

## STANDARDIZATION OF GERMINATION TEST AND EVALUATING SEED QUALITY PARAMETERS IN KASANI (*CICHORIUM INTYBUS* L.) SEED

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### SUMMARY

The study was conducted to optimize the germination test and assess seed quality parameters in Kasani during 2022 in the laboratories of the Department of Seed Science and Technology, aimed. The results revealed maximum germination was recorded in Top of Paper (66.67%) method at 25°C and lowest germination (38.67%) was recorded at 30°C in sand substrate. Maximum hard seeds (17.33%) were recorded in between paper at 30°C and lowest hard seeds (7.33%) at 20°C in top of the paper. The other seed quality parameters viz., seedling length, seedling dry weight, vigour Indices and radicle emergence were also observed maximum at 20°C. The radicle emergence was started on 1<sup>st</sup> day in all temperatures except at 15°C temperature. From 2<sup>nd</sup> day radicle emergence was observed in all temperatures and till the final day the maximum radicle emergence (74.67%) was observed at 20°C. Thus, the study concludes that for conducting the germination test in Kasani crop, the optimum temperature is 20°C, and both the top of the paper (TP) and between paper methods are suitable.

**Key word:** Germination, hard seeds, *Cichorium intybus*, radicle emergence test, seed quality, temperatures

Kasani, also known as Chicory (*Cichorium intybus* L.), belongs to the Asteraceae family and is a winter (rabi) season crop native to Europe and Asia (Bala *et al.*, 2021). It is a traditional herb with applications in various medicinal sectors, including Ayurveda, Unani, and Siddha medicinal systems (Choudhary *et al.*, 2021). These seeds contain 4.7% oil, with a composition of saturated (21.7%) and unsaturated (78.3%) fatty acids, along with polyphenols (Abbas *et al.*, 2015). Kasani is recognised as a substitute forage crop in livestock farming due to its ability to generate substantial biomass yields and its high palatability to animals (Cichota *et al.*, 2020; Arya *et al.*, 2020). Under favourable conditions during the warm season, forage chicory produces a significant amount of high-quality feed. Animal performance on Kasani is equivalent to that on legumes and surpasses that on grass-based pastures. (Li and Kemp, 2005; Kirti and Arya, 2019).

Germination represents the beginning of a plant's life and develops in three distinct phases. The initial stage involves imbibition, where dormant seeds absorb water, initiating the hydrolysis process. Following imbibition, the second phase of germination unveils as

a regulatory process. This stage is marked by the initiation of ATP synthesis through glycolysis, the Krebs cycle, respiratory chain activities, and the translation of stored mRNA. Subsequently, the third phase signifies the peak of germination, wherein the radicle emerges from the seed coat, forming a root. Simultaneously, plumules develop into a shoot system capable of harnessing inorganic matter, water, and light energy, facilitating robust and healthy growth (Nonogaki *et al.*, 2010). The delayed receipt of seed testing reports from laboratories poses a challenge to the rapid marketing of seeds. Therefore, substituting radicle emergence for germination can be employed to expedite the process and save time. Seed germination represents a crucial event in the life cycle of plants. Subsequent to seed germination, the emergence of seedlings becomes an important phenological event that significantly influences the successful establishment of plants, particularly in annual species. The germination pattern of seeds and the growth of seedlings in many plant species with non-dormant seeds are often greatly influenced by various environmental factors, including temperature, water potential, substrate, and burial depth (Szczerba *et al.*, 2021). From a physiological perspective, germination

emerges as a complex process, intricately regulated by a multitude of signals and subject to the influence of both internal and external factors. Internally, factors such as seed dormancy and the presence of accessible food reserves play a crucial role. Externally, key factors involve water availability, temperature, oxygen levels, light exposure, and relative humidity (Makena *et al.*, 2016).

