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SEED DEVELOPMENT AND INDUCTION OF GERMINATION OR DORMANCY, AND STORAGE POTENTIAL IN PROSO MILLET

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ABSTRACT

Proso millet (*Panicum miliaceum*) short duration crop, hence adopts the quality to escape end-of-season drought. Cultivation of millets is necessary in hill agro-ecosystem, as they are climate resilient crops. Production of proso millet in India is low; to enhance production and productivity, it is necessary to study seed quality as seed is the main carrier of technology. This study deals with analysis of seed quality during development and post-harvest storage. Such as, anthesis, seed development through various stages including physiological and harvest maturity. Analysis of induction of seed germination and dormancy, and seed vigour potential at different development stages including during storage were performed. Results showed that proso millet seed require acquisition of desiccation tolerance to express full germination and vigour. In addition, seed exhibited highest vigour at physiological maturity, and immediately after physiological maturity induction of dormancy appeared, and increased up to 50% at harvest maturity. Thus, at harvest maturity, seed lots showed heterogeneity in physiological seed quality. Overall, there was 50% reduction in in seed quality due to dormancy. Hence, it is suggested that proso millet seeds may be harvested at physiological maturity. Otherwise, seed collected at harvest maturity may remain 50% dormant, however, seed dormancy is released slowly during storage. Moreover, this study has been conducted on single genotype, need to be repeated with large number varieties and germplasm to improve seed quality through conventional breeding.

***Keywords* :** Days after anthesis, physiological maturity, proso millet, harvest maturity, seed vigour index-I, speed of germination.

Introduction

Millets are most ancient crops; however, their cultivation was neglected for a long time, probably due to extensive cultivation of Wheat-Rice, under high input conditions, for higher productivity as the requirement of that time. This has lead loss of sustainability of various agro-ecosystem and nutritional security in human diet. Again, cultivation of millets is being encouraged by several national and international agencies. For example, declaration of millet year 2023 by the UNO and Government of India by providing several incentives to farmers. Also, cultivation of millet is imperative to explore large biodiversity to ensure nutritional food security and sustainability (FAO, 2023). Among the millets proso millet (*Panicum miliaceum*) is one of the important minor millets. In India, the crop is predominantly cultivated in the states of Tamil Nadu, Karnataka, Andhra Pradesh, Bihar, Madhya Pradesh, Orissa, Himanchal and Uttarakhand, under rain-dependent conditions. Proso millet is having several characteristic features, such as, C4 photosynthetic pathway, short crop duration, i.e., between 65 and 85 days, high temperature and water-deficit stresses tolerance. In addition, it is tolerance to various biotic stresses. These features make it as resilient crop for the climate change impacts. In addition, being short crop duration, it has the tendency to escape end-of-season drought. As millets are reported to be highly nutritious, the nutraceutical profile of proso millet is highly beneficial in maintaining high blood pressure. In addition, it provides adequate amount of zinc, iron and vitamin B6. Moreover, it is free from gluten, hence, its consumption is beneficial for human being to remain healthy (Das *et al*., 2019). In spite of having all these characteristic features yield levels of proso millet are very low, i.e., between 531 and 600 kg ha^{-1} and India ranks ninth in the world production (Tonapi *et al*., 2015). Though, several varieties have been developed but farmers in Uttarakhand are still growing landraces, which are poor in yield. Since, seed is important carrier of technology and plays a crucial role in enhancing productivity, seed related issues are less addressed in this crop (Gupta *et al*., 2012, Ragupathi *et al*., 2017; and Raj *et al*., 2024). With the present status of knowledge on the crop, it is necessary to find out important issues related with low productivity. Seed development, such as, induction of germination or dormancy, desiccation tolerance and vigour status at the physiological and harvest maturity are important parameters determining seed quality and ultimate productivity (Bewley and Black *et al*., 1994; Nautiyal *et al*., 2023). Hence, it was thought of interest to investigate these issues related with proso millet seed development in variety TNAU-202.

