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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN HYBRID MAIZE SEED QUALITY DURING AMBIENT STORAGE IN DIFFERENT PACKAGING MATERIALS

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ABSTRACT

The present study investigated the impact of seed moisture content and packaging materials on the quality of maize hybrid PMH 1 seeds under ambient storage conditions. Maize seeds harvested four months prior were desiccated to various moisture levels (12%, 10%, and 8%) and stored in different packaging materials, including laminated polypropylene bags, non-laminated polypropylene bags, and high-density polyethylene (HDPE) bags. Seeds with 12% moisture content stored in cloth bags served as the control. The study found that seeds stored in laminated polypropylene bags, non-laminated polypropylene bags, HDPE bags, and cloth bags maintained germination rates above 90% for up to 13 months, meeting the Indian Minimum Seed Certification Standards (IMSCS). However, germination rates gradually declined with extended storage, and no storage treatment could maintain germination above IMSCS after 13 months. Seed moisture content exhibited fluctuations corresponding to the relative humidity of the environment, with greater variation observed in seeds stored in cloth and polypropylene bags compared to those in HDPE bags. There was a consistent decline in α-amylase activity and antioxidant enzyme levels with prolonged storage, regardless of the packaging treatment. Food reserves, including sucrose, starch, oil, and total soluble proteins, decreased over time, while total free amino acids increased. Protein profiling revealed that the intensity of electrophoretic bands corresponding to polypeptides of molecular weights 44 and 52 kDa was higher in fresh seeds compared to seeds stored for 15 months under various packaging treatments. Despite statistical parity in germination rates across different packaging materials, polypropylene bags (both laminated and non-laminated) emerged as cost-effective and durable alternatives to cloth and HDPE bags, owing to their lower cost and higher flexural strength.

Keywords: Antioxidant enzymes, Germination, Polypropylene, Protein profiling, Seed moisture

Quality seed production is crucial for ensuring high crop production and productivity. Achieving a bountiful harvest relies on seeds meeting high-quality criteria, including germination and vigour (Patil, 2013). Seeds, being delicate living entities, depend on various biotic and abiotic environmental factors for their storability. If seed viability and vigour are not properly maintained during storage, seeds become unsuitable for the next planting season. Seed storage poses a significant challenge in India due to its tropical and subtropical climate, where high relative humidity and temperatures can lead to a decline in seed quality.

Hygroscopy, or the ability of seeds to absorb moisture, significantly affects seed quality and storage duration. Storing seeds in open containers is problematic due to their hygroscopic nature, which can lead to deterioration. Research indicates that seed deterioration can be mitigated by using proper packaging materials and maintaining appropriate seed moisture levels (Abba and Lavato, 1999). Seeds should be dried to low moisture levels and stored in moisture-proof bags to manage fluctuations in seed moisture content and maintain equilibrium with the ambient relative humidity. Proper containers and packaging materials are essential to prevent direct interactions between the storage environment and seed materials, thereby preserving seed viability over extended periods.

Seed aging leads to DNA degradation, which impairs the transcription process and results in faulty enzyme synthesis crucial for initial germination (Kapoor *et al.*, 2010). During seed germination, the breakdown of reserve materials is facilitated by proteolytic, lipolytic, and amylolytic enzymes, with the resulting products transported to the growing seedlings for their development (Bhadauria *et al.*, 2019). Research has shown that α-amylase activity is suppressed during storage, leading to a reduced availability of sugars essential for germination in wheat and chickpea

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seeds (Chauhan *et al.*, 2011). Elevated activities of antioxidant enzymes, such as catalases, peroxidases, and superoxide dismutases, are observed when the production of reactive oxygen species is increased due to biotic or abiotic stresses (Yücel *et al.*, 2018). Membrane damage and the impairment of cellular machinery are associated with reduced seed viability during storage (Bashir *et al.*, 2007). Traditionally, seeds of maize hybrid PMH 1 are stored in cloth bags for sale and storage purposes. This study aimed to standardize the seed moisture content and packaging materials for the storage of maize hybrid PMH 1 seeds and to evaluate the physiological and biochemical changes occurring in seeds stored under ambient environmental conditions.