Temperature stands as a crucial environmental factor in the germination of seeds. The speed and efficiency of the germination process, crucial for water absorption, can be influenced when temperatures deviate from the optimal range. In ideal conditions, the absorption process occurs rapidly. Several research studies have indicated a linear relationship between temperature and the number of germinated seeds, with an increase up to an optimal level, followed by a linear decrease as temperatures surpass the upper limit (Steinmaus *et al.*, 2000). Temperature significantly influences both biochemical and physiological metabolic processes. Temperature can impact seed germination by modulating enzyme activities and either promoting or inhibiting the synthesis of hormones that play a role in the germination process (Xu *et al.*, 2017). Seed germination is influenced by the interaction between environmental conditions and seed quality. To ensure the production of high-quality Kasani seeds, it is crucial to possess data on the optimal germination potential, the temperature at which the highest germination percentage is achieved, and the specific duration for recording germination percentages. Using suitable substrates signifies a valuable approach for enhancing seed germination. Assessing the impact of different substrates on Kasani seed germination is a crucial aspect in the standardization of germination testing. In the current study, we investigated the influence of three distinct substrates on the germination of Kasani seeds. These facts play a key role in assessing seed quality in testing laboratories. In consideration of this, the current study was conducted to standardize the germination test and analyse the optimum temperature for various seed quality parameters in Kasani seeds.

#### MATERIALS AND METHODS

The study was conducted on freshly harvested seeds of Kasani variety HCI-13 which were procured from Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding. In order to evaluate the effect of different temperature and substrate on seeds of Kasani (*Cichorium intybus* L.) were carried out by factorial experiment based on

complete randomized block design with three replications at the laboratory of Department of Seed Science and Technology, CCS Haryana Agricultural University, Hisar during 2022. 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). 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, 2019 rules. 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 6<sup>th</sup> day for Kasani.

$$\text{Germination (\%)} = \frac{\text{No. of normal seedlings}}{\text{Total number of seeds kept for germination}} \times 100$$

Hard seeds were observed and expressed in percentage in between paper and top of the paper substrates. 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 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. Vigour indices were calculated using the formula recommended by Abdul-Baki and Anderson (1973) in all substrates, which is outlined as follows:

$$\text{Vigour index-I} = \text{Germination (\%)} \times \text{Average seedling length (cm)}$$

$$\text{Vigour index-II} = \text{Germination (\%)} \times \text{Average seedling dry weight (mg)}$$

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 analysis of data collected during the study was done by using the factorial complete randomized design as described by Panse and Sukhatme (1985). All the values described as mean of the replicates with the evaluation of CD at 5% level of significance by using software OPSTAT.

## RESULTS

In case of Kasani, analysis of variance of the factorial design of various laboratory parameters showed a significant effect of temperature and substrates on germination, hard seeds, seedling length, seedling dry weight, vigour index-I and vigour index-II. Analysis of variance has also significant effect of temperature and duration on radicle emergence of the Kasani seeds (Table 1).

The data presented in Table 2 for germination illustrates how Kasani seeds perform under various temperatures (15°C, 20°C, 25°C, 30°C, and 20-30°C alternate temperature) when placed in different substrates. The maximum germination percentage (66.67%) was observed at 20°C in top of the paper method, followed by germination percentage (62.00%) at 20-30°C in top of the paper, while the lowest germination percentage (38.67%) was recorded in sand at 30°C. From all the mean of different temperatures, the highest germination percentage (62.44%) was recorded at 20°C, at par in germination percentage (57.33%) at 20-30°C, whereas the lowest germination percentage (43.11%) was observed at 30°C temperature. In case of substrates, the maximum germination (56.67%) was observed when seeds were placed in top of the paper, while the lowest germination (49.20%) was observed when seeds were placed in sand. Hard seeds of Kasani at various temperatures (15°C, 20°C, 25°C, 30°C, and 20-30°C) when placed in different substrates is presented in table 2. The maximum hard

seeds were observed at 30°C (17.33%) in between paper method, followed by (16.67%) hard seeds at 25°C in between paper and minimum hard seeds was found at 20°C (7.33%) in top of the paper. Among all different temperatures, the maximum hard seeds observed at 30°C (16.00%), followed by (15.33%) hard seeds at 25°C and lowest hard seeds was recorded (8.33%) at 20°C. From both the substrata, the highest hard seeds (13.47%) were recorded in between paper and lowest (11.73%) hard seeds were recorded in top of the paper.

