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Effects of Dormancy-Breaking Treatments on Seed Quality Parameters in Medicinal Herb Tulsi (*Ocimum tenuiflorum* L.)

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ABSTRACT: Tulsi, holds a revered status in various cultures around the world for its medicinal, culinary, and spiritual significance. This study was conducted for optimizing the germination test and seed quality parameters at alternate temperature of 20-30°C in Tulsi during 2022 in the laboratory of Department of Seed Science and Technology, CCS HAU Hisar. Various dormancy-breaking treatments were used during the study such as, GA_3 @ 0.05 and 0.1% for 24 hours, KNO_3@ 0.2% for 24 hours, Pre-chilling (5-10°C for 7 days), and Light treatments (16 hours light followed by 8 hours darkness). The results revealed that the germination percentage, seedling length, seedling dry weight, vigour index-I and vigour index-II was recorded maximum in top of the paper (TP) with the GA_3 0.1% treatment and the minimum was recorded with control in sand substrate at 20-30°C alternate temperature. The radicle emergence was recorded maximum with GA_3 @ 0.1% at 7th day at 20-30°C alternate temperature. The dormant seeds were observed maximum with control in BP and the minimum dormant seeds were recorded with GA_3 0.1% in TP at 20-30°C alternate temperature. The research reveals significant effects of these treatments on seed germination and other crucial seedling parameters. Seeds treated with GA_3 0.1% exhibited substantial improvements in germination percentage and overall seedling quality. These findings highlight the efficacy of GA_3 treatment in enhancing the germination process and seedling establishment of Tulsi under specified temperature conditions.

Keywords: Alternate temperature, germination testing , *Ocimum tenuiflorum,* substrates, Tulsi, hard seeds

INTRODUCTION

The demand for medicinal plants is currently on the rise, with a growing acceptance of their importance. Medicinal herbs, in particular, have consistently served as indicators of overall ecosystem health. Human beings have valued and considered the significance of medicinal plants since ancient times [1]. Tulsi, an aromatic shrub belonging to the basil family Lamiaceae, is believed to have its origins in north central India. Scientifically, it is known as *Ocimum tenuiflorum* L. In Ayurveda, Tulsi is recognized as “The Incomparable One,” “Mother Medicine of Nature,” and “The Queen of Herbs.” It holds a revered status as an “elixir of life,” unparalleled in both its medicinal and spiritual [2]. It is commercially cultivated for its leaves in hot and humid parts of India. In laboratory investigations, it has been revealed that Tulsi offers protection against injury induced by toxic chemicals. This is achieved by elevating the levels of antioxidant molecules, such as glutathione, within the body. Additionally, Tulsi enhances the activity of antioxidant enzymes like superoxide dismutase and catalase. These enzymes play a crucial role in safeguarding cellular organelles and membranes, mitigating the harmful effects of free radicals generated due to oxygen deficiency and exposure to various toxic agents [3-4].

In the realm of crop production, the stage of seed germination and seedling growth emerges as the most susceptible phase to environmental stresses. These stresses encompass factors like cold, salt, or drought, which can particularly delay the initiation and diminish the seed germination. The germination test holds particular significance as it ensures the reproducibility, reliability, and uniformity of seeds. Germination is a sequential series of morphogenetic events leading to the transformation of an embryo into a seedling. Although all plant seeds possess germination potential, various

factors such as seed coat thickness, hard seed coat, underdeveloped embryos, over-ripeness, the presence of growth inhibitors, insufficient water, lack of oxygen, and unfavourable environmental conditions can obstruct the germination process.

The dormancy and germination of seeds are influenced by genetic factors and environmental conditions during the seed growth period, as well as storage conditions. This variability contributes to diverse reports across various species, genotypes, ecotypes, and environments. The International Seed Test Associations (ISTA) has suggested various techniques to overcome dormancy and promote germination. Key methods include stratification (both mechanical and chemical), the use of germination stimulator solutions (such as gibberellin, potassium nitrate and nitric acid), exposure to light cycles, and specific temperature regimens. Gibberellins, a plant hormone found naturally, initiate the germination of seeds by facilitating their sprouting [5]. Gibberellic acid (GA_3) can break seed dormancy in various species and facilitate the germination of some plants that typically require cold stratification, exposure to light or post-ripening conditions [6]. Potassium nitrate is extensively documented as a compound that enhances the germination of photo dormant seeds. KNO_3 raises the ambient oxygen levels by reducing the availability of oxygen for the citric acid cycle [7]. Cold treatment is supposed to modify the balance between inhibitors and promoters [8].