MATERIALS AND METHODS

The seeds of PMH1 were obtained from Punjab Agricultural University, Ludhiana. Initial assessments were made to determine the germination rate and seedling vigour index of the seed lot. The seeds were then desiccated to various moisture levels using fused $CaCl_2$ as a desiccant. Following desiccation, seed samples were stored in different packaging materials, as detailed subsequently, and kept at room temperature.

The experiment was conducted using a factorial completely randomized design (CRD). The different treatments included: seeds with 12% moisture packed in cloth bags (control); seeds with 12% moisture packed in laminated polypropylene bags, non-laminated polypropylene bags, and HDPE bags; seeds with 10% moisture packed in laminated polypropylene bags, non-laminated polypropylene bags, and HDPE bags; and seeds with 8% moisture packed in laminated polypropylene bags, non-laminated polypropylene bags, and HDPE bags. The dimensions of the bags used were 32x40 cm for cloth bags, 35x40 cm for laminated and non-laminated polypropylene bags, and 57.5x41.5 cm for HDPE bags (Table 1). The thickness of the polyethylene bags was 600 gauge.

Physiological parameters were recorded monthly throughout the storage period of the seeds. Seed moisture content was measured using the moisture analyzer AND MX-50. Seed germination was assessed according to ISTA guidelines (2020), and the Seedling Vigour Index (SVI) was determined as per Abdul-Baki and Anderson (1973). The speed of germination was evaluated following AOSA procedures (1983), while mean germination time was calculated according to Ellis and Roberts (1981). Membrane leakage was measured based on the method described by Fletcher and Drexler (1980).

The α-amylase activity in four-day-old seedlings was assessed using the method described by Sadasivam and Manickam (1996), with the enzyme activity expressed as mg maltose per gram of fresh weight

Table 1. Seed storage treatments

Sr. No.	Treatments	Cost of one 5kg capacity bag (Rs.)	Cost of 7,23,000 bags* (Rs.)
T_1	Seeds with 12% MC stored in cloth bags (control) (32×40cm)	10.88	78,66,240
T_2	Seeds with 12% MC stored in laminated polypropylene bags (35×47cm)	7	50,61,000
T_3	Seeds with 10% MC stored in laminated polypropylene bags (35×47cm)	7	50,61,000
T_4	Seeds with 8% MC stored in laminated polypropylene bags (35×47cm)	7	50,61,000
T_5	Seeds with 12% MC stored in non-laminated polypropylene bags (35×47cm)	7	50,61,000
T_6	Seeds with 10% MC stored in non-laminated polypropylene bags (35×47cm)	7	50,61,000
T_7	Seeds with 8% MC stored in non-laminated polypropylene bags (35×47cm)	7	50,61,000
T_8	Seeds with 12% MC stored in polyethylene bags (57.5×41.5cm)	13.57	98,11,110
T_9	Seeds with 10% MC stored in polyethylene bags (57.5×41.5cm)	13.57	98,11,110
T_{10}	Seeds with 8% MC stored in polyethylene bags (57.5×41.5cm)	13.57	98,11,110

*In Punjab, an area of 144.6 thousand ha was under cultivation of maize in *kharif* 2021. As 25 kg seed/ha is needed which requires 7,23,000 bags of 5kg capacity for 36,150 quintal maize seed; MC-moisture content.

(FW). The activities of antioxidant enzymes, including catalases, peroxidases, and superoxide dismutases, were measured using the methods outlined by Aebi (1983), Claiborne and Fridovich (1979), and Marklund and Marklund (1974), respectively.