The seedling length performance of the Kasani is presented in table 2. The maximum seedling length was observed in between paper method (12.09 cm) at the temperature 20°C, followed by seedling length (11.38 cm) at 20°C in sand and lowest seedling length (6.54 cm) was recorded in top of the paper at 15°C. Among all temperatures, the maximum seedling length (11.51 cm) was found at 20°C, at par seedling length (10.44 cm) at 25°C and lowest seedling length (6.69 cm) at 15°C was recorded. From all the substrata the maximum seedling length was recorded (9.97 cm) in the between paper and minimum recorded in top of the paper (9.28 cm). The data of seedling dry weight in the table 3 indicates the effect of different temperatures on Kasani in different substrates. The highest seedling dry weight (1.43 mg) was observed with the 20°C temperature in sand, followed by seedling dry weight (1.40 mg) at 25°C in sand, whereas the lowest seedling dry weight (0.94 mg) was recorded at 15°C in the top of the paper. When considering the mean of all temperatures, the maximum seedling dry weight (1.29 mg) was recorded at 20°C, at par seedling dry weight (1.27 mg) at 25°C, whereas the lowest seedling dry weight (0.96 mg) was recorded at 15°C. Among the substrates, the maximum seedling dry weight (1.27 mg) was recorded in the sand substrate and the lowest seedling dry weight (1.08 mg) was recorded in top of the paper.

TABLE 1

Analysis of variance for various seed quality parameters in Kasani seeds as influenced by different temperatures and substrates

Source of variation	d.f.	Mean sum of squares						
		Germination (%)	Hard Seed (%)	Seedling length (cm)	Seedling dry weight (mg)	VI- I	VI- II	Radicle emergence
T	2	226.49**	22.53*	1.80**	0.14**	10,880.42**	56.09*	3,549.95**
S	4	540.36**	59.80**	29.10**	0.17**	190,180.63**	1,730.28**	588.21**
T×S	8	14.49	1.53	0.11	0.012**	683.12	72.56**	359.38**
Error	30	6.58	5.07	0.14	0.002	1332.87	16.96	26.13

\*\*Significant at  $p=0.01$ ; \*Significant at  $p=0.05$ , T=Temperature, S=Substrates/Duration, T×S = Interaction between Temperature and Substrates/Duration, DF= Degree of freedom, VI-I= vigour index I, VI-II= vigour index II.

The data in the table 3 shows the vigour index-I of Kasani seeds when germinate at different temperatures. The highest vigour index-I (736) recorded in top of the paper with the 20°C temperature, followed by vigour index-I (733) at 20°C in between paper and the lowest vigour index-I (316) was observed at 15°C in sand. Among all the mean of various temperatures, the highest vigour index-I (718) at 20°C, followed by (569) vigour index-I was observed at 20-30°C and at 15°C the lowest vigour index-I (345) was recorded. In all three substrate, the “top of the paper” substrate recorded the highest vigour index-I (527), whereas the “sand” substrate showed the lowest vigour index-I (476). In table 3 data shows the vigour index-II of Kasani seeds at various temperatures and placed in different substrates. The highest vigour index-II (86) was observed when Kasani seeds were sown in sand method at 20°C, followed by vigour index-II (79) at 20°C in between paper. The lowest vigour index-II (44) was recorded in sand at 30°C. When taking into account the mean of all the temperatures, the vigour index-II (80) recorded maximum at 20°C, followed by (69) vigour index-II at 20-30°C and the lowest vigour index-II (47) were recorded at 30°C. When analyzing the different substrates, the “sand” substrate recorded the highest vigour index-II (63), and in “between paper” substrate recorded the lowest vigour index-II (59).

