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STANDARDIZATION OF PRE-GERMINATION TREATMENT, TEMPERATURE AND MEDIA FOR GERMINATION TESTING IN ISABGOL (*PLANTAGO OVATA* FORSK.)

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ABSTRACT

Isabgol (*Plantago ovata* Forsk.) is an important medicinal crop valued for its high mucilage content, yet its seed testing is difficult due to very small seed size and physiological dormancy, leading to inconsistent germination results. This study aimed to standardize germination testing procedures using five released varieties. Experiments were conducted at Seed unit out at the University of Horticultural Sciences, Bagalkot and ICAR–Indian Institute of Seed Science Regional Station, GKVK, Bengaluru. Pre-germination treatment with KNO_3 @ 0.1 per cent was most effective in breaking dormancy and significantly improved germination over GA_3 and untreated control. Among testing conditions, the Top of Paper (TP) method at 20–27°C recorded the highest germination percentage, fastest germination speed, and better seedling growth, while sand medium and 30°C showed poor performance. The first and final count days were standardized to the 3rd and 8th day. The optimized pretreatment and germination conditions developed in this study provide a reliable, crop-specific protocol for accurate germination assessment and improved seed quality evaluation in Isabgol.
Keywords: Physical purity , standard germination test, moisture estimation.

Introduction

India is a major producer and exporter of medicinal plants, and the demand for plant-based therapeutics has increased significantly in recent years. Among the commercially important species, Isabgol (*Plantago ovata*) holds high industrial value due to its mucilaginous seed husk used across pharmaceutical, nutraceutical and food sectors (Maqbool *et al.,* 2023). Although Gujarat and Rajasthan account for most of the national production, the crop's cultivation has not expanded proportionately to market demand, primarily due to seed-related limitations (Panda and Giri, 2024). Germination is a major constraint in Isabgol cultivation. The crop exhibits inherent seed dormancy, rapid viability loss during storage and generally low and erratic germination, all of which lead to poor field establishment and inconsistent plant stands (Anon, 2023). These biological constraints reduce yield stability and discourage large-scale cultivation. The problem is intensified by the lack of crop-specific seed certification standards and the absence of validated germination testing protocols. Although improved varieties have been released by research institutions, they have not entered the formal seed chain due to the unavailability of minimum germination standards and reliable testing procedures.

Existing research on Isabgol has focused heavily on dormancy and seed physiology, with limited emphasis on developing standardized, practical approaches for evaluating germination performance. Given that germination percentage is the most critical determinant of seed quality and field establishment in this crop, the development of robust and crop-specific germination testing protocols is essential. Such protocols would support accurate assessment of seed viability, enable the formulation of minimum seed certification standards and facilitate the large-scale multiplication and distribution of quality seed material

(Das and Trivedi, 2021). Strengthening germination testing frameworks is therefore a key requirement for improving seed quality assurance and supporting the expansion of Isabgol cultivation in response to increasing industrial demand.

Material and Methods

Fresh seeds of five released Isabgol varieties Mayuri, Niharika, Vallabh Isabgol-1, Vallabh Isabgol-2 and Gujarat Isabgol-1 procured from CIMAP, Lucknow and DMAPR, Anand, were used to optimize germination temperature and testing media for developing a crop-specific germination protocol. All the experiments were conducted at at Seed unit out at the University of Horticultural Sciences, Bagalkot located at 16 °10′ North latitude, 75°42' East longitudes, and 542.0 m above mean sea level (MSL) under Northern dry zone of Karnataka (Zone- III). and at The Regional Station of the Indian Institute of Seed Science, Bengaluru (13.010°N, 77.570°E), following Factorial Completely randomized design (FCRD).

Pre-germination treatments

Due to inherent physiological dormancy in Isabgol seeds, pre-germination treatments were evaluated to improve germination uniformity. Fresh seeds of five varieties (Mayuri, Niharika, Vallabh Isabgol-1, Vallabh Isabgol-2 and Vallabh Isabgol-3) were subjected to different concentrations of KNO_3 and GA_3 prior to testing. Blotter papers were soaked in the respective solutions and seeds were tested using the top-of-paper method in a germinator for 8 days.

Treatments

KNO_3 (0.1%, 0.2%), GA_3 (100, 200, 300 ppm) and untreated control.