Biochemical reserves, including sucrose, starch, total soluble proteins, and total free amino acids, were determined using established methods: sucrose and starch by Dubois *et al*. (1956) and ROE (1934), total soluble proteins by Lowry *et al*. (1951), and total free amino acids by Lee and Takahashi (1966). Oil content was estimated using the NMR MQC from Oxford Instruments. Proteins from fresh seeds (one month old) and 15-month-old seeds stored under various packaging treatments were extracted with 2 ml of an extraction buffer composed of 0.25 M sodium chloride, 1% sodium dodecyl sulfate (SDS), 1% mercaptoethanol, and 0.05 M sodium phosphate (monobasic). Protein samples, containing 90 µg of protein per well, were mixed with an equal volume of sample buffer and heated at 95ºC for 3 minutes in heating blocks. Protein profiling was conducted using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as outlined by Laemmli (1970).

Seed quality parameters, including seed moisture content, germination, seedling vigour index, germination speed, and mean germination time, were recorded monthly over a 12-month period. Biochemical parameters were assessed every three months throughout the same period. The experiment was conducted using a factorial completely randomized design, and critical difference values were determined through analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Seed moisture content

Maize hybrid PMH 1 seeds, desiccated to moisture levels of 12%, 10%, and 8% and stored in various packaging materials, exhibited notable changes in moisture content over the storage period. After four months of harvest, the moisture content of seeds decreased at six (October) and seven months (November) of storage due to a reduction in ambient relative humidity. This was followed by an increase in moisture content during eight to nine months (December to January) as relative humidity rose. In the subsequent period from ten to twelve months (February to April), the moisture content again decreased due to a drop in relative humidity. From May to August, the rising relative humidity led to increased moisture content in the seeds. Seeds stored in cloth bags and polypropylene bags exhibited greater fluctuations in moisture content compared to those stored in high-density polyethylene (HDPE) bags. This discrepancy highlights the porous nature of cloth and polypropylene bags, which are less effective at maintaining stable moisture levels. On the other hand, the moisture content in HDPE bags remained more consistent due to their moisture-impervious properties. Some variations in moisture content in polyethylene bags could be attributed to the seeds' metabolic activity. Likhayo *et al.* (2018) similarly observed that moisture content in maize seeds stored in hermetic bags remained stable, while it decreased in polypropylene bags.

Germination and seedling vigour index

Germination percentages for maize hybrid PMH 1 seeds were assessed over a 12-month period, starting

