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Genotype-Independent Germination-Testing Procedure for Isabgol

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Isabgol (Plantago ovata Forsk.) is an important medicinal crop cultivated on approximately 150,000 ha in India. The objectives of the present study were to establish genotype-independent germination-testing conditions, viz., in situ (Petri dish) and ex situ (two sub-conditions, i.e., vermicompost and vermiculite) and to determine the days of first and final count for in situ seed germination and final count for ex situ conditions using two Isabgol varieties, viz., 'Niharika' and 'Mayuri'. Data were recorded on percent germination, germination energy, and germination period. Under in situ conditions, day 3 was appropriate for the first count and day 5 for the final count, whereas day 12 was suitable for the final count under ex situ conditions. The mean percent germination (82.50% in Petri dish, 43.00% in vermicompost and 44.46% in vermiculite) and germination energy (20.63% in Petri dish, 10.75% in vermicompost and 11.12% in vermiculite) were recorded at final count day for in situ and ex situ conditions. The germination period was found to be 5 d for in situ and 12 d for ex situ (i.e., vermicompost and vermiculite in earthen pots) test conditions. For percent germination, test conditions, varieties and days differed significantly. Variety 'Niharika' had the maximum percent germination (93.67% in Petri dish, 46.00% in vermicompost and 56.00% in vermiculite), followed by 'Mayuri' (71.33%, 40.00% and 33.33%, respectively).

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Differential behavior of the two varieties, viz., 'Niharika' and 'Mayuri', under in situ and ex situ conditions in the absence of any interacting effects indicated the germination capacity of the varieties was attributable to their genetic make-up, but first and final count day was genotype-independent.

KEYWORDS Czabator's index, Plantago ovata, *percentage germination, germination energy, seed viability, varieties, genotypes*

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) is an important medicinal crop in India, which is cultivated on approximately 150,000 ha in northern Gujrat, Rajasthan, and Haryana, with an annual production of about 95,000 tons of Isabgol seed. New areas are emerging in certain pockets of Uttar Pradesh, Bihar, and Madhya Pradesh states.

The seeds of Isabgol are cooling, demulcent, and extensively used to treat habitual constipation. Its husk is used to treat dysentery and irritation of the intestinal tract (Lal, Misra, and Kumar 2000; Khanuja et al. 2004).

Standard laboratory germination tests are conducted to determine the percent germination and suitability of seeds for producing a crop (Johnson and Wax 1978; Yaklich and Kulik 1979). The standard germination test is an excellent measure of viability (Sherf 1953; Athow and Caldwell 1956; Tekrony 1973). Healthy and viable seeds germinate easily and a number of external and internal factors influence the germination process (Farooqi and Chandra 1983). The objectives of the present research were to determine the best time for first and final count for seed germination and associated germination parameters of commercially cultivated Isabgol varieties under different test conditions.

MATERIALS AND METHODS

Seed Collection

The seeds of two varieties of *Plantago ovata* L., viz., 'Niharika' and 'Mayuri' (Patra and Kumar 2005) used in this experiment, were harvested in April, 2006 from the crop grown at Research Farm, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow. The seeds were cleaned in May–June, 2006 and stored in paper bags at room temperature (15–25°C). These varieties are being maintained and are available at the National Gene Bank of Medicinal and Aromatic Plants, CIMAP, Lucknow.

Germination Tests

Germination tests were conducted in February 2007 using 15 cm sterile Petri dishes lined with a high-quality mill-made filter paper and moistened with sterile distilled water to ensure adequate moisture for seed germination. According to International Seed Testing Association (ISTA) rules, treatments were arranged in a split-plot design, with four replicates of 100 seeds each for the two varieties under *in situ* (Petri dish) and *ex situ* (i.e., vermicompost and vermiculite in earthen pots) test conditions. The substrate used for Isabgol seeds was the top of the filter paper (TP) in the case of Petri dishes. The Petri dishes and earthen pots were placed at room temperature (minimum 11.75°C and maximum 24.01°C), with relative humidity varying from 56.76–84.91% (Figure 1).

Data Collection and Analysis

The seeds were observed daily and considered "germinated" when the essential structures (root system, shoot axis, cotyledons) were visible, and the number of germinated seeds was recorded (ISTA Rule 2006). The data on germination were recorded from the fifth day onwards until maximal germination was reached (Kumar et al. 2008a, 2008b). To calculate percentage

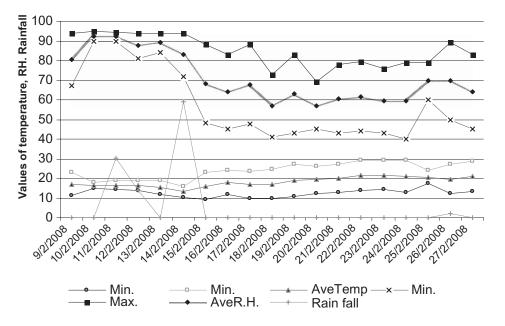


FIGURE 1 Temperature, relative humidity and rainfall during experimentation.

germination, germination energy (%) and germination period, Czabator's index (1962) was used:

Percent germination (G%) =	Total number of seeds germinated Total number of seeds in all replicates	
	Fotal number of seeds in all replicates	
	1/4 of maximum number	
Germination energy (GE) =	$\frac{\text{of seeds germinated in a day}}{\text{Total number of seeds in all replicates}} \times 100$	
	Total number of seeds in all replicates	
Germination period (GPd) = Days from planting to when maximum		
	number of seeds had germinated.	