The initial and crucial step in evaluating the quality and maximizing yields from a specific seed lot is seed germination. Seed testing procedures for certain medicinal crops can be found in the ISTA. These rules provide guidelines for substrate, temperature, test duration, and dormancy-breaking treatments. There is a lack of information regarding the temperature and substrate conditions required for seed germination in *O. tenuiflorum* L. spp., as per the ISTA. Seed dormancy is a condition where seeds do not germinate even when exposed to favourable conditions of moisture, temperature, and oxygen for germination. Therefore, it is essential to investigate the existence of dormancy in Tulsi seeds prior to planting. The objective of this research was to analyse the germination test for various seed quality parameters in Tulsi seeds and to determine the impacts of various treatments for breaking seed dormancy that can stimulate and improve the germination of the Tulsi seeds.

MATERIALS AND METHODS

The *Ocimum tenuiflorum* L. variety Hisar Selection-1, which was procured from Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, was used for the experiment. The study was conducted in the laboratories of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during 2022.

Dormancy-breaking treatments

In the experiment, seeds were treated with dormancy-breaking treatments of GA_3 @ 0.05 and 0.1% for 24 hours, KNO_3@ 0.2% for 24 hours at ambient conditions, Pre-chilling (5-10^{o}C for 7 days), and Light treatments (16 hours light followed by 8 hours darkness). The ambient conditions (Temperature Maximum= 23.2^{o}C, Minimum= 8.1^{o}C and Relative Humidity Maximum= 94%, Minimum= 51%). After the dormancy-breaking treatments seed were kept in germination chamber at various temperatures. Three substrates *i.e.,* between paper (BP), top of the paper (TP) and sand were used for assessing the seed quality parameters at various temperatures such as 15°C, 20°C, 25°C, 30°C and alternate temperature of 20-30°C (20°C for 16 hrs. and 30°C for 8 hours), where the relative humidity was maintained as per ISTA procedures. The final count of germination percentage was recorded on 8th day of germination test, where the maximum germination was recorded.

Germination (%)

Germination test was conducted by placing 100 seeds in three replications in between paper method, top of the paper and sand. On the day of final count, the seedlings were evaluated for germination percentage. Normal seedlings, abnormal seedling, dead and hard seeds were evaluated as per ISTA rules [9]. The seeds were observed on a daily basis for the emergence of both the radicle and plumule, and the final count of such seeds was recorded until maximum germination was achieved *i.e.* on 8th day for Tulsi.

$$\text{Germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds kept for germination}} \times 100$$

Hard Seeds (%)

These seeds were not able to imbibe water under the growing conditions and remain hard. Hard seeds were observed and expressed in percentage in between paper and top of the paper substrates.

Seedling length (cm)

To determine the average seedling length, ten normal seedlings were chosen randomly from each of the three replications and expressed in the centimeter from all the substrates. The seedling length was assessed from the root tip to the tip of the primary leaf.

Seedling dry weight (mg)

To record seedling dry weight, the same 10 seedlings were used for the seedling dry weight and dried at 80°C±2 for 24 hours in the hot air oven and then recorded average seedling dry weight in mg. Thereafter, the seedlings were taken out and allowed to cool in a desiccator for one hour before being weighed using an electronic balance.

Vigour indices

Vigour indices were calculated using the formula recommended by Abdul-Baki and Anderson [10] in all substrates, which is outlined as follows:

Vigour index-I = Germination (%) × Average seedling length (cm)
Vigour index-II = Germination (%) × Average seedling dry weight (mg)

Radicle emergence test (%)

Radicle emergence is the appearance of a radicle after breaking through the seed coat. A radicle emergence test was performed using the "Top of the paper" method. The count of seeds that exhibited radicle emergence of at least 2 mm in length was recorded at 24-hour intervals, starting from the initiation of radicle emergence and continuing for maximum radicle emergence for each of the replicates.

$$\text{Radicle emergence (\%)} = \frac{\text{No. of seeds with 2 mm radicle length}}{\text{Total no. of seeds sown}} \times 100$$

Statistical analyses

Statistical analyses of the obtained data from various experiments were analysed by using the factorial complete randomized design as described by Panse and Sukhatme [11]. All the values described as mean of the replicates with the evaluation of CD at 5% level of significance probability level and wherever the value is non-significant it is denoted by 'NS' by using software OPSTAT by Sheoran [12].

