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After Ripening Period Impacts the Seed Germination of Berseem

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ABSTRACT: An experiment was set up to identify the minimum duration of seed storage after harvest for completion of after-ripening processes in berseem seeds (*Trifolium alexandrinum*). The fresh seeds of three berseem cultivars were stored under ambient conditions for 0, 30, 60, 90, 120, 150, 180, 210 and 240 days after harvest (DAH). At the end of each storage period, the seeds were tested for germination. All the three cultivars of berseem had 40-55% germination immediately after harvest maturity, which indicated medium level of seed dormancy. The after-ripening period significantly enhanced the germination of all the berseem cultivars up-to 150 DAH. Wardan and Bundel Berseeem -2 showed 80% germination (required for certified seed of berseem by Indian Minimum Seed Certification Standard (IMSCS) at only 60 DAH whereas, whereas Bundel Berseeem -3 seed took 120 days to reach at standard germination (80%). Higher fraction of dead seeds than dormant seeds across all cultivars were observed after 240 DAH, which indicates the complete breakdown of seed dormancy. The results of this experiment can help plan the time of seed testing and planting for full realization of the germination potential of berseem cultivars under subtropical conditions.

Keywords: Berseem, *Trifolium alexandrinum*, Seed dormancy, After-ripening period, Seed germination

Berseem (*Trifolium alexandrinum* L.) is an important green fodder crop as it is highly palatable, succulent and nutritious due to the presence of high total digestible nutrients (62%) and crude protein (20%), besides its fast growing nature [1]. However, inadequacy of high quality berseem seed in the market impedes realization of full potential of berseem forage production, which results in fodder scarcity during winter. Additionally, farmers are not excited to prolong the berseem crop for seed production as they have to sacrifice summer forage to opt for seed [2].Beginning of winter season is considered as the ideal climatic condition for berseem seed germination under the agro-ecological conditions of central India including Bundelkhand region. The berseem seed, usually harvested at the end of April to first fortnight of May, shows endogenous dormancy and require a period of dry storage to trigger germination. Therefore, the endogenous dormancy in berseem is considered as bane for immediate seed testing and certification processes. As a result, traders have to wait for few months to know the quality of seed produced/sold in the market. The quality of berseem seed available in the market is very poor due to limited involvement of reputed seed companies and seed lots sold in local markets contain alarming level of physical impurities [3]. Therefore, monitoring and maintenance of physiological and physical quality is of utmost importance for better winter fodder supply.

Storing of dry seeds in an ambient (warm) environment, usually in darkness, before sowing is an ex situ dormancy alleviating treatment and is more commonly known as dry after-ripening [4]. Dry after-ripening may bring about changes in the levels of growth inhibitors or growth promoters within the seed embryo followed by encouraging the seed germination [5]. When after-ripening is completed, further seed storage induces degradation processes, such as peroxidation of polyunsaturated fatty acids, and damage of cell membranes and DNA, causing germination to decrease [6]. Berseem and related species are endowed with hardseededness or physical dormancy which generates from deposition of complex and condensed compounds in the palisade layers. The after-ripening requirement for breaking dormancy, which is very common in legumes, is the result of an ecological adaptation. However, there are very little or no information available on dormancy loss during after-ripening process in the genus *Trifolium*. Hence, an experiment was conducted to find out the duration of endogenous seed dormancy in berseem.

MATERIALS AND METHODS

The experiment was set up with freshly harvested seeds of the three berseem cultivars *viz.,* Wardan, Bundel Berseem-2 (BB-2) and Bundel Berseem-3 (BB-3) at Division of Seed Technology, ICAR-IGFRI, Jhansi. The seeds of all cultivars were harvested at full maturity stage during *Rabi* 2015-16. Collected seeds were cleaned manually, placed in paper bags and stored under ambient conditions for 0, 30, 60, 90, 120, 150, 180, 210 and 240 days after harvest (DAH). After each of these storage periods, 4 replicates of 100 seeds of each variety were tested on filter paper (top of paper) for germination according to the ISTA rules [7].The germination test was performed in seed germinator (Labsol make) with 8h light and 16h dark cycle per day at constant temperature. After the 7th day of germination, seeds were evaluated for normal seedling, abnormal seedling, hard seed and dead seed as per ISTA rules [7]. The seedlings which had well-developed essential structures were considered as normal whereas, seedling with damage, deformed and decayed were considered as abnormal seedlings. The hard and dormant seeds were differentiated based on water imbibition level: hard seed did not imbibe any water whereas; dormant seed imbibed water but did not show radicle protrusion. The data were analyzed by ANOVA and differences between the means were compared by the least significant difference (LSD) test at the $P<0.05$ level. To correct for non-normality of the germination percentage values, the statistical analyses were realized on arcsine transformed values.

