

# Influence of Salicylic Acid and Gibberelic Acid on Germination and Growth of Bitter Gourd, *Momordica Charantia* L.

## ABSTRACT

**Aim:** Uniform and rapid germination and growth is a major barrier to successful crop production of bitter gourd, a major summer vegetable of our country. Seed priming of different signaling molecules can efficiently confer this problem. Therefore, the present study was conducted to explore the potentiality of different signaling molecules such as salicylic acid (SA) and gibberelic acid (GA<sub>3</sub>) to increase germination and growth of bitter gourd.

**Place and Duration of the Study:** The study was conducted in the Department of Seed Science and Technology, Bangladesh Agricultural University, from September-October, 2022.

**Methodology:** The bitter gourd (cv. Dhakaiya) seeds were soaked in 1mM, 2 mM and 3mM GA<sub>3</sub>, and 3mM, 6mM and 9mM SA solutions for 1 hour. The untreated seeds were used as control. Seeds were germinated in petri dishes and data on germination was collected. Then seedlings were transplanted to pot after 7 days to record growth parameters at 15<sup>th</sup> day of establishment.

**Results:** The experiment's findings indicated that lower concentrations of SA and GA<sub>3</sub> had a beneficial effect, however greater levels significantly reduced the bitter gourd's ability to germinate and develop in comparison to the control. Findings of the study showed priming concentrations of 3mM SA, 6mM SA, and 1mM GA<sub>3</sub>, markedly improved the germination percentage, shoot and root length, seedling vigour, and fresh and dry weight of the shoot and root, RWC of bitter gourd.

**Conclusion:** Pretreatment with SA and GA<sub>3</sub> was observed to be relatively more efficient in increasing germination of bitter gourd compared with control. Overall, this study suggests that bitter gourd seed priming 3mM SA, 6mM SA, and 1mM GA<sub>3</sub> can improve germination and growth.

Key words: Salicylic acid, Gibberelic acid, Germination, Growth, Bitter gourd

## 1. INTRODUCTION

A balanced diet for humans depends heavily on vegetables, which also provide farmers with a source of revenue. *Momordica charantia*, a cucurbitaceous vegetable known for its bitter taste and therapeutic properties, is one of the most consumed cucurbitaceous vegetables in Bangladesh. It is widely grown around the nation, primarily in the summer. Its immature fruit, which also functions as a blood purifier and is very helpful to diabetics, is a great source of nutritional fiber, minerals, and vitamins (C and A) [1]. It also possesses anti-carcinogenic qualities and can be employed as a cytostatic drug against several cancer types [2]. Additionally, it is employed in conventional therapy to treat conditions like perlipidemia, digestive issues, menstrual irregularities, and a number of microbiological diseases [2].

An essential component of production, quality, and ultimately the profitability of vegetable farmers is uniform and quick germination. Even though bitter gourd seeds have a high germinability, rapid and uniform emergence is usually problematic because of the thick seed coat that causes the seed to slowly consume water and produce delayed germination [3]. Priming is one of the strategies that have been used to address this issue. Increasing crop performance through seed priming is an easy, inexpensive and efficient technique. It is referred to as a physiological technique that hydrates and dries seeds without radicle protrusion into water or the solution of additional priming chemicals to enhance the pre-germinative metabolic process [4, 5, 6]. It has been demonstrated that several horticultural and agricultural crops profit from it in terms of seed germination, seedling establishment and ultimately productivity [7, 8, 9, 10, 11]. Thus, it has been suggested for a long time as a possible means of enhancing crop performance [12]. Additionally, primed seeds exhibit improved uniformity in the emergence of seedlings and a higher rate of germination, both of which aid in the regular establishment of crops and subsequently the production. Better plant water status management and an improved ability for nutrient uptake are linked to the primed plants' rapid development [13, 14].

