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Seed Germination and Seedling Vigor Index in *Bixa orellana* and *Clitoria ternatea*

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ABSTRACT

In the present investigation with seeds of two important medicinal plants viz. Bixa orellana L. (Annatto) and Clitoria ternatea L., optimal conditions for seed germination in Annatto were determined by subjecting the seeds to varied concentration of plant growth regulators earlier but hardseededness could not be broken. Thereafter, acid scarification (AS) with H_2SO_4 was done. Freshly harvested seeds (FH) and one year old (OYO) seeds were taken for the study. The study on effectivity of sulfuric acid at elevated moisture content (60 %) has been amply demonstrated in enhancing germination and reducing MGT in B. orellana. In C. ternatea, a 3×4×3 factorial experiment was set up to test the effects of moisture content with three levels of moisture of 12%, 10% and 5%, storage duration at 4 levels of 3, 6, 9 and 12 months and storage temperature at 3 levels of 30ºC, 20ºC and -20ºC on the seed germination. All pretreatments significantly reduced the percentage of hard seeds as compared to non treated control seeds in Annatto. The percentage of hard seed decreased gradually as the pretreatment duration increased for all samples. The final germination percentage of all accessions, was significantly increased by the pretreatments. The total increase on average was 43.5 percent point for all samples (from 36.7 % of control to 80.2 % of pretreatments) in Annatto. The study suggests that the seeds of C. ternatea may possess physiological dormancy and seeds are moisture sensitive and therefore, altering moisture level is an effective method for breaking its physiological dormancy.

Key words*: *Bixa orellana, Clitoria ternatea, Seeds, Germination, Dormancy.

INTRODUCTION

Species of some plant families exhibit erratic germination due to seed dormancy in *Ferula assafoetida*[1], *Plantago* species and Asalio[2], Asoka[3], *Artemisia annua*[4,5], Chickpea[6]. They readily germinate within the native environment, but fail to show good germination under alien condition depending on the plant species and type of dormancy. To break dormancy in order to induce germination, several methods were used[1,6,7].

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Seeds of *Bixa orellana* L. is commonly known as Annatto belonging to Bixaceae family are economically important because of the pigments present in the seed coat, which are used by cosmetic and food industries. This species is used as an antioxidant, hepatoprotective (liver protector), hyperglycemic and also used as a food-coloring agent. Seeds of *B. orellana* show orthodox seed storage behaviour. *Clitoria ternatea* L. commonly known as Aparajita is a valuable medicinal plant belonging to family Fabaceae. Its roots, seeds and leaves have tremendous medicinal value[8]. The limited cultivation and insufficient attempts for its replenishment, the wild stock of this species has been markedly depleted. In Annatto, hardseededness, induced by drying seeds below 40% moisture content, is the main cause of its slow germination[9].

In *C. ternatea*, the major problem is poor seed germination and seedling vigor. The consequence is possible extinction of the species and this provides justification for conservation and propagation of this valuable germplasm. Our study showed that the seeds of *C. ternatea* exhibited physical dormancy due to hard seed coat and chemical scarification using sulphuric acid is effective in breaking dormancy. However, no information is available on the physiological dormancy status of *Clitoria* seeds. Such information is scanty for *C. ternatea*. Therefore, the present study aims to examine (i) dormancy status of *C. ternatea* seeds, its moisture-sensitivity, and to determine the optimal storage conditions; i.e., seed moisture content, storage temperature and storage period for the species and to investigate (ii) the effect of different pretreatments with sulfuric acid to break the hard seededness in Annatto seeds to accelerate and increase the seed germination.