At the end of the experiment, data were subjected to an analysis of variance and mean separation. The statistical package used was based on Panse and Sukhatme (1976).

RESULTS AND DISCUSSION

A seed is regarded as germinated following the emergence and development of a seedling from the seed embryo (Willan 1985). Germination of a seed in a laboratory test is the emergence and development of a seedling to a stage where the aspect of its essential structures (root system, shoot axis, cotyledons, terminal buds) indicates whether or not it is able to develop further into a satisfactory plant under favorable soil conditions (ISTA Rule 2006).

The varieties (genotypes), test conditions, and number of days to counting affected the germination of Isabgol seeds, as in the case of *Tagetes minuta* and *T. erecta* (Kumar et al. 2008a, 2008b). Variation caused by these three main factors was significant, as was the variation attributable to interactions between/among the three factors, except the varieties \times days interaction (Table 1). According to ISTA rules, first and final count in the case of Plantago lanceolata could be on days 4-7 and day 21, respectively, at 20–30°C temperature (ISTA Rule 2006). In the present study, percentage germination was recorded from day 3 onwards. Assuming the interactions of varieties with other factors to be negligible, we used the overall means for comparisons. The mean *in situ* percent germination was 92.24% for 'Niharika' and 67.43% for 'Mayuri', whereas under ex situ conditions (i.e., vermicompost and vermiculite), percent germination for 'Niharika' was 45.95% (vermicompost) and 52.9% (vermiculite). For 'Mayuri', percent germination was 39.28% (vermicompost) and 32.47% (vermiculite). Counts were made on day 9 under *in situ* conditions and day 18 under *ex situ* conditions. For percent germination, under *in situ* conditions, Isabgol varieties

Sources of variation	df	Mean square
Replications	2	84.09
Conditions	2	19729.36**
Error (1)	4	41.24
Varieties	1	9955.56**
Conditions × Varieties	2	995.10**
Error (2)	6	75.73
Days	6	93.65**
Conditions × Days	12	35.61**
Varieties × Days	6	3.41
Conditions × Varieties × Days	12	13.12**
Error (3)	72	5.51
Total	125	

TABLE 1 Analysis of Variance of Seed Germination in Isabgol

[†]Conditions: Petri dish (*in-situ*), Vermicompost (*ex-situ*), Vermiculite (*ex-situ*), Varieties: 'Niharika', 'Mayuri'; Days: Day on which counts of germinated seeds were taken.

 $LSD = Conditions: 3.891; Conditions \times Varieties: 5.265; Varieties: 3.794; Conditions \times Days: 4.079; Days: 1.559; Varieties \times Days: 4.042; Conditions \times Days \times Varieties: 23.24.$

favored day 3 for the first count and day 5 for the final count, and under ex situ conditions, day 12 for the final count. In a previous study with P. ovata, day 5 was best for first day count and day 7 for final day count at 20°C with 82–95% seed germination (Parihar and Kumar 2006). Similarly, the germination energy, which is the expression of speed of germination, was 23.06% for 'Niharika' and 16.86% for 'Mayuri' under in situ conditions, whereas under ex situ conditions, 'Niharika' showed 11.49% (in vermicompost) and 13.23% (in vermiculite) and 'Mayuri' 9.82% (in vermicompost) and 8.12% (in vermiculite) when counted on day 9 (in situ) and day 18 (ex situ). The weight of 1,000 seeds of variety 'Niharika' was recorded as 1.626 g and 'Mayuri' as 1.208 g. A significant positive correlation (p = 0.01) was observed between 1,000-seed weight and mean percent germination and germination energy, irrespective of the test conditions (*in situ* and *ex situ*). The result suggested that the vigor/vitality of the seed depended on seed weight. Other investigators also recorded such observations (Kandya 1978; Chauhan and Raina 1980; Singh, Bhagat, and Singh 1990).

