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Prabhat Tiwari
Department of Silviculture and Agroforestry, College of Horticulture & Foretsry, RLBCAU, Jhansi, Uttar Pradesh, India

Rakesh Kumar
Department of Silviculture and Agroforestry, College of Horticulture & Foretsry, RLBCAU, Jhansi, Uttar Pradesh, India

Corresponding Author:
Prabhat Tiwari
Department of Silviculture and Agroforestry, College of Horticulture & Foretsry, RLBCAU, Jhansi, Uttar Pradesh, India

Effects of pre-sowing seed treatments on germination and seedling growth performance of *Ocimum basilicum* L.

Prabhat Tiwari and Rakesh Kumar

Abstract
Ocimum basilicum is an important medicinal plant throughout the world. The present investigation focuses on influence of various inorganic seed pre-treatment on germination and seedling vigour in Basil. The mature seeds of *Ocimum basilicum* were collected from Dr. Y.S. Parmar, UHF, Nauni district- Solan (H.P.) India. The seeds were subjected to 4 pretreatments, which included soaking in normal and boiling water and soaking in GA3 in different concentration and duration. The results of the study indicated that the highest germination percentage (24.27) was observed in T_1 (Soaking of seeds in 20 ppm GA3 for 45 min.) and lowest (0.40) in T_3 (Soaking of seeds in hot water for 10-15 min.). Boiling water treatments decreased the germination percentage of the seeds than control. With regards to speed of germination, soaking the seeds in 10 ppm GA3 for 45 min. (T_2) recorded the highest speed of germination (10.40) followed by seeds soaked in 20 ppm GA3 (T_1). In the present investigation, pre-treatment with GA3 emerged as the best pre-treatment as proven by earlier workers, but the second best treatment was soaking in normal water, especially 24 hours duration. This is a low cost pre-treatment compared to GA3 and hence can be widely used by farmers in nurseries. However, the definite pattern of influence of pre-treatments visible in seedling traits observed after four weeks.

Keywords: Germination value, germination percentage, speed of germination, *Ocimum basilicum*, seedling vigour

Introduction
Basil (*Ocimum basilicum* L.) belonging to the plant family Lamiaceae, subfamily Nepetoideae, and the genus sensu lato, comprises 65 species. Basil is an annual, 20-60 cm long, white-purple flowering plant, which is originally native to India and other regions of Asia [22]. Depending on the species and cultivar, the leaves may taste somewhat like anise, with a strong, pungent, often sweet smell. Basil is most commonly used fresh in cooked recipes. In general, it is added at the last moment, as cooking quickly destroys the flavour. The fresh herb can be kept for a short time in plastic bags in the refrigerator, or for a longer period in the freezer, after being blanched quickly in boiling water. The dried herb also loses most of its flavour, and what little flavour remain tastes very different, with a weak coumarin flavour, like hay. When soaked in water, the seeds of several basil varieties become gelatinous. It is mainly used for culinary or medicinal purposes because of its high concentration of antioxidant phenolic compounds, such as rosmarinic acid and other caffeic acid derivatives (CADs) [20, 16]. and due to the typical aroma derived from its EOs. The distinction between the numerous basil varieties is largely based on their EO composition, which is of the utmost importance in consumers' preference. The large consumption of basil as a food ingredient makes it a possible candidate for bio fortification purposes.

The study of seed germination and seedling growth of medicinal plant species like *Ocimum basilicum* has received special attention from the scientific community due to the increased demand for these plants in the medicinal, herbal and pharmacological industry, coupled with the need to make rational crops for the production of herbs [13]. High seed quality and seedling establishment are the cornerstones of profitable, efficient, and sustainable crop production [8]. Seed dormancy is defined as the failure of an intact viable seed to complete germination under favorable conditions and is controlled by several environmental factors such as light, temperature, and the duration of seed storage [19]. Dormancy is an important component of physiological seed quality and so plants with a long history of domestication and plant breeding generally have lower seed dormancy than wild or more recently domesticated species [4]. Depending on the plant species and type of dormancy, various methods like scarification, pretreatment with plant growth regulators (PGRs), Inorganic pretreatments and temperature shocks are used to break dormancy [4, 14].

