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Revitalizing Tulsi growth: Impact of pre-germination treatments on seed quality and seedling performance in Uttar Pradesh's semi-arid zones

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Abstract
Tulsi revered across cultures for its medicinal, culinary, and spiritual significance, is a plant of considerable importance. This study, conducted in 2020 at the RLBCAU nursery in Jhansi, Uttar Pradesh, sought to enhance the germination process and seed quality of Tulsi through various pre-germination treatments. The treatments tested included gibberellic acid (GA3) at 0.05% and 0.1%, potassium nitrate (KNO_3) at 0.05% and 0.1%, indole-3-butyric acid (IBA) at 0.05% and 0.1%, hot water treatment, and an untreated control. Among these, the application of 0.1% GA3 yielded the highest germination percentage at 32.1%, along with a significantly enhanced seedling vigour index of 20.21. The study underscores the substantial impact of pre-germination treatments on seed germination rates and seedling traits. Seeds treated with 0.1% GA3 demonstrated notable improvements in both germination rate and overall seedling quality, highlighting the effectiveness of GA3 in promoting robust seedling development. These findings suggest that GA3 treatment at 0.1% concentration is particularly beneficial for improving the germination and early growth of Tulsi seedlings, offering a promising approach for optimizing cultivation practices in semi-arid regions like Jhansi.

Keywords: Dormancy, germination, *Ocimum tenuiflorum*, GA_3, KNO_3, IBA, seedling vigour index

Introduction
Tulsi (*Ocimum tenuiflorum* L.), an aromatic shrub from the basil family *Lamiaceae*, is believed to have originated in north-central India. Highly revered in Ayurveda, Tulsi is often referred to as "The Incomparable One," "Mother Medicine of Nature," and "The Queen of Herbs." It is celebrated as an "elixir of life" for its profound medicinal and spiritual significance. Beyond its extensive therapeutic properties, Tulsi plays a crucial role in spiritual and cultural practices, making it a cherished herb in both health and religious contexts. The essential oil extracted from Tulsi is highly valued in various industries, including food, pharmaceuticals, cosmetics, and aromatherapy, owing to its distinctive flavor, aroma, and recognized antimicrobial and insecticidal properties.

The rising demand in the pharmacological industry and the need for efficient cultivation of medicinal plants has spurred significant interest in the study of seed germination for species like Tulsi. High seed quality and successful seedling establishment are essential for sustainable and profitable crop production. However, seed dormancy—defined as the failure of an intact, viable seed to complete germination under favorable conditions—often poses a significant challenge. Environmental factors such as light, temperature, and seed storage duration influence dormancy. To overcome this barrier, various methods, including scarification, pre-treatment with plant growth regulators (PGRs), and temperature shocks, are employed depending on the species and type of dormancy.

Ocimum tenuiflorum is known for its high yields of raw materials and active ingredients, including total phenols, flavones, chlorophyll, and L-ascorbic acid. In Western Europe, herb growers have extensively used plant growth regulators for over a decade. These regulators promote metabolic processes; enhance seed and fruit production, and increase resistance to fungal and bacterial diseases. PGRs are commonly employed to improve seed germination capacity, increase biomass yield, and enhance resistance to diseases and adverse growth conditions.

Gibberellins (GAs), a type of PGR, are naturally synthesized by seeds and play a crucial role in germination by hydrolyzing storage nutrients and directly influencing embryo growth. The external application of PGRs to seeds has been shown to break seed dormancy and enhance seedling establishment in many aromatic and medicinal plants.

Among the various PGRs, Gibberellic Acid (GA3) is particularly notable for its broad physiological effects. GA3 has been shown to positively influence various aspects of plant life, including promoting plant vigour and stimulating the biosynthesis of secondary metabolites. Its application can lead to improved growth, increased biomass, and enhanced production of compounds essential for plant health and agricultural productivity.