RESULTS AND DISCUSSION

Germination potential stands as the fundamental prerequisite for all seeds. Viability and vigour represent the dual facets of seed quality and are intricately linked when assessing seed quality. In the present study, the results revealed that there was a significant difference in various seed quality parameters of Tulsi seeds due to substrates and dormancy-breaking treatments at 20-30°C alternate temperature. The enhancement in germination of Tulsi seeds due to dormancy-breaking treatments indicates that the dormancy observed in this study may be attributed to the presence of hard seed coats obstructing water absorption. Among the treatments, GA_3 0.1% treatment showed the maximum germination in Tulsi seeds, whereas the control exhibited the lowest seed performance, resulting in the least improvement in various seed quality parameters for Tulsi seeds.

As mentioned in table 1, the maximum germination was recorded in top of the paper (TP) (33.33%) with the GA_3 0.1% treatment, followed by TP (28.67%) with the GA_3 0.05% treatment and lowest germination was observed in sand substrate (13.33%) with the control. While considering the mean of all dormancy-breaking treatments, the highest germination (27.56%) was recorded in GA_3 0.1% treatment and lowest germination (16.67%) was observed in control. Among the mean of all substrates, the highest germination (25.33%) was observed in top of the paper and lowest germination (16.94%) was observed in sand. The enhanced germination of Tulsi seeds with dormancy-breaking treatments indicates that the dormancy observed in the Tulsi under study is recognized to the presence of hard seed coats, preventing the entry of water into the seeds. Several works have documented the positive effects of GA_3 treatment on seed germination in medicinal plants, highlighting its potential as a valuable tool in seed priming and enhancement strategies. Zhang *et al.* [13] reported that GA_3 increased the germination and showed the significant effects on *Gentiana rigescens*, a medicinal plant. Rout *et al.* [14] also highlighted the significance of GA_3 for the germination in *Cassia fistula* L.

Maximum dormant seeds were recorded in BP (56.00%) with the control followed by (53.33%) in between paper with light treatment and lowest hard seeds were observed in TP (36.67%) with the GA_3 0.1% treatment. From all dormancy-breaking treatments, maximum dormant was seed observed in control (54.33%) followed by (51.67%) dormant seeds in light treatment and minimum dormant seeds were recorded (40.00%) with the treatment GA_3 0.1%. From both the substrata the maximum dormant seeds were observed in between paper (49.78%) and

Table 1. Effect of dormancy-breaking treatments and growing substrata on germination percentage, dormant seeds (%) and seedling length (cm) in Tulsi at 20-30°C alternate temperature

	Germination (%)				Dormant seeds (%)			Seedling length (cm)			
	Substrate (S)				Substrate (S)			Substrate (S)			
Treatments	BP	TP	Sand	Mean	BP	TP	Mean	BP	TP	Sand	Mean
GA_3 0.1 %	28.00	33.33	21.33	27.56	43.33	36.67	40.00	5.73	5.97	5.50	5.73
	(31.90)	(35.24)	(27.49)	(31.54)	(41.15)	(37.25)	(39.20)				
GA_3 0.05 %	25.33	28.67	18.67	24.22	46.67	42.67	44.67	5.54	5.82	5.26	5.54
	(30.20)	(32.36)	(25.58)	(29.38)	(43.07)	(40.77)	(41.92)				
KNO_3 0.2%	24.67	27.33	18.00	23.33	48.67	44.67	46.67	5.29	5.50	5.07	5.29
	(29.76)	(31.51)	(25.07)	(28.78)	(44.22)	(41.92)	(43.07)				
Pre-chilling	21.33	22.67	15.67	19.89	50.67	48.00	49.33	4.99	5.28	4.70	4.99
	(27.49)	(28.42)	(23.28)	(26.40)	(45.36)	(43.84)	(44.60)				
Light Treatment	18.67	20.67	14.67	18.00	53.33	50.00	51.67	4.78	4.96	4.54	4.76
	(25.56)	(27.02)	(22.50)	(25.03)	(46.89)	(44.98)	(45.94)				
Control	17.33	19.33	13.33	16.67	56.00	52.67	54.33	4.66	4.81	4.38	4.62
	(24.59)	(26.05)	(21.40)	(24.01)	(48.43)	(46.51)	(47.47)				
Mean	22.56	25.33	16.94		49.78	45.78		5.17	5.39	4.91	
	(28.25)	(30.10)	(24.22)		(44.85)	(42.54)					
	CD (P=0.05)		S.Em±		CD (P=0.05)		S.Em±	CD (P=0.05)		S.Em±	
S	1.243		0.432		1.325		0.451	0.063		0.022	
T	1.757		0.610		2.295		0.782	0.089		0.031	
S x T	NS		1.057		NS		1.106	NS		0.053	

#Values in the parenthesis are arc-sine transformed of the original

minimum dormant seeds were recorded (45.78%) in top of the paper. Around 40-50% dormant seeds during a germination test, even with the dormancy-breaking treatments, could be attributed to several factors such as poor seed quality, genetic factors and environmental factors during seed development. It is reported that the GA_3 contributes to the development of the embryo, breaks the mechanical constraints of the seed coat and promotes the radicle protrusion in recalcitrant *P. notoginseng* seeds.