Plant seed germination depends on both intrinsic and extrinsic factors. The principle factors that influence seed dormancy include certain PGRs and notably among them the abscisic acid (ABA) is involved in germination inhibition while gibberellins (GAs) participate in the termination of seed dormancy [7, 12]. Commonly, PGRs improve seed germination capacity, increase biomass yield, and confer resistance to diseases and adverse growth conditions [21]. GAs is generally synthesized by seeds and their role in germination is thought to be hydrolysis of storage nutrients in seeds and a direct effect on embryo growth [18]. External application of PGRs to seeds could break seed dormancy and enhance seedling establishment of many aromatic and medicinal plants [1, 9, 11, 15, 24, 26].

In the present study, we investigated seed germination and seedling growth of medicinal plant species *viz*. sweet basil (*O. basilicum* L.) because previous studies on seed germination of these plant species using PGRs and inorganic pretreatments are very scarce. Therefore, we investigated presowing treatments with different PGRs including GA_3, hot water treatment at different concentrations with the aim to improve seed germination and seedling growth of these medicinally important plant species.

Materials and Methods

Seed collection

The mature seeds of *Ocimum basilicum* were collected from Dr. Y.S. Parmar, UHF, Nauni district- Solan (H.P.) during August to September 2019. The seeds were separated from the inflorescences, cleaned, and dried for a week at room temperature (25 ± 2 °C). The seeds were surface sterilized in 70% ethanol for 1 min followed by 5% hypochlorite for 10 min and then washed with sterile distilled water before an experimental procedure to prevent contamination. For one day and kept for storage. The evaluation of seed reflected that the average number of seeds per kg was 1, 00,000 and weight of sown seed ranged from 50-60g.

Pretreatment and nursery practices

In order to hasten the germination of *Ocimum basilicum* seeds, four pretreatments were selected which include soaking in tap and boiling water and soaking in GA3 in different concentrations and duration (Table 1). Two thousand four hundred seeds in six replications were prepared for each pretreatment. After subjecting to pretreatment, the seeds belonging to respective treatments were sown in prepared raised nursery beds and the following cultural practices were adopted.

Watering: The regular supply of clean water is essential to plant growth. Plants are made out of more than 90% water. When grown in containers, nursery plants have only a limited volume of substrate and do not have the ability of mature trees to search for water from below the soil surface; hence the bed was watered daily against protection from scarcity of water.

Shading: Construct a shade to protect the seeds from direct sunlight for two to three weeks by using locally available materials such as grass, mats, or banana fibbers for shade construction.

Weeding: Weeds are a threat to healthy seedlings development. They compete with seedlings for nutrients, water and light, hence they must be controlled. With our own hand removed all the weeds around the beds unless ensure that this can be converted to compost.

Mulching: Just after seed sowing until germination mulching is done and it reduces evaporation, retain moisture, and prevent soil erosion. After planting the soil surface of the planting holes is covered with some materials (dry grass/leaves/twigs) to avoid evaporation or help the plant to retain water.

Table 1: The various pre-treatments adopted prior to sowing of *Ocimum basilicum* seeds

Treatments	Presowing Parameters
T_1	Soaking in 20 ppm GA3 for 45 min.
T_2	Soaking in 10 ppm GA3 for 45 min.
T_3	Soaking in hot water for 10-15 min..
T_4	Soaking in normal water for 24 hrs. (control)

Germination evaluation

The daily germination counts were made on the seeds until no further germination occurred. The following observations *viz*. the imbibition period, the germination percentage, speed of germination, mean daily germination (MDG), peak value of germination (PV) and germination value (GV) were also made as suggested by Czabator [5].

Imbibition period: Number of days from sowing to commencement of germination of the seeds.

Speed of germination: Speed of germination was calculated by the following formula

Speed of germination= n1/d1+n2/d2+n3/d3+----------

Where, n = number of germinated seeds, d= number of days.

Mean daily germination (MDG): Mean daily germination can be calculated by the following formula

MDG = Total number of germinated seeds/ Total number of days

Peak Value (PV): Peak value was calculated by the following formula

PV = Highest seed germinated/ Number of days

Germination Value (GV): Germination value was calculated by the following formula

GV = PV X MDG

Germination percentage: Number of germinated seeds / Total number of seeds × 100

Seedling vigour

The germinated seedlings belonging to different pretreatments were grown under similar environmental condition for 30 days. The seedlings were evaluated as described in Seedling Evaluation Handbook [2]. At the end of the experiment all seedlings were measured for shoot length, root length and total height. The seeds showing the higher seedling growth are considered to be more vigorous.