Plant hormones, which have tiny molecules and are present in minuscule amounts in cells, are essential for controlling the growth processes of plants [13]. Salicylic acid (SA; o-hydroxy benzoic acid) is one of the crucial plant growth regulators (PGRs) that contributes significantly to the development and growth of plants. For instance, SA priming of cereal seeds has been shown to positively affect plant growth and development in maize [15] and also in rice [16, 17, 18, 19, 20]. Therefore, SA has the potential to enhance yield components as well as biological, physiological and morphological indices in plants [13]. Gibberellins (GA), another significant phytohormone that is a member of the tetracyclic diterpenoid carboxylic acid group, can stimulate plant growth and development by encouraging seed germination and releasing dormancy [8, 21]. They have an impact on growth and development throughout the entire plant life cycle as tiny plant growth molecules. A number of studies to have better germination and growth of bitter gourd has been done to see the efficiency of these growth regulators but the ideal concentration is yet to be determined. Therefore, the goal of this study is to investigate the ideal SA and GA<sub>3</sub> concentration for boosting bitter gourd seed germination uniformity and seedling growth.

## **2. MATERIALS AND METHODS**

The experiment was accomplished using local bitter gourd variety Dhakaiya at the Department of Seed Science and Technology in Bangladesh Agricultural University. Initially, uniform-sized seeds were sterilized for 5 minutes with 1% sodium hypochlorite, then washed repeatedly with double-distilled water. Firstly, for priming, the seeds were soaked in 1mM, 2 mM and 3mM GA<sub>3</sub>, and 3mM, 6mM and 9mM SA in separate screw-capped bottles. For control, untreated seeds were used. Ten bitter gourd seeds were then placed on petri dishes (150\*20 diameter) with three layers of Whatman filter papers moistened with 20 ml distilled water and stored in standard laboratory conditions (room temperature was 25±1<sup>0</sup>C and relative humidity was 95%). Three

independent duplicates of the experiment were run in a completely randomized block design. 7 days old seedlings were transplanted in hydroponic solution in pots and grown for further collection of data.

**Table 1:** The following treatments were maintained:

Treatment	Concentration of priming agent
T1	Control
T2	3mM SA
T3	6mM SA
T4	9mM SA
T5	1mM GA <sub>3</sub>
T6	2mM GA <sub>3</sub>
T7	3mM GA <sub>3</sub>

**2.1 Determination of germination and growth parameters**

Germination percentage, vigor index, root length, shoot length, fresh and dry weight and relative water content of leaf were considered as germination and growth parameters and determined in control as well as plants under treatment.

**2.2 Germination percentage**

The number of sprouted seeds were counted daily commencing from the 1st day to 7th day. After 7th day, final count was done and Germination Percentage (GP) of final day was calculated by the following formula stated in [22]:

$$\text{Percent germination (PG)} = (\text{Total no. of seeds germinated}) / (\text{Total no. of seeds taken for germination}) \times 100 \dots\dots\dots [1]$$

**2.3 Shoot and root length**

Shoot and root length of all sprouting’s from each replication were measured on the 15th day. Shoot length was measured from shoot base to the tip of the longest leaf and root length was measured from root base to the root tip.

**2.4 Fresh and dry weight of shoot and root**

Fresh weight (g) of root and shoot were measured just after harvesting. The dry weight (g) of root and shoot were determined by drying the sample in an oven at 80±2°C for three days’ till attaining a constant weight.

**2.5 Relative water content (RWC)**

The procedures of Mostofa and Fujita were used to determine the relative water content (RWC) [23]. After 14 days of planting, leaf samples were collected, and fresh weights (FW) of the leaves were taken. After the leaves had been submerged in water for one or two hours, their turgid weights were measured. Following the removal of excess water from the turgid leaves, the

turgid weight (TW) was immediately recorded. To determine the leaves' dry weight, they were subsequently oven-dried for 48 hours at 70 degrees Celsius (DW). The RWC was examined using the following equation:

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100 \dots \dots \dots [2]$$

## **2.6 Statistical analysis**

Utilizing the statistical program Minitab 17.0, a one-way ANOVA was performed on the data gathered for each parameter (Minitab Inc., State College, PA, USA). Pair-wise comparisons revealed statistical differences between the means of various treatments (P 0.05).