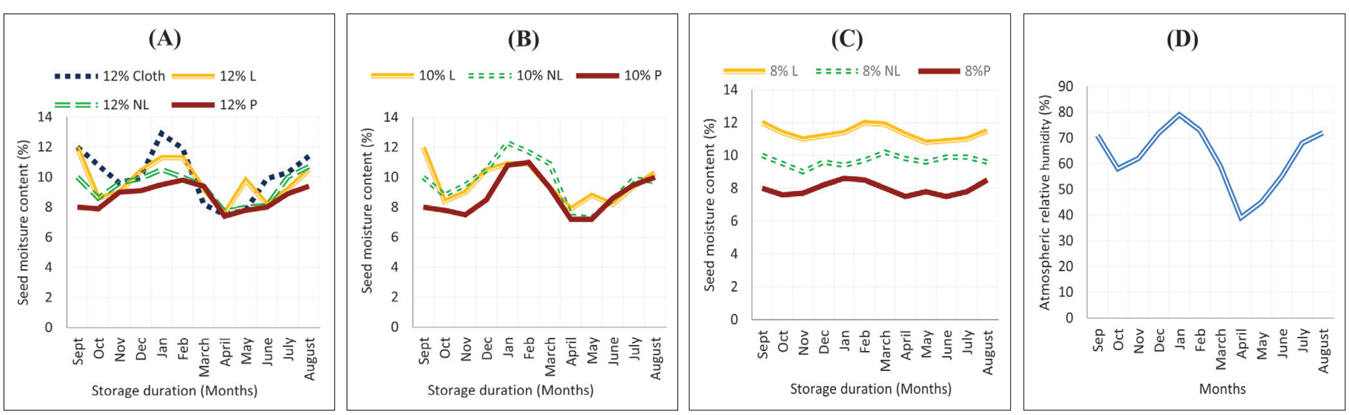

Fig. 1. Effect of different seed storage treatments on moisture content (%) of maize hybrid PMH 1 seeds over time (A, B, C). (A) Seeds with 12% moisture content stored in different packaging materials, (B) Seeds with 10% moisture content stored in different packaging materials, (C) Seeds with 8% moisture content stored in different packaging materials. (D), Mean atmospheric relative humidity from September 2020 to August 2021. Packaging materials: L = laminated polypropylene bags, NL = non-laminated polypropylene bags, P = HDPE bags.

five months after harvest and extending up to 16 months. A gradual decline in both germination percentage and seedling vigour index was observed as the storage duration increased. All seed storage treatments maintained germination rates above 90% for up to 13 months, thereby meeting the Indian Minimum Seed Certification Standards (IMSCS). However, no storage treatment was able to sustain germination above IMSCS beyond 13 months (Table 2). The observed decrease in seedling vigour index was associated with reductions in both germination percentage and seedling length during storage. Similar trends were reported by Shabana *et al.* (2015), who found that the germination percentage of the maize cultivar "Three Way Cross 324" declined with extended storage periods, regardless of the type of packaging material used, including paper, cotton cloth, woven polyethylene, cotton plus polyethylene, high-density polyethylene, and low-density polyethylene.

Germination speed and mean germination time

At the 5-month mark of seed storage, all seed packaging treatments exhibited the highest germination speed values. As the seeds aged, a gradual decline in germination speed was observed across all storage treatments (Table 2). On the other hand, the mean germination time was at its lowest at the 5-month storage period for the various packaging treatments, but it consistently increased with longer storage durations (Table 2). This trend is consistent with findings from Abdoli *et al.* (2015), who reported a significant increase in mean germination time with seed ageing in six maize hybrids subjected to accelerated ageing treatments.

Table 2. Effect of storage treatments on seed quality parameters of maize hybrid PMH 1 over different storage durations

Storage duration (Months)	Germination (%)	Speed germination	Mean germination time (Days)	Seedling vigour index
5 (September, 2020)	96.27	5.34	3.65	1770
6 (October, 2020)	96.20	5.18	3.80	1671
7 (November, 2020)	97.70	5.01	3.88	1601
8 (December, 2020)	96.73	4.98	3.95	1507
9 (January, 2021)	97.67	4.70	4.07	1462
10 (February, 2021)	96.67	4.45	4.31	1402
11 (March, 2021)	97.43	3.89	4.41	1331
12 (April, 2021)	97.33	3.68	4.46	1179
13 (May, 2021)	96.46	3.44	4.50	1085
14 (June, 2021)	84.93	2.94	4.52	950
15 (July, 2021)	83.07	2.74	4.70	854
16 (August, 2021)	78.10	2.31	4.83	754
SEm(±)	0.36	0.08	0.05	13.11
LSD ($p \leq 0.05$)	1.00	0.23	0.13	36.55
Storage treatments				
T_1	92.72	3.97	4.17	1272
T_2	93.61	4.00	4.26	1318
T_3	94.41	4.07	4.31	1330
T_4	93.17	4.04	4.24	1269
T_5	93.28	4.10	4.26	1298
T_6	93.39	4.08	4.31	1274
T_7	92.61	4.08	4.24	1297
T_8	92.61	4.04	4.33	1311
T_9	93.86	4.05	4.23	1324
T_{10}	92.31	4.10	4.29	1278
SEm(±)	0.33	0.07	0.04	11.97
LSD ($p \leq 0.05$)	NS	NS	NS	NS
Interaction LSD	NS	NS	NS	NS

Similarly, Garoma *et al.* (2017) observed an increase in mean germination time in four maize parental lines stored under ambient conditions.

