



Effects of seed moisture content, temperature, and storage period on various physiological and biochemical parameters of *Lallemantia iberica* Fisch. & C.A.Mey

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Abstract

Alterations in physiological and biochemical parameters due to unfavorable storage conditions cause delayed germination, decreased germination rate, and even loss of viability of stored seeds. Therefore, these alterations constitute major problems for seed scientists, farmers, seed bank practitioners, and seed companies involved in seed production and storage. This research aimed to understand the mechanisms of seed ageing by the changing physiological and biochemical properties of *Lallemantia iberica* seeds. The seeds were stored with different moisture contents (5, 10, and 15%) at different temperatures (15 °C, 25 °C, 35 °C, and 45 °C) for 0, 30, 60, 90, and 120 days. Seed germinability was reduced from 100 to 0% by increasing seed moisture content, temperature, and storage period, while membrane integrity, as assayed by electrical conductivity (EC), K^+/Na^+ of the seed leachates, and malondialdehyde (MDA), was gradually compromised. Seed deterioration was characterized by exacerbation of membrane impairment, reduction of total soluble sugar, α -amylase and β -amylase activities as well as catalase (CAT) and ascorbate peroxidase (APX) activities. The results indicated a negative correlation of EC, K^+/Na^+ , and MDA with germination indexes, vigor index, α -amylase and β -amylase activities, total soluble sugar, CAT, and APX levels. Generally, seed storage with 5% moisture content at 15 °C from 0 to 30 days had relatively high α -amylase and β -amylase activities, total soluble sugar, antioxidant enzyme activities, and low extents of membrane impairment. The results suggest that these storage conditions can be considered favorable for maintaining *L. iberica* seed quality; however, higher levels of the storage factors facilitate deterioration during storage.

Keywords Seed ageing · Germination · Antioxidant enzyme · Malondialdehyde · Total soluble sugar

Introduction

As important germplasm for plant propagation and breeding, seeds are the basic genetic resource for future agricultural innovations and developments (Chen et al. 2021). For this reason, preserving seed quality during storage is critical for the seed industry and farming community (Sperling et al. 2020). However, changes in seed moisture content, oxygen level, storage period and temperature during storage constitute major problems which may hasten seed ageing reactions and lead to the decline in viability and quality of seeds (Ebene et al. 2020). Inopportune storage conditions not only lead to alterations of biochemical and physiological indexes in stored seeds but also cause a decrease in germination ability and even death of the stored seed (Lin et al. 2022). Consequently, these changes affect seedling growth, establishment, and crop yield and have a significant economic impact (Kurek et al. 2019).

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The decrease in quality of seeds during storage in most species studied to date can be attributed to the reactive oxygen species (ROS) generation (Adetunji et al. 2021b; Lee and Hay 2020). Reduced germinability following storage or seed ageing may be associated with structural alterations in various organelles such as chloroplasts, mitochondria, the plasma membrane, peroxisomes, apoplast, the endoplasmic reticulum, and the cell wall (Garcia-Caparrós et al. 2021). Of these structures, mitochondria, due to the presence of the respiratory chain, are the largest center for ROS production (Klupczyńska et al. 2022) and play an important role in cell signaling (Małecka et al. 2021). The production of ROS such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), and hydroxyl radicals ($\bullet OH$), which are toxic oxidants cause biochemical, mechanical, and physical lesions in cell components of stored seeds (Adetunji et al. 2021c).

The ROS accumulated in storage attacks major biomolecules such as lipids, nucleic acids, proteins, polysaccharides, and metabolites. It leads to the loss of enzyme activity, alteration of the molecular structure of the nucleic acids (Kijowska-Oberc et al. 2021; Li et al. 2022; Stegner et al. 2022), cellular membrane disruption, and reduced respiration (Kurek et al. 2019). For instance, ROS-induced lipid peroxidation can cause severe degradation of the membrane, inactivation of enzymes, total loss of membrane-bound protein activities, and cell death (Adetunji et al. 2021a). Lipid peroxidation is culpably involved in the loss of viability of seed in many crop species (Małecka et al. 2021). In addition, it brings about mitochondrial swelling, increased membrane viscosity, and solute leakage of seeds during storage.