## **3. RESULTS**

### **3.1 Effect of priming on germination and seedling growth of bitter gourd**

#### **3.1.1. Germination percentage**

The effects of SA priming and GA<sub>3</sub> priming on germination of bitter gourd seedlings are presented in Table 2. Seed priming with SA and GA<sub>3</sub> increased germination percentage considerably. Priming with 3 and 6 mM SA showed 100% germination and with 9mM 87.51%. Also priming with 1 and 2 mM SA showed 100% germination and with 3mM 87.5%. All the concentrations showed increased germination percentage compared with control which only accounted for 62.33%. Germination percentage improved between 40-60% with both priming agents compared with control.

#### **3.1.2. Seedling growth and vigour index**

To evaluate the consequences of SA and GA<sub>3</sub> priming on the growth of bitter gourd seedlings and to find out the effect of these agents on seedling vigor, the shoot and root length was monitored (Table 2).

Priming concentrations had both positive and negative effects on shoot and root length of bitter gourd seedlings. The priming with 1 mM GA<sub>3</sub> showed the highest shoot length and the shoot length was recorded as 24.3cm. Again, priming with 2 and 3mM GA<sub>3</sub> increased the shoot length significantly by 40% and 7%, respectively, in comparison with control plants. Moreover, seed priming with 3 and 6 mM SA enhanced shoot length by 14% and 13% but priming concentration 9mM SA decreased shoot length by 17% compared to control. Likewise, root length showed significant variation with different priming treatments. Priming with 6 mM SA had the highest root length which accounted for 16.35cm and priming with 3 mM SA had the second highest root length which was 15.5cm. All other treatments had decreased value compared with control. Priming with every agent, significantly increased SVI both for SA and GA<sub>3</sub> primed conditions compared with control. Among the treatments, the highest SVI was recorded for priming with 1mM GA<sub>3</sub> and the lowest result was found for 9 mM SA.

**Table 2:** Effect of concentrations of plant growth regulators i.e salicylic acid and gibberelic acid on germination and seedling properties of bitter gourd