Membrane leakage

There was a gradual increase in membrane leakage as the storage period extended, regardless of the packaging treatments. The lowest membrane leakage values for maize seeds were recorded at the 6-month storage period across all packaging types (Table 3). This rise in membrane leakage can be attributed to the deterioration of membrane integrity over time with seed ageing, as noted by Umesha *et al.* (2017). Bhandari *et al.* (2017) found that maize seeds of variety QPM1 stored in hermetic containers had lower electrical conductivity, indicating better membrane integrity, compared to seeds stored in fertilizer bags and earthen bins after 12 months.

α-Amylase activity

There was a consistent decrease in α-amylase activity with the prolongation of seed storage duration, regardless of the packaging treatments. Specifically, the activity of α-amylase decreased threefold after 15 months of seed storage compared to that observed at 6 months (Table 3). α-Amylase plays a crucial role in germination by breaking down complex carbohydrates like starch into simpler sugars, which are then transported to developing seedlings. The reduced α-amylase activity in aged seeds can be attributed to diminished expression of amylase genes, which is often linked to changes in the tissue's response to gibberellins (Bernal-Lugo *et al.*, 1999).

Antioxidant enzymes

There was a noticeable decline in the activity of three key antioxidant enzymes—superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)—as the storage period extended across all packaging treatments. The highest enzyme activities were observed at the initial storage period (6 months), while the lowest activities were recorded at the end of the storage period (15 months) (Table 3). These antioxidant enzymes are crucial for neutralizing reactive oxygen species generated during seed ageing. Their decreased activity over time suggests a reduced capacity to manage oxidative stress associated with prolonged storage. Radha *et al.* (2014) similarly reported a

Table 3. Effect of storage treatments on membrane leakage, α-amylase activity, and antioxidant enzyme activity in maize hybrid PMH 1 seeds over different storage durations

Storage duration (Months)	Membrane leakage (%)	α-amylase activity (mg maltose/ g FW)	SOD activity (ΔA/min/g FW)	POD activity (ΔA/min/g FW)	CAT activity (ΔA/min/g FW)
6	43.15	4.65	131.11	38.00	1.63
9	52.63	3.79	106.51	25.40	1.17
12	56.68	2.73	90.90	16.22	0.90
15	64.56	1.55	63.85	9.26	0.56
SEm(±)	1.06	0.10	2.53	1.38	0.04
LSD($p \leq 0.05$)	2.99	0.28	7.13	3.91	0.12
Storage treatments					
T_1	55.50	3.10	101.34	23.34	1.09
T_2	56.49	3.15	100.24	21.09	1.10
T_3	56.66	3.00	100.63	23.67	1.09
T_4	54.19	3.26	96.12	19.20	1.06
T_5	53.92	3.20	96.95	22.22	1.05
T_6	54.65	3.12	106.74	24.17	1.06
T_7	52.33	3.24	94.16	22.07	1.05
T_8	53.87	3.24	98.61	22.55	1.06
T_9	52.76	3.25	92.78	23.06	1.11
T_{10}	52.17	3.23	93.38	20.82	1.08
SEm(±)	1.67	0.16	3.99	2.19	0.07
LSD($p \leq 0.05$)	NS	NS	NS	NS	NS
Interaction LSD	NS	NS	NS	NS	NS

Table 4. Effect of storage treatments on biochemical reserves in maize hybrid PMH 1 seeds over different storage durations