Materials and Methods

Field experiments were conducted with proso millet (variety TNAU-202) during *kharif* season of 2023 at the Department of Seed Science and Technology, HNB Garhwal University, Srinagar Garhwal, Uttarakhand. Seeds of the variety were obtained from ICAR-IIMR, Hyderabad. Field experiment was conducted in randomized block design (RBD) with a spacing of 30 x 10 cm, row to row and plant to plant, respectively. The plot size was 3 x 4 m and replicated three times. Observations on field emergence, general plant growth and development was recorded after field emergence to harvest maturity. Such as, days to field emergence, first leaf, second leaf stage, days to 50% flowering and 100% flowering were recorded. At final harvest plant length, number of tillers, number of leaves $tiller^{-1}$, number of seed $panicle^{-1}$. Data on plant vegetative weight, biomass, seed yield, grain yield were m^{-2}. Harvest index was calculated based on biomass and economic yield.

Inflorescence growth and basic floral biology were studied in 20 selected plants from each replicate. Plants were tagged by random selection in the beginning of anthesis. For studying time of anthesis, flower on panicle were marked and the opened flowers were counted between 8:00 am and 2:00 pm at hourly intervals daily until completion of anthesis. The opened flowers were recorded and mark at each interval in order to avoid recounting of the flowers and calculated as the percentage of opened flower (Harish *et al*., 2017). Plant reached to anthesis within 50 days after sowing and sampled for analysing of seed, such as, moisture, dry mass, seed germination and/or dormancy, speed of germination and seedling vigour. These observations were recorded during seed development at an interval of 5 days until 40 days after anthesis (DAA). For sampling of seed development three plants were selected and collected samples were brought to the laboratory in ice box. In the laboratory samples were divided into three parts, the first part was analysed for seed germination as fresh seed (F), i.e., without desiccation (T1), the second part was desiccated for 48 hours at room temperature (FD 48h, T2) while, the third part was desiccated at room temperature for 30 days (FD) and thereafter was analysed for seed germination (T3). At each sampling date seeds were analysed for moisture, dry mass, germination and/or dormancy. Seed moisture content was determined gravimetrically in hot-air oven following ISTA protocols (2021). Also, observations on panicle length, width, fresh weight, dry weight and 1000-seed weight (i.e., test weight) were recorded at each sampling date following Ragupathi *et al*. (2017).

Seed germination study was conducted in petri plates of 5 cm diameter in three replicates for each treatment. Whatman filter paper No 1 was placed at the bottom of each petri plate and twenty seeds were arranged over the Whatman filter paper. After wetted the filter paper with double distilled water, seeds were incubated at 28 ±1°C. In petri plated water levels were checked every day and maintained daily to provide optimum water content. At final count (i.e., 7 days) 10 germinated seedlings were selected randomly from each replicate and treatment and seedling length and dry mass were measured to calculate seedling vigour I and II following (Abdul-Baki and Anderson 1973). Tetrazolium test was conducted to check the non-germinated seeds, whether they are viable or not and viable seeds not sowing germination were considered as dormant (ISTA, 2021). Seeds after through drying and processing were stored in cotton bags at ambient laboratory conditions. Seed lots were analysed for germination and vigour, both before and after storage at different time intervals, i.e., 0, 3, 6 and 9 months. Speed of germination was calculated by recording seed germination daily for seven days (Maguire, 1962).

Results

Vegetative growth

In proso millet vegetative growth development was recorded at field emergence to final harvest.

Coleoptile emergence was recorded three days after sowing (DAS) and first leaf and second leaf were recorded on four and seven DAS, respectively. First flower initiand at 46 DAS and 50% and 100% flowering was recorded at 50 and 55 DAS, respectively. Plant growth and development were optimum at final. At final harvest seed yield was recorded 155 g m^{-2}, vegetative yield 357 g m^{-2} and biomass 524 g m^{-2} with HI of 32% (Table 1).