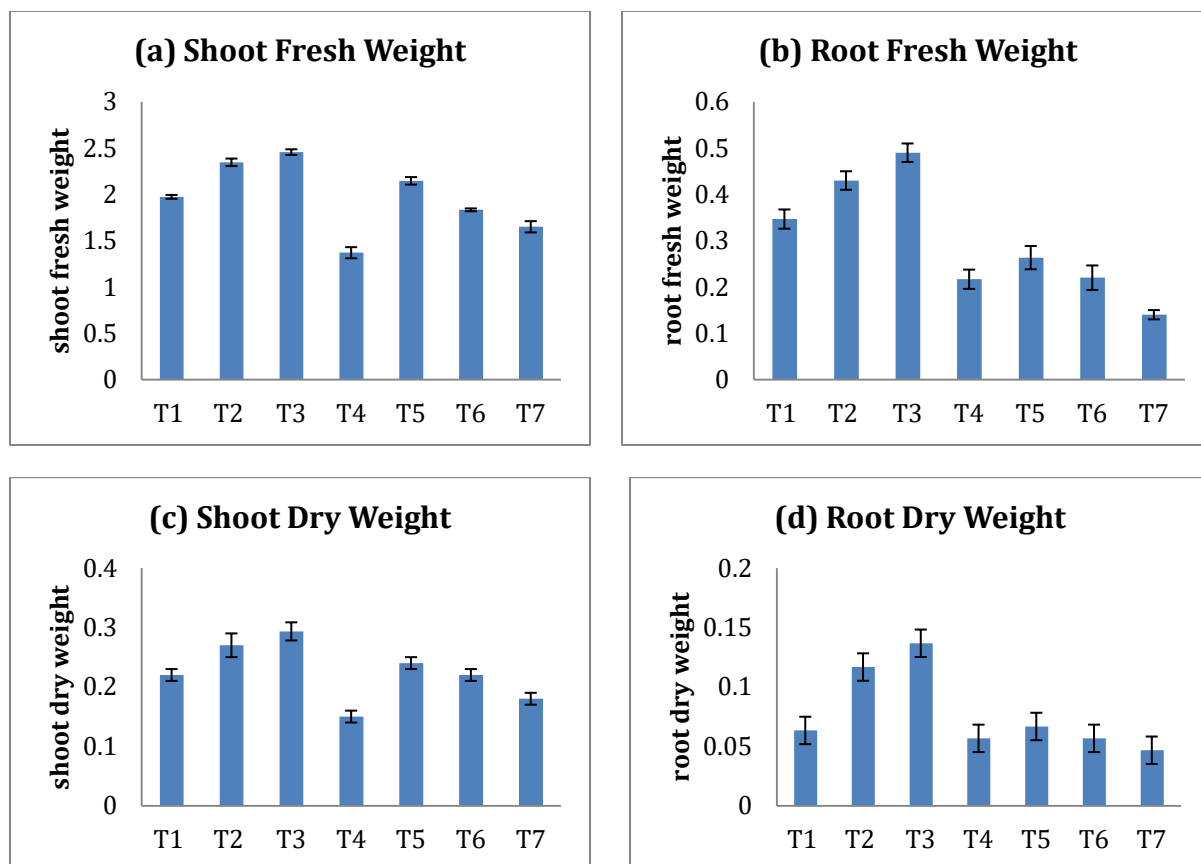
Treatment	Germination Percentage (%)	Shoot Length (cm)	Root Length (cm)	Vigour Index
T1 (Control)	62.33c	15.26667e	13.44c	1789.418
T2 (3mMSA)	100a	17.5c	15.5b	3300c
T3 (6mMSA)	100a	17.3c	16.35333a	3365.333c
T4 (9mMSA)	87.50	12.57333f	10.53f	2021.726e
T5 (1mM GA3)	100a	24.3a	12.3d	4660a
T6 (2mM GA3)	100a	21.44b	11.29667e	4273.667b
T7 (3mM GA3)	87.5b	16.4d	9.27g	2858.633d
Level of Significance	*	*	*	*

In a column, values having similar letter(s) do not differ significantly at 5% level of probability by LSD0.05 test. \* indicates significant at 5% level of significance

### 3.1.3. Fresh and dry weight

A considerable variation in fresh and dry weight was observed as a result of priming of bitter gourd seeds (Fig.1). The highest shoot and root fresh weight was observed with priming concentration of 6mM SA which was 2.45g and 0.49g, respectively. Priming with 3mM SA and 1 mM GA<sub>3</sub> increased shoot fresh weight by 18% and 8%, respectively where a decrease in fresh weight of shoot was observed for other treatment compared with control. Similarly priming with 3 and 6mM SA increased root fresh weight by 63% and 86%, and priming with 1mM GA<sub>3</sub> increased by 31% compared with control.