This study focused on investigating the seed germination and seedling growth of the medicinal plant species *Ocimum tenuiflorum* L., given the limited research on the seed germination of this species using PGRs and inorganic pre-treatments. To address this gap, we explored various pre-sowing treatments, including different concentrations of GA3, IBA, KNO_3, and hot water treatments, to enhance seed germination and seedling growth in this medicinally significant plant species. The findings from this research are expected to provide valuable insights into optimizing the cultivation practices of Tulsi, ensuring improved germination rates and healthier seedlings, which are crucial for meeting the growing demand for this revered herb across various industries.

Materials and Methods

Seed Collection and Surface Sterilization

The experiment was conducted in 2020 at the nursery of Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh. Seeds were collected from the nursery of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan district, Himachal Pradesh. The seeds underwent surface sterilization by immersing them in 70% ethanol for 1 minute, followed by a 10-minute treatment with 5% hypochlorite. Afterward, the seeds were thoroughly rinsed with sterile distilled water to eliminate any contaminants. The sterilized seeds were then stored for 24 hours before commencing the experimental procedures. Evaluation showed that the seeds averaged 100,000 per kilogram, with the weight of the sown seeds ranging between 50 and 60 grams.

Treatment for Seed Germination

The seeds were carefully selected using the sinker and floater method, as described by Mandal (2000) [41]. In this process, seeds that sank in water were chosen for the experiment, as they are typically more viable. The study aimed to evaluate the effects of different pre-sowing treatments on Tulsi seed germination, specifically using Gibberellic Acid (GA3), Indole-3-butyric Acid (IBA), and Potassium nitrate (KNO_3) at concentrations of 0.1%, 0.05%, as well as hot water soaking. Uniform-sized seeds were immersed in these plant growth regulator (PGR) solutions for 24 hours to assess their impact on germination. Distilled water was used as the control treatment to provide a baseline for comparison.

Observation of Seed Germination

Seed germination was monitored daily for 30 days. Germination was defined as the point when the tip of the radicle had emerged from the seed coat. Germination parameters like Germination percentage were recorded as per Esechie (1994) [13]. The following observations *viz*. the imbibition period, speed of germination, mean daily germination (MDG), and the peak value of germination (PV) and germination value (GV) were also made as suggested by Czabator (1962) [10].

Table 1: Different pre-sowing treatments of *Ocimum tenuiflorum* L

Treatment code	Treatment	Duration and Concentration of pre-sowing parameter
T_1	GA_3	0.1% for 45 min
T_2	GA_3	0.05% for 45 min
T_3	KNO_3	0.1% for 45 min
T_4	KNO_3	0.05% for 45 min
T_5	IBA	0.1% for 45 min
T_6	IBA	0.05% for 45 min
T_7	Hot water soaking	10-15 min
T_8	Control	24 hrs

Table 2: Various parameters studied under the experiment

S. No.	Parameter	Formula
1	Imbibition Period	The number of days from sowing to the commencement of germination.
2	Speed of Germination:	Speed of Germination=n1/d1+n2/d2+n3/d3+… Where n represents the number of germinated seeds and d represents the number of days.
3	Mean Daily Germination (MDG)	MDG=Total number of germinated seeds/ Total number of days
4	Peak Value (PV)	PV=Highest number of seeds germinated/ Number of days
5	Germination Value (GV)	GV=PV×MDG
6	Germination Percentage	Germination Percentage=Number of germinated seeds/ Total number of seeds×100

Attributes of Seedling Vigour

Seedling length

To determine the average seedling length, ten normal seedlings were randomly selected from each of the three replications. The seedling length was carefully measured in centimeters, starting from the root tip and extending to the tip of the primary leaf. This measurement provided a quantitative assessment of seedling growth performance under various experimental conditions. Each seedling was evaluated based on specific parameters, including shoot length, root length, and total height, following the guidelines outlined in the Seedling Evaluation Handbook (reference). Seedlings that exhibited greater shoot and root lengths, as well as overall height, were considered to demonstrate superior vigor. This superior vigor is indicative of better overall growth performance, reflecting the effectiveness of the different treatments applied during the experiment.