Data analysis

Variation in germination parameters due to different seed pre-treatments was compared by using analysis of variance. The data obtained were subjected to statistical analysis where ever necessary as per the procedure suggested by Gomez and Gomez [10]. However, the effects exhibit significance at 5 per cent level of probability, the critical difference (CD) was calculated. Analysis was carried out on computer using the package "STATISTICS"

Results and Discussion

Germination parameters

Observations on germination pattern of the seeds indicated that the germination started 6 days after sowing and continued

up to 28 days. Different pre-sowing treatments significantly affected various germination parameters of the seeds (Table 2). The imbibition period of the seeds varied strikingly with pre-treatments applied. The fastest germination i.e. the least imbibition period (4 days) was observed in seeds soaked in normal water (control) for 24 hours (T_4) whereas; the highest imbibition period (7 days) was recorded in T_3 (soaked in hot water for 10-15 min.). The variation in germination percentage of the Basal seeds due to different pre-treatment is given in Table 2. With regard to germination percentage, the highest value (24.27 %) was observed in T_1 (soaking of seeds in 20 ppm GA3 for 45 min.) and lowest (0.40 %) was in T_3 (soaking of seeds in hot water for 10-15 min.). Soaking of seeds in 10 ppm GA3 for 45 min (T_2) resulted in lower germination percentage compared to control. The MDG, PV and GV of the seeds subjected to different pre-treatments are given in Table 2. The highest MDG was observed in T_1 (3.60) and T_4 (2.92) and the lowest MDG was recorded in T_3 (0.05). Similar to germination per cent, the highest MDG was recorded in seeds treated with 20 ppm GA3 and the lowest was in hot water treatment. Peak value of germination also showed somewhat similar trend, with seeds treated with normal water (T_4) recording the highest value (0.64), followed by 20 ppm GA3 (0.46) and the lowest was in T_3 (0.06). Germination value which is product of GV and MDG also showed similar trend, the highest germination value was recorded in T_4 (1.88) followed by T_1 (1.66) and the lowest was in T_3 (0.003). The variation in speed of germination of the basal seeds is given in the table 2. The treatment with 10 ppm GA3 produced the highest speed of germination (9.96) and the lowest speed of germination was recorded in control (0.25) treatments. Germination of Basil due to different pre-treatments is clearly reflected in figure 1.

Table 2: The variation in germination parameters of *Ocimum basilicum* seeds due to pre-treatment

Treatments	Germination Parameters					
	Imbibition period (days)	Speed of germination	Peak value	Mean daily germination	Germination value	Germination percentage (%)
T_1	6	9.96	0.46	3.60	1.66	24.27
T_2	7	10.40	0.31	1.86	0.58	12.58
T_3	6	8.24	0.06	0.05	0.003	0.40
T_4	4	0.25	0.64	2.92	1.88	19.70
S. Em.	0.53	0.64	0.04	0.09	0.05	0.71
C.D. @ 5	1.12	1.36	0.08	1.19	0.10	1.52

Seedling growth

The variation in seedling growth attributes as affected by pre-treatments at the end of four weeks are presented in the Table 3. Analysis of variance revealed significant difference in the growth attributes of the seedlings due to pre-treatment at five per cent significance level. The mean shoot length of the seedlings under various treatments was the highest (24 & 23 cm) in T_4 (control) and T_2 (treated with 10 ppm GA3) whereas, least was observed in T_3 (hot water treatment, 19.33 cm). Highest root length was recorded in T_4 & T_1 (13.6 & 12.33 cm) and lowest in T_1 (7.6 cm). The highest seedling height after 28 days were observed in the seedling obtained from seeds soaked in normal water for 24 hours (37.66 cm) and the lowest (24.66 cm) for the seeds soaked in 20 ppm GA3 for 45 min. Hence, a definite pattern with regard to change in growth parameters due to seed pre-treatment cannot be deduced.

