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Standardization of seed enhancement techniques for healthy production of radish microgreens

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Abstract

The research work entitled “Standardization of seed enhancement techniques for healthy production of radish microgreens” was carried out at the Seed Technology Research Unit, AICRP on Seed (Crops), UAS, Bangalore. Among 16 different seed enhancement techniques, by considering microgreen quality parameters tested, the best three seed enhancement treatments are hydropriming for 6 hours, priming with H₂O₂ (0.3%) for 12 hours and priming with beejamrutha for 6 hours. Hydropriming of radish seeds for 6 hours showed improved tray emergence (90.0%), speed of emergence (34.0), seedling height (21.6 cm), shoot mass (368.3 mg), seedling mass (412.2 mg) and microgreen yield (162.0 g/tray) compared to control. Since hydropriming is best and cheapest seed enhancement method it could be used for commercial production of radish microgreens. Among 12 different growing media by considering radish microgreen quality parameters tested, the best growing media was coco peat (90%) + vermicompost (10%) combination. In conclusion, hydropriming for 6 hours with growing media coco peat (90%) + vermicompost (10%) could be used commercially for growing radish microgreens.

Keywords: Radish, Microgreens, Seed enhancement techniques, Media

Introduction

The increasing incidence of lifestyle-related disorders such as cancer, diabetes, and obesity has accelerated the demand for functional and nutraceutical foods. In this context, microgreens have emerged as a promising natural dietary source due to their high nutritional value and health-promoting properties. Microgreens are tender, edible seedlings of vegetables and herbs harvested at the cotyledon stage, typically within 7-21 days after germination, and are known to contain higher concentrations of phytonutrients such as ascorbic acid, β -carotene, phyloquinone and tocopherols compared to their mature counterparts (Sharma *et al.*, 2020) [9].

Their short growth cycle, minimal input requirement, and suitability for urban and peri-urban cultivation have increased their commercial importance. Microgreens can be grown in soilless media such as cocopeat, peat and vermicompost, with cocopeat being widely preferred due to its superior water-holding capacity and aeration, resulting in improved germination and yield (Ramya *et al.*, 2022) [6]. Among various crops, radish (*Raphanus sativus* L.) microgreens are particularly popular due to their rapid growth, ease of cultivation, desirable flavor, and rich nutritional profile.

Despite their advantages, the success of microgreen production largely depends on seed quality, as seeds represent a major production cost and directly influence germination, uniformity, and seedling vigor. Moreover, ensuring seed health is critical, as contaminated seeds may harbor pathogenic microorganisms. Therefore, appropriate seed sanitation and enhancement techniques are essential, especially under organic and chemical-free production systems.

Seed enhancement techniques such as hydropriming, matrix priming, and pre-germination treatments have been reported to improve germination rate, uniformity, and early seedling growth, thereby reducing the production cycle and enhancing crop performance (Lee *et al.*, 2004) [4]. However, standardization of these techniques for microgreens, particularly in radish, remains limited. Hence, the present study was undertaken to standardize seed enhancement

techniques for improving seed performance and ensuring healthy production of radish microgreens.

Material and Methods

The experiment was conducted at AICRP on Seed (Crops), UAS, GKVK, Bengaluru, using a Completely Randomized Design (CRD) with 16 treatments and 3 replications. The seeds of radish are collected from Ravi hybrid seeds, Bangalore used for the present study. The collected seeds of radish were fresh and untreated, stored under ambient conditions and recorded 85.0 percent and 6.0 percent of initial germination and moisture content respectively. Seeds were sown in trays (15 × 10.5 inches) filled with cocopeat at a seed rate of 20 g per tray.

Priming: Seeds were surface sterilized by dipping the seeds in sodium hypochlorite (5%) solution for 5 minutes and dried on filter paper. Priming solutions are prepared according to the dosage specific for the treatment. The duration of priming varies with the priming material used for the treatment.

During priming, the seeds were kept in dark condition @ 25±2 °C. After respective priming treatments for specific period, seeds were washed with distilled water thoroughly. Then primed seeds were dried at room temperature on filter paper for 24 hours, later to their original moisture contents. Dried seeds were then packed in polythene bags and stored for further use.