Reproductive growth

Anthesis and pollination: Proso millet is self-pollinated crop and anthesis is chasmogamous in this process flower bud gets open after pollination. Sequence of flower bud opening was recorded basipetal. In this study, maximum anthesis was recorded between 10:00 and 11:00 am, while minimum between 1:00 pm and 2:00 pm (Fig.1). It indicated that for anthesis high humidity and lower temperatures are required with a bright sunshine. In addition, the process of anthesis was recorded active for 12–15 days, after initiation of first flower. The florets remained open for about 10–15 minutes.

Panicle development: Panicle length became double from 5 to 35 DAA, while width increased gradually from 2.1 cm at 5 DAA to 5.2 cm at 30 DAA. On the other hand, panicle fresh weight increased from 1.0 g at 5 DAA to 4.0 g at 30 DAA. Thereafter, a decrease in panicle fresh weight was recorded at harvest maturity (Table 2). Similarly, panicle dry weight increased with maturity mainly because of increase in seed mass. The increase was from 0.26 g at 5 DAA to 2.0 g at 40 DAA (harvest maturity). Panicle moisture came down to 22% at harvest maturity, however, decrease in panicle moisture was sharp, i.e., 85% at initial development to 23% at d harvest maturity (Table 2). Thousand seed mass (or test weight) increased up to 30 DAA, thereafter, it decreased slightly between 35 and 40 DAA. In addition, increase in 1000-seed mass was recorded in an exponential manner between 20 and 25 DAA (Table 2). This indicated that seed filling rate was quite high during this period.

Seed moisture content and dry mass accumulation: During seed development typical trends in moisture loss and dry mass accumulation were recorded. Such as, at the initial stage (5 DAA) seed dry mass was about 15%, while seed moisture content was 85%. Thereafter, seed moisture content decreased continuously through different stages of development and reached to 25% at 40 DAA. On the other hand, seed dry mass increased rapidly and reached to 77% at 40 DAA (Fig. 2). The trends of seed dry mass accumulation and loss of moisture content, crossed each other at a mid-point, after 25 days of anthesis. In addition, the gap between dry mass accumulation and moisture loss were 70% at initial (5 DAA) and 50% at harvest maturity (40 DAA) (Fig. 2). The difference in gain of moisture percentage and loss of moisture were significant at all the stages of seed development.

Physiological maturity: Seed physiological maturity was assessed by dry mass accumulation and 1000-seed weight was recorded at each stage of development, i.e., right from 10 DAA until 40 DAA (Table 2). Seed mass was recorded higher at 30 DAA (5.3 g), and reduced to 5.1 and 5.0 g at 35 and 40 DAA, respectively (Table 2). Thus, physiological maturity in proso millet could be considered at 30 DAA as it is defined at the maximum dry mass accumulation.

Induction germination/dormancy and acquisition of desiccation tolerance: Seed during various stages of development were evaluated for germination or dormancy. Seed germination studied at different time intervals with the seed as fresh (F), desiccated for 48 h (F48) and fully desiccated seed at room temperature for one month (FD) showed significant variation in germination behaviour (Table 3,). For example, seed either fresh, desiccated for 48 h and fully desiccated, did not show any germination up to 10 DAA. Seed exhibited germination only 15 DAA, that too the fully desiccated seeds with 35% germination (Table 3). Seed germination in fresh seeds was not recorded until 20 DAA, while, 7% germination was recorded in 48 h desiccated seed. In addition, germination in fully desiccated seed was much higher (65 %) at this stage as compared to the rest of the two treatments. Further, seed germination fully desiccated seed at 30 DAA reached up to 92%, while 10% in T1 and 20% in T2. (Table 3). Seed germination percentage reduced in fully desiccated seeds at 30 DAA (from 92 to 45 %) and a further reduction was noticed at 35 DAA (68 %). Reduction in germination percentage was continued in fully desiccated seeds even up to final maturity and germination reached to 45% indicating a dormancy of 55%.

