**43.448**	**42.708**
C.V.		**2.864**	**3.290**	**2.314**	**3.785**

Note: T_1 (control), T_2 (GA_3 30ppm), T_3 (GA_3 50ppm), T_4 (GA_3 70ppm), T_5 (Kinetin 2 ppm), T_6 (Kinetin 4 ppm), T_7 (Kinetin 6 ppm)

Germination energy: Significantly highest germination energy was observed in T_6 (98.00) which was followed by T_3, T_2, and T_5. But all these four priming materials were not statistically significant varied. But control T_1 (95.00) had the lowest magnitude which was preceded by T_7 and T_4. With the following few exceptions, the genotype response of rice to seed priming followed a pattern that was identical to the average influence of priming: T_4 and T_7 were unable to have a substantial impact on control subjects. Seed priming with Kinetin improved the germination and growth of different species, such as rice (Roohi and Jameson 1991).

Mean germination time (days): Lowest mean germination time was noted in T_6 (1.414 days). But some treatments were non-significant difference viz. T_2 and T_3; T_3 and T_6. While, maximum mean germination time taken for control T_1 (9.86 days) followed by T_4, T_7 and T_5 but non-significant difference was noticed among T_5 and T_7; T_4 and T_7. A similar outcome was observed in tomatoes by Ray and Bordolui (2022a).

Germination Index: Significantly highest germination index was recorded for T_3 (94.967) after that the treatments were T_2, T_6 and T_4. But T_2, T_6 and T_4 were non-significantly varied. Control or control i.e., T_1 (88.833) was observed lowest germination index preceded by T_7 and T_5, though non-significant difference was observed between T_5 and T_7. According to Roohi and Jameson (1991), the speed of germination in rice was enhanced by seed priming with gibberellic acid.

50% Germination time (hours): T_3 (12.5hours) took the least time to germinate 50% of the provided seed, which was preceded by T_4 and T_7, however these three were not statistically significant difference. While control T_1 (14.34 hours) shown the maximum time to 50% germination followed by T_6, T_5, and T_2. None of the three (T_2, T_5, and T_6) showed statistically significant differences. Similar outcomes were observed in carrots primed with Ag-nano particles by Kundu and Bordolui (2024).

Table 3: Effect of different hormonal priming on germination index, 50% germination time, mean germination time and germination energy.

Sr. No.	Priming material	GE	GI	MGT	50% GT
1	T_1	95.000	88.833	9.860	14.340
2	T_2	97.000	93.500	2.243	12.930
3	T_3	97.333	94.967	2.134	12.500
4	T_4	,96.000	93.333	5.008	12.650
5	T_5	97.000	92.250	4.260	13.220
6	T_6	98.000	93.500	1.414	13.500
7	T_7	95.000	91.583	4.510	12.800
CD(0.01)		**1.682**	**0.956**	**0.806**	**0.734**
SEm (±)		**0.549**	**0.312**	**0.263**	**0.240**
SE(d)		**0.777**	**0.441**	**0.372**	**0.339**
C.V.		**0.986**	**0.584**	**10.843**	**3.159**

Note: T_1 (control), T_2 (GA_3 30ppm), T_3(GA_3 50ppm), T_4(GA_3 70ppm), T_5 (Kinetin 2 ppm), T_6 (Kinetin 4 ppm), T_7 (Kinetin 6 ppm); GE=Germination Energy; GI= Germination Index; MGT= Mean Germination Time (days); 50% GT= 50% Germination Time (hrs)

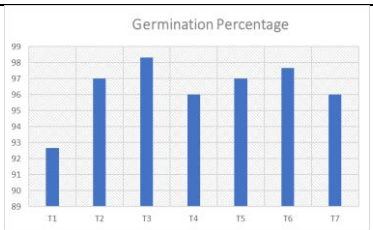

Fig. 1. Effect of different hormonal priming on germination perventage.

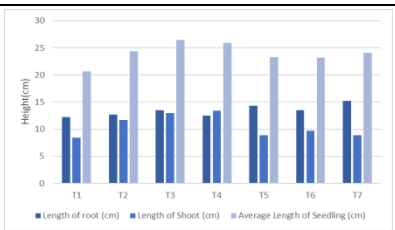

Fig. 2. Effect of different hormonal priming on root length (cm), shoot length(cm) and average seedling length(cm).

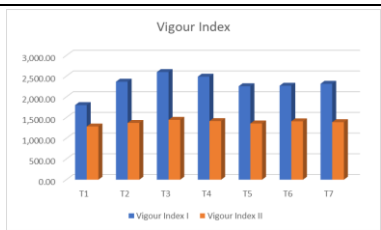

Fig. 3. Effect of different hormonal priming on vigour index I and Vigour index II.

CONCLUSIONS

Phytohormone priming of seeds breaks dormancy and increases viability, ensuring hermonized germination. For rice, seed priming with phytohormones has become a successful seed treatment technique; however, there are still limitations to this approach, and treatment conditions and techniques vary from phytohormone to phytohormone. For instance, if seeds are exposed to a hormonal solution for an extended period of time during priming, they may lose their ability to withstand desiccation, which lowers their viability. The primed seed with GA @ 50 ppm had the best germination, vigour, and seedling characteristics among all the priming materials. It could therefore be chosen as the hormonal priming material with the best performance. The beneficial effects of GA @50 ppm on germination, seedling growth, and development were noted; these findings may be the main reason to recommend seed priming with GA @ 50 ppm for the establishment of rice crops.

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