Storage duration (Months)	Sucrose (mgg^{-1} FW)	Starch (mgg^{-1} FW)	Total free amino acids (mgg^{-1} FW)	Total soluble proteins (mgg^{-1} FW)	Oil (%)
6	21.25	336.12	0.55	3.49	4.26
9	17.00	304.74	0.82	2.97	4.16
12	12.09	271.01	0.94	2.62	4.02
15	7.93	227.59	1.25	2.36	3.76
SEm(±)	0.39	5.58	0.01	0.06	0.06
LSD($p≤0.05$)	1.09	15.75	0.02	0.17	0.16
Storage treatments					
T_1	13.84	262.18	0.87	3.07	4.05
T_2	14.94	255.29	0.81	3.07	4.03
T_3	16.31	271.87	0.90	2.85	4.05
T_4	15.22	293.75	0.91	2.72	4.02
T_5	13.21	282.99	0.83	2.89	3.99
T_6	13.48	287.93	0.88	2.70	4.08
T_7	12.90	311.75	0.96	2.81	4.04
T_8	14.59	290.04	0.83	2.94	4.08
T_9	14.49	287.88	0.94	2.92	4.08
T_{10}	16.25	304.97	0.95	2.59	4.09
SEm(±)	0.61	8.83	0.01	0.09	0.09
LSD($p≤0.05$)	NS	NS	NS	NS	NS
Interaction LSD	NS	NS	NS	NS	NS

gradual decline in peroxidase and amylase activity with increased storage duration in maize hybrid Hema under both natural and accelerated ageing conditions. Abdoli *et al.* (2015) also found that the activities of catalase, peroxidase, and superoxide dismutase were significantly lower in seeds subjected to accelerated ageing compared to non-aged seeds across six maize hybrids.

Biochemical reserves

The content of various biochemical reserves, including sucrose, starch, total soluble proteins, and oil, gradually decreased with an extended storage period. After 15 months of storage, the lowest levels of these reserves were recorded across all packaging materials, with no significant interaction effect observed between packaging types and storage duration. On the other hand, the total free amino acids increased as the storage period progressed (Table 4). The reduction in sucrose content over time results in a decreased availability of respiratory substrates crucial for seed germination (Lugo and Leopold, 1991). Starch, which is a major energy source for germination, also decreased due to hydrolysis by enzymes such as α-amylase, β-amylase, and α-glucosidase, and its subsequent use as a respiratory substrate. This reduction aligns with findings by Rehman *et al*. (2002), who noted decreases in starch content in maize grains stored for 6 months. Simic *et al.* (2007) also observed a decrease in oil content in maize, soybean, and sunflower seeds with extended storage periods. The increase in total free amino acids during prolonged storage is attributed to the breakdown of proteins, reflecting the ongoing biochemical changes within the seeds.

Protein profiling

Protein profiling of maize hybrid PMH 1 seeds, both fresh (1 month old) and 15 months old, stored in various packaging treatments, was conducted using SDS-PAGE. The analysis revealed electrophoretic bands corresponding to polypeptides with molecular weights of 10, 16, 18, 30, 33, 42, 44, 52, 62, and 74 kDa across all treatments. Notable differences in band intensities were observed among the packaging treatments. Specifically, the intensities of bands corresponding to polypeptides of 44 and 52 kDa were higher in fresh seeds compared to the 15-month-old seeds stored in different packaging materials (Fig. 2). Wu *et al.* (2011)