The main antioxidant defense systems is enzymic, and includes ascorbate peroxidase (APX), catalase (CAT),

peroxidase (POD), and superoxide dismutase (SOD). They effectively protect cells from oxidative injuries even when present in low quantity (Garcia-Caparrós et al. 2021). They can scavenge the over-accumulated ROS and maintain its dynamic balance in seeds (Lin et al. 2022). Some studies have reported a close relationship between seed ageing and a decline in antioxidant enzymes activity in *Fagus sylvatica* (Małecka et al. 2021), *Glycine max* (Lin et al. 2022), and *Avena sativa* (Sun et al. 2022) seeds. Usually, a decrease in these enzymes activity in seeds leads to a reduction in respiratory potential in aged seeds, resulting in a decrease in energy (ATP) production and food reserve for seed germination (Zhang et al. 2021). ROS-mediated degradation of fats, proteins, and carbohydrates under suboptimal storage conditions can inhibit the provision of energy for the activity of germination enzymes such as α -amylase and β -amylase (Adetunji et al. 2021c). Therefore, assessing physiological and biochemical processes remains paramount for giving insight into the complex mechanisms of seed ageing and the development of new methods for seed conservation under different storage conditions (Gerna et al. 2022; Zhang et al. 2021).

The present study selected *Lallemantia iberica* ((M.Bieb.) Fisch. & C.A.Mey) as sample species to elucidate further the ageing process of seeds at different moisture levels, temperatures and storage periods. *Lallemantia iberica* is an annual herb, belongs to the Lamiaceae family and is cultivated in Southwestern Asia and Europe. Because of their high mucilage and oil content, *L. iberica* seeds have high economic values, particularly to the food and pharmaceutical industries (Paravar et al. 2022). It has been reported that *Lallemantia* spp oil have high fatty acid components, especially linolenic

Table 1 Log worth of storage treatments effects on germination indices, seedling growth, and physio-chemical properties in *L. iberica*

Treatment	Log Worth		p-Value
Storage period	115.050		0.00000
Temperature	62.734		0.00000
Seed moisture content	61.306		0.00000
Seed moisture content × Storage period	24.266		0.00000
Storage period × Temperature	20.601		0.00000
Seed moisture content × Temperature	15.754		0.00000
Seed moisture content × Storage period × Temperature	2.666		0.00216

acid (67–74%), which possesses important beneficial health effects (Paravar et al. 2021). However, several seed lots of *L. iberica* are naturally aged due to inappropriate storage conditions by most farmers. Accordingly, the overarching aim of this study was to evaluate the ageing-induced physiological and biochemical changes in stored seeds of *L. iberica* under different moisture contents, temperatures, and storage periods.

Material and methods

Seed sample

Mature seeds of *Lallemantia iberica* Fisch. & C.A.Mey were collected from plants grown in November 2019 at the seed production site in Shahed University, Tehran, Iran. The seeds (approximately 5% moisture content) were stored at -5°C in tightly sealed containers prior to the experiments to avoid moisture variation.

Storage treatments

A completely randomized factorial design with three replicates was used to evaluate the effects of storage temperatures (15°C , 25°C , 35°C , and 45°C), seed moisture contents (5, 10, and 15%), and storage periods (0, 30, 60, 90, and 120 days) in *L. iberica* seeds.

Moisture content

For measurement of initial seed moisture, three replications of 5 g seeds were placed in an oven at 103°C for 17 h and reweighed. The moisture level was measured based on fresh weight. The initial seed moisture content (SMC) was approximately 5%. For higher moisture contents (10 and 15%), water was added to the seeds inside aluminium foil bags using Eq. (1): (ISTA 2013).

$$W_2 = \frac{(A - B)}{100 - A} \times W_1 \quad (1)$$

where A was the target moisture content, B was the initial SMC (%), W_1 was the initial weight of seed mass (g), and W_2 was mass of water to add. After that, approximately 18 g of seeds with different MC (5, 10 and 15%) were sealed in aluminium foil bags and kept in 10°C for a 24-h period to achieve moisture equilibration within the sample. Then, three bags of seeds (at least 400 seeds in each bag) at each target moisture content were used to determine the seed moisture content. Other seeds stored in bags were aged in incubators (Heraeus, Thermo Fisher Scientific Inc, Hanau, Germany) at 15, 25, 35 and 45°C . Samples were taken out every 30 days for up to 120 days.