Treatment details

Seeds of were primed with following treatments and laboratory and microgreen tray observation were recorded. Treatments were T₁-Control, T₂-Hydro priming (6 hrs), T₃-UV rays treatment (15 minutes), T₄-Dry heat treatment (80 °C for 5 minutes), T₅-Soaking with H₂O₂ (0.3%, 20 °C, 6 hrs), T₆-Soaking with tri sodium phosphate (0.5%, 20 °C, 6 hrs), T₇-Biopriming with *Trichoderma viridae* (6 g/kg), T₈-Sterilizing with 70% alcohol, T₉-Priming with lime water (10 minutes), T₁₀-Priming with humic acid (2%, 20 °C, 2 hrs), T₁₁-Matrix priming (20 °C, 12 hrs with cocopeat), T₁₂-Priming with cow urine (30% for 6hrs), T₁₃-Priming with α Tocopherol (100 mg/litre with 0.1% tween-20 for 4 hrs soaking), T₁₄-Priming with beejamrutha (2% for 6 hrs), T₁₅-Seed coating with red soil (5 g/kg of slurry treatment), T₁₆-Seed coating with kaolinite (5 g/kg slurry treatment)

Observations recorded

Seed germination was assessed using the between paper method as per International Seed Testing Association rules (Anon., 2019), with 400 seeds (100 seeds × 4 replications) incubated at 25 °C and counts recorded on the 5th and 14th day; germination percentage was expressed based on normal seedlings. Speed of emergence was calculated by daily counting of emerged seedlings up to the 8th day using Maguire's index (Maguire, 1962). Tray emergence (%) was recorded on the 8th day using 1000 seeds per treatment and expressed on emerged seedling basis (Anon., 2019). Growth parameters such as mean seedling height, shoot mass and seedling mass were measured from ten randomly selected seedlings on 8 days after sowing (DAS). Microgreen yield was recorded at harvest (appearance of first true leaves) by cutting seedlings above the media surface and measuring fresh weight using a digital balance.

Results and Discussion

Different seed enhancement treatment was carried out to know its effect on seed and radish microgreen quality parameters. The results showed that different seed enhancement technique had significantly different effects on tray emergence, speed of emergence, seedling height, shoot mass, seedling mass and microgreen yield. Among 16 different seed enhancement techniques, by considering microgreen quality parameters tested, the best three seed enhancement treatments are hydropriming for 6 hours, priming with H₂O₂ (0.3%) for 12 hours and priming with beejamrutha for 6 hours.

Table 1 shows that hydropriming of radish seeds for 6 hours showed improved seed germination (94.0%), tray emergence (90.0%), speed of emergence (34.0) and seedling height (21.6 cm) of radish microgreens compared to control. Significantly highest radish seed germination in primed seeds (90.0%) compared to control (84%) might be due to soaking of radish seeds which increases enzyme activation, translocation and utilization of reserve food materials. Amylase and lipase are the storage-activating enzymes that are stimulated and produced by soaking. Soaking also mobilizes anti-oxidant enzymes that inhibit oxidation in seeds, maintaining seed vigour and causing early emergence. While rehydration affects pre-germinative germination processes, which in turn induces early emergence (Farooq *et al.*, 2006) [1].

Rapid and synchronized germination by an activation of pre germinative process that induce quantitative and qualitative biochemical changes such as membrane repair, a strong synthesis and activation of the enzymes involved in the degradation and mobilization of reserves might be the reason for significantly increased tray emergence percentage (90.9%) of radish microgreens compared to control (85.0%). In comparison to unprimed seeds, hydroprimed seeds causes early emergence and seedling establishment, which speeds up emergence and results in a much greater overall tray emergence. These similar results were also observed by Singh *et al.* (2014) [10].

Increased speed of emergence (34.0) of radish microgreens in primed seeds compared to control (28.4) attributed to the completion of pre-germination processes like DNA replication, increased RNA and protein synthesis, increased ATP availability, quicker embryo growth, repair of damaged seed parts, reduced leakage of metabolites, decreased lipid peroxidation and increased antioxidant activities by enhancing all these activities it increases the speed of germination. Increased germination and speed of emergence of primed seeds are caused by metabolic repair.

