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Mechanism and duration of seed dormancy in linseed genotypes

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Abstract
Seeds are the primary and essential starting point for a wide range of agronomic important plants. However, the success in germination seems to be highly dependent on genotype, environment, cultivated treatments and their interactions. Degree of seed dormancy and Abscisic acid (ABA) on inducing seed dormancy in ten linseed genotypes were investigated. Linseed genotypes were classified into three groups *viz.,* Week, Moderate and strong dormancy. The initial seed germination of all linseed genotypes was varies from 15 to 33 % indicating that the dormancy is imposed by the seed coat factors. ABA in those genotypes also varied from 4.931 to 6.142 Pico moles per gram fresh weight (pmol/g fw) of seed and was sufficient to induce the dormancy.

Keywords: dormancy, abscisic acid and linseed

1. Introduction
Linseed or flax (*Linum usitatissimum* L.) is an important crop of tropical as well as temperate zone of the world. If it is grown only for seed, it is called as oil flax, seed flax or linseed and when cultivated for fibre purpose, it is called fibre flax. Long stemmed linseed produces a high quality fibre and short stemmed linseed bears larger seeds of high oil content. The major linseed growing countries are India, Russia, Argentina, Canada, United States America, China, Egypt and Brazil *etc.* The total world linseed production is of 2.05mt with a productivity of 82.6 Kg ha^{-1} in an area of 24.85 m ha. While in India, it occupies an area of 296 thousand ha with a production of 1.55 lakh t and a productivity of 408 kg per ha. Rajasthan is the highest producer of linseed in India.
Dormancy is problematic in agriculture as it affects plant establishment but it is the ability of the seeds to delay their germination until the time and place are right reflecting an important survival mechanism in plants. Seed dormancy is a complex and genetically inherited trait whose intency is modified by environment during seed development and commonly found in many species.
Another interesting physiological phenomenon observed in linseed is presence of dormancy from immediate harvest of crop up to 25-30 days depending on genotypes. Knowledge on the duration of seed dormancy is very much useful to seed analysts while testing seed samples. Dormancy is one mechanism by which seeds maintain their viability in unfavourable condition. In spite of this advantage, dormancy creates problem for seed analysts and seed producers, especially when germination percentage of seed lot must be determined in few weeks after harvesting.

Material and Methods
The seed material used in the present study consisted of ten linseed genotypes with different duration of maturity obtained from Main Agricultural Research Station (MARS), University of Agricultural Sciences, Raichur, Karnataka, India.
Between paper methods of germination test as prescribed by the International Seed Testing Association was followed. Four replication of 100 seeds each were randomly counted and placed on the germination paper at uniform spacing of 25 mm between seeds in row. The rolled paper towels with seeds were secured at both the ends with rubber bands and placed vertically in cabinet of seed germinator by maintaining a constant temperature of 25±1°C and relative humidity of 90%. The germination was recorded on 7th day and based on normal seedlings produced the germination per cent was worked out.

Duration of dormancy

Germination was recorded periodically at weekly interval from harvest up to the stage where the germination reached to Minimum Seed Certification Standard (80%). The dormancy duration was computed as the period from harvest till the germination reached to 80% in each entry. Based on dormancy duration, linseed genotypes were classified into three categories viz., week (14 days), Moderate (21 days) and strong (28 days).

Mechanism of dormancy

This experiment was conducted after about 2 days of harvest to study the dormancy duration behaviour in linseed genotypes. The germination test was conducted by between paper at 25°C. After 7 days, seedlings were evaluated and germination percentage was calculated on the basis of normal seedlings.

Abscisic acid (ABA) estimation

ABA was extracted from 0.1 g of seed samples with 500 µl buffer solution (80 ml methanol, 2 ml glacial acetic acid and 18 ml double distilled water). The ground seeds were homogenized using the homogenizer and samples were centrifuged at 10,000 rpm for 10 minutes and then supernatant was lyophilized using the lyophilizer. The tracer solution was prepared by adding 5 ml of 1X TBS buffer to each ABA tracer vials and replaced with the cap and contents were mixed by inverting the bottle several times and solution was kept for five minutes before use.