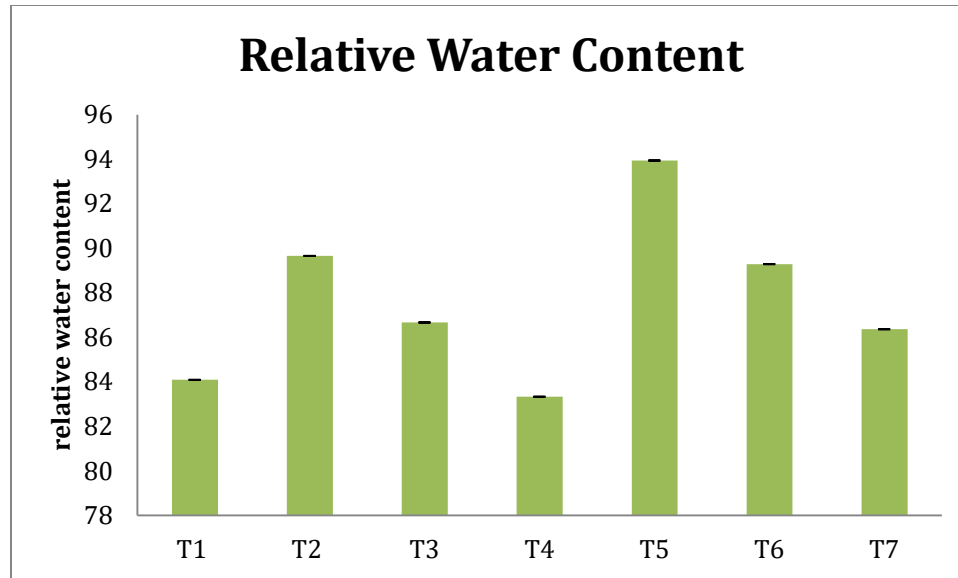
In case of dry weight the highest shoot and root weight was observed with priming concentration of 6mM SA, which was 0.29g and 0.13g, respectively. Priming with 3mM SA and 1 mM GA<sub>3</sub> increased dry weight of shoot by 22% and 9%, respectively where a decrease in shoot dry weight was observed for other treatment compared with control. Moreover root dry weight showed an similar pattern as of increase 84% and 5% with 3mM SA and 1 mM GA<sub>3</sub> priming compared with control. Other concentrations of priming decreased in root dry weight compared with control.



**Figure 1.** Effect of concentrations of plant growth regulators i.e salicylic acid and gibberellic acid on (a) shoot fresh weigh (b) root fresh weight, (c) shoot dry weight, (d) root dry weight of bitter melon. Vertical bars are SEM (n=3).

### 3.1.4 Relative water content (RWC)

A considerable variation of relative water content was observed as a result of priming seeds (Fig. 2). All the concentrations except 9mM SA relative water content increased between 2-11% whereas in 9mM SA RWC decreased by 0.9% compared to control. The highest RWC was observed in pretreatment with 1mM GA<sub>3</sub>.



**Figure 2.** Effect of concentrations of plant growth regulators i.e salicylic acid and gibberelic acid on relative water content of bitter gourd. Vertical bars are SEM (n=3).

#### 4. DISCUSSION

The process of seed germination is the first and most critical stage in the growth of a plant because it helps seedlings adapt to their constantly changing environment and increase their production [4, 24, 25]. Due to this feature, several pre-treatment application procedures are frequently utilized to promote seedling germination and establishment. Because the bitter gourd's seed coat is so thick, it takes longer for the seed to soften before germination, which causes a delayed emergence [3]. By assisting the biological processes necessary for germination and weakening the seed coat before to sowing, phytohormone pre-sowing promotes early germination [26]. Early seed germination cause seedlings to grow quickly and become taller. According to the results of our study, 3mM, 6mM, and 1 mM SA and GA<sub>3</sub> increased germination %, seedling vigor, shoot and root length, fresh weight and dry weight of seedlings, and RWC which positively supports the above statements. On the other hand, bitter gourd germination and growth were negatively impacted by greater SA and GA<sub>3</sub> concentrations. These findings are in line with several studies that have demonstrated the effectiveness of low quantities of signaling molecules in promoting germination and growth [5, 13, 27, 28, 29, 30]. Plants grown from primed seeds grow more quickly than those grown from unprimed plants, resulting in taller seedlings (Table 2). Similar to this, [31, 32, 33] reported that cucurbits had longer shoots when they were sown after priming than when they were not. The positive impact of priming on plant growth can be attributed to better root development and, as a result, an increased capacity for utilizing nutrients that permits a higher relative growth rate [14] and better plant water status regulation [13]. The direct impact of pretreatment on the regulation of the cell cycle and the mechanisms of cell elongation can also be the reason.

#### 5. CONCLUSION

It has been determined that the seed priming concentration has a substantial positive and negative impact on the bitter gourd's quick germination and seedling growth. The germination percentage, shoot and root length, seedling vigor, and fresh and dry weight of the shoot and root all significantly increased with priming concentrations of 3mM SA, 6mM SA, and 1mM GA<sub>3</sub>. So it stands to reason that these concentrations might be utilized to ensure consistency in boosting the production of bitter gourd. To confirm our findings, it is strongly advised to carry out the same experiment on field level.

