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Studies on duration of seed dormancy and dormancy breaking methods on seed quality in lamb's quarters (*Chenopodium album* L.) seeds

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Abstract
The laboratory experiment was conducted during 2020-21 having 19 treatments with 4 replications planned with CRD design. Freshly harvested lamb's quarters variety Bathua - Pusa Green seeds were exposed to various physico-chemical dormancy breaking methods. For physical methods seeds were exposed to a different treatments and duration. For chemical treatments soaking duration was 48 hrs. Results revealed that, soaking of lamb's quarters seeds in ethrel @ 100 ppm (T_{19}) showed significantly highest normal seedlings (84.00%), root length (3.25 cm), shoot length (3.90 cm), seedling dry weight (3.15 mg) and seedling vigour index I (600) followed by seeds soaking in thiourea @ 1 per cent (T_{17}) recorded normal seedlings (79.00%), root length (3.09 cm), shoot length (3.86 cm), seedling dry weight (3.05 mg) and seedling vigour index I (549) and lowest was observed in control (T_1). For duration of seed dormancy results showed that, lamb's quarters variety "Bathua - Pusa Green" exists up to 80 to 85 days after harvest with germination of 68.25 to 75 per cent, respectively.

Keywords: dormancy, lamb's quarters, germination, vigour index

Introduction
Lamb's quarters (*Chenopodium album* L.) is a minor winter green leafy vegetable belongs to the family Amaranthaceae with a chromosome no. 2n=36. It is extensively cultivated and consumed in Northern India as a food crop, cultivated for leafy vegetable. It is native to Europe and extensively distributed in different parts of world *viz.*, West Indies, South America, North America, Africa, Australia, Oceania and India. In India, it is usually found in Upper Gangetic plains, Kashmir, Punjab, West Bengal, Kumaon (Uttaranchal), Maharashtra, Tamil Nadu, Karnataka and Peninsular India. The lamb's quarters are tolerant to cold, drought and salinity and have potential for cultivation in marginal lands. Where, winter season is most suitable.

It is a fast growing annual plant, grown well in tropical and sub-tropical regions with soil rich in nitrogen. Economically, the leaves and stems are used as vegetable either raw or cooked and the tender leaves are used in many Indian dishes. As a seedling, it has two long, linear-shaped cotyledons and the first ovate-shaped true leaves are opposite. True leaves eventually become more distinctly alternate and may be purplish on the underside with both surfaces covered with white granules or a mealy substance.

The leaves constitute an important and inexpensive source of moisture (89.65%), protein (3.70%), fat (0.40%), other carbohydrates (2.90%), minerals (2.60 g), calcium (15 g), phosphorus (8 g), iron (4.20 g), thiamine (0.01 mg), niacin (0.60 mg), vitamin C (35 mg), carotene (1.47 µg) per 100 g along with traces of iodine, fluorine, and vitamin K. The essential amino acids of leaf proteins were as follows leucine, isoleucine, lysine, methionine, phenylalanine, threonine, valine and tryptophan. Being cheap source of proteins and amino acids, it can compensate for the amino acid deficient food of the poor community (Pande *et al.*, 2009) [20].

Seeds of lamb's quarters were dried and were ground into flour for bread, cakes or gruel. Flour made of lamb's quarters seed is dark coloured from the blackish seed coats but bakes up into a tasty and nutritious product. The seeds taste like buckwheat and are delicious raw, but are tiny and hard to chew because the seeds are small and smooth. They had to be boiled, mashed and dried before grinding. It is nutraceutical food, an alternative source for nutrients. The root contains saponin and two flavonoids *viz.*, 'kampferol' and 'quercetin'.

Therefore, it is widely used in folk medicine around the world. Particularly, it is used in the treatment of rheumatism, bug bites, sun stroke, urinary problems, skin problems *etc*., Also, the plant has medicinal values like anthelmintic, antiphlogistic, antirheumatic, contraceptive, laxative, odontalgic property and act as a blood purifier and anti-ulcer agent (Sanwal, 2008) [23].