Design: Factorial CRD with two replications.

Observations

Germination (%) was recorded on the 8th day as the proportion of normal seedlings from 100 seeds tested (Table 1.)

$$Germination\ (\%) = \frac{Number\ of\ seeds\ produced\ normal\ seedling}{Total\ number\ of\ seeds\ sown} \times 100$$

Standardization of optimum temperature and media for germination test in Isabgol

To identify suitable media and temperature conditions for the standard germination test, seeds of five varieties (Mayuri, Niharika, Vallabh Isabgol-1, Vallabh Isabgol-2 and Gujarat Isabgol-1) were evaluated across three media and four temperature regimes. Four hundred seeds per variety were tested for each treatment combination using the top-of-paper, between-paper and sand methods for 10 days in a germinator.

Treatments

Media: Top of paper (TP), Between paper (BP), Sand
Temperature: 20°C, 27°C, 30°C, 20/30°C (alternate)
Design: Factorial CRD with two replications

Observations

First count:

Day on which germination exceeded 50% of final germination and normal seedlings were clearly distinguishable.

Final count:

Day on which no further increase in germination was observed.

Speed of germination:

Daily counts of radicle-emerged seeds were recorded up to the 10th day, and speed of germination was calculated using Maguire's (1962) formula. Higher value indicates faster emergence.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \cdots + \frac{X_n - X_{n-1}}{Y_n}$$

Where,

X1 -Number of seeds germinated at first count.
X2- Number of seeds germinated at second count.
Xn- Number of seeds germinated on nth day.
Y1-Number of days from sowing to first count.
Y2- Number of days from sowing to second count.
Yn- Number of days from sowing to nth count.

Germination (%) :

Percentage of normal seedlings obtained on the 10th day from 100 seeds tested.

$$\text{Germination (\%)} = \frac{\text{Number of seeds produced normal seedling}}{\text{Total number of seeds sown}} \times 100$$

Seedling length (cm):

Ten normal seedlings from each treatment were measured on the final count day; mean length was expressed in centimetres.

Results

The effect of seed pre-germination treatments on germination in Isabgol is presented in Table 1. It shows that all pre-germination treatments significantly enhanced germination compared to the untreated control. KNO_3 @ 0.1% recorded the highest germination (79%), closely followed by KNO_3 @ 0.2% (77.0%) and GA_3 @ 100 ppm (73.8%), while the control showed the lowest germination (67.4%). Among the varieties, Niharika showed the highest

germination (89.33%), followed by Vallabh Isabgol-2 and Vallabh Isabgol-1, whereas Mayuri showed the lowest (31.33%). The interaction (Treatment × Variety) also differed significantly, with Niharika + KNO_3 (0.1% or 0.2%) showing the maximum germination (94%), while Mayuri under control recorded the minimum (20%).

Table 1 : Influence of pre-germination seed treatments on germination of Isabgol varieties

Germination (%)							
Varieties (A)	**Pre-treatments (B)**						
	KNO_3 (0.1%)	**KNO_3 (0.2%)**	**GA_3 (100 ppm)**	**GA_3 (200 ppm)**	**GA_3 (300ppm)**	**Control**	**Mean**
Mayuri	38.00	34.00	35.00	34.00	27.00	20.00	**31.33**
Niharika	94.00	92.00	90.00	89.00	87.00	84.00	**89.33**
Vallabh Isabgol 1	88.00	87.00	86.00	81.00	80.00	73.00	**82.50**
Vallabh Isabgol 2	89.00	87.00	86.00	85.00	86.00	73.00	**84.33**
Gujrat Isabgol 1	86.00	85.00	72.00	71.00	70.00	87.00	**78.50**
Mean	**79.00**	**77.00**	**73.80**	**72.00**	**70.00**	**67.40**	**73.20**
	S.Em±	**C.D.@1%**					
Factor (A)	1.376	2.828					
Factor (B)	1.507	3.098					
Interaction (AXB)	3.370	6.927					

First Count Day (nth day)

As shown in Table 2, more than 50% of the final germination was achieved by the 3rd day, and seedling structures were clearly distinguishable. Thus, the 3rd day was taken as the first count day.