## **DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

## **REFERENCES**

1. Asna AC, Joseph J, Joseph John K. Botanical Description of Bitter Gourd BT—The Bitter Gourd Genome. In: Kole C, Matsumura H, Behera TK, editors. Cham: Springer International Publishing; 2020. p. 7–31. [https://doi.org/10.1007/978-3-030-15062-4\\_2](https://doi.org/10.1007/978-3-030-15062-4_2)
2. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: A review. *J Ethnopharmacol.* 2004; 93(1):123–32. <https://doi.org/10.1016/j.jep.2004.03.035> PMID: 15182917
3. Baig KK, Ara N, Ali S, Khan BP, Wahab A, Rabbani U. Effect of seed priming on bitter gourd with different sources of phosphorus at various soaking durations. *Pure Appl Biol.* 2020; 9(1):80–90.
4. Rhaman MS, Rauf F, Tania SS, Khatun M. Seed priming methods: Application in field crops and future perspectives. *Asian Journal of Research in Crop Science.* 2020a;25:8-19.
5. Sung Y, Cantliffe DJ, Nagata RT, Nascimento WM. Structural changes in lettuce seed during germination at high temperature altered by genotype, seed maturation temperature, and seed priming. *J Am Soc Hortic Sci.* 2008; 133(2):300–11.
6. Yibchok-Anun S, Adisakwattana S, Yao CY, Sangvanich P, Roengsumran S, Hsu WH. Slow acting protein extract from fruit pulp of *Momordica charantia* with insulin secretagogue and insulinomimetic activities. *Biol Pharm Bull.* 2006; 29(6):1126–31.
7. Bruce TJA, Matthes MC, Napier JA, Pickett JA. Stressful “memories” of plants: Evidence and possible mechanisms. *Plant Sci.* 2007; 173(6):603–8.

8. Farooq M, Basra SMA, Rehman H, Saleem BA. Seed priming enhances the performance of late sown wheat (*Triticum aestivum* L.) by improving chilling tolerance. *J Agron Crop Sci.* 2008; 194(1):55–60.
9. Farooq M, Usman M, Nadeem F, Rehman HU, Wahid A, Basra SMA, et al. Seed priming in field crops: Potential benefits, adoption and challenges. *Crop Pasture Sci.* 2019; 70(9):731–71.
10. Gopalan C, Sastri B. R, Balasubramanian SC. Nutritive Value of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research (ICMR); 1989.
11. Sher A, Sarwar T, Nawaz A, Ijaz M, Sattar A, Ahmad S. Methods of seed priming. In: *Priming and Pretreatment of Seeds and Seedlings.* Springer; 2019. p. 1–10.
12. Chen K, Arora R. Priming memory invokes seed stress-tolerance. *Environ Exp Bot* [Internet]. 2013; 94:33–45.
13. Ahmed M, Qadeer U, Ahmed ZI, Hassan FU. Improvement of wheat (*Triticum aestivum*) drought tolerance by seed priming with silicon. *Arch Agron Soil Sci.* 2016; 62(3):299–315.
14. Muhammad I, Kolla M, Volker R, Gunter N. Impact of Nutrient Seed Priming on Germination, Seedling Development, Nutritional Status and Grain Yield of Maize. *J Plant Nutr.* 2015; 38(12):1803–21.
15. Ruan S, Xue Q, Tylkowska K. The influence of priming on germination of rice (*Oryza sativa* L.) seeds and seedling emergence and performance in flooded soil. *Seed Sci Technol.* 2002;30:61–67.
16. Basra SMA, Farooq M, Tabassam R, Ahmad N. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (*Oryza sativa* L.). *Seed Sci Technol.* 2005; 33(3):623–8.
17. Farooq M, Basra SMA, Ahmad N. Improving the performance of transplanted rice by seed priming. *Plant Growth Regul.* 2007; 51(2):129–37.
18. Mahboob W, Rehman HU, Basra SMA, Afzal I, Abbas MA, Naeem M, Abbas M. Seed priming improves the performance of late sown spring maize (*Zea mays*) through better crop stand and physiological attributes. *Int J Agric Biol.* 2015;17(3):491–498. doi: 10.17957/IJAB/17.3.14.283.
19. Rehman H, Iqbal H, Basra SMA, Afzal I, Farooq M, Wakeel A, Ning W. Seed priming improves early seedling vigor, growth and productivity of spring maize. *J Integr Agric.* 2015;14(9):1745–1754. doi: 10.1016/S2095-3119(14)61000-5.
20. Szalai G, Pál M, Árendás T, Janda T. Priming seed with salicylic acid increases grain yield and modifies polyamine levels in maize. *Cer Res Commun.* 2016;44:537–548. doi: 10.1556/0806.44.2016.038.
21. Farooq M, Basra SM, Wahid A, Ahmad N. Changes in Nutrient-Homeostasis and Reserves Metabolism During Rice Seed Priming: Consequences for Seedling Emergence and Growth. *Agric Sci China* [Internet]. 2010; 9(2):191–8.