Seed dormancy indicates the inability of the seeds to germinate even under favourable conditions. This condition may be due to any one or several causes due to immature embryos, seed coat impermeable to water and gases, inhibitors, light sensitivity and mechanical restriction by seed coat. Presence of seed dormancy is both problematic as well as advantageous. It is problematic for post-harvest seed testing and it is advantageous in avoiding viviparous germination in tropical cultivars grown during monsoon season.

Dormancy creates problems for seed analysts and seed producers, especially when the germination percentage of seed lot must be determined in a few weeks after harvest. Sometimes even if the seeds germinate readily at harvest due to unfavourable environmental conditions during storage or germination, secondary dormancy may develop. Farmers mistake this secondary dormancy as non-viability.

Lamb's quarters are mainly propagated through seeds. However, the heteromorphic differences were observed in seed morphology and dormancy (Williams and Harper, 1965). In which, brown seeds were large, non-dormant and more salt tolerant and germinate rapidly. Whereas, black seeds are salt-sensitive and a large proportion of seeds are dormant (Sun *et al.,* 2005) [29]. Machabee and Saini, (1991) [15] found that the presence of primary dormancy in Chenopodium is due to physiological inhibiting mechanism. This inhibiting mechanism is mainly due to the presence of high level of inhibitors (abscisic acid) and low level of growth promotors (ethylene and gibberellins). However, the dormancy breaking treatments are essentially required for getting higher germination at immediate use.

It is necessary to study the duration of seed dormancy, methods to overcome dormancy and improve the performance in lamb's quarters. It is known that *Chenopodium* species exhibit physiological dormancy and the depth of dormancy depends on the seed coat thickness, which in turn is determined by the environment experienced by the mother plant during seed maturation (Penfield and Mac Gregor, 2017). Knowledge on the duration of seed dormancy is very much useful to the farmers who take up seed production or crop production immediately after harvest. While, understanding mechanism or nature of dormancy will help the scientists to find out cost effective dormancy breaking methods.

Material and Methods

The seed material of lamb's quarters variety Bathua-Pusa Green used for the present investigation to know the duration of seed dormancy and the effect of dormancy breaking methods on seed quality was obtained from Indian Agricultural Research Institute, New Delhi. The laboratory experiment was carried out at the Department of Seed Science and Technology, College of agriculture Raichur, University of Agricultural Science, Raichur during the year 2020-21 and data was analyzed using completely randomized design (CRD) with four replications. The fresh seeds of lamb's quarters variety Bathua - Pusa Green were extracted from mature fruits grown during December 2020. The half filled and empty seeds, which floated when soaked in water were discarded and tested for germination as per ISTA (Anon., 2013) [3] rules to asses duration of seed dormancy from immediately after harvest (0 day) up to 85 days after harvest with five days of interval.

Seed germination (%)

The standard germination test was carried out by following petri plate method as per ISTA procedure. Twenty five seeds in sixteen replications were taken from each treatment and placed on blotter paper uniformly. The petri plates were kept in germination chamber maintained at 25 ± 2 ℃ temperature and 90 ± 5 per cent relative humidity. Then the final count was taken on 8th day. The number of normal seedlings from each replication was counted and the mean germination was expressed in percentage (Anon., 2013) [3].

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total no. of seed}} \times 100$$

For dormancy breaking the following various physical and chemical dormancy breaking treatments were imposed to break the dormancy of freshly harvested seeds.