MATERIAL AND METHODS

Bixa orellana L. (Annatto)

Seeds of *Bixa orellana* were collected from Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat. Optimal conditions for seed germination were determined by subjecting the seeds to varied concentration of plant growth regulators earlier but hardseededness could not be broken. Thereafter, acid scarification (AS) with H_2SO_4 was done. Freshly harvested seeds (FH) and one year old (OYO) seeds were taken for the study. Seed samples of Annatto were harvested in the month of January for the study for both FH and OYO seeds. Average fresh weight and dry weight of seeds were determined. The seeds were then treated with concentrated H_2SO_4 for varying durations. The seeds were acid scarified in a 500 ml Borosil Beaker by soaking the seeds in concentrated H_2SO_4 for different duration (5 s, 1 m, 3 m, 6 m, 9 m, 12 m and 15 m.) after which the acid was decanted and seeds were rinsed thoroughly (five times) with running tap water and then with distilled water (DW) before placing for germination test. Non scarified seeds were used as the control. Each pretreatment had four replicates of 100 seeds each which were randomly arranged and incubated at 20, 25 and 30 ^{0}C in an incubator without light. The parameters recorded were germination rate, final germination percentage and percentage of hard seed.

Clitoria ternatea

Seeds of *Clitoria ternatea* were collected from Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat. To reach to the level of targeted moisture content seeds were subjected to initial moisture content of 5% and increasing to 10 and 12% target moisture levels. The corresponding target mass for each target moisture content was determined by using standard equation of WF = (100-MI)/100-MF*)* × *W1*, where WF is mass of the seed (g) at target moisture content, MI is initial moisture content, MF is the target moisture content and WI is initial seed mass (g). The water loss in desiccated seeds was monitored by weighing the seeds at regular intervals. Once seed samples attained the target moisture content, they were subjected to germination tests without further pretreatment. A 3×4×3 factorial experiment was set up to test the effects of moisture content with three

levels of moisture of 12%, 10% and 5%, storage duration at 4 levels of 3, 6, 9 and 12 months and storage temperature at 3 levels of 30ºC, 20ºC and -20ºC on the germination of *C. ternatea*. Vigor indexes I and II (sign of multiplication germination percentage x seedling length and germination percentage x seedling dry weight respectively) were measured after 10 days. Mean germination time (MGT) was calculated as MGT= Σ (*nd*)/N; where *n* is the number of seeds which germinated after each incubation period in days *d* and N is the total number of seeds emerged at the end of the test.

Statistical analysis: The experiment for laboratory germination was laid in complete randomized block design with vertical rows as treatments and horizontal blocks as replications. For all other purposes, Analysis of variance was applied for all the experiments. Least significant difference (LSD) was estimated separately for comparison of treatments[10].

RESULTS AND DISCUSSION

Germination is a vital phenomenon during the life cycle of a plant[11]. In both these species tried, seed germination had indicated a trend towards seedling establishment which could be a part of continuation of the study in future. In Annatto, results revealed that all pretreatments significantly ($P<0.05$) reduced the percentage of hard seeds as compared to non treated control seeds. The percentage of hard seed decreased gradually as the pretreatment duration increased for all samples (Table 1). The final germination percentage of all accessions, was significantly increased ($P<0.05$) by the pretreatments (Table 2). The total increase on average was 43.5 percent point for all samples (from 36.7 % of control to 80.2 % of pretreatments).

In Annatto, all pretreatments increased the germination rate markedly. The time to 50 % germination (T_{50}) was 9 days for seeds treated with sulfuric acid for 15 min in contrast to the control seeds, which had not yet reached T_{50} at the end of the test, which was after 14 days. The effectivity of the pretreatment declined as the pretreatment period decreased from 15 min to 5 sec. This supports the findings that the pretreatment length when using sulfuric acid is critical and moisture content[12,13]. It has been reported that sulfuric acid pretreatment has the potential to kill seeds in accessions or populations[12] and similar damage was found in this present study also when sulfuric acid was used alone beyond 15 min (30 min and 1 hour: data not shown). In this study also some damage was caused due to sulfuric acid leading to less percentage of germination. However, this study on effectivity of sulfuric acid at elevated moisture content (60 %) has amply demonstrated the enhancement of germination and reducing mean germination time (MGT) in *B. orellana*. Results are in conformity with several other legume crops for removing hard-seededness[12,13,14].