Final Count Day (nth day)

Daily germination observations (Table 2) indicated that maximum germination was completed by the 8th day, after which no further increase occurred. By this stage, seedlings could be clearly classified into normal, abnormal, and fresh ungerminated (FUG) categories. Therefore, the 8th day was taken as the final count day.

Table 2 : First and final count day for germination studies in Top of the paper (TP) method in Isabgol

Temperatures	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
20 ºC	0	72	76	78	80	82	82	82	82	82
27 ºC	11	68	72	75	75	78	79	80	80	80
30 ºC	0	13	24	41	49	51	54	54	54	54
20 ºC / 30ºC	0	58	64	66	70	76	76	76	76	76
Mean	**3**	**53**	**59**	**65**	**69**	**72**	**73**	**73**	**73**	**73**

Speed of germination showed a wide range (0.85–83.86) and was significantly influenced by variety, media, and temperature (Table 3). Faster germination occurred at 20°C and 27°C, while 30°C recorded the lowest values. BP medium favoured higher germination speed compared to TP and sand. Among varieties, Niharika and Gujarat Isabgol-1 exhibited higher speeds, whereas Mayuri was the poorest performer. The highest speed of germination was observed in Gujarat Isabgol-1 under TP at 20/30°C. All main and interaction effects were significant at 1%, confirming strong treatment influence on germination speed. Germination percentage also varied significantly across treatments (Table 4), ranging from 6% to 93%. Niharika recorded the highest germination, while Mayuri showed the lowest. Temperatures of 20°C and 27°C supported higher germination, whereas 30°C resulted in reduced germination across varieties. Among media, TP produced maximum germination, followed by BP, with sand showing the least response. All main and interaction effects were significant at the 1% level, indicating strong influence of treatment combinations. Seedling length differed significantly due to variety, media, and temperature (Table 5). Gujarat Isabgol-1 produced the longest seedlings, comparable to Vallabh Isabgol-1, while other varieties showed shorter seedlings. The 20/30°C alternate temperature produced the maximum seedling length, followed by 20°C, whereas 30°C resulted in reduced growth. Among media, BP supported maximum elongation, and sand recorded minimum length. All main and interaction effects were significant at 1%, confirming the strong combined influence of treatments.

Table 3 : Effect of temperature and testing media on speed of germination of Isabgol varieties

Speed of germination						
		Temperatures (C)				
Varieties (A)	**Media(B)**	**20 ºC**	**27 ºC**	**30 ºC**	**Alternate temp (20 ºC/ 30 ºC)**	**Mean (Varieties)**
Mayuri	TP	20.45	16.32	9.15	11.49	14.35
	BP	14.21	15.60	5.84	16.43	13.02
	SAND	2.91	2.36	2.32	2.87	2.61
	Mean	**12.52**	**11.43**	**5.77**	**10.26**	**9.99**
Niharika	TP	90.29	85.21	46.78	69.38	72.91
	BP	75.82	77.46	29.67	65.52	62.12
	SAND	7.26	8.17	5.22	6.63	6.82
	Mean	**57.79**	**56.94**	**27.22**	**47.18**	**47.28**
Vallabh Isabgol 1	TP	82.91	77.05	29.82	75.34	66.28
	BP	75.19	53.21	4.38	61.05	48.46
	SAND	5.66	5.49	5.38	6.86	5.85
	Mean	**54.59**	**45.25**	**13.19**	**47.75**	**40.19**
Vallabh Isabgol 2	TP	81.19	83.84	26.95	77.15	67.28
	BP	82.02	72.02	15.23	60.71	57.49
	SAND	6.30	6.62	6.70	6.60	6.55
	Mean	**56.50**	**54.16**	**16.29**	**48.15**	**43.78**
Gujarat Isabgol 1	TP	77.49	97.69	39.53	83.86	74.64
	BP	78.10	76.05	14.80	70.70	59.91
	SAND	5.68	0.85	7.04	6.44	5.00
	Mean	**53.75**	**58.19**	**20.45**	**53.67**	**46.52**
Grand mean (Temperature)		**47.03**	**45.19**	**16.59**	**41.40**	**37.55**
Mean (Testing Media)		**TP**	**BP**	**Sand**		
		59.09	46.20	5.36		
		S.Em ±	**C.D.@1%**			
Factor (A)		0.920	2.603			
Factor (B)		0.713	2.016			
Factor (C)		0.823	2.328			
Interaction AXB		1.593	4.508			
Interaction BXC		1.425	4.032			
Interaction AXC		1.840	5.206			
Interaction AXBXC		3.187	9.017			

Table 4 : Effect of temperature and testing media on germination (%) in Isabgol varieties.