Seed storability: Seed moisture content during storage showed no significant loss and maintained between 9.85 and 10.5%. The initial germination percentage was 76 and increased to > 90 % after 8 months of storage. As germination percentage is direct indicator of seedling vigour, it also showed similar trend in SVI I and SVI II (Table 5, Fig. 5). The influence of storage period on selling vigour indices were significant.

Discussion

Seed development is a complex process and regulated mainly through genotype by environment interaction, this study has demonstrated induction of germination and dormancy, acquisition of desiccation tolerance, differences in seed vigour at physiological and harvest maturity and their relationship with seed quality in proso millet variety. As these processes play crucial role in determining seed quality, therefore needed to be studied for identification of trait(s) for its association with vigour. This study showed that seed germination and seedling vigour were highest at physiological maturity, that was expressed at 35 DAA. To achieve this stage seed passed through various morphological, physiological and molecular changes. For example, the morphology of seed/panicle size and colour changed at different stages of development, and colour became yellow-brown at both physiological and harvest maturity. In general, seed quality is considered highest at physiological maturity. To achieve physiological maturity seed goes through the process of expression of phytohormones, transcription factors that play different roles during seed development (Barake, 2018). In this variety, physiological maturity was attained when 1000-seed mass was highest at 30 DAA, thereafter seed mass reduced, however, the reduction in seed mass was statistically non-significant. At physiological maturity higher germination percentage and seedling vigour were recorded. In general, it is considered that seed at physiological maturity shows maximum dry mass, high potential of germination and vigour (Bewley and Black 2012). Induction of germination, i.e., 32% was found at an early stage of seed development (i.e., 15 DAA), in the seed exposed to full desiccation (seed mc around 10-12%). While, in non-desiccated and partially desiccated seeds, it ranged between 8 and 11%, only. Thus, it is concluded that for expression of full germination, acquisition of desiccation tolerance is a must.

This variety showed induction of dormancy immediately after attaining physiological maturity, and degree of dormancy increased until harvest maturity about 50%. Thus, the seed lot obtained at harvest maturity exhibited heterogeneity in their physiological quality. In most of the millets such heterogeneity in physiological seed quality have been reported (Burton, 1969; Ragupathi *et al*. 2017). In addition, seed dormancy is regulated by ABA released during the process of acquisition of decision tolerance, while higher concentration of GA3 than ABA, induce germination (Nautiyal *et al*., 2023). In nature, seed remain dormant until the external environment become favourable for seed germination (Bareke, 2018) however, in crop cultivation this character was improved accordingly to meet agronomical requirements. With the induction of dormancy seed germination and seedling vigour including speed of germination were also influenced. Thus, seed lot having both dormancy and germinability in equal proportion, it could be difficult to evaluate vigour, immediately after harvest. In addition, such behaviour of seed may be related with post-harvest maturation. In this case, seed dormancy persisted for about three months and seed showed full germination after six months. Thus, there was uniformity in germination and seedling vigour came in to existence at the time of sowing for next season crop. On the other hand, seed dormancy is inherited character from ancestors and is crucial for survival of a population in ecosystem (Nautiyal *et al.* 2023). In addition, deposition of storage compound such as heat-shock proteins, LEAs and antioxidants during seed development and maturation were found associated with desiccation tolerance (Sripathi and Groot, 2023). Among the storage compound phytic acid, the seed storage reserve of phosphorus was identified as important compound for early seedling growth. Therefore, all the above-mentioned processes including desiccation are important in maintaining germinability and vigour during dry storage (Pereira *et al*., 2017). Proso millet seeds in this study showed desiccation tolerance before physiological maturity, but it was of low degree (8-20%), such behaviour was also reported in other crop species (Bewley and Black 2012). Thus, understanding of seed development and maturation is important in quality seed production. As harvest of seed before physiological maturity or over maturity may lead poor quality. Thus, several components of seed quality including desiccation tolerance, and physiological maturity, after ripening have potential influence on seed quality.