T_1: Control
T_2: Keeping in refrigerator at 4 ℃ for 2 days
T_3: Keeping in refrigerator at 4 ℃ for 4 days
T_4: Water soaking for 24 hrs
T_5: Water soaking for 48 hrs
T_6: Hot water treatment at 50 ℃ for 10 minutes
T_7: Hot water treatment at 60 ℃ for 40 minutes
T_8: Sun drying for 24 hrs
T_9: Sun drying for 48 hrs
T_{10}: Hot air drying treatment at 50 ℃ for 2 days
T_{11}: Hot air drying treatment at 60 ℃ for 3 days
T_{12}: Soaking in KNO_3 (1%)
T_{13}: Soaking in KNO_3 (2%)
T_{14}: Soaking in GA_3 (500 ppm)
T_{15}: Soaking in GA_3 (1000 ppm)
T_{16}: Soaking in thiourea (0.5%)
T_{17}: Soaking in thiourea (1%)
T_{18}: Soaking in ethrel (50 ppm)
T_{19}: Soaking in ethrel (100 ppm)

For the physical methods exposed the seeds to the respective treatments for respective duration and for the chemical treatments, soaking duration was 48 hrs and the treated lamb's quarters seeds were surface dried and tested for germination. Seeds were germinated in petri plates with a diameter of 9 cm containing two sheets of blotter paper moistened initially. Each treatment was replicated four times. The petri plates were kept in walk in seed germinator for 8 days at 25 ± 2 ℃. The germination was recorded on 8th day and based on normal seedlings produced. The germination percentage was worked out.

Seedling vigour index (SVI)

The seedling vigour index I was determined by employing the formula given by (Abdul-Baki and Anderson, 1973) [1].

SVI - I = Germination (%) × Total seedling length (cm)

Results and Discussion

The germination was about 14.25 per cent when tested immediately after the harvest (0 day), which increased steadily from 14.25 to 75 per cent after 85 days of harvest

which was above the Minimum Seed Certification Standards (70%) (as lamb's quarters belongs to Amaranthaceae family) indicating complete release of dormancy. This suggested that, the period of dormancy in lamb's quarters variety Bathua - Pusa Green seeds was upto 85 days after harvest (Table 1). Similar findings were noticed by Sindhuja *et al.*, 2020 in lamb's quarters (*Chenopodium album* L.) var. Ooty 1 (Ck 1), freshly harvested seed possess dormancy for about 30 days. Thereafter, seed started to germinate minimally (2%) and reached 100 per cent on 165 days after harvest. The outcomes are in line with expectations. According to Kulheri *et al.*, 2019 [11], fresh seed dormancy in bunch type groundnut seeds lasted more than 4 weeks. Freshly harvested seeds of cucumber variety "Swarna Sheetal" demonstrated dormancy for 35-40 days following harvest, according to Kavya *et al.*, 2019 [9]. In sunflower cultivars EC 69874 and EC68415, Kumar and Shastry, 1975 [13] discovered a dormancy period of 45 to 80 days. The results are in agreement with Mathur *et al.*, 2000 [16] in groundnut, Sebastian, 2014 in foxtail and Proso millet, Kulsumbi, 2018 [12] in spinach.

The lamb's quarters seeds were exposed to various pre-treatments to break dormancy which showed significant differences on seed physiological parameters while lowest seed physiological parameters were found in untreated seeds, which indicates different pre-treatments are necessary to overcome the dormancy and to improve the seedling emergence and uniform plant stand.