The percent germination happens to be one of the most vital characteristics of the seed to be used commercially; hence it would be desirable on the part of anyone who is working with seed to have precise information about the maximum mean percent germination within a specific time period. Further, effective stand after the germination (associated germination parameter like germination energy, germination period, etc.) is another important characteristic that gives an idea about the final population. Thus, it would be desirable to have information regarding these parameters for

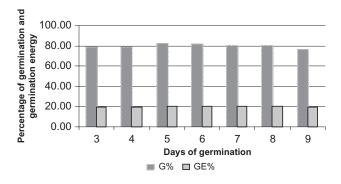


FIGURE 2 Mean percent germination (G) and germination energy (GE) of Isabgol varieties under *in situ* conditions.

developing quality seed. Detailed presentation of percent germination and germination energy percent from the day of first count till the end of the experiment for every successive day in respect to *in situ* and *ex situ* conditions is depicted in Figures 2–9 for a thorough understanding of these two parameters. The mean percent germination (78.66% on first count day and 82.50% on final count day in Petri dish; 43.00% in vermicompost and 44.46% in vermiculite on final count day) and germination energy (19.67% on first count day and 20.63% on final count day in Petri dish; 10.75% in vermicompost and 11.12% in vermiculite on final count day) were recorded (Figures 2–3). Variety 'Niharika' had the maximum seed percentage germination (93.67%), followed by 'Mayuri' (71.33%) at final count day under *in situ* conditions, whereas in *ex situ* (i.e., vermicompost and vermiculite) conditions, 'Niharika' had maximum (46.00% in vermicompost and 33.33% in vermiculite) at the final count day (Figures 4–6). The first and final count day under *in situ*

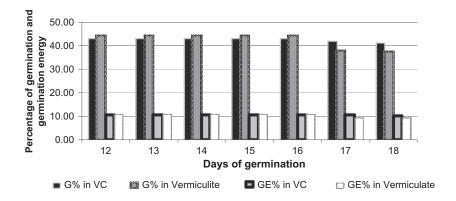


FIGURE 3 Mean percent germination (G) and germination energy (GE) of Isabgol varieties under *ex situ* conditions.

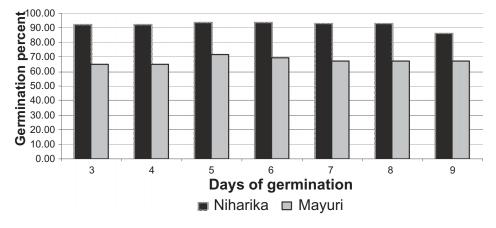


FIGURE 4 Percent germination of Isabgol varieties under in situ (Petri dish) conditions.

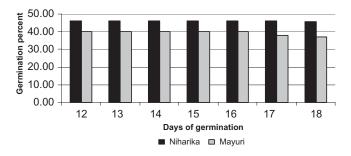


FIGURE 5 Percent germination of Isabgol varieties under *ex situ* (Vermicompost in earthen pot) conditions.

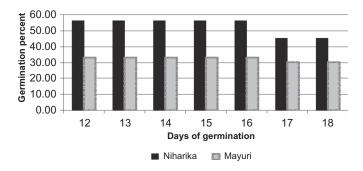


FIGURE 6 Percent germination of Isabgol varieties under *ex situ* (Vermiculite in earthen pot) conditions.

conditions and final count day for *ex situ* conditions were the same irrespective of the variety used, thereby indicating that first and final count day of germination was more of a physiological characteristic in Isabgol. Similarly, the germination energy was maximum (23.42%) in 'Niharika', followed by

'Mayuri' (17.83%) at final count day under *in situ*, whereas in case of *ex situ* (i.e., vermicompost and vermiculite) conditions, 'Niharika' had maximum (11.50% in vermicompost and 14.00% in vermiculite), followed by 'Mayuri' (10.00% and 8.33%), at final count day (Figures 7–9). The germination period (i.e., the period during which maximum number of seeds could be obtained) was found to be 5 d under *in situ* and 12 d under *ex situ* conditions.

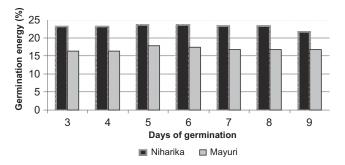


FIGURE 7 Germination energy of Isabgol varieties under in situ (Petri dish) conditions.

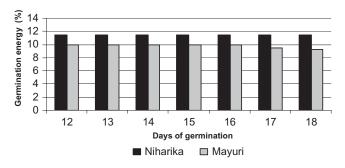


FIGURE 8 Germination energy of Isabgol varieties under ex situ (Vermicompost) conditions.

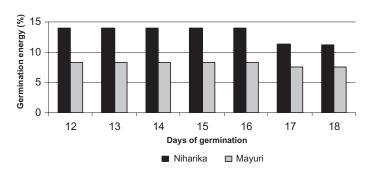


FIGURE 9 Germination energy of Isabgol varieties under ex situ (Vermiculite) conditions.

CONCLUSIONS

The present study revealed that the Isabgol variety 'Niharika' showed 93.67% and 56.00% and 'Mayuri' showed 71.33% and 40.00% seed germination with an optimum germination time of 5 d under in situ and 12 d under *ex situ* conditions. These results would benefit growers; anything other than pure germinable seed is waste. The results would also be useful for Isabgol-producing industry and researchers in developing the quality standards for quality seed and farmers in producing good yield/ unit area.

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