Allometric index
The shoot length and root length recorded 30 days after sowing were used to calculate the allometric index using the formula described by Hosseini *et al.* (2013) [17].

Allometric index = Root length/ Shootlength

Seedling dry weight (mg)
To measure seedling dry weight, the same ten seedlings used for length measurements were carefully dried in a hot air oven at 80 °C ± 2 °C for 24 hours. Once fully dried, the seedlings were allowed to cool in a desiccator for one hour to stabilize their weight. After cooling, the seedlings were weighed using an electronic balance, and the average seedling dry weight was recorded in milligrams, providing valuable data on the biomass accumulation of the seedlings under different treatments.

Vigour indices
Vigour indices were determined using the formulas established by Abdul-Baki and Anderson for all substrates.

Vigour Index = Germination Percentage (%) × Average Seedling Dry Weight (mg)

Statistical analysis
The experimental data were analyzed using a completely randomized design, as outlined by Panse and Sukhatme. The results are presented as the mean values of the replicates, with statistical significance determined by calculating the critical difference (CD) at a 5% significance level. The analysis was conducted using R software, ensuring rigorous statistical evaluation of the experimental outcomes.

Results and Discussion
Influence of Treatments on Germination Parameters
Various pre-sowing treatments were applied to assess their impact on germination parameters and seedling vigor. The treatments significantly influenced these parameters, as shown in Table 3. Germination of Tulsi seeds began four days after the experiment's initiation. The imbibition period, indicating the time taken for seeds to absorb water and swell, varied notably based on the pre-treatments applied. The shortest imbibition period of five days was observed in seeds soaked in 0.1% GA3, indicating faster germination. In contrast, the control group, with no treatment, exhibited the longest imbibition period of 12 days.

The germination percentage was highest in seeds treated with GA3 at both 0.1% and 0.05% concentrations, yielding 32% and 28% germination, respectively. These findings are consistent with Dzayi (2010) [11], who highlighted the role of GA3 in promoting cell growth by facilitating calcium release from the cell wall into the cytoplasm. This process enhances water absorption and cell expansion, which are crucial for germination. After this growth phase, GA3 is deactivated, and calcium is reabsorbed into the cell wall, leading to cell stiffening. During water absorption, the embryo generates GA3, triggering the production of hydrolytic enzymes like α- and β-amylase in the aleurone cells. These enzymes break down starch into glucose, which the embryo absorbs for nourishment. Additionally, GA3 influences protein synthesis for mRNA production, aiding DNA replication and the breakdown of endosperm materials in the seed, as described by Lahuti *et al.* (2003) [23].

Similar effects of GA3 on germination have been reported in *Atropa belladonna* (Genova *et al.* 1997) [16] and *Pimpinella anisum* (Shahrajabian *et al.*, 2019) [34]. The speed of germination was enhanced across all pre-sowing treatments compared to the control. The highest Peak Value (PV) was observed in seeds treated with 0.1% GA3, followed by hot water treatment, while the lowest PV and Germination Value (GV) were recorded in the control group, at 0.13 and 0.09, respectively. The highest GV was noted in seeds treated with 0.1% GA3 (3.033), followed by 0.05% GA3 (2.33). IBA treatments at both 0.1% and 0.05% concentrations showed similar effects. Prabhat and Tiwari (2019) reported comparable results, further supporting the findings of this study.

Table 3: The variations in germinatation parameters of *Ocimum tenuiflorum* L. due to pre-sowing treatments