Total soluble sugars

To determine the total soluble sugars, 0.3 g of dry seeds were homogenized with 10 ml of 80% ethanol, then placed in a water bath and heated at 80°C for 40 min. The homogenate was centrifuged at 12,000 g for 15 min. Total soluble sugars were determined spectrophotometrically at 490 nm (Steinfeld et al. 2015).

Enzymes activities of α -amylase and β -amylase

Enzyme extract was prepared with 400 mg of a mixture of endosperm and embryonic axis of the seeds from each treatment. Samples were frozen with liquid nitrogen and homogenized in 8 ml of 50 mM potassium phosphate buffer (pH 7.0) including 0.1 ethylenediaminetetraacetic acid, 2 mM phenylmethylsulfonyl fluoride, and 2% (w/v) polyvinylpyrrolidone. After centrifuging it at 12,000 g for 20 min at 4°C , supernatants were used to determine α -amylase and β -amylase (Bernfeld 1955).

Germination test after storage

The seed quality at different storage conditions was measured by a standard germination test (ISTA, 2013). Four 50-seed replication were used. The seeds were germinated on four layers of water-saturated filter paper in Petri dishes with a diameter of 90 mm at 10°C in a germination room, with a photoperiod of 8 h light: 16 h dark and 85% RH for 14 days (Paravar et al. 2018). Seed germination count was taken every day. Afterwards, the seed germination percentage (GP) and germination rate (GR) were calculated using Eqs. (2) and (3), respectively (Aghighi Shahverdi et al. 2019).

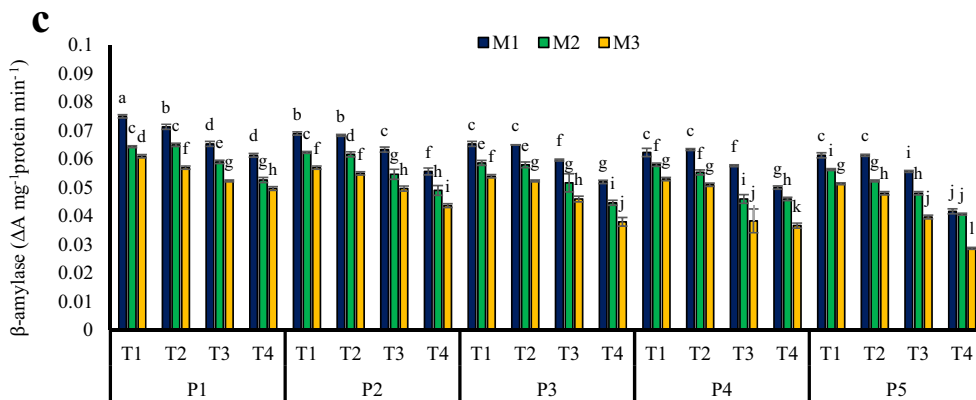
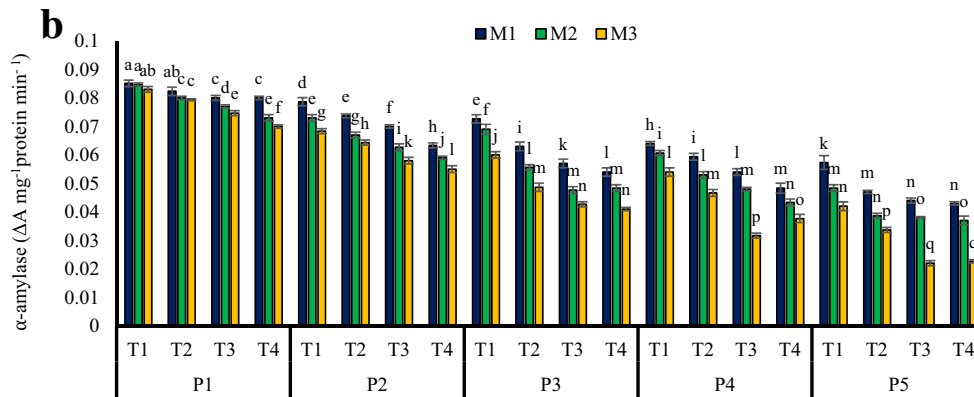
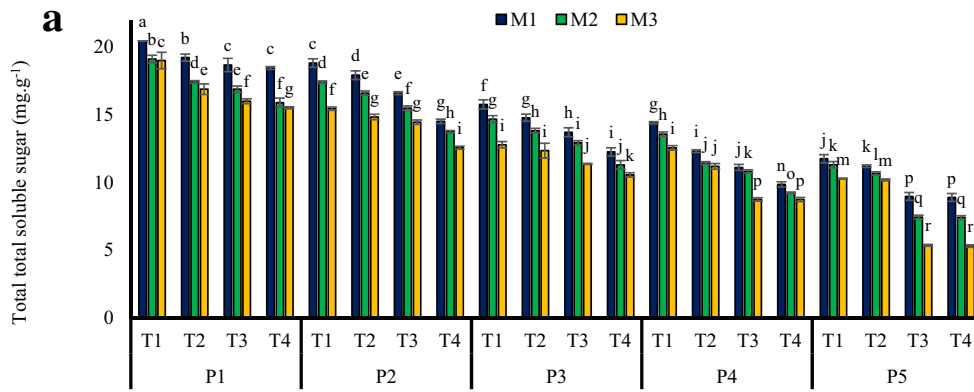
$$\text{GP} = (N \times 100)/M \quad (2)$$

