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# Effect of Seed Priming with Liquid Organic on Germination, Seedling Development and Enzymatic Activity of Wheat (*Triticum aestivum* L.)

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## ABSTRACT

A laboratory experiment was conducted on wheat variety UP-2526 to evaluate the herbal *kunapajala* concentration to improve seed germination and seedling vigour. The experiment consisted of five treatments viz; no priming (control), hydropriming, 10%, 25% and 50% *kunapajala* priming for 16 hours. Results revealed that significantly higher germination % was observed from 25% *kunapajala* primed and hydroprimed seeds jointly which was at par with 10% *kunapajala* primed seeds. Hydropriming resulted into highest speed of germination whereas, the mean germination time and days taken to 50% emergence was found to be lowest with hydropriming and it was followed by 25% *kunapajala* priming. Development of seedling measured in terms of shoot and root length, seedling length, dry weight of seedling and seedling vigour index-I and seedling vigour index-II was significantly higher with 10% *kunapajala* primed seeds than rest of the treatments. Significantly superior imbibition rate and α-amylase activity were observed with 25% *kunapajala* primed seeds whereas the maximum seed metabolic efficiency was found with 10% *kunapajala* primed seed among all priming treatments. So, seed priming with either 10% or 25% herbal *kunapajala* is most effective method to improve germination, seedling growth and biochemical parameter of wheat seeds.

**Keywords** Herbal *kunapajala*, Seed germination, Seedling vigour, Seed priming, Wheat.

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## INTRODUCTION

Crop establishment is an important factor depends upon optimum plant population and uniform emergence. Proper crop establishment depends upon quality of the seed in terms of its germination and seedling vigour. Seed priming is the most widely used seed invigoration technique to improve field emergence and crop establishment under adverse environment condition. It is a pre-sowing seed treatment where the seeds are soaked in priming media upto the second phase of germination which may vary from crop to crop and subsequently dried in shade. This process may improve uniform emergence and synchronized crop growth. It has the potential to enhance the seedling vigour and germinability of fresh seeds and also the excellent ability to revive the partially aged seeds and improves the field emergence (Thejeshwini *et al*. 2019). Hydropriming is one of the oldest practices used for seed invigoration. This practice has been used widely to stimulate the speed, uniformity of emergence and improve the final plant stand. Nowadays, researchers are focusing on eco-friendly seed invigoration techniques to improve seed germination and uniform crop establishment. In vrikshayurveda, herbal based *kunapajala* is used to enhance the biological efficiency of crop as well as soil. The liquid manure *kunapajala* (jala=water) or *kunapambu,* is derived from the Sanskrit word *'kunapa*' meaning "smelling like a dead body, stinking" and it is a fermented liquid manures prepared from flesh, animal urine, marrow, (Kumar *et al.* 2020). Nowadays, herbal based *kunapajala* is used as nourishment of seed, crop and soil. It is very effective in nourishing plants at various stages as soil and foliar application (Kavya *et al.* 2020, Sudhakar *et al.* 2010) and also can be used as a seed priming technique because of having properties of growth stimulation, nutrition and antimicrobial activity. This liquid organic contains essential macro and micro nutrients (Ankad *et al.*2017), vitamins, growth hormones (IAA and $GA_3$), essential amino acids (Sudhakar *et al.* 2010) and beneficial microbes like nitrogen fixing symbiotic and free-living bacteria and phosphorus solubilizing bacteria (Chakraborty *et al.* 2019). So, *kunapajala* is a promising and eco-friendly plant stimulant for sustainable crop production and safe agro ecosystem (Kavya and Ushakumari 2020). Hence, the present study was proposed to evaluate the impact of herbal *kunapajala* concentration, a liquid organic on seed germination and seedling development of wheat.

## MATERIALS AND METHODS

The laboratory study was conducted at seed physiology laboratory, Department of Agronomy, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar (29°1' N latitude, 79°29'E longitude and elevation of 231 m above MSL) during *rabi* season of 2020-21. The experiment was laid out in completely Randomized Design with five treatments (no priming, hydropriming, 10%, 25% and 50% *kunapajala* priming) and six replications. Wheat seeds of variety UP-2526 were soaked in herbal *kunapajala* at 10%, 25% and 50% concentrations and tap water (hydropriming) for 16 hours in 1: 2 :: seed: priming media ratio and seeds were shade dried up to original moisture content (11.8%). For determination of water imbibition rate and α-amylase activity, seeds are soaked in different priming media for 8, 12, 16 and 24 h and 12, 16 and 24 h, respectively.