This study clearly showed that seed filling rate was highest between 25 and 30 DAA which has contributed significantly in seed size or test weight, but the time period could not be sufficient to produce quality seed, hence need to be prolonged to a a reasonable seed filling period to enhance productivity. This period seems to be too short as compared to the cereal crops. The crop itself is small millet and seed size is too small, in general, test weight, is considered an important parameter of seed vigour, which is direct measurement of seed filling rate and period (Yalamalle *et al*., 2023). One way to enhance seed mass is to enhance harvest index (HI) and there are reports that mobilization of pre-anthesis stem reserves is possible to the developing seed in several cereals and especially, monocot crops under water-deficit stress during seed maturation (Blum, 1988,). For this purpose, germplasm including landraces of proso millet should be evaluated for enhancing HI. Finally, seed storage study showed that proso millet seed could be stored safely for a period of eight months with maintaining seed certification standards (Navami *et al.*, 2022).

Table 1: Crop morphological and phenological parameters including grain/seed yield and yielding attributes in proso millet variety TNAU-202, in *kharif* season, 2023.

Parameters	Range
Coleoptile emergence	3-4
First leaf	4-5
Second leaf	7-8
Flowering start	45-47
50 % flowering (DAS)	49-50
100% flowering (DAS)	54-56
Total days of anthesis	10-15
Plant hight (cm)	90-120
Number of tillers plant^{-1}	6-12
Number of leaves tiller^{-1}	5-7
Number of seed panicle^{-1}	280-320
Grain yield (g m^{-2})	166
Seed yield (g m^{-2})	155
Vegetative yield (g m^{-2})	357
Biomass (g m^{-2})	524
Harvest index (%)	32

Table 2: Panicle length (cm), width (cm), fresh mass (g), dry mass (g), panicle moisture content (%), 1000-seed mass (test weight) and panicle colour at different seed development stages in proso millet variety TNAU-202, in *kharif* season, 2023.

Days after anthesis (DAA)	Panicle length (cm)	Panicle width (cm)	Panicle fresh weight (g)	Panicle dry weight (g)	Panicle moisture content (%)	1000-seed weight (g)	Seed colour (visual observation)
5	26.2	2.14	1.21	0.26	78.2	0.0	-
10	29.5	2.82	2.40	0.55	77.0	1.8	Green
15	36.3	3.53	3.48	1.26	63.9	2.0	White milky
20	42.2	4.04	3.85	1.65	58.3	3.7	White milky
25	43.9	4.70	4.31	1.87	56.1	4.6	Yellow
30	47.9	5.17	4.44	1.97	55.6	5.3	Yellow
35	50.3	5.10	3.21	2.04	36.1	5.1	Yellow Brown
40	50.3	5.00	2.63	2.09	20.0	5.0	Yellow Brown
CD	0.287	0.026	0.040	0.017	3.48	0.055	-

Table 3: Seed moisture content (%), germination percentage (%) and dormancy (%) at different seed development stages in proso millet grown during *kharif* season of 2023 at HNBGU, Chauras campus.

Days after anthesis	Seed moisture content (%)			Seed germination (%)			Dormancy (%)		
	*T1	*T2	*T3	T1	T2	T3	T1	T2	T3
5	85	43	6	0	0	0	0.0	0.0	0.0
10	75	39	8	0	0	0	0.0	0.0	0.0
15	65	36	9	0	0	35	0.0	0.0	0.0
20	58	30	10	0	8	65	0.0	0.0	0.0
25	47	26	11	8	20	79	66	52	2
30	38	25	11	10	20	92	78	70	2
35	30	20	12	20	28	68	78	64	28
40	24	16	12	21	22	45	72	70	50
Average	53	29	9.9	7.49	12.33	48.12	73	64	20
	CD			CD			CD		
Days (D)	1.09			3.33			1.248		
Treatment(T)	0.671			2.41			0.764		
D X T	1.89			5.77			2.161		

*T1= non-desiccated seed (fresh seed), T2= 48 h desiccated seed, T3= Fully desiccated seed

Table 4: Speed of germination, seedling dry weight (g- 10 seedling), seed vigour index-I and II at different seed development stages in proso millet grown during *kharif* season of 2023 at HNBGU, Chauras campus.