Early germination and emergence of seedling from hydroprimed seeds causes radish microgreens to grow quickly and become taller. Radish microgreens grown from primed seeds grow more quickly than those grown from unprimed seeds, resulting in taller seedlings (21.6 cm) compared to control (14.9 cm). The present results were in tune with the findings of Junpatri and Sangpituk, 2019 different soaking treatments had significantly enhanced the germination percentage, microgreen height and fresh weight of chia microgreens. Increased seedling height by primed seeds also observed by Shakuntala *et al.* (2020) [8].

Table 2 shows that hydropriming of radish seeds for 6 hours showed improved shoot mass (368.3 mg), seedling mass (412.2 mg) and yield (162.0 g/tray) of radish microgreens

compared to control. Priming speed up the germination process and increasing the rate and synchronization, as well as the lengths of the shoots and roots so hydropriming increases the fresh and dry mass of radish microgreens. Early emergence and seedling establishment in treated seeds might be the reason for increased shoot mass (368.3 mg) and seedling mass (412.2 mg) of radish microgreens compared to control 358.1 mg and 393.0 mg respectively. These results are in same line with Lee *et al.* (2005)^[3] who revealed that 'primed-germinated' treatment increased shoot dry weights by 49, 20 and 84 percent for kale, radish and amaranth respectively, compared to those from non-treated seeds.

Primed seedlings showed a two-fold increase in sucrose phosphate synthase-specific activity in their cotyledons. Amylase activity was shown to be greater in the shoots of hydroprimed seedlings, which accelerated the shoot's rapid hydrolysis of transient starch. This hydrolysis increases the quantity of glucose that is available for the growth of the microgreen shoot and resulting in an increase radish microgreen yield (162.0 g/tray) compared to control (156 g/tray). These results are in agreement with Li *et al.* (2021)^[5] that seed soaking increase the dry weight percentage in daikon radish, broccoli, cabbage and kohlrabi microgreens as compared with the unsoaked treatment. Hydroprimed seeds reported to be effective in early germination, better establishment and increased yield in barley (Rashid *et al.*, 2006)^[7].

Priming of radish microgreens with H₂O₂ (0.3%) for 12 hours showed improved tray emergence (90.5%), speed of emergence (33.8), seedling height (21.1 cm), shoot mass (363.7 mg), seedling mass (408.3 mg) and microgreen yield (161.7 g/tray). H₂O₂ has antiseptic and antibacterial properties so it reduces the mycoflora activity in the seeds and increases the number of healthy seedlings. H₂O₂ seed

treatment has the potential to improve the seed germination by increasing the number of healthy seedlings by reducing the population of microorganisms. The seed coat softens by soaking seeds in a hydrogen peroxide solution, allowing the seeds to absorb more oxygen. This results in increased germination speed. Hydrogen peroxide encourages healthy root growth because of the extra oxygen molecule. Oxygen can help plant roots absorb nutrients from the soil. Therefore, this extra bit of oxygen better enables the roots to absorb more nutrients, which means faster, healthier, and more vigorous growth of microgreens. Seed priming with H₂O₂ is known to enhance seedling height in individual effect through increased water imbibition and activation of enzymes (amylases, xylanase and dehydrogenases) and numerous reactive oxygen species (ROS)-scavenging antioxidants. Seeds absorb more oxygen during the imbibition stage, which causes an accumulation of ROS and changes the redox state. It also increases the activity of antioxidant enzymes such as super oxidase dismutase (SOD), catalase (CAT), ascorbate oxidase (AOX) and peroxidase (POX) in seedlings.

Beejamrutha for 6 hours showed improved tray emergence (90.0%), speed of emergence (33.5), seedling height (20.0 cm), shoot mass (363.2 mg), seedling mass (406.2 mg) and microgreen yield (161.6 g/tray). The reason behind the better performance of Beejamrutha treatment might be due to presence of macro as well as micro nutrients, many vitamins, essential amino acids, growth promoting factors like Indole Acetic Acid (IAA), gibberellic acid (GA) and beneficial microorganisms in it. Swaminathan (2005)^[11] showed that naturally occurring beneficial microorganisms mainly bacteria, yeasts, actinomycetes, photosynthetic bacteria and certain fungi were detected in cow dung which is one of component of Beejamrutha.