The lamb's quarters seeds soaked in ethrel @ 100 ppm (T_{19}) recorded significantly maximum normal seedlings (84.00%), followed by thiourea @ 1.00 per cent (T_{17}) (79.00%) and minimum (14.50%) was observed in control (T_1) (Table 2.). In dormant seeds, abscisic acid (ABA) was found to inhibit ethylene production and germination. Therefore, growth of dormant embryonic axis and cotyledon was improved by exogenous application of ethylene (Satoh and Esashi, 1980) [24]. Thus, the increase in germination and seedling vigour in chenopodium might be due to ethylene interaction with growth inhibitor (abscisic acid), growth promotor (gibberellin) and also involve in enzyme synthesis (α-amalyse) (Ketring, 1977; Matilla, 2000) [10, 17]. In addition, ethylene production results in accumulation of 1-aminocyclopropane-1-carboxilic acid (ACC) which accompanied with improved activity of endo β - mannanase, a cell-wall enzyme that weakens the endosperm and allows seed to germinate and also related to amino acid accumulation in seeds (Esashi *et al.*, 1996; Nascimento, 2003) [4]. These results are in conformity with the findings of Karssen, 1976 [8] in lamb's quarters, Pallavi *et al.*, 2010 [19] in sunflower, Halloin, 1976 [6] in cotton, Leubner-Metzger *et al.*, 1998 [14] in tobacco, Stephen *et al.*, 1981 [28] in wild oat and Seiler *et al.*, 1998 [26] in wild sunflower. Exposure of freshly harvested seeds to hot air drying treatment at 60 ℃ for 4 days (T_{11}) recorded significantly maximum number of abnormal seedlings (10.50%), followed by seeds exposed to sun drying for 48 hr (T_9) (9.50%). whereas, seeds kept in refrigerator at 4 ℃ for 2 days (T_2) showed minimum (3.25%) number of abnormal seedlings (Table 2). The highest number of abnormal seedlings obtained due to exposure of seed to hot air drying treatment for longer period in which damage to the embryo and seed coat may resulting into more leachates by increasing cracks in the seed coat or reducing the peroxidase activity in the seed covering structures thereby promoting the degradation and evaporation of short chain saturated fatty acids (SCSFAs) from the dormant seeds thereby increasing the abnormal seedlings. These results are similar Janaiah *et al.*, 2006 [7] in bitter gourd. Exposure of freshly harvested seeds to hot water treatment at 60 ℃ for 40 min (T_7) recorded significantly maximum (5.50%) number of fresh ungerminated seeds, followed by seeds exposed to hot water treatment at 50 ℃ for 10 min (T_6) (5.25%). whereas, seeds exposed to hot air drying treatment (T_{11}), seeds soaked in KNO_3 @ 2 per cent (T_{13}) and soaked in thiourea @ 1 per cent (T_{17}) showed minimum (2.00%) number of fresh ungerminated seeds. Seeds soaked in ethrel @ 100 ppm (T_{19}) recorded significantly minimum (3.50%) number of hard seeds. whereas, the control (T_1) recorded the maximum (70.25%) number of hard seeds. Exposure of freshly harvested seeds to hot air drying treatment at 60 ℃ for 4 days (T_{11}) recorded significantly maximum (13.50%) number of dead seeds. whereas, the seeds exposed to sun drying for 24 hr (T_8) showed significantly minimum (3.00%) number of dead seeds. More number of dead seeds were obtained with seeds exposed to hot air drying treatment for longer period may cause damage to the embryo and endosperm due to excessive heat exposure (Vari *et al.*, 2007 in Sesbania species) [30].

Significantly highest root length, shoot length and seedling length (3.25 cm, 3.90 cm and 7.15 cm respectively) were observed in case of seeds soaked in ethrel @ 100 ppm (T_{19}). However, lowest was observed in control (T_1) (Table 3). This increase in root length, shoot length and seedling length due to the involvement of ethrel in stimulating the hydrolytic enzymes that are needed for the degradation of the cells surrounding the radicle and plumule and makes it available to growing tips and causes rapid cell division and cell elongation which results in higher root and shoot growth. These results are in conformity with Gowda, 2014 [5] in groundnut and Meharunnisanarejo *et al.*, 2012 [18] in sunflower.

Similarly with respect to seedling dry weight and seedling vigour index-I were significantly highest (3.15 mg and 600 respectively) in ethrel @ 100 ppm (T_{19}) and lowest was observed in control (T_1) (Table 4). The increase in seedling vigour index I due to increase in germination and seedling length. Soaking seeds in ethrel @ 100 ppm (T_{19}) obtained the best result. This treatment increase germination and proved good growing characteristics in either vegetative or generative organs which in turn improve the seed vigour. Sebastain, 2014 [25] in prosomillet and foxtail millet got similar results.