RESULTS AND DISCUSSION

The germination test results at different DAH are shown in table 1. All the three cultivars showed a medium level of seed dormancy as only 52.8% seeds germinated immediately after harvest and many seeds remained fresh. Additional duration of the storage period resulted in a continuous increment in percent germination in all the three cultivars of berseem. In addition, fraction of dormant seed gradually declined with increasing duration of seed storage. The results indicated that warm and dry storage (after-ripening) increased germination of berseem cultivars due to loss of dormancy which is likely to occur during the summer dry season as maximum change in germination was occurred just after the harvest (30 and 60 DAH) in May and June months when temperature was higher. Therefore, in berseem high temperature regime during warm and dry storage period break the dormancy. For example, in Wardan, germinated fraction was only 40%, whereas 56% seeds were dormant at immediately after harvest (0 DAH) (Figure 1a). However, at 240 DAH the seed germination increased to 93.3% with only 1.3% seed remaining dormant. The dead seed% in Wardan was 4-7% throughout the after ripening period. The results indicated that Wardan seed germination reached >80% (Minimum seed germination in IMSCS) at 60 DAH. The improved berseem cultivars (BB-2 and BB-3) showed higher germination just after harvesting than old and check variety (Wardan). The seed germination in BB-2 and BB-3 was 54.67% and 54%, respectively, whereas dormant

Table 1. Average percentage of germinated, dormant and dead seed in berseem during different days after harvest (DAH)

DAH	Berseem Seed Percentage		
	Germinated	Dormant	Dead
0	52.83	43.50	3.67
30	71.17	23.50	5.33
60	77.17	16.83	6.00
90	83.17	10.00	6.83
120	88.50	5.33	6.17
150	91.00	3.17	5.83
180	91.67	2.33	6.00
210	93.50	2.17	4.50
240	94.33	0.83	4.83
CD 5%)	4.19	3.82	NS

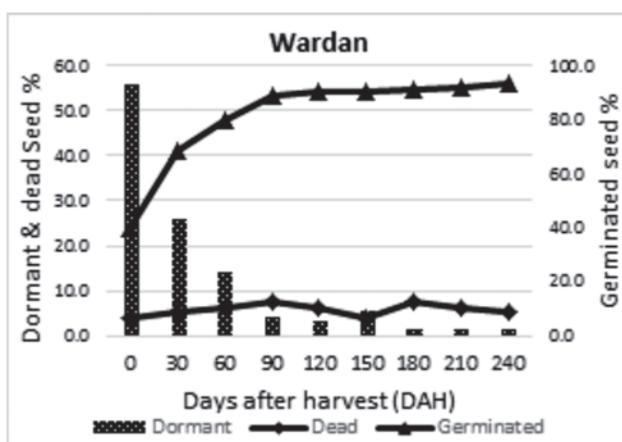

Figure 1a. Mean of germinated, dormant and dead seed percent in berseem variety Wardan during different days after harvest (DAH)

seeds were found to be 41.33% and 42% in BB-2 and BB-3, respectively at 0 DAH (Figure 1b & 1c).The maximum germination (94.67 and 93.33%) was recorded at 240 DAH in both BB-2 and BB-3, respectively. The dead seed fraction (%) in Wardan was found similar (4-7%) to BB-2 and BB-3 throughout the storage period. The major dormancy breaking effect of after-ripening obviously appeared at 120, 150 and 120 DAH, (Wardan, BB-2 and BB-3, respectively) indicated by a significant increase of the final germination between two consecutive after-ripening dates.

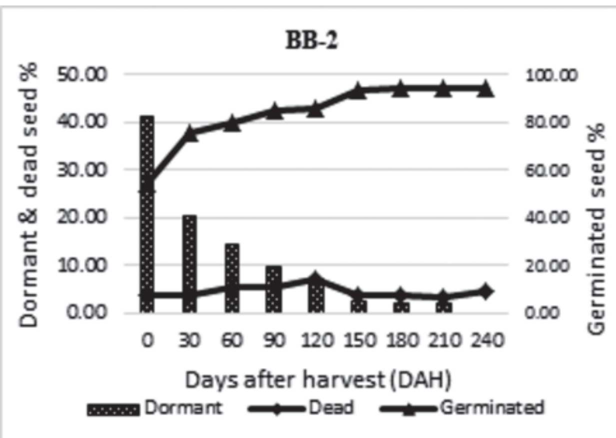

Figure 1b. Mean of germinated, dormant and dead seed percent in berseem variety BB-2 during different days after harvest (DAH)