The data indicates the effect of different temperatures in Kasani seeds in different days in the

table 4. The radicle emergence was started on 1<sup>st</sup> day in all temperatures except 15°C. At 1<sup>st</sup> day, the radicle emergence was recorded highest (54.67%) at 30°C, followed by (43.33%) at 25°C and lowest was recorded (14.00%) at 20-30°C and no radicle emergence was recorded at 15°C on this day. From 2<sup>nd</sup> day radicle emergence was also observed at 15°C. On 2<sup>nd</sup> day, the highest radicle emergence (68.67%) was recorded at 20°C, followed by radicle emergence (56.67%) was observed at 20-30°C and lowest (50.00%) recorded at 25°C. Till the 5<sup>th</sup> day, the maximum radicle emergence was completed. On 5<sup>th</sup> day, the highest radicle emergence (74.67%) was observed at 20°C, followed by (64.00%) at 20-30°C and lowest (59.33%) at 25°C. when considering the mean of each temperature, the highest radicle emergence (62.13%) was recorded at 20°C, followed by (57.20%) at 30°C and lowest (45.73%) at 15°C. While comparing the mean of all days, the 5<sup>th</sup> day recorded the highest radicle emergence (64.00%) and the 1<sup>st</sup> day recorded the lowest radicle emergence (26.67%).

## DISCUSSION

The regulation of plant growth and development is dependent on the crucial factors of temperature and substrate. Above 20°C, there is a linear decrease in germination, while abnormal seedlings, hard seeds, and dead seeds increase proportionally with rising temperatures. At 15°C, there is a slight decrease in germination, and there is an increase in abnormal

TABLE 2  
Effects of substrata, temperature on germination, hard seeds, seedling length (cm) in Kasani seeds

Temp. (T)	Germination (%)				Hard seeds (%)			Seedling length (cm)			
	Substrate (S)				Substrate (S)			Substrate (S)			
	BP	TP	Sand	Mean	BP	TP	Mean	BP	TP	Sand	Mean
15°C	48.00 (43.83)	59.33 (50.36)	47.33 (43.45)	51.56 (45.88)	12.67 (20.83)	12.00 (20.22)	12.33 (20.52)	6.84	6.54	6.68	6.69
20°C	60.67 (51.15)	66.67 (54.72)	60.00 (50.76)	62.44 (52.21)	9.33 (17.62)	7.33 (15.46)	8.33 (16.54)	12.09	11.05	11.38	11.51
25°C	46.67 (43.07)	49.33 (44.60)	45.33 (42.30)	47.11 (43.32)	16.67 (24.03)	14.00 (21.93)	15.33 (22.98)	10.92	10.12	10.27	10.44
30°C	44.67 (41.92)	46.00 (42.69)	38.67 (38.43)	43.11 (41.01)	17.33 (24.59)	14.67 (22.50)	16.00 (23.54)	9.64	9.25	9.56	9.49
20-30°C	55.33 (48.04)	62.00 (51.93)	54.67 (47.66)	57.33 (49.21)	11.33 (19.65)	10.67 (18.94)	11.00 (19.29)	10.37	9.45	9.97	9.93
Mean	51.07 (45.60)	56.67 (48.86)	49.20 (44.52)		13.47 (21.34)	11.73 (19.81)		9.97	9.28	9.58	
	C. D. (P=0.05)			S. Em±	C. D. (P=0.05)		S. Em±	C. D. (P=0.05)		S. Em±	
S	1.922			0.662	1.727		0.581	0.277		0.095	
T	2.481			0.855	2.730		0.919	0.357		0.123	
S x T	NS			1.481	NS		1.300	NS		0.213	

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

TABLE 3  
Effects of substrata, temperature on seedling dry weight, vigour index-I, vigour index-II in Kasani seeds

Temp. (T)	Seedling dry weight (mg) Substrate (S)				Vigour index-I Substrate (S)				Vigour index-II Substrate (S)			
	BP	TP	Sand	Mean	BP	TP	Sand	Mean	BP	TP	Sand	Mean
15°C	0.96	0.94	0.98	0.96	329	388	316	345	46	56	47	50
20°C	1.31	1.15	1.43	1.29	733	736	684	718	79	76	86	80
25°C	1.29	1.13	1.40	1.27	511	499	465	492	60	56	63	60
30°C	1.08	1.05	1.15	1.10	431	426	369	409	48	48	44	47
20-30°C	1.13	1.12	1.37	1.20	574	587	545	569	62	69	75	69
Mean	1.15	1.08	1.27		516	527	476		59	61	63	
	C. D. (P=0.05)			S. Em±	C. D. (P=0.05)			S.Em±	C. D. (P=0.05)			S. Em±
S	0.034			0.012	27.357			9.426	NS			1.063
T	0.044			0.015	35.318			12.169	3.983			1.373
S x T	0.076			0.026	NS			21.078	6.900			2.377