Seedling length was recorded at the 8th day (at the time of final count of germination test). The maximum seedling length was recorded in top of the paper (5.97 cm) with the treatment GA_3 0.1% followed by seedling length (5.82 cm) with the GA_3 0.05% treatment in top of the paper and minimum seedling length was recorded in sand (4.38 cm) with the control. From all the mean of dormancy-breaking treatments, maximum seedling length was found (5.73 cm) with the treatment GA_3 0.1% at par seedling length (5.54 cm) with GA_3 0.05% and minimum in control (4.62 cm). Among all the substrata the maximum seedling length was recorded (5.39 cm) in top of the paper and minimum recorded in sand (4.91 cm) mentioned in table 1. The maximum seedling dry weight (0.56 mg) was observed with the GA_3 0.1% treatment in top of the paper and followed by (0.53 mg) seedling dry weight recorded with GA_3 0.1% treatment in between paper, whereas the minimum seedling dry weight (0.37 mg) was recorded with control in top of the paper. When considering all the dormancy-breaking treatments, the maximum seedling dry weight of (0.53 mg) was observed with the GA_3 0.1% treatment and followed by (0.50 mg) seedling dry weight in GA_3 0.05%, whereas the minimum seedling dry weight (0.39 mg) was recorded in control. Among the various substrates, the maximum seedling dry weight (0.48 mg) was recorded in top of the paper method, while the minimum seedling dry weight (0.44 mg) was observed in the sand mentioned in table 2. Yadavannavar *et al.* [15] also reported that the same results in Stevia (*Stevia rebaudiana* Bertoni.) and found that GA_3 recorded the maximum root length, shoot length and seedling dry weight.

The maximum vigour index-I recorded (199) in top of the paper with treatment GA_3 0.1% followed by (167) vigour index-I with GA_3 0.05% in top of the paper and the minimum vigour index-I (58) with the control in sand. When considering all the dormancy-breaking treatments, the GA_3 0.1% treatment recorded the maximum vigour index-I (159) at par with GA_3 0.05% (135), while the control showed the minimum vigour index-I (77). Among all three substrate, the “top of the paper” substrate

Table 2. Effect of dormancy-breaking treatments and growing substrata on Seedling dry weight, Vigour index-I and Vigour index-II in Tulsi at 20-30ºC alternate temperature

Temp. (T)	Seedling dry weight (mg)				Vigour index-I				Vigour index-II			
	Substrate (S)				Substrate (S)				Substrate (S)			
	BP	TP	Sand	Mean	BP	TP	Sand	Mean	BP	TP	Sand	Mean
GA_3 0.1 %	0.53	0.56	0.51	0.53	160	199	117	159	15.00	18.67	10.67	14.78
GA_3 0.05 %	0.50	0.52	0.48	0.50	141	167	98	135	12.67	15.33	9.33	12.44
KNO_3 0.2%	0.46	0.49	0.45	0.46	130	150	91	124	11.33	13.33	8.00	10.89
Pre-chilling	0.43	0.45	0.41	0.43	106	120	74	100	9.33	10.33	6.33	8.67
Light Treatment	0.40	0.43	0.39	0.41	89	102	67	86	7.33	9.00	5.67	7.33
Control	0.39	0.42	0.37	0.39	81	93	58	77	6.67	8.00	5.00	6.56
Mean	0.45	0.48	0.44		118	139	84		10.39	12.44	7.50	
	CD (P=0.05)		S.Em±		CD (P=0.05)		S.Em±		CD (P=0.05)		S.Em±	
S	0.006		0.002		6.479		2.250		0.606		0.210	
T	0.008		0.003		9.163		3.182		0.857		0.297	
S x T	NS		0.005		15.871		5.511		1.484		0.515	

recorded the maximum vigour index-I (139), whereas the "sand" substrate showed the minimum vigour index-I (84). The maximum vigour index-II (18.67) was observed when Tulsi seeds were treated with GA_3 0.1% and placed in top of the paper and followed by vigour inex-II (15.33) with treatment GA_3 0.05% in the top of the paper and the minimum vigour index-II (5.00) was observed with control in sand. When taking into account all the dormancy-breaking treatments, the GA_3 0.1% treatment resulted in the maximum vigour index-II (14.78) followed by vigour index-II (12.44) with the treatment GA_3 0.05%, whereas in control the minimum vigour index-II (6.54) was recorded. While analyzing the different substrates, the "top of the paper" substrate recorded the maximum vigour index-II (12.44), and in sand recorded the minimum vigour index-II (7.50) mentioned in table 2. Fazeli Kakhki *et al.* [16] also observed that the gibberellin has a significant effect on increasing germination and vigour index in scallion (*Allium fistulosum*) seeds.