Germination (%)						
		Temperatures (C)				
Varieties (A)	**Media (B)**	**20 ºC**	**27 ºC**	**30 ºC**	**Alternate temp (20 ºC/ 30 ºC)**	**Mean (Varities)**
Mayuri	TP	27.00	38.00	18.00	20.00	25.75
	BP	17.00	20.00	10.00	24.00	17.75
	SAND	7.00	7.00	6.00	16.00	9.00
	Mean	17.00	**15.67**	**11.33**	**20.00**	**16.00**
Niharika	TP	93.00	89.00	80.00	89.00	87.75
	BP	92.00	90.00	57.00	92.00	82.75
	SAND	21.00	22.00	14.00	16.00	18.25
	Mean	68.67	**67.00**	**50.33**	**65.67**	**62.92**
Vallabh Isabgol 1	TP	89.00	81.00	48.00	84.00	75.50
	BP	80.00	64.00	11.00	70.00	56.25
	SAND	17.00	16.00	14.00	20.00	16.75
	Mean	62.00	**53.67**	**24.33**	**58.00**	**49.50**
Vallabh Isabgol 2	TP	86.00	85.00	47.00	86.00	76.00
	BP	86.00	79.00	31.00	82.00	69.50
	SAND	24.00	21.00	18.00	17.00	20.00
	Mean	65.33	**61.67**	**32.00**	**61.67**	**55.17**

Gujarat Isabgol 1	TP	84.00	90.00	60.00	91.00	81.25
	BP	82.00	85.00	30.00	77.00	68.50
	SAND	16.00	17.00	17.00	15.00	16.25
Mean		60.67	64.00	**35.67**	**61.00**	**55.33**
Grand mean (Temperature)		54.73	53.30	30.73	53.27	**48.01**
Mean (Testing Media)		**TP**	**BP**	**Sand**		
		69.25	58..95	16.05		
		S.Em ±	**C.D.@1%**			
Factor (A)		0.956	2.705			
Factor (B)		0.740	2.095			
Factor (C)		0.855	2.419			
Interaction AXB		1.656	4.685			
Interaction BXC		1.481	4.190			
Interaction AXC		1.912	5.410			
Interaction AXBXC		3.312	9.370			

Table 5 : Effect of temperature and testing media on seedling length (cm) in Isabgol varieties.

Seedling length(cm)						
		Temperatures (C)				
Varieties (A)	**Media (B)**	**20 ºC**	**27 ºC**	**30 ºC**	**Alternate temp (20 ºC/ 30 ºC)**	**Mean (Varieties)**
Mayuri	TP	3.99	3.84	3.90	5.23	4.24
	BP	5.35	6.53	5.24	6.79	5.98
	SAND	3.61	3.81	3.56	4.08	3.77
Mean		**4.32**	**4.73**	**4.23**	**5.37**	**4.66**
Niharika	TP	6.02	4.21	3.51	5.33	4.77
	BP	6.50	6.60	6.95	7.35	6.85
	SAND	4.03	4.10	3.84	4.03	4.00
Mean		**5.52**	**4.97**	**4.77**	**5.57**	**5.21**
Vallabh Isabgol 1	TP	6.11	3.70	3.90	6.50	5.05
	BP	5.40	6.62	5.20	6.68	5.98
	SAND	5.09	5.15	4.20	5.24	4.92
Mean		**5.53**	**5.16**	**4.43**	**6.14**	**5.32**
Vallabh Isabgol 2	TP	6.70	3.82	3.90	6.23	5.16
	BP	6.33	6.34	5.47	7.30	6.36
	SAND	4.39	4.25	4.03	4.45	4.28
Mean		**5.81**	**4.80**	**4.47**	**5.99**	**5.27**
Gujarat Isabgol 1	TP	5.88	4.25	3.92	7.18	5.31
	BP	5.84	5.82	5.26	6.21	5.78
	SAND	5.48	5.24	4.75	5.52	5.25
Mean		**5.73**	**5.10**	**4.64**	**6.30**	**5.45**
Grand mean (Temperature)		**5.38**	**4.95**	**4.51**	**5.87**	**5.18**
Mean (Testing Media)		**TP**	**BP**	**Sand**		
		4.91	6.19	4.44		
		S.Em ±	**C.D.@1%**			
Factor (A)		0.050	0.141			
Factor (B)		0.039	0.109			
Factor (C)		0.045	0.126			
Interaction AXB		0.086	0.244			
Interaction BXC		0.077	0.219			
Interaction AXC		0.100	0.282			
Interaction AXBXC		0.173	0.489			