Treatments	Germination Parameters					
	Germination percentage	Speed of germination	Imbibition period	MDG	PV	GV
GA_3 0.1% (T_1)	32.10a	15.23a	5g	4.10a	0.74a	3.03a
GA_3 0.05% (T_2)	28.31b	14.40b	6f	3.70b	0.63c	2.33b
KNO_3 0.1% (T_3)	26.41c	13.13c	7e	3.20c	0.33f	1.05c
KNO_3 0.05% (T_4)	25.31c	12.50d	7e	2.90d	0.27g	0.78d
IBA 0.1% (T_5)	21.44d	10.00e	8d	2.00e	0.54d	1.08c
IBA 0.05% (T_6)	18.63e	9.77e	9c	1.6f	0.44e	0.70d
Hot water soaking (T_7)	15.31f	8.20f	11b	1.10g	0.70b	0.76d
Control (T_8)	11.01g	5.70g	12a	0.70h	0.13h	0.09e
$LSD_{p<0.05}$	1.13	0.54	0.32	0.15	0.57	0.09

MDG: Mean daily germination, PV: Peak Value, GV: Germination Value
Note: Mean Values followed by different alphabets (a,b,c…) in a column varied significantly ($p<0.05$)

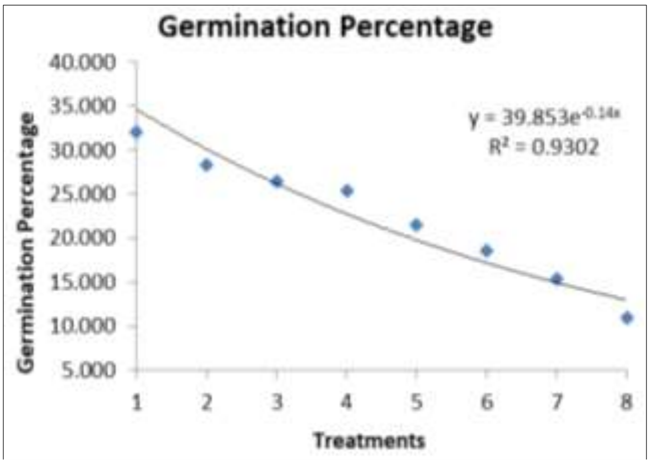

Fig 1: Relationship between different pre-sowing treatment with germination percentage and speed of germination

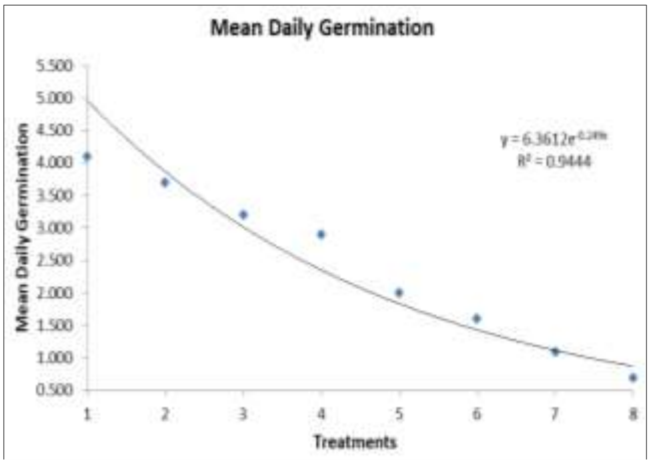

Fig 3: Relationship between different pre-sowing treatment with imbibition period