For preparing herbal *kunapajala,* the ingredients (10 kg cow dung, 10 L cow urine, 2 L sour butter milk, 2 kg jaggery, 2 kg sprouted urd, 2 kg mustard cakes, 3 L rice bran water, 1 L fresh milk, 3-4 pieces of dry cow dung, 10 kg nettle grass and 10 kg leaves of other grasses) were mixed into a plastic drum of 200 L capacity and the volume of solution was made up to 200 L with water. Then it was shaken properly with a stick and the lid was closed. The contents of the drum were mixed properly with stick twice a day (morning and evening). The day when the bubbles stopped appearing in the drum, the process was completed and herbal *kunapajala* was ready. Then it was sieved properly with cloth for further use.

The standard germination test was evaluated according to the method given by ISTA (2004). Fifty seeds of wheat in each of six replications were placed on two layers of moist germination paper and covered with another sheet of moist germination paper. Then, it was rolled carefully and fastened with the rubber band. The rolled paper samples were kept in incubator at 20±2°C temperature. Physiological parameters i.e., Germination % (Kala and Eswari 2019) at $8^{th}$ day, speed of germination (Maguire 1962), mean germination time (Ellis and Robert 1981), days to 50% germination (Farooq *et al.* 2019), root length, shoot length and dry weight of seedling, seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II) (Abdul-Baki and Anderson 1973), seed metabolic efficiency (Sikder *et al.* 2009) at $4^{th}$ and $8^{th}$ days after incubation and biochemical parameter like the α-amylase activity of wheat seeds (Mazumdar and Majumder 2017), were evaluated. The rate of

**Table 1**. Effect of different seed invigoration treatments on germination indices and seedling vigour parameters of wheat.

| Treatments | Germination (%) | SOG (seedlings /day) | MGT (days) | $T_{50}$ (days) | Shoot length (cm) | Root length (cm) | Seedling length (cm) | Seedling dry weight (mg) | SVI-I | SVI-II |
|---|---|---|---|---|---|---|---|---|---|---|
| No priming | 97.7 | 27.4 | 1.92 | 1.43 | 10.0 | 8.33 | 18.9 | 17.8 | 1850 | 1742 |
| Hydropriming | 99.7 | 36.2 | 1.57 | 1.14 | 11.8 | 9.72 | 21.5 | 20.7 | 2145 | 2063 |
| 10% KJ priming | 99.3 | 31.9 | 1.75 | 1.27 | 12.5 | 10.9 | 23.5 | 23.0 | 2334 | 2281 |
| 25% KJ priming | 99.7 | 32.4 | 1.72 | 1.26 | 11.5 | 9.37 | 20.7 | 22.6 | 2062 | 2256 |
| 50% KJ priming | 97.3 | 27.6 | 1.92 | 1.41 | 10.3 | 8.86 | 19.2 | 18.3 | 1867 | 1784 |
| SEm (±) | 0.6 | 0.7 | 0.03 | 0.03 | 0.5 | 0.23 | 0.5 | 0.6 | 46 | 56 |
| CD (p=0.05) | 1.9 | 2.1 | 0.09 | 0.09 | 1.4 | 0.74 | 1.5 | 1.8 | 153 | 180 |

KJ: *Kunapajala;* SOG: Speed of germination; MGT: Mean germination time; $T_{50}$: Days taken to 50% germination; SVI-I: Seedling vigour index-I; SVI-II: Seedling vigour index- II

water imbibition was calculated by method given by Tian *et al*. (2014) with modification. The collected experimental data from laboratory experiment was analyzed by OPSTAT software for Completely Randomized Design (CRD). The critical difference was calculated at 5% level of significance.

## RESULTS AND DISCUSSION

### Effect of seed priming on germination indices and seedling vigour parameters

In the present experiment, hydro primed seeds and 25% *kunapajala* primed seeds jointly recorded maximum germination percentage (99.7%) which was statistically at par 10% *kunapajala* priming (99.3%) (Table 1). The highest speed of germination (36.2 seedlings/ day) was observed with hydropriming among all the priming treatments and it was followed by priming with 25% and 10% *kunapajala* (32.4 and 31.9 seedlings/day, respectively) which remained at par to each other. The lowest speed of germination was recorded in no priming treatment (27.4 seedlings/ day) which was at par with 50% *kunapajala* primed seeds (27.6 seedlings/ day). Significantly lower mean germination time and days to 50% emergence (1.57 days and 1.14 days, respectively) were observed with hydropriming than rest of the priming treatments and it was followed by priming with 25% and 10% *kunapajala* which remained at par to each other. The maximum mean germination time and days to 50% emergence were recorded with no priming treatment (1.92 days and 1.43 days, respectively) which remained at par with 50% *kunapajala* priming treatment (1.92 days and 1.41 days, respectively).