$$\text{GR} = \sum N_i/T_i \quad (3)$$

where, N is the sum of germinated seeds at the end of the experiment, M is the total of placed seeds in a Petri dish, and T is the number of days after germination.

After germination, seedling length (SL) was manually determined with a ruler, the seedling dry weight (SDW) was measured after drying at 70°C for 24 h, and the vigor index (VI) was analyzed using Eqs. (4) (Aghighi Shahverdi et al. 2019).

$$\text{VI} = \text{GP} \times \text{SL} \quad (4)$$



Source of variation	Mean squares		
	TSS	α-amyl	β-amyl
Seed moisture (SM)	73.99**	0.002**	0.0026**
Storage period (SP)	436.81**	0.008**	0.0009**
Temperature (T)	110.74**	0.002**	0.002**
SM × SP	1.7**	0.00007**	0.000001n.s
SM × T	0.53**	0.00003**	0.0000158**
SP × T	3.05**	0.00004**	0.0000192**
SM × SP × T	0.73**	0.00001**	0.0000082**

Fig. 1 Effects of different storage treatments on total soluble sugar (a), α -amylase (b), and β -amylase enzyme activity (c) of *L. iberica*. The bars indicate means \pm SEM of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Seed moisture contents (M1: 5%, M2: 10%, and M3: 15%); Temperatures (T1: 15 °C, T2: 25 °C, T3: 35 °C, and T4: 45 °C); Storage periods (P1: 0, P2: 30 days, P3: 60 days, P4: 90 days, and P5: 120 days). The sources of variance for TSS, α -amylase, and β -amylase enzyme activity were as follows: seed moisture, storage period, temperature and interaction between three factors. *ns* no significant difference, **significantly different at 0.01 probability level. *Significantly different at 0.05 probability level. TSS: total soluble sugar; α -amyl: α -amylase; β -amyl: β -amylase

Antioxidant enzymes assays

To measure the activity of an enzyme, 20 mg seeds were homogenized into 20 ml of phosphate buffer (0.1 M, pH 7.0) using a mortar and pestle at 4 °C. The homogenate was centrifuged at 16,000 g for 20 min. Catalase activity (CAT; EC 1.11.1.6) was measured spectrophotometrically following H_2O_2 consumption at 240 nm (Bailly et al. 1998). The enzyme assay contained 10 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 200 μ L of enzyme extract. Ascorbate peroxidase (APX; EC 1.11.1.7) activity was measured at the absorbance of 290 nm (Nakano and Asada 1981). The reaction mixture contained 50 mM phosphate (pH 7.0), 1 mM sodium ascorbate, 2.5 mM H_2O_2 , and 50 μ L of enzyme extract.

Electrical conductivity

Electrical conductivity was performed using three replicates of 100 seeds in 50 ml deionized water after 24 h of soaking at 20 °C. Results are expressed as μ S mg^{-1} of dry seeds (ISTA 2013).

Inorganic ions

To analyze the inorganic ions such as potassium (K^+) and sodium (Na^+), 0.20 g of dry seed samples were ashed at 500–600 °C and afterwards dissolved in 2.0 M HCl. (Berry and Johnson 1966). The K^+ and Na^+ contents were analyzed using a flame photometer (BWB XP Technologies, Newbury, UK) (Stuffins 1967).