Discussion

Isabgol seeds possess physiological dormancy (McNeil and Duran, 1992), making pretreatment essential for reliable germination testing. In the present study, KNO_3 and GA_3 treatments significantly improved germination over the untreated control. KNO_3 @ 0.1% recorded the highest germination (78%), compared to the control (67.40%), while GA_3 (73.80%) was moderately effective. The superior response to KNO_3 is attributed to enhanced enzyme activation, improved oxygen uptake, and increased seed coat permeability, which collectively help break dormancy (Ali *et al.,* 2010; Verma and Solanki, 2022). These results are consistent with earlier reports supporting nitrate-based dormancy alleviation (Ali *et al.,* 1988; Qadir and Khan, 2019). Among varieties, Niharika showed the highest germination, indicating stronger seed vigour, whereas Mayuri recorded the lowest (31.33%), likely due to harder seed coats or deeper dormancy (Kumar *et al.,* 2009).Overall, findings confirm that KNO_3 @ 0.1% is the most effective pretreatment for breaking dormancy and improving germination in Isabgol.(Table 1)

The first and final germination counts in the present study occurred on the 3rd and 8th day, respectively, showing a slightly delayed response compared to earlier reports (Poojar, 2000), likely due to differences in seed vigour, environment, and media properties (Table 2).

The speed of germination was strongly influenced by temperature, media, and varietal differences (Table 3). Higher values were recorded at 20 °C and 27 °C, while 30 °C resulted in the lowest speed, reflecting reduced metabolic activity under higher temperatures. Paper-based media (TP and BP) showed markedly faster germination than sand, attributable to better moisture–air balance. These findings agree with earlier studies reporting optimum germination at 20–25 °C and reduced performance at higher temperatures (Najafi and Rezvani, 2003; Tabrizi *et al.,* 2005)

Germination percentage in Isabgol was strongly influenced by temperature, medium, and varietal differences. The highest germination was obtained at 20 °C on top of paper (TP), attributed to optimal metabolic activity, efficient imbibition, and better aeration, whereas 30 °C and sand medium resulted in the lowest germination due to thermal stress and poor moisture–air balance. These results agree with earlier findings that germination of *Plantago ovata* declines beyond the optimum temperature range and performs better on paper-based substrates (Najafi and Rezvani, 2003; Tabrizi *et al.,* 2005; Sousa *et al.,* 2008; Yadav, 2021) (Table 4).

Seedling length also varied significantly with temperature, media, and variety (Table 5). The longest seedlings occurred under 20/30 °C alternating temperature, followed by 20 °C, while 30 °C restricted elongation. BP medium supported maximum seedling growth due to favourable aeration and moisture, whereas sand performed poorly. Among varieties, Gujarat Isabgol 1 showed the greatest elongation. These results are consistent with earlier findings highlighting the benefits of moderate temperatures and suitable substrates for early seedling growth (Yadav, 2021).

Conclusion

The study established reliable conditions for standard germination testing in *Plantago ovata*. Pretreatment with KNO_3 @ 0.1 per cent effectively broke physiological dormancy and consistently improved germination across varieties. Among the testing conditions, 20 °C and paper-based media, especially Top of Paper (TP), produced the highest germination percentage, fastest germination speed, and better early seedling growth. The first and final counts were standardized on the 3rd and 8th day, respectively. Varietal differences were evident, with Niharika performing best and Mayuri the poorest. Overall, KNO_3 pretreatment combined with TP medium at 20°C provides an optimal and crop-specific protocol for accurate and uniform germination testing in Isabgol.

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