Data presented in Table 1 depicted the shoot and root length was significantly varied due to all seed invigorated treatments except 50% *kunapajala* priming. Significantly longer shoot was recorded with 10% *kunapajala* priming (12.5 cm) which remained at par with hydropriming (11.8 cm) and 25% *kunapajala* priming (11.5 cm). The longest root and seedling length were obtained with 10% *kunapajala* priming (10.9 and 23.5 cm, respectively) among to all treatments and it was followed by hydropriming (9.72 and 21.5 cm, respectively) and 25% *kunapajala* priming (9.37 and 20.7 cm, respectively) which was statistically at par with each other. Non-primed seeds showed the shortest shoot, roots and seedling length (10, 8.33 and 18.9 cm, respectively) which was at par with 50% *kunapajala* priming (10.3, 8.86 and 19.2 cm, respectively). Significantly improved dry weight of seedling and seedling vigour index-II were obtained from 10% *kunapajala* priming (23.0 mg and 2281, respectively) which was at par with 25% *kunapajala* priming (22.6 mg and 2256, respectively). The maximum seedling vigour index-I (2334) was observed with 10% *kunapajala* priming among all seed invigoration treatments. The lowest dry weight of seedling, SVI-I and II were recorded with no priming (17.8 mg, 1850 and 1742, respectively) which was par with 50% *kunapajala* priming (18.3 mg, 1867 and 1784, respectively).

Rapid accumulation of germination metabolites and metabolic repair due to priming, promotes quick translocation and supply of food reserve to developing seedlings (Farooq *et al*. 2019). Thus, germination process of primed seeds is completed in short time. The enhancement of seedling growth parameters in 10% *kunapajala* primed seeds might be due to the quick commencement in enzymatic activities and breakdown of reserved food materials. Seeds invigorated with 2% panchagavya fortified seeds (*Jatropha curcas* and *Pongamia pinnata*) showed higher germination percentage which might be resulted from the activity of microbes and growth promoters like $GA_3$ and IAA in liquid concoction (Srimathi *et al*. 2013). Ankad *et al.* (2017) observed that germination percentage of kalamegha and ashwagandha seeds treated with 10% *kunapajala* and *Panchagavya* showed significant over no priming. Similar findings are reported by Kala *et al*. (2019) and Sumangala and Patil (2009). The improved seedling length from kunapajala priming might be obtained because of presence of plant growth promoting factors produced by microbes that are present in *kunapajala* (Kavya *et al*. 2020). However, it was reduced with increasing concentration of kunapajala (50%). The enhancement in seedling growth parameters and seedling vigour through seed priming with liquid organics also has been noticed by Kala and Eswari (2019), Ankad *et al.* (2017), Srimathi *et al.* (2013) and Sumangala and Patil (2009). However, the reduction in germination indices and seedling growth parameters with increased concentration levels of liquid organic could be because of the optimum dose of organic products which is generally specific to particular crops (Henig-Sever *et al*. 2006).

Water imbibition rate was found to be highest with 25% *kunapajala* priming among all the priming treatments in all the soaking duration viz, 8,12,16 and 24 hours (Table 2). Among different soaking periods, the maximum rate of imbibition was observed between 12 to 16 hours. The rate of imbibition was 50.7% and 62.9% higher in 12 hours soaking period than that of 8 and 24 hours of soaking durations. The increment in imbibition rate of 25% *kunapajala* primed seeds was 57.9% and 20.6% at 12 and 16 hours of soaking, respectively than hydropriming. These results are in close agreement with Bormashenko *et al.* (2012) and Ling *et al.* (2014). The lower imbibition rate of water during initial hours was observed compared to successive hours as starch slowly absorbs water than protein. Solutes produced as results of α-amylase activity, enhanced the movement of water into the seeds because of osmotic potential of seed (Baron 1979). However, the imbibition rate was decreased in increasing *kunapajala* concentration (50%) and the magnitude of decrement were 55.0% and 41.7% than 25% *kunapajala* priming at 12 and 16 hours of soaking. The decline in water uptake with increasing *kunapajala* concentration might be resulted from modification of seed coat due to accumulation of inhibitory hydrophobic substances.