Days after anthesis	Vigour test											
	Speed of germination			Seedling dry weight (g 10 seedling)			Vigour index-I			Vigour index-II		
	T1*	T2*	T3*	T1	T2	T3	T1	T2	T3	T1	T2	T3
5	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	4	0	0	0.009	0	0	340	0	0	0.032
20	0	0.4	8	0	0.005	0.011	0	24	780	0	0.003	0.070
25	0.2	1	11	0.012	0.013	0.013	107	286	1375	0.009	0.025	0.124
30	0.7	1	12	0.015	0.014	0.014	174	292	1283	0.016	0.029	0.113
35	1	1	8.5	0.017	0.013	0.013	355	364	932	0.034	0.023	0.091
40	1	1.2	5.5	0.015	0.012	0.013	337	278	458	0.033	0.028	0.059
Average	0.36	0.5	6.9	0.007	0.007	0.009	121	155	646	0.092	0.013	0.061
CD												
Days (D)	0.56			0.001			67			0.005		
Treatment(T)	0.34			0.001			41			0.003		
D X T	0.97			0.001			116			0.009		

*T1= non-desiccated seed (fresh seed), T2= 48 h desiccated seed, T3= Fully desiccated seed

Table 5: Seed moisture content (%), germination Percentage (%) speed of germination Seed vigour index-I and II during different storage period (i.e., October-June, 2023-2024) in proso millet variety TNAU-202 at ambient laboratory conditions.

Storage period in months	Seed moisture (%)	Germination (%)	Speed of germination	Seed vigour index-I	Seed vigour index-II
0	10.16	75	14.6	826	0.08
3	9.85	82	16.0	1019	0.10
6	10.51	87	19.2	1152	0.12
9	10.23	98	42.4	1323	0.17
CD	0.38	4.57	1.80	112	0.007

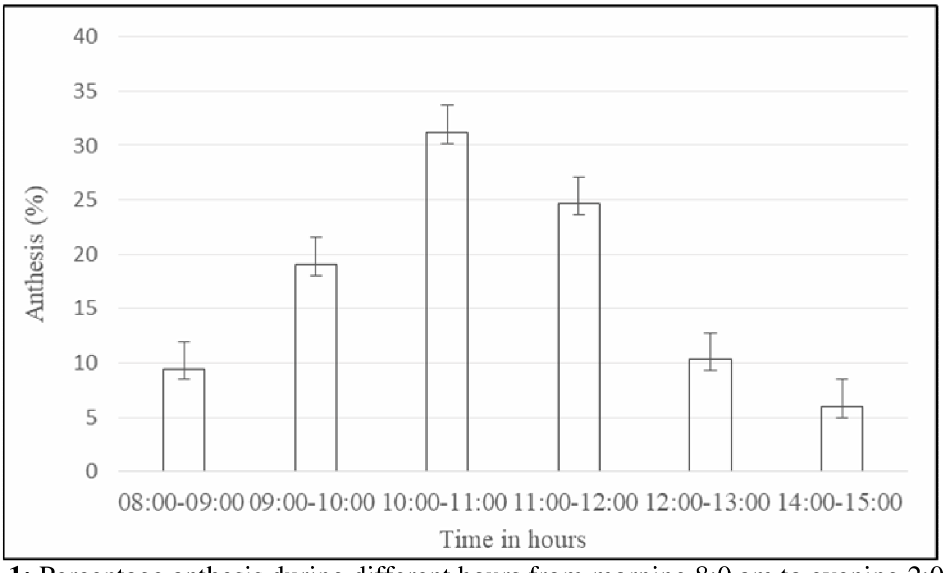

Fig. 1: Percentage anthesis during different hours from morning 8:0 am to evening 2:0 pm of proso millet during *kharif* season, 2023 (Mean error is shown over the bars).