TABLE 4  
Effect of temperatures on radicle emergence (%) of Kasani seeds

Duration	Temperatures					
	15°C	20°C	25°C	30°C	20-30°C	Mean
1 <sup>st</sup> day (24hr)	0.00 (0.00)	21.33 (27.48)	43.33 (41.15)	54.67 (47.67)	14.00 (21.93)	26.67 (27.65)
2 <sup>nd</sup> day (48hr)	53.33 (46.89)	68.67 (55.96)	50.00 (44.98)	56.00 (48.44)	56.67 (48.88)	56.93 (49.03)
3 <sup>rd</sup> day (72hr)	55.33 (48.05)	72.67 (58.48)	57.33 (49.20)	57.33 (49.21)	58.67 (50.03)	60.27 (50.99)
4 <sup>th</sup> day (96hr)	58.00 (49.59)	73.33 (58.90)	57.33 (49.20)	58.00 (49.60)	59.33 (50.45)	61.20 (51.55)
5 <sup>th</sup> day (120hr)	62.00 (51.94)	74.67 (59.77)	59.33 (50.36)	60.00 (50.76)	64.00 (53.19)	64.00 (53.20)
Mean	45.73 (39.29)	62.13 (52.12)	53.47 (46.98)	57.20 (49.14)	50.53 (44.90)	
C. D. (P=0.05)	S=3.760		T=3.760		SxT=8.409	
S. Em (±)	S=1.320		T=1.320		SxT=2.951	

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

seedlings, hard seeds, and dead seeds. The optimal temperature of 20°C led to the maximum germination but the fast radicle emergence was observed at 25°C and 30°C than 20°C temperature on 1<sup>st</sup> day. From 2<sup>nd</sup> day, the maximum radicle emergence was observed at 20°C temperature. Bhuker *et al.* (2020) also reported that the radicle emergence test, employing the top of the paper method, can serve as a rapid assessment technique for predicting germination percentages in small-seeded crops such as pearl millet and mustard. Later on the abnormal seedlings were observed at 25°C and 30°C which result low germination percentage. High temperatures of 25°C and 30°C result in the inhibitory effect of the increased temperature. However, germination was also delayed at low temperature of 15°C, extending the germination duration for longer time and resulting in a slow radicle emergence from the Kasani seeds. Lower temperatures lead to a slower metabolism in seeds, resulting in delayed growth. These results were in accordance with Luo *et al.* (2018) who observed that the germination speed of rapeseed declined as temperature decreased. Suriyasak *et al.* (2020) found that seeds

exposed to high temperature causes delayed germination of heat-stressed seeds of paddy. Sharavdorj *et al.* (2021) revealed that the germination occurred over a wide range of temperature from 15 to 30°C, but the highest germination recorded between at 15 to 20°C in various forage crops. Butler *et al.* (2014) showed that germination of cool-season forage legume was recorded highest at 20°C temperature and the germination of forage legumes like, ‘Devine’ little burr medic (*Medicago minima* L.), ‘Apache’ arrow leaf clover (*Trifolium vesiculosum* Savi) and ‘Armadillo’ burr medic (*Medicago polymorpha* L.), experienced significant reduction when exposed to temperature above 30°C. Wei *et al.* (2020) revealed that the maximum germination percentage 87.72% was recorded at temperature of 21°C in Lettuce (*Lactuca sativa* L.). However, at 28°C, the average germination percentage dropped to 42.84% and at 35°C, the germination declined to 1.01%. It can be concluded from this study that with an increase in temperature, there is a decrease in germination. Sharma *et al.* (2022) reported that the seedling vigour parameters were reduced at 30°C and 35°C.

## CONCLUSION

Maximum germination percentage and other seed quality parameters such as seedling length, seedling dry weight, radicle emergence and vigour indices with minimum number of hard seeds were recorded at 20°C. Maximum germination percentage was recorded in top of the paper followed by between paper method. Hence, the study concludes that the germination test for Kasani should be carried out at a temperature of 20°C, using either the top of the paper (TP) or between paper method.

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