Seed metabolic efficiency is a measurement of translocation of food reserve from endosperm to growing parts of seedlings. Perusal of data presented in Table 2, revealed that after 4th and 8th day of incubation, the highest seed metabolic efficiency was recorded with 10% *kunapajala* priming (2.99 and 0.96 g/g, respectively) which remained at par with 25% *kunapajala* priming (2.98 and 0.92 g/g, respectively) as compared to rest of the treatments (Table 2). However, the lowest values of seed metabolic efficiency at both periods were obtained with control (1.92 and

**Table 2.** Effect of different seed invigoration treatments on water imbibition rate, seed metabolic efficiency and α-amylase activity of wheat.

| Treatments | Water imbibition rate (mg/g dry seed/h) | | | | Seed metabolic efficiency (g/g) | | α-amylase activity (mg of starch hydrolyzed/g of seeds) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 8 hours | 12 hours | 16 hours | 24 hours | 4th DAI | 8th DAI | 12 hours | 16 hours | 24 hours |
| No priming | 2.01 | 14.2 | 12.1 | 3.4 | 1.92 | 0.44 | 21.5 | 21.5 | 21.5 |
| Hydropriming | 3.20 | 17.6 | 16.5 | 6.1 | 2.80 | 0.64 | 23.2 | 24.9 | 23.1 |
| 10% KJ priming | 8.23 | 23.4 | 16.5 | 7.9 | 2.99 | 0.96 | 24.0 | 25.2 | 23.0 |
| 25% KJ priming | 13.70 | 27.8 | 19.9 | 10.3 | 2.98 | 0.92 | 24.1 | 27.1 | 24.6 |
| 50% KJ priming | 1.24 | 12.5 | 11.6 | 1.8 | 2.00 | 0.54 | 19.8 | 21.3 | 20.7 |
| SEm (±) | 0.96 | 1.2 | 0.9 | 0.3 | 0.07 | 0.04 | 0.6 | 0.6 | 0.3 |
| CD (p=0.05) | 3.06 | 3.9 | 2.7 | 1.1 | 0.21 | 0.11 | 1.9 | 1.9 | 1.1 |

KJ: *Kunapajala*; DAI: Day after incubation.

0.44 g/g, respectively) which were at par with 50% *kunapajala* priming (2.00 and 0.54 g/g, respectively). Similar finding was reported by Pal *et al*. (2017). The reduction in 50% *kunapajala* primed seeds could be due to less uptake of water by seeds and reduction in $GA_3$ and other enzymes involved in hydrolytic process during germination (Marambe *et al.* 1992).

**Effect of seed invigoration treatments on biochemical parameter**

The α- amylase activity was significantly influenced by different seed invigoration treatments and soaking durations (Table 2). The α-amylase activity of primed seeds increased up to 16 hours of soaking and after that it reduced in all the priming treatments. The maximum α-amylase activity was observed with 25% *kunapajala* primed seeds at 12, 16 and 24 hours of priming durations (24.1, 27.1 and 24.6 mg of starch hydrolyzed/g of seeds, respectively) which was statistically at par with 10% *kunapajala* priming at 12 hours of priming duration (24.0 mg of starch hydrolyzed/g of seeds) as compared to other treatments. The expression of *RAmy1A* is triggered by transported $GA_3$ and it induced the α-amylase activity in endosperm (Kaneko *et al.* 2002). Amino acids, vitamins and growth regulators (IAA and $GA_3$) which are present in herbal *kunapajala* (Sudhakar *et al*. 2010) may induce the α-amylase activity in primed seeds and thus endosperm starch is hydrolyzed to supply the energy necessary for germination. Marambe *et al.* (1992) reported significant correlation between the α-amylase activity and water absorption by seeds. Those results were in close agreement of Farooq *et al.* (2019) and Ashraf and Foolad (2005). However, α-amylase activity was reduced with increasing the concentration levels of *kunapajala* and it was 17.8%, 15.9% and 21.4% lower in 50% *kunapajala* priming as compared to 25% *kunapajala* priming after 12, 16 and 24 hours of incubation. This may be due to negative impact on synthesis of gibberellic acid which eventually affected the starch hydrolysis.

## CONCLUSION

From the above findings, it may be concluded that seed priming with either 10% or 25% herbal based *kunapajala* is an eco-friendly seed invigoration technique to improve emergence, seedling development and biochemical activity of wheat over hydropriming and no priming. However, seed priming with higher concentration of priming media i.e., 50% herbal *kunapajala* should be avoided as it negatively impacted all the germination and growth parameters of wheat seedlings.

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