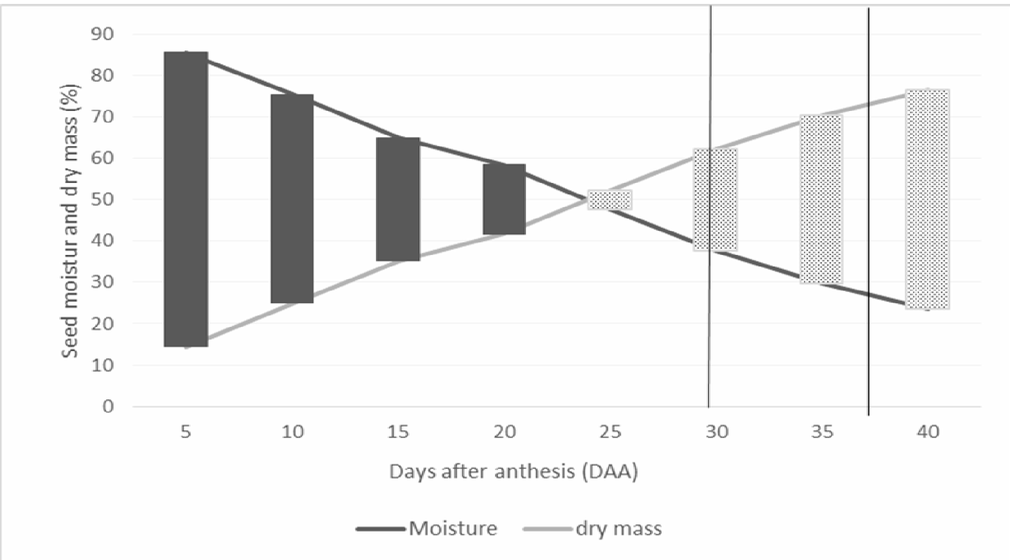

Fig. 2: The accumulation of seed dry mass and loss of moisture content in developing seeds of proso millet at different days after anthesis.

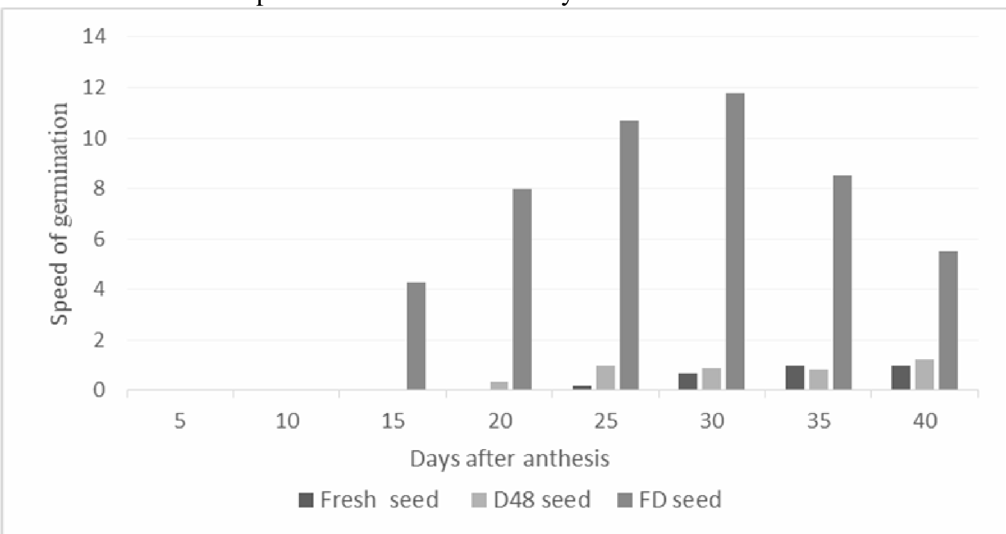

Fig. 3: Speed of seed germination in seeds of different treatments, i.e., T1= non-desiccated seed (fresh seed), T2= 48 h desiccated seed, T3= Fully desiccated seed in proso millet variety TNAU-202, (cd values write here in parenthesis).