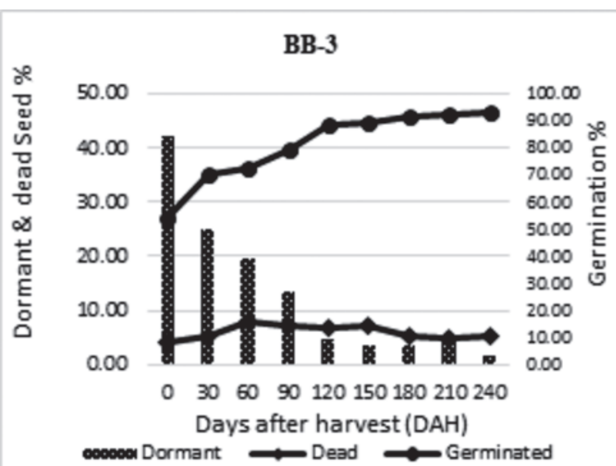

Figure 1c. Mean of germinated, dormant and dead seed percent in berseem variety BB-3 during different days after harvest (DAH)

Overall, there was a strong and positive relationship between the duration of the storage period and germination (R^2= 0.7523) and negative relationship between after ripening period and dormant seed percent (R^2= -0.7674) (Figure 2). The need of sufficient after-ripening period has been reported by several authors in different crops, such as Veatch-Blohm and Koutavas [9] in Arabidopsis which is a model crop for plant study; Zhang et al. [10] in *Stipa bungeana*, a perennial grass; Schutz *et al.* [11] in four species of Asteraceae family, and many more. Trifolium seeds have been reported to have hard seed coats and they require a considerable after-ripening period to soften the testa [12]. Though different classes of seed dormancy exist in plant species, hardseededness or physical dormancy is the most prevalent in legumes including berseem. It involves development of water impermeable seed coat caused by the presence of condensed tannins, phenolic and suberin impregnated layers of palisade cells, pectic substances, and higher proportion of cellulose and hemicellulose [13]. After-ripening promotes dormancy release by break down of those condensed compounds with time, resulting in softened seed coat which ultimately allows enough water imbibition for germination to start [14]. In berseem and related species, seed scarification (a technique to physically damage the seed coat to reduce hard seed while keeping the seed viable) and temperature treatment have been proved to be germination hastening methods [15]. In other way, when seed producers don't want to treat the seeds at all, simply storing them for a certain period (after-ripening period) will result in germination. The study showed that berseem seed needs on an average120-150 DAH for complete breakdown of embryo dormancy. A scope of intervening through scarification or temperature treatment at this point to realize the full germination potential before natural date would be an important area of research.

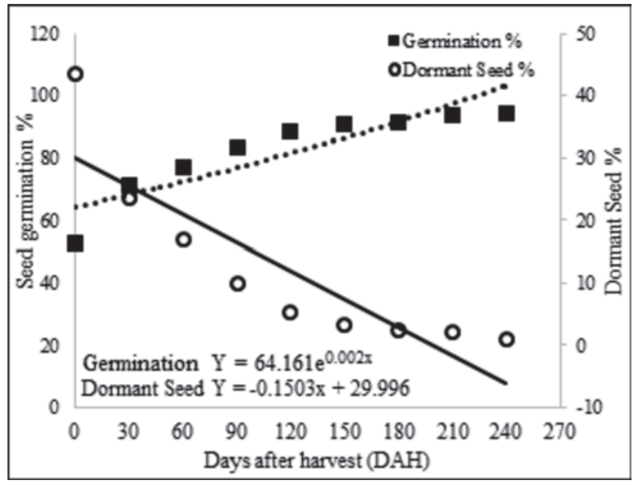

Figure 2. Correlation of seed germination and dormant seed percent with different days after harvest (DAH) in berseem

The treatments pertaining to germination enhancement in freshly harvested berseem seeds have mainly been focused in research purposes. Moreover, identifying the exact period of after-ripening in this species has been limited to research and development rather than for commercial purposes. As a consequence, dearth of knowledge in this regard sometimes results in wrong interpretation of germination results due to non-realization of full germination potential. The results in this study will help understand the correct after-ripening period required for berseem seed, which will further the research activities to shorten this period. Therefore, the patterns of germination measured in this study provide another aspect for deciding about optimum seed testing dates, best sowing date and, in particular, about the minimum time period between harvests and sowing of berseem seed.

CONCLUSION

In conclusion, after-ripening improved the final germination in all the three cultivars of berseem with a decreasing degree of seed dormancy. A significant dormancy-breaking effect was found at 60, 60 and 120 DAH for *Wardan*, BB-2 and BB-3, respectively. At least five months of after-ripening were required before maximum germination in the cultivars.

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