Table 1: Duration of seed dormancy in lamb's quarters seeds

Days	Seed germination (%)
T_1 - At 0 Day (Immediately after Harvest)	14.25
T_2 - 5 Days after Harvest	16.50
T_3 - 10 Days after Harvest	19.50
T_4 - 15 Days after Harvest	21.25
T_5 - 20 Days after Harvest	24.75
T_6 - 25 Days after Harvest	26.50
T_7 - 30 Days after Harvest	31.50
T_8 - 35 Days after Harvest	34.75
T_9 - 40 Days after Harvest	40.75
T_{10} - 45 Days after Harvest	45.50
T_{11} - 50 Days after Harvest	48.50
T_{12} - 55 Days after Harvest	50.25
T_{13} - 60 Days after Harvest	53.50
T_{14} - 65 Days after Harvest	57.75
T_{15} - 70 Days after Harvest	60.75
T_{16} - 75 Days after Harvest	64.25
T_{17} - 80 Days after Harvest	68.25
T_{18} - 85 Days after Harvest	75.00

Table 2: Effect of dormancy breaking methods on normal seedlings, abnormal seedlings, fresh ungerminated, hard and dead seeds imposed immediately after harvest in lamb's quarters seeds

Treatments	Normal seedlings (%)	Abnormal seedlings (%)	Fresh ungerminated Seeds (%)	Hard Seeds (%)	Dead Seeds (%)
T_1 – Control	14.50	6.00	3.50	70.25	5.75
T_2 - Keeping in refrigerator at 4 ℃ for 2 days	59.00	3.25	2.50	31.50	3.75
T_3 - Keeping in refrigerator at 4 ℃ for 4 days	67.00	3.75	2.75	22.50	4.00
T_4 - Water soaking for 24 hr	50.75	6.25	3.75	32.25	7.00
T_5 - Water soaking for 48 hr	53.50	6.00	4.75	29.50	6.25
T_6 - Hot water treatment at 50 ℃ for 10 minutes	33.00	6.25	5.25	51.00	4.50
T_7 - Hot water treatment at 60 ℃ for 40 minutes	29.00	6.75	5.50	53.00	5.75
T_8 - Sun drying for 24 hr	25.75	6.25	4.25	60.75	3.00
T_9 - Sun drying for 48 hr	28.75	9.50	3.00	53.75	5.00
T_{10} - Hot air drying treatment at 50 ℃ for 2 days	58.25	9.25	3.75	19.50	9.25
T_{11} - Hot air drying treatment at 60 ℃ for 4 days	64.00	10.50	2.00	10.00	13.50
T_{12} - Soaking in KNO_3 (1%)	71.25	4.25	2.25	16.50	5.75
T_{13} - Soaking in KNO_3 (2%)	68.25	5.00	2.00	19.25	5.50
T_{14} - Soaking in GA_3 (500 ppm)	61.00	5.25	2.25	26.75	4.75
T_{15} - Soaking in GA_3 (1000 ppm)	64.25	5.50	2.75	20.50	7.00
T_{16} - Soaking in thiourea (0.5%)	74.25	7.00	2.50	10.50	5.75
T_{17} - Soaking in thiourea (1%)	79.00	5.75	2.00	5.50	7.75
T_{18} - Soaking in ethrel (50 ppm)	76.75	4.50	3.25	8.75	6.75
T_{19} - Soaking in ethrel (100 ppm)	84.00	5.00	2.25	3.50	5.25
Mean	55.91	6.11	3.17	28.70	6.12
S.Em±	0.48	0.54	0.59	0.57	0.49
CD @ 1%	1.36	1.54	1.68	1.62	1.39

Table 3: Effect of dormancy breaking methods on root length, shoot length and seedling length imposed immediately after harvest in lamb's quarters seeds