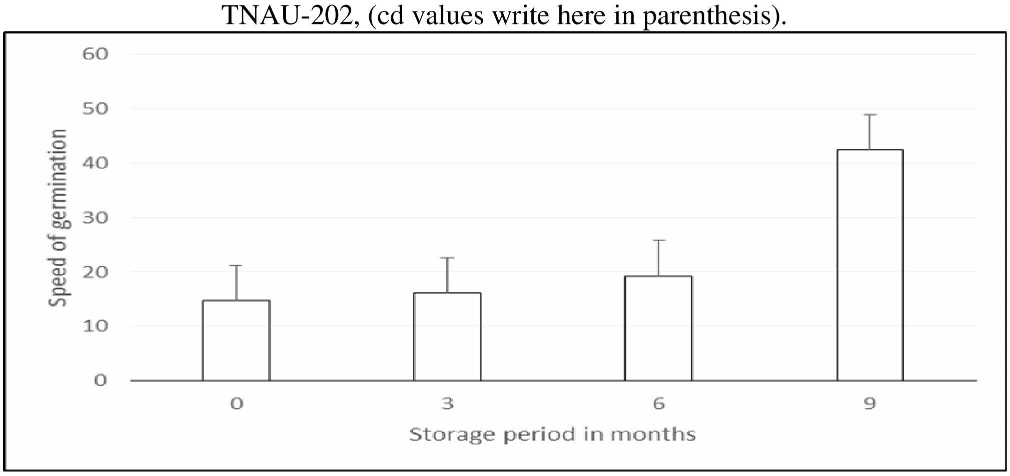

Fig. 4: Speed of germination during different storage period (i.e., October-June, 2023-2024) in proso millet variety TNAU-202 at ambient laboratory conditions.

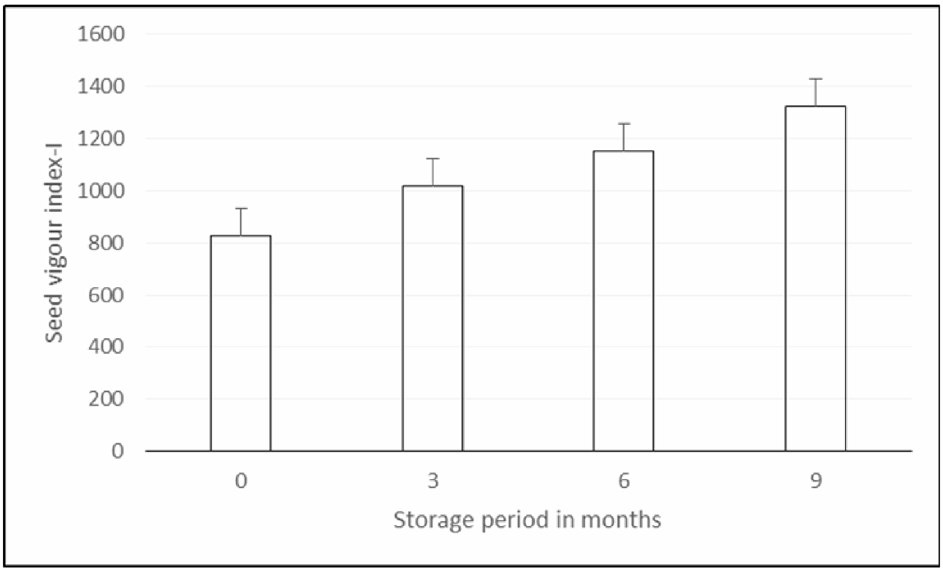

Fig. 5: Seed vigour index-I during different storage period (i.e., October-June, 2023-2024) in proso millet variety TNAU-202 at ambient laboratory conditions.

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