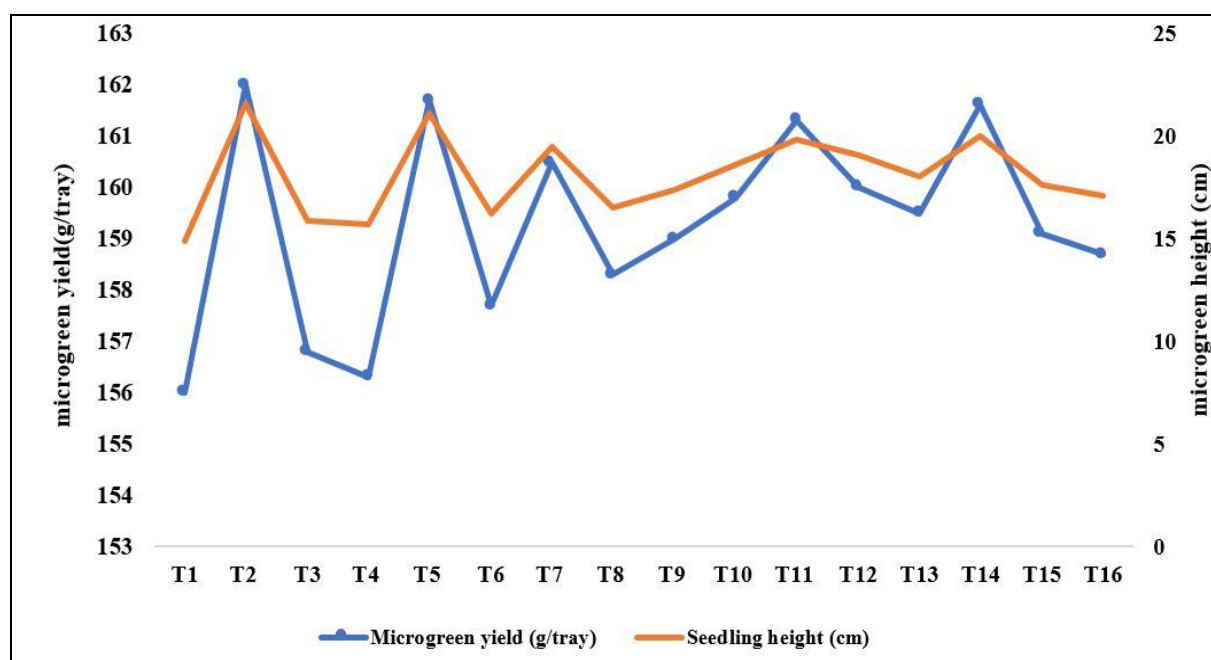
Table 1: Influence of seed enhancement techniques on microgreen yield, tray emergence, speed of emergence and seedling height of radish microgreens

Treatments	Microgreen yield (g/tray)	Tray emergence (%)	Speed of emergence	Seedling height (cm)
T ₁ -Control	156.0	85.0	28.4	14.9
T ₂ -Hydro priming for 6 hrs	162.0	90.9	34.0	21.6
T ₃ -UV rays for 15 minutes	156.8	86.1	28.9	15.9
T ₄ -Dry heat @ 80 °C for 5 minutes	156.3	85.2	28.6	15.7
T ₅ -Soaking with H ₂ O ₂ (0.3%, 20 °C, 12 hrs)	161.7	90.5	33.8	21.1
T ₆ -Soaking with tri sodium phosphate (0.5%, 20 °C, 12 hrs)	157.7	86.5	32.0	16.2
T ₇ -Biopriming with <i>Trichoderma viridae</i> (6 g/kg)	160.5	89.2	29.3	19.5
T ₈ -Sterilizing with 70% alcohol	158.3	85.5	29.3	16.5
T ₉ -Lime water @ 10 minutes	159.0	86.7	30.1	17.4
T ₁₀ -Humic acid @ 2%, 20 °C, 2 hrs	159.8	88.2	31.0	18.6
T ₁₁ -Matrix priming (20 °C, 12 hrs with cocopeat)	161.3	89.6	32.8	19.8
T ₁₂ -Cow urine @ 30% for 6hrs	160.0	88.5	31.5	19.1
T ₁₃ -α Tocopherol @ 100 mg for 16 hrs	159.5	87.8	30.9	18.0
T ₁₄ -Beejamrutha (6 hrs)	161.6	90.0	33.5	20.0
T ₁₅ -Seed coating with red soil (5 g/kg slurry treatment)	159.1	87.9	30.6	17.6
T ₁₆ -Seed coating with kaolinite (5 g/kg slurry treatment)	158.7	86.9	29.8	17.1
Mean	158.0	87.7	30.8	18.0
S.E.m±	2.24	3.09	0.66	0.34
CD (p = 0.01)	6.46	8.90	1.90	0.99
CV (%)	2.44	6.09	3.70	3