The radicle emergence data indicates the effect of different dormancy-breaking treatments in Tulsi seeds at different days in table 3. There was no radicle emergence on 1st, 2nd and 3rd day in all treatments. The radicle emergence was started on 4th day in all dormancy-breaking treatments at 20-30ºC. At 4th day, the maximum radicle emergence (9.33%) was recorded with GA_3 0.1% treatment followed by (6.67%) recorded with GA_3 0.05% and minimum was recorded (3.33%) in control. Till the 7th day, the maximum radicle emergence was achieved. On 7th day, the maximum radicle emergence (32.00%) was observed with GA_3 0.1% followed by (30.67%) in GA_3 0.05% and minimum (21.33%) in control. When considering the mean of each treatment, the maximum radicle emergence (13.62%) with GA_3 0.1% followed by (11.81%) radicle emergence was observed in GA_3 0.05% and the minimum (6.76%) radicle emergence was recorded with control. While comparing all days, the maximum radicle emergence (26.00%) was recorded at 7th day at par (23.33%) at 6th day and minimum (5.78%) radicle emergence was recorded at 4th day. Ahmadi *et al.* [17] reported that gibberellin increased the length of radicle and stem, seedling dry weight, seedling vigour index and catalase activity in *Kelussia odoratissima*, a medicinal herb.

These results were supported by study of Vieira *et al.* [18]; Amri *et al.* [19] highlighted that the GA_3 exerts a stimulating effect on seed germination by acting as a plant growth regulator, primarily targeting seed dormancy breakdown and facilitating early seedling development. Its mechanism involves the promotion of hydrolytic enzyme synthesis, especially α-amylase, which plays a crucial role in converting stored seed reserves into soluble forms essential for seedling nourishment. Moreover, GA_3 regulates diverse physiological processes such as cell elongation and division, crucial for the emergence and establishment of seedlings.

Based on the research conducted at alternating temperature of 20-30°C, it is evident that various

Table 3. Effect of dormancy-breaking treatments on radicle emergence (%) of Tulsi at 20-30°C alternate temperature

Duration	Treatments (T)						
	GA3 0.1 %	GA3 0.05 %	KNO_3 0.2%	Pre-chilling	Light Treatment	Control	Mean
1st day (24hr)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2nd day (48hr)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
3rd day (72hr)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
4th day (96hr)	9.33 (17.76)	6.67 (14.79)	6.00 (14.04)	5.33 (13.29)	4.00 (11.53)	3.33 (10.40)	5.78 (13.64)
5th day (120hr)	22.67 (28.39)	16.67 (24.08)	14.67 (22.50)	8.67 (17.09)	6.67 (14.92)	6.00 (14.04)	12.56 (20.17)
6th day (144hr)	31.33 (34.02)	28.67 (32.36)	26.00 (30.65)	19.33 (26.05)	18.00 (25.07)	16.67 (24.05)	23.33 (28.70)
7th day (168hr)	32.00 (34.43)	30.67 (33.61)	27.33 (31.51)	22.67 (28.42)	22.00 (27.95)	21.33 (27.48)	26.00 (30.56)
Mean	13.62 (16.37)	11.81 (14.98)	10.57 (14.10)	8.00 (12.12)	7.24 (11.35)	6.76 (10.85)	
C.D. (P=0.05)	S=0.837		T=0.775		SxT=2.049		
SE.m (±)	S=0.297		T=0.275		SxT=0.727		

#Values in the parenthesis are arc-sine transformed of the original

dormancy-breaking treatments exerted significant effects on both seed germination percentage and other quality parameters of Tulsi. Particularly, seeds treated with GA_3 at a concentration of 0.1% recorded noticeable enhancements in germination percentage as well as other crucial seedling parameters. These findings underscore the efficacy of GA_3 treatment in improving the germination process and overall seedling quality of Tulsi under the specified temperature conditions. The results suggest that incorporating GA_3 treatment protocols into Tulsi cultivation practices could potentially optimize seedling establishment and contribute to improved crop yields.

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Conflict of Interest

The authors declare no conflicts of interest in relation to the research presented in this paper.

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