22. Rauf F, Sagar A, Rahman T, Hossain MA, Kabir H, Hossain AK. Seed Quality Status of Rice Varieties Based on Physical Properties, Seed Health and Proximate Composition. *Int. J. Plant Soil Sci.* 2020;32:14-24.
23. Mostofa, M.G., Fujita, M. Salicylic acid alleviates copper toxicity in rice (*Oryza sativa* L.) seedlings by up-regulating antioxidative and glyoxalase systems. *Ecotoxicology* **22**, 959–973 (2013). <https://doi.org/10.1007/s10646-013-1073-x>
24. Sadeghian SY, Yavari N. Effect of water-deficit stress on germination and early seedling growth in sugar beet. *J Agron Crop Sci.* 2004; 190(2):138–44.
25. Tania SS, Rhaman MS, Hossain MM. Hydro-priming and halo-priming improve seed germination, yield and yield contributing characters of okra (*Abelmoschus esculentus* L.). *Tropical Plant Research.* 2020;7:86-93.
26. Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf.* 2005;60(3):324–349. doi: 10.1016/j.ecoenv.2004.06.010.
27. Ahmad I, Basra SMA, Wahid A. Exogenous application of ascorbic acid, salicylic acid and hydrogen peroxide improves the productivity of hybrid maize under at low temperature stress. *Int J Agric Biol.* 2014;16:825–830.
28. Jamil E, Zeb S, Ali QS, Ahmad N, Sajid M, Siddique S, et al. Effect of Seed Soaking On Seed Germination and Growth of Bitter Gourd Cultivars. *Pure Appl Biol.* 2016; 5(1):31–6.
29. Pill, W.G. and E. A. Kilian. 2000. Germination and emergence of parsley in response to osmotic or matricseed priming and treatment with gibberellin. *HortSci*, 35(5):907-909.
30. Yarnia, M. and E. F. M. Tabrizi. 2012. Effect of Seed Priming with Different Concentration of GA3, IAA and Kinetin on Azarshahr Onion Germination and Seedling Growth. *J. Basic. Appl. Sci. Res.*, 2(3):2657-2661.
31. Ogbuehi HC, Madukwe DK, Ashilonu P. Assessment of Hydro Priming of Seeds on Performance of Morphological Indices of Bambara Groundnut (*Vigna Subterrenea* Linn.) Landrace. *Glob J Biol Agric Heal Sci.* 2013; 2(2):17–22.
32. Shakuntala NM, Kavaya KP, Macha SI, Kurnalliker V, Patil M. Studies on standardization of water soaking duration on seed quality in cucumber (*Cucumis sativus* L.) seeds. 2020; 9(4):1400–4.
33. Mehta DK, Kanwar HS, Thakur AK, Thakur S, Thakur KS. Standardization of seed hydro-priming duration in bitter gourd, *Momordica charantia* l. *Int J Bio-resource Stress Manag.* 2014; 5(1):98.