Table 2: Influence of seed enhancement techniques on microgreen yield, seedling mass and shootmass of radish microgreens

Treatments	Microgreen yield (g/tray)	Seedling mass (mg)	Shoot mass (mg)
T ₁ -Control	156.0	393.0	358.1
T ₂ -Hydro priming for 6 hrs	162.0	412.2	368.3
T ₃ -UV rays for 15 minutes	156.8	394.7	358.9
T ₄ -Dry heat @ 80 °C for 5 minutes	156.3	394.1	358.5
T ₅ -Soaking with H ₂ O ₂ (0.3%, 20 °C, 12 hrs)	161.7	408.3	363.7
T ₆ -Soaking with tri sodium phosphate (0.5%, 20 °C, 12 hrs)	157.7	395.4	359.2
T ₇ -Biopriming with <i>Trichoderma viridae</i> (6g/kg)	160.5	403.7	362.2
T ₈ -Sterilizing with 70% alcohol	158.3	396.8	359.6
T ₉ -Lime water @ 10 minutes	159.0	397.4	360.0
T ₁₀ -Humic acid @ 2%, 20 °C, 2 hrs	159.8	399.5	361.5
T ₁₁ -Matrix priming (20 °C, 12 hrs with cocopeat)	161.3	403.9	362.4
T ₁₂ -Cow urine @ 30% for 6hrs	160.0	402.3	361.7
T ₁₃ - α Tocopherol @ 100 mg for 16 hrs	159.5	399.3	361.2
T ₁₄ -Beejamrutha (6 hrs)	161.6	406.2	363.2
T ₁₅ -Seed coating with red soil (5 g/kg slurry treatment)	159.1	398.7	360.1
T ₁₆ -Seed coating with kaolinite (5 g/kg slurry treatment)	158.7	397.0	359.8
Mean	158.0	400.1	361.1
S.E.m $\pm$	2.24	14.09	12.73
CD(p = 0.01)	6.46	NS	NS
CV (%)	2.44	6.10	6.11

Note: NS-Non-significant

**Fig 1:** Influence of seed enhancement techniques on microgreen yield (g/tray) and microgreen height (cm) of radish microgreens

Conclusion

Results conclude that hydropriming for 6 hour seed enhancement technique showed improved seed and microgreen quality parameters in radish. Among 16 different seed enhancement techniques, by considering microgreen quality parameters tested, the best three seed enhancement treatments are hydropriming for 6 hours, priming with H₂O₂ (0.3%) for 12 hours and priming with beejamrutha for 6 hours. Hydropriming of radish seeds for 6 hours showed improved tray emergence (90.0%), speed of emergence (34.0), seedling height (21.6 cm), shoot mass (368.3 mg), seedling mass (412.2 mg) and microgreen yield (162.0 g/tray). Highest seed germination in primed seeds might be due to soaking of radish seeds which increases enzyme activation, translocation and utilization of reserve food materials. Rapid and synchronized germination by an activation of pre germinative process that induce

quantitative and qualitative biochemical changes such as membrane repair, a strong synthesis and activation of the enzymes involved in the degradation and mobilization of reserves might be the reason for increased tray emergence percentage of radish microgreens. Primed seedlings showed a two-fold increase in sucrose phosphate synthase-specific activity in their cotyledons.

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