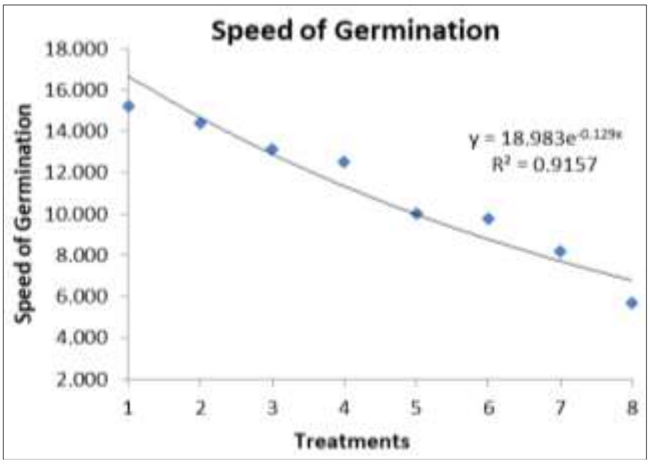

Fig 2: Relationship between speed of germination and treatment

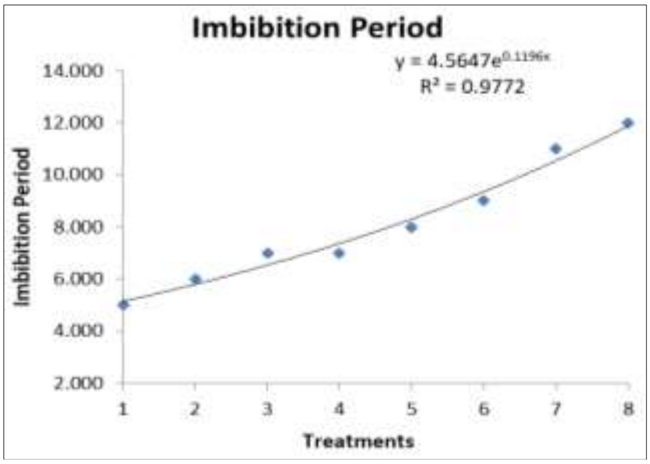

Fig 4: Relationship between different pre-sowing treatment with mean daily germination

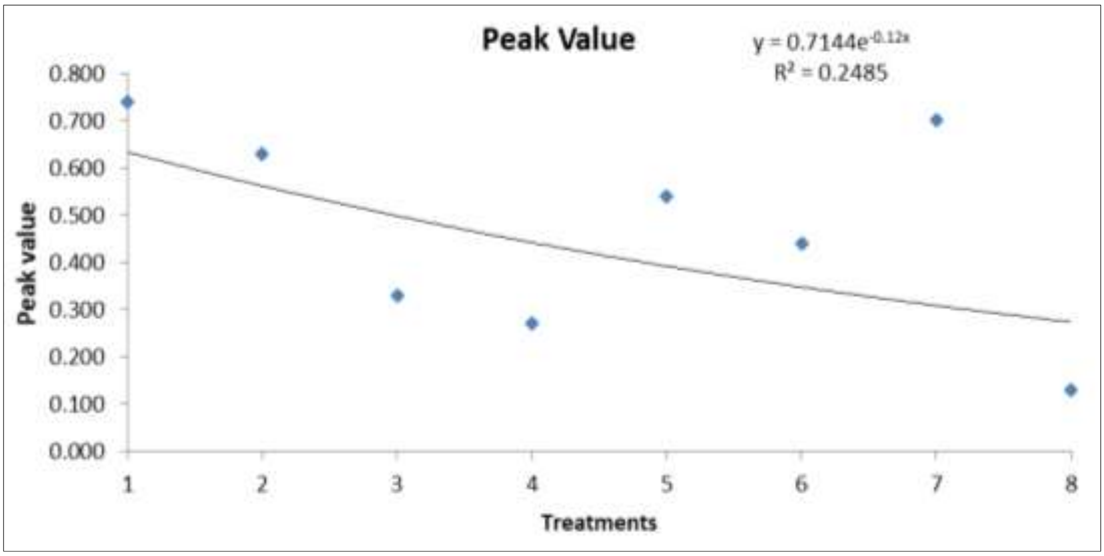

Fig 5: Relationship between different pre-sowing treatment with Peak value

Seedling Vigour

The study investigated the effects of various concentrations of Gibberellic Acid (GA3) on seedling growth characteristics and biomass, as shown in Table [reference]. The data reveal that different GA3 concentrations significantly influenced both shoot and root development. The highest shoot length of 15.31 cm was achieved with 0.1% GA3 treatment, while the shortest shoot length of 7.40 cm was recorded in the 0.05% GA3 treatment. Similarly, the longest root length of 9.40 cm was observed with 0.1% GA3, whereas the control group exhibited the shortest root length of 3.32 cm. This increase in root length, particularly with the 0.1% GA3 treatment, correlates with the enhanced above-ground growth, indicating a parallel improvement in root development.