Malondialdehyde concentration

Malondialdehyde as a product of lipid peroxidation was analyzed. Two mg of seeds were homogenized with 5 ml of 0.5% (w/v) thiobarbituric acid (TBA) in 20% (w/v) trichloroacetic acid. The homogenate was incubated at 95 °C for 30 min, then cooled immediately and centrifuged at 16,000 g for 30 min. The supernatants were used to determine MDA (Heath and Packer 2018).

Data analysis

Data were subjected to normality and homogeneity tests with Minitab software (version 17). As the data had normal distribution and homogeneity of variances, no data transformation was performed. Data were subjected to a mixed analysis of variance (ANOVA) using SAS (SAS version 9.2, SAS Institute, Cary, NC, USA). Duncan's multiple range test (0.05%) was used to compare the sets of means. Size effects (Log-worth) and the correspondent p-values were additionally calculated. Origin Pro Software (OriginLab Corporation, Northampton, MA, USA) was also used to plot graphs and run Pearson correlation. Principal Component Analysis (PCA) was used to explore the correlations among measured variables and storage treatments.

Results

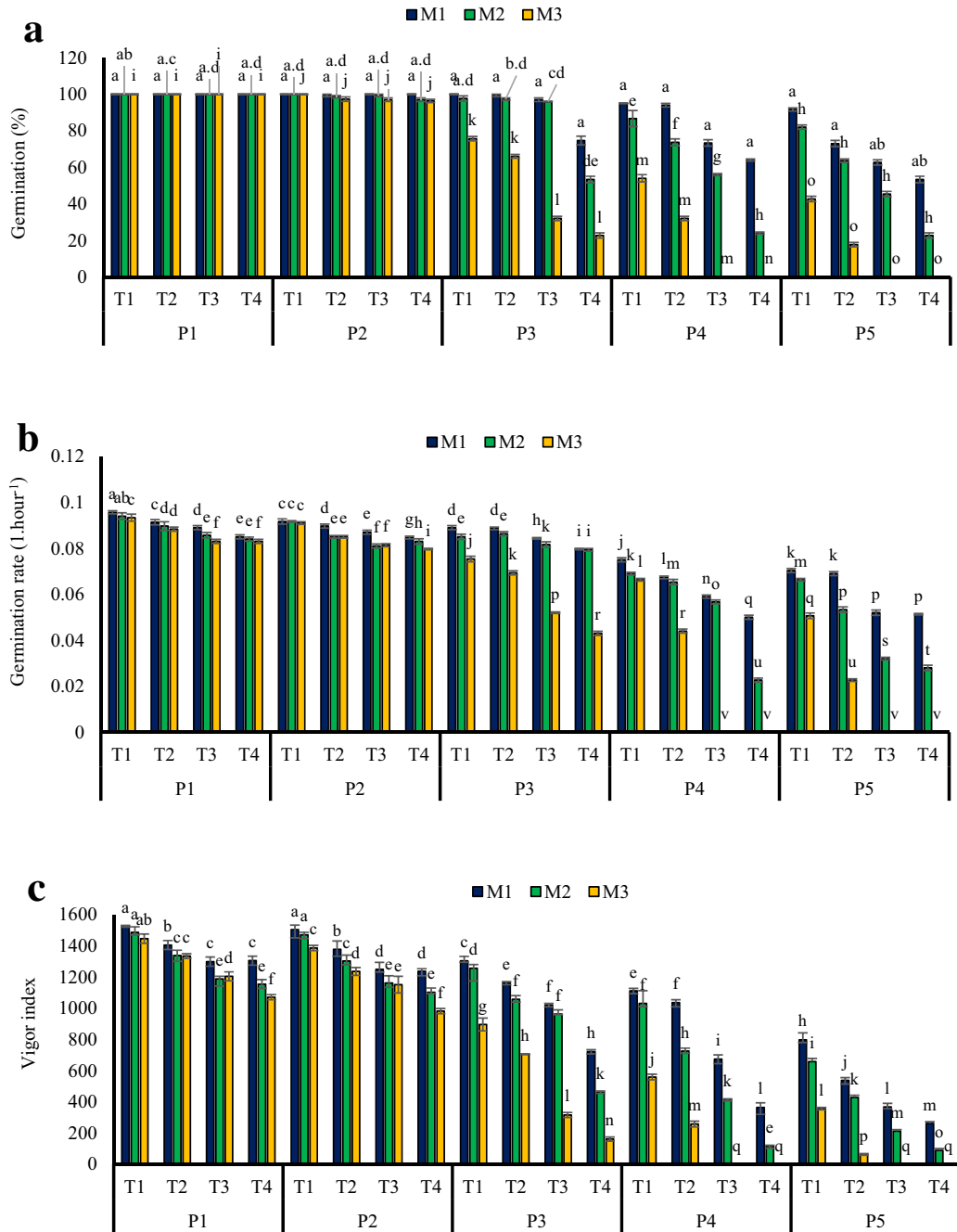
The effect of the interactions of seed moisture content, temperature and storage period on physiological and biochemical changes of *L. iberica* were significant ($p < 0.0001$, Table 1). Log-worth values showed that storage period had the greatest effect (log-worth = 115.05), followed by temperature (62.37) and SMC (61.30). Thus, the long-period storage caused higher changes in physiological and biochemical structures of stored seeds.