Seed dormancy is the major riddle in the planting stock production of herbal plants. Various presowing treatments are tried on seeds all over the world to get quick and uniform germination. There are scarcity for reports on seed dormancy and pre-sowing treatments on Basal. Basal normally takes 3-4 weeks' time to complete germination. In the present study, the germination commenced from second week onwards and was continued up to 4 weeks. In the present investigation, the treatment with pre-treatment with 20 ppm GA3 emerged as the best one as proven by earlier workers. Seed germination is promoted by gibberellin (GA) in many plant species. Abscisic Acid (ABA) is an example of a hormone (endogenous), which inhibits seed germination, while gibberellic acid (GA3) is known to promote seed germination [23]. In general, application of GA3 increased seed emergence percentage of many plant species including *Arbutus unedo* [6], black gram and horse gram [3] and coriander [17]. However, the stimulatory effects of GA3 on seed germination have been reported to be species/dose dependent. The seed germination rate of coriander increased by 19.13% as GA3 concentration increased from 3.5 to 35 mg L^{-1} [17]. GA3 had deleterious effect on seed germination of *Oroxylum indicum* where the low concentration (17-35 mg L^{-1}) reduced seed germination and the high concentration (52-70 mg L^{-1}) produced abnormal seedlings [25]. This study also reveals that control treatment somewhat better as compare to other treatments. The treatment with hot water reduced the germination percentage compared to control; hence, these treatments can be avoided. Other than enhancing the germination many authors have reported the enhanced seedling growth due to presowing treatments. Our results demonstrate that presowing seed treatment with GA3 and treated with normal water are the most effective germination stimulant for seed germination of sweet basil, indicating that the seeds possess physiological dormancy. The present study has established a successful methodology for overcoming seed dormancy and optimizing seed germination and seedling vigour in sweet basil to satisfy the demand for their medicinal parts in the pharmacological industry.

Table 3: Growth attributes of the *Ocimum basilicum* seedlings as affected by different pre-treatment.

Treatments	At the end of 28 Days		
	Shoot length (cm)	Root length (cm)	Total seedling height (cm)
T_1	17	7.6	24.66
T_2	23	12.33	35.33
T_3	19.33	13	32.33
T_4	24	13.6	37.66
S. Em.	0.47	0.74	1.03
C.D. @ 5	1.01	1.58	2.19

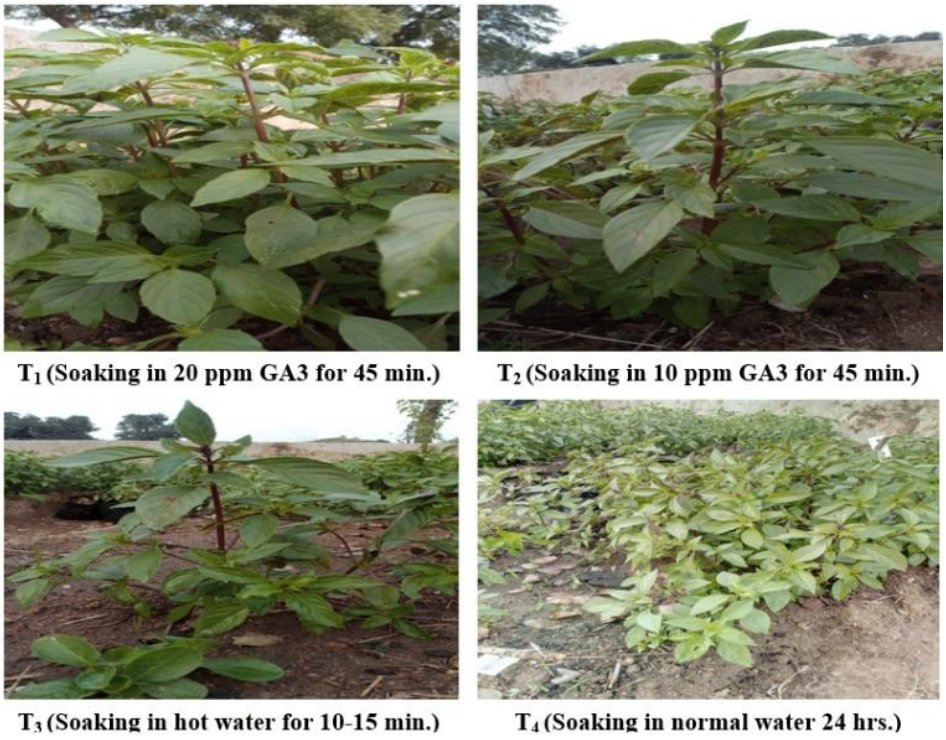

Fig 1: The variation in germination of *Ocimum basilicum* seeds due to pre-treatment

Conclusion

The summarization of the present study indicates that the presowing treatments affected the germination parameter of the Basal seeds and seedling growth also. The treatment with GA3 once again proved to be the best pre-treatment; while, boiling water treatment reduced the germination of the seeds below the control. However, the significant influence of pre-treatments on seed germination was visible in seedling traits observed after four weeks.

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