Seedling dry weight was also affected by the treatments, with GA3 and Potassium Nitrate (KNO_3) showing superior performance compared to the control, which had the lowest dry weight of 0.25 g. This suggests that plant growth regulators (PGRs) such as GA3 and KNO_3 can mitigate adverse effects on growth, potentially due to their influence on endogenous growth regulators (Thomas *et al*., 2005) [39]. Previous research supports that GA3 treatment promotes growth by activating physiological processes involved in seedling development (Taiz and Zeiger, 2010) [38].

Among all dormancy-breaking treatments, the longest seedling length was achieved with 0.1% GA3, measuring 24.71 cm, followed closely by 0.05% GA3 at 23.20 cm. The control group had the shortest seedling length of 10.72 cm, consistent with findings by Singh *et al*. (2002) [35]. The enhanced performance of GA3-treated seeds is likely due to its role in breaking seed dormancy, which facilitates earlier and more vigorous germination by promoting the diffusion of endogenous auxins and gibberellin-like substances (Gurung *et al*., 2014) [19]. All GA3 treatments effectively enhanced seedling growth compared to the control, likely due to the activation of amylase, which converts carbohydrates into simpler sugars, thus providing energy and nutrients for rapid seedling growth. Previous studies have also observed increased plant growth resulting from GA3 treatment (Lee *et al*., 1999; Rout *et al*., 2016) [25, 32].

The allometric index, a measure of growth efficiency, was highest with 0.05% GA3 (0.646), statistically comparable to 0.1% GA3 (0.615), and followed by KNO_3 0.05% (0.661). GA3 0.05% and IBA 0.1% treatments were statistically similar. Among all treatments, the highest vigor index of 20.218 was recorded with GA3 0.1%, followed by 15.33 with GA3 0.05%. The control had the lowest vigor index of 2.753. These results align with Singh *et al.* and are consistent with findings by Fazeli Kakhki *et al*., who reported that gibberellins significantly enhance both germination and vigor index in scallion (*Allium fistulosum*) seeds.

Table 4: The variations in seedling vigor of *Ocimum tenuiflorum* L. due to pre-sowing treatments

Treatments	Seedling Vigor					
	Shoot length (cm)	**Root length (cm)**	**seedling length (cm)**	**seedling weight (mg)**	**Vigor index**	**Allometric index**
GA_3 0.1% (T_1)	15.3a	9.40a	24.71a	0.63a	20.21a	0.61c
GA_3 0.05% (T_2)	14.10b	9.10a	23.20b	0.58b	16.40b	0.64bc
KNO_3 0.1% (T_3)	12.60c	7.13d	19.73c	0.51c	13.47c	0.56d
KNO_3 0.05% (T_4)	11.45d	7.56c	19.01d	0.46d	11.64d	0.66bc
IBA 0.1% (T_5)	10.00e	8.33b	18.33e	0.38e	8.14e	0.83a
IBA 0.05% (T_6)	9.20f	6.37e	15.57f	0.33f	6.15f	0.69b
Hot water soaking (T_7)	8.60g	4.60f	13.20g	0.29g	4.43g	0.53d
Control (T_8)	7.40h	3.32g	10.72h	0.25h	2.75h	0.44e
$LSD_{p<0.05}$	0.56	0.32	0.53	0.02	0.57	0.04

Note: Mean Values followed by different alphabets (a,b,c…) in a column varied significantly ($p<0.05$)

Conclusion

The findings of the study indicate that Gibberellic Acid (GA3) has a substantial effect on both seed germination and seedling growth in *Ocimum tenuiflorum* L. The results demonstrate that a 0.1% GA3 pre-treatment significantly enhances germination rates, accelerates seedling growth, and improves overall seedling quality. Given these outcomes, it is recommended to use a 0.1% GA3 solution for pre-treating seeds of *Ocimum tenuiflorum* L. This treatment optimizes germination, promotes robust seedling development, and ensures better quality seedlings, thereby benefiting cultivation practices for this important medicinal plant species.

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