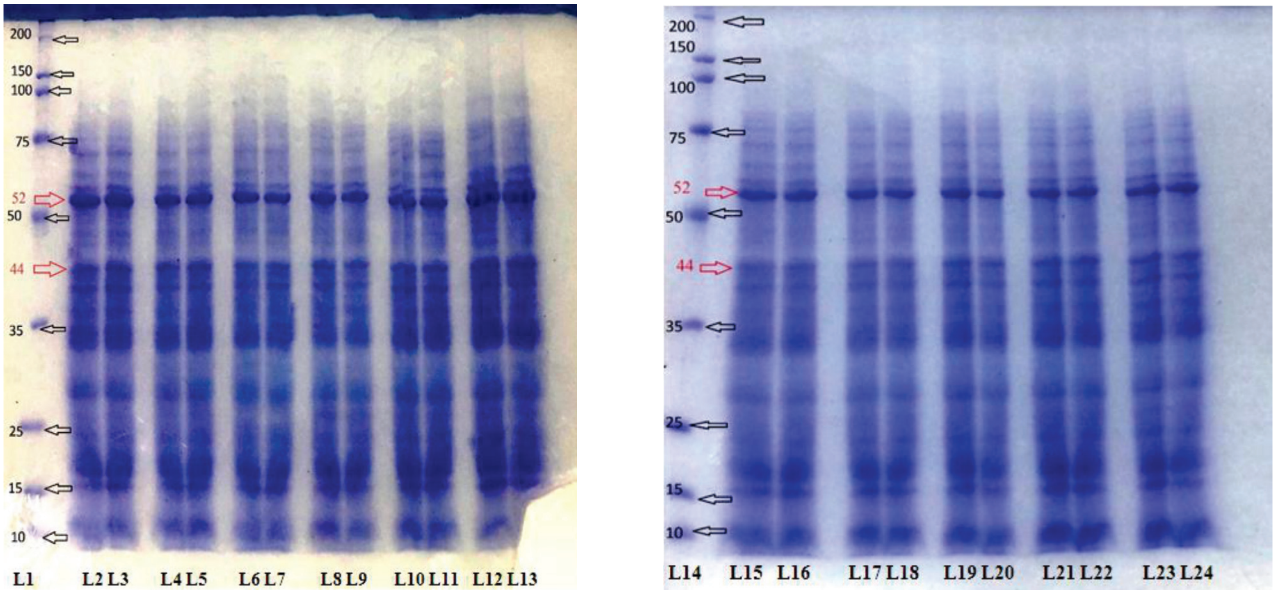

Fig. 2. SDS-PAGE Protein profiling of fresh (1 Month Old, L2-L3) and 15-month aged seeds (L4-L13 and L15-L24) of maize hybrid PMH 1. Lane 1 and 14: Molecular markers; Lane 2-3: Fresh seeds; Lane 4-5: Seeds with 12% moisture content stored in cloth bags (control); Lane 6-7: 12% moisture content, laminated polypropylene bags; Lane 8-9: 10% moisture content, laminated polypropylene bags; Lane 10-11: 8% moisture content, laminated polypropylene bags; Lane 12-13: 12% moisture content, non-laminated polypropylene bags; Lane 15-16: 10% moisture content, non-laminated polypropylene bags; Lane 17-18: 8% moisture content, non-laminated polypropylene bags; Lane 19-20: 12% moisture content, polyethylene bags; Lane 21-22: 10% moisture content, polyethylene bags; Lane 23-24: 8% moisture content, polyethylene bags. Protein patterns of embryos changed with artificial aging, with reduced protein abundance over time.

also reported changes in protein patterns with aging, noting a reduction in the abundance of the 12.2 kDa protein in accelerated-aged maize seeds.

CONCLUSION

During the *kharif* 2021 season, maize was cultivated over 144.6 thousand hectares in Punjab, requiring approximately 36,150 quintals of seed at a rate of 10 kg per acre. To package this amount of seed, around 7,23,000 bags of 5 kg capacity are needed. As detailed in Table 1, the cost per bag is Rs. 10.88 for cloth bags, Rs. 7 for laminated/non-laminated polypropylene bags, and Rs. 13.57 for HDPE bags. Therefore, the total cost for 7,23,000 cloth bags is approximately Rs. 78,66,240, while laminated/non-laminated polypropylene bags would cost about Rs. 50,61,000, and HDPE bags would cost around Rs. 98,11,110. All packaging treatments maintained germination rates above 90% for up to 13 months. However, polypropylene bags are more durable than HDPE bags due to their higher tensile and flexural strength, and they are also more cost-effective compared to cloth and HDPE bags. Therefore, laminated or non-laminated polypropylene bags are recommended as cost-effective alternatives to cloth and HDPE bags for short-term seed storage and sale, given their lower cost and superior strength.

Authors' contribution

Conceptualization and designing of the research work (NK, RS, GK, TPS); Execution of lab experiments (SK); Analysis of data and interpretation (SK, NK, SS, GK); Preparation of manuscript (SK, NK).

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