Treatments	Root length (cm)	Shoot length (cm)	Seedling length (cm)
T_1 – Control	2.53	3.17	5.70
T_2 - Keeping in refrigerator at 4 ℃ for 2 days	2.77	3.52	6.29
T_3 - Keeping in refrigerator at 4 ℃ for 4 days	2.91	3.65	6.56
T_4 - Water soaking for 24 hr	2.74	3.43	6.17
T_5 - Water soaking for 48 hr	2.75	3.43	6.18
T_6 - Hot water treatment at 50 ℃ for 10 minutes	2.72	3.42	6.14
T_7 - Hot water treatment at 60 ℃ for 40 minutes	2.66	3.42	6.08
T_8 - Sun drying for 24 hr	2.58	3.35	5.93
T_9 - Sun drying for 48 hr	2.59	3.36	5.95
T_{10} - Hot air drying treatment at 50 ℃ for 2 days	2.76	3.45	6.21
T_{11} - Hot air drying treatment at 60 ℃ for 4 days	2.82	3.61	6.43
T_{12} - Soaking in KNO_3 (1%)	3.05	3.73	6.78
T_{13} - Soaking in KNO_3 (2%)	3.03	3.67	6.70
T_{14} - Soaking in GA_3 (500 ppm)	2.82	3.57	6.39
T_{15} - Soaking in GA_3 (1000 ppm)	2.86	3.63	6.49
T_{16}- Soaking in thiourea (0.5%)	3.06	3.81	6.87
T_{17} - Soaking in thiourea (1%)	3.09	3.86	6.95
T_{18} - Soaking in ethrel (50 ppm)	3.08	3.84	6.92
T_{19} - Soaking in ethrel (100 ppm)	3.25	3.90	7.15
Mean	2.85	3.57	6.42
S.Em±	0.06	0.02	0.07
CD @ 1%	0.17	0.07	0.19

Table 4: Effect of dormancy breaking methods on seedling dry weight and seedling vigour index I imposed immediately after harvest in lamb's quarters seeds

Treatments	Seedling dry weight (mg)	Seedling vigour index -I
T_1 – Control	1.25	82
T_2 - Keeping in refrigerator at 4 ℃ for 2 days	2.03	371
T_3 - Keeping in refrigerator at 4 ℃ for 4 days	2.35	439
T_4 - Water soaking for 24 hr	1.50	313
T_5 - Water soaking for 48 hr	1.63	330
T_6 - Hot water treatment at 50 ℃ for 10 minutes	1.48	202
T_7 - Hot water treatment at 60 ℃ for 40 minutes	1.45	176
T_8 - Sun drying for 24 hr	1.38	152
T_9 - Sun drying for 48 hr	1.40	171
T_{10} - Hot air drying treatment at 50 ℃ for 2 days	1.85	361
T_{11} - Hot air drying treatment at 60 ℃ for 4 days	2.08	411

T_{12} - Soaking in KNO_3 (1%)	2.68	483
T_{13} - Soaking in KNO_3 (2%)	2.58	457
T_{14} - Soaking in GA_3 (500 ppm)	2.08	389
T_{15} - Soaking in GA_3 (1000 ppm)	2.33	416
T_{16} - Soaking in thiourea (0.5%)	2.70	510
T_{17} - Soaking in thiourea (1%)	3.05	549
T_{18} - Soaking in ethrel (50 ppm)	3.00	531
T_{19} - Soaking in ethrel (100 ppm)	3.15	600
Mean	2.10	365
S.Em±	0.10	5.99
CD @ 1%	0.29	16.95

Conclusion

The duration of seed dormancy in lamb's quarters variety "Bathua – Pusa Green" exists up to 80 to 85 days after harvest with germination percent of 68.25 to 75 per cent, respectively which was above the Minimum Seed Certification Standards (70%), as lamb's quarters belongs to Amaranthaceae family. Among the different dormancy breaking treatments soaking of lamb's quarters seeds in ethrel @ 100 ppm (T_{19}) showed significantly highest normal seedling per cent, root length, shoot length, seedling length, seedling dry weight and seedling vigour index-I when compared to other treatments and control followed by thiourea @ 1 per cent hence considered as the best dormancy alleviation treatment in lamb's quarters seeds.

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