For standard preparation, the standard strip was diluted with true buffer solution (TBS) per l ml as per the instruction in the Phytodetek ABA Test Kit. The strip was cut at the arrows so that the filter fit inside and dispensed 1 ml of IX TBS buffer and closed the tube, vortexed the solution for an additional 30 seconds. The solution approximately contained 1000 Pico moles per ml (nm) ABA. The standards were serially diluted in IX TBS buffer. The standard contained an enantiomeric mixture of (+/–) ABA. To the test wells 100 µl of standard or sample extract was added in duplicate. 100 µl of diluted tracer prepared was dispensed in each well and mixed the contents by gently tapping the plate. Test wells were covered with plate sealer and placed in a humid box (air tight plastic box lined with damp paper towel). Test wells were incubated in the refrigerator at 4°C for three hours. At the end of the incubation period, substrate was prepared by dissolving one substrate tablet in 5 ml of substrate diluents buffer '100 µl of substrate was added to each test wells and incubated for 3 hours. The test wells were removed from the refrigerator and contents were expelled from the test wells. The test wells were washed twice with IX PBST wash buffer. 200 µl of substrate solution was added to each well using a multichannel pipette, then covered the test wells with the plate sealer and placed in a humid box, and incubated at 37°C for 60 minutes. The absorbance values were recorded at 405 nm by using an enzyme-linked immunosorbent assay (ELISA) reader. All determinations were carried out in dim light. The ABA levels were consistent with the dilution made and no interference from impurities was detected when ABA standards were added to diluted extracts. Results are expressed as picomol per gram fresh weight (pmol g-1 fw).

Results and Discussion

The duration of dormancy in 10 genotypes ranged from 7 to 28 days with an initial germination percentage of 15 to 33 when they were tested immediately after harvest (Table 1). Among the ten genotypes, four genotypes *i.e.*, Padmini, NL-115, Indraalsi and Ruchi were weak (2 weeks), three genotypes (T-397, Suyog and S-36) as moderate (3 weeks) and three genotypes (Shikha, RLV-6 and Parvathi) were found as strong (4 weeks) in dormancy duration. The highest dormancy duration of 28 days was observed in two genotypes *viz.*, RLV-6 and Parvathi and less dormancy was noticed in the genotype NL-115.

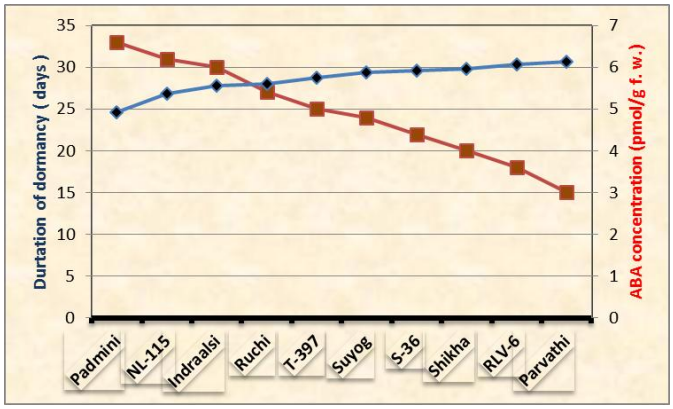

Fig 1: Duration of dormancy and ABA concentration (pmol/g fw) in linseed genotypes

ABA concentration in the linseed genotypes ranged from 4.931 to 6.142 Pico moles per gram fresh weight (pmol/g f. w.) of seed (Table 1). Even though there was no much variation in ABA concentration between the genotypes, it was sufficient to induce the dormancy. Highest ABA concentration was found in Parvathi (6.142 pmol/g f. w.) followed by RLV-6 and Shikha (6.061 and 5.958 pmol/g f. w. respectively). Whereas, lowest ABA concentration of 4.931 pmol/g f. w. was recorded in Padmini followed by NL-115 (5.365 pmol/g f.w.). Many scientists have estimated abscisic acid (ABA) using various methodologies and have correlated it with dormancy. Sergio Mapelli *et al.*(1995) [6] reported total bound ABA in rice and wheat seeds were 3.916 and 1.650 µg per gram of dry seed, Anil (2015) [3] reported the total ABA in proso millet genotypes varies from 3.199 to 3.404 Pico moles per gram fresh weight (pmol/g fw) of seed respectively and Hanumantappa *et al.* (2016) [4] reported total ABA in rice genotypes varies from 39.30 to 53.45 Pico moles per gram fresh weight (pmol/g fw) of seed Thus it can be concluded that the presence ABA in linseed genotypes induce dormancy. A linear relationship can be established between the germination per cent and ABA concentration.

Table 1: Duration of dormancy and ABA concentration in linseed genotypes

Genotypes	ABA concentration (pmol/g f. w.)	Per cent seed germination					Dormancy duration (days)/ type of dormancy
		Weeks after harvest					
		Immediately after harvest	1	2	3	4	
Padmini	4.931	33	78	83	87	89	14 (Weak dormancy)
NL-115	5.365	31	76	85	89	92	14 (Weak dormancy)
Indira Alsi-32	5.564	30	74	82	86	88	14 (Weak dormancy)

Ruchi	5.599	27	70	81	85	87	14 (Weak dormancy)
T-597	5.755	25	60	74	82	86	21 (Moderate dormancy)
Suyog	5.874	24	58	73	83	88	21 (Moderate dormancy)
S-36	5.920	22	61	70	81	85	21 (Moderate dormancy)
Shikha	5.958	20	52	68	76	83	28 (Strong dormancy)
RLV-6	6.061	18	44	65	74	84	28 (Strong dormancy)
Parvathi	6.142	15	36	62	73	81	28 (Strong dormancy)

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