In *C. ternatea*, the moisture content in the seed was found to be 6.8 %. The percentage of germination and seedling vigor index happen to be the most important characteristics of the seed to be used for cultivation. Only those seeds which germinate rapidly and vigorously under the favourable situation under controlled conditions are likely to be capable of producing vigorous seedlings in field conditions. The experiment shows that seed moisture content in *C. ternatea* can be altered to 5-12 % without any detrimental effect on germinability. The data on germination percentage, mean germination time and vigor index suggests that 10 % moisture level and storage at -20 and 20ºC is more suitable for improving seed quality and for long term storage (Table 3). Similar studies were conducted on seeds of *Stychnos coculiods* and *S. nuxvomica* and found that 20-10 % moisture level can significantly improve germination[15]. Storage of seeds of *Clitoria ternatea* at -20, 20 and 30ºC after increasing moisture level significantly enhance germination percentage which gradually increased during each three month interval up to one year. This suggests that the seeds of *C. ternatea may* possess physiological dormancy. Present study shows that the seed of *C.*

ternatea is moisture sensitive and therefore, altering moisture level is an effective method for breaking its physiological dormancy. However, further investigation using plant hormones is required to confirm the physiological dormancy in the seeds of *Clitoria*.

Present study indicates that seeds with 10 % moisture level stored at 20ºC are more suitable for better seedling emergence timing in *C. ternatea*. The increase in vigor index has been parallel to the corresponding enhancement of germination percentage during each time interval (3 months) at different moisture levels studied. The gradual increase in germination percentage and vigor index within seed population collected during same period can be attributed to intraspecific variation in the physiological maturity of seeds. The extent of emergence as well as its timing both within a season and between seasons can vary depending on the origin of seeds[16,17].

Table 1: Effect of sulfuric acid scarification pretreatments (of varying lengths of time) on breaking hard seed dormancy in Annatto seed lots of two years

Percentage of hard seed								
Seed lot	**Control**	**5 s**	**1 min**	**3 min**	**6 min**	**9 min**	**12 min**	**15 min**
Fresh harvested seeds	73.4	52.3	38.5	30.8	26.4	20.3	17.3	12.3
One year old seed	69.4	48.4	33.6	24.3	22.1	18.5	13.4	10.4
Mean	71.4	50.4	36.1	27.6	24.2	19.4	15.3	11.3
LSD (P=0.05)	2.13	2.33	3.14	4.11	3.12	ns	2.87	ns

Table 2: Effect of sulfuric acid scarification pretreatments on final germination percentage in Annatto seed lots of two years

Final germination percentage								
Seed lot	**Control**	**5 s**	**1 min**	**3 min**	**6 min**	**9 min**	**12 min**	**15 min**
Fresh harvested seeds	33.2	50.2	57.4	62.4	69.8	72.3	75.2	78.7
One year old seed	40.2	54.2	60.1	66.5	71.2	75.4	78.2	81.8
Mean	36.7	52.2	58.7	64.4	70.5	73.8	76.7	80.2
LSD (P<0.05)	5.43	3.03	2.77	3.11	ns	ns	ns	ns

Table 3: Germination percentage and seedling vigor index of *Clitoria ternatea* in response to storage temperature, moisture content and storage duration

Temperature	**Germination (%)**			**Seedling Vigour Index (includes both I & II)**				
	Storage Duration (Months)							
	3	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Ambient	49.3	54.6	56.0	64.0	2002	2111	2141	2762
-20^{0}C	59.1	64.0	52.4	64.4	1882	2377	2036	2721
20^{0}C	68.0	66.2	61.7	64.0	2212	2417	2595	2945
30^{0}C	64.8	64.0	59.1	40.0	1994	2348	2379	1584
LSD	6.3	7.28	8.93	9.42	334.4	365.1	484.6	492.9
SE	2.17	2.49	3.04	3.22	115.7	125.1	166.0	168.8
P-value	<0.001*	0.015*	0.185	<0.001*	<0.001* 0.300	0.003* 0.560	0.101	<0.001*

*Significant difference following Turkey's test (α=0.05), p= Probability value

CONCLUSION

This study on effectivity of sulfuric acid at elevated moisture content (60 %) has amply demonstrated the enhancement of germination and reducing mean germination time (MGT) in *B. orellana*. Present study also indicates that seeds with 10 % moisture level stored at 20ºC are more suitable for better seedling emergence timing in *C. ternatea*. However, future study is required in these two important

medicinal species under field condition to compare the findings under laboratory to develop seed standards.

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