Total soluble sugar and amylase enzymes content

ANOVA results showed that total soluble sugar, α -amylase and β -amylase activities depended on seed moisture content, temperature, and storage period and the interaction between three factors (Fig. 1). Increasing SMC significantly reduced total soluble sugar levels of *L. iberica* seeds (Fig. 1a). The highest and lowest total soluble sugar levels were observed in stored seeds with 5% and 15% SMC, respectively. Total soluble sugar was gradually reduced in the stored seeds from 15 °C to 45 °C. Increasing the period storage caused the reduction of total soluble sugar. The α -amylase (Fig. 1b) and β -amylase (Fig. 1c) activities significantly decreased with increasing SMC, temperature of the storage environment, and storage period.

Germination and vigor index

ANOVA results showed that germination percentage, germination rate and vigor index depended on seed moisture content, temperature, and storage period and the interaction between three factors (Fig. 2). Generally, seed germination (Fig. 2a), germination rate (Fig. 1b), and vigor index (Fig. 2c) of *L. iberica* decreased with increasing ageing treatments. The germination percentage (Fig. 2a),



Source of variation	Mean squares		
	GP	GR	VI
Seed moisture (SM)	16504.8**	0.01**	1954174.55**
Storage period (SP)	21986.68**	0.02**	7018540.03**
Temperature (T)	6612.5**	0.01**	2269203.14**
SM × SP	2709.99**	0.001**	158736.75**
SM × T	406.41**	0.0005**	17251.03**
SP × T	1163.38**	0.0005**	79124.49**
SM × SP × T	95.88**	0.0001**	16390.23**

Fig. 2 Effects of different storage treatments on germination (a), germination rate (b), and vigor index (c) of *L. iberica*. The bars indicate means \pm SEM of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Seed moisture contents (M1: 5%, M2: 10%, and M3: 15%); Temperatures (T1: 15 °C, T2: 25 °C, T3: 35 °C, and T4: 45 °C); Storage periods (P1: 0, P2: 30 days, P3: 60 days, P4: 90 days, and P5: 120 days). The sources of variance for GP, GR, and VI were as follows: seed moisture, storage period, temperature and interaction between three factors. *ns* no significant difference, **significantly different at 0.01 probability level. *Significantly different at 0.05 probability level. *GP* germination percentage, *GR* germination rate, *VI* vigor index

germination rate (Fig. 2b), and vigor index (Fig. 2c) were lowest in stored seeds of *L. iberica* at highest SMC (15%) and temperature (45 °C). Similarly, the greatest reduction in germination percentage, germination rate, and vigor index was observed in 60 to 120 days of ageing treatments.

Seedling growth

Statistical analysis by ANOVA showed that seedling growth significantly decreased with rising seed moisture content, temperature, and storage period and as a result of an interaction between three factors (Fig. 3). The seedling length (Fig. 3a) and seedling dry weight (Fig. 3b) of *L. iberica* decreased as moisture content increased. Similarly, the seedling length and seedling dry weight of *L. iberica* were significantly decreased by the increasing temperature of the storage environment with the most reduced seedling length and seedling dry weight observed in seeds stored at 45 °C. The highest and lowest seedling length were observed in seeds stored for 0 and 120 days, respectively (Fig. 3a). Seed storage for 60 to 120 days significantly reduced seedling length (Fig. 3a) and seedling dry weight (Fig. 3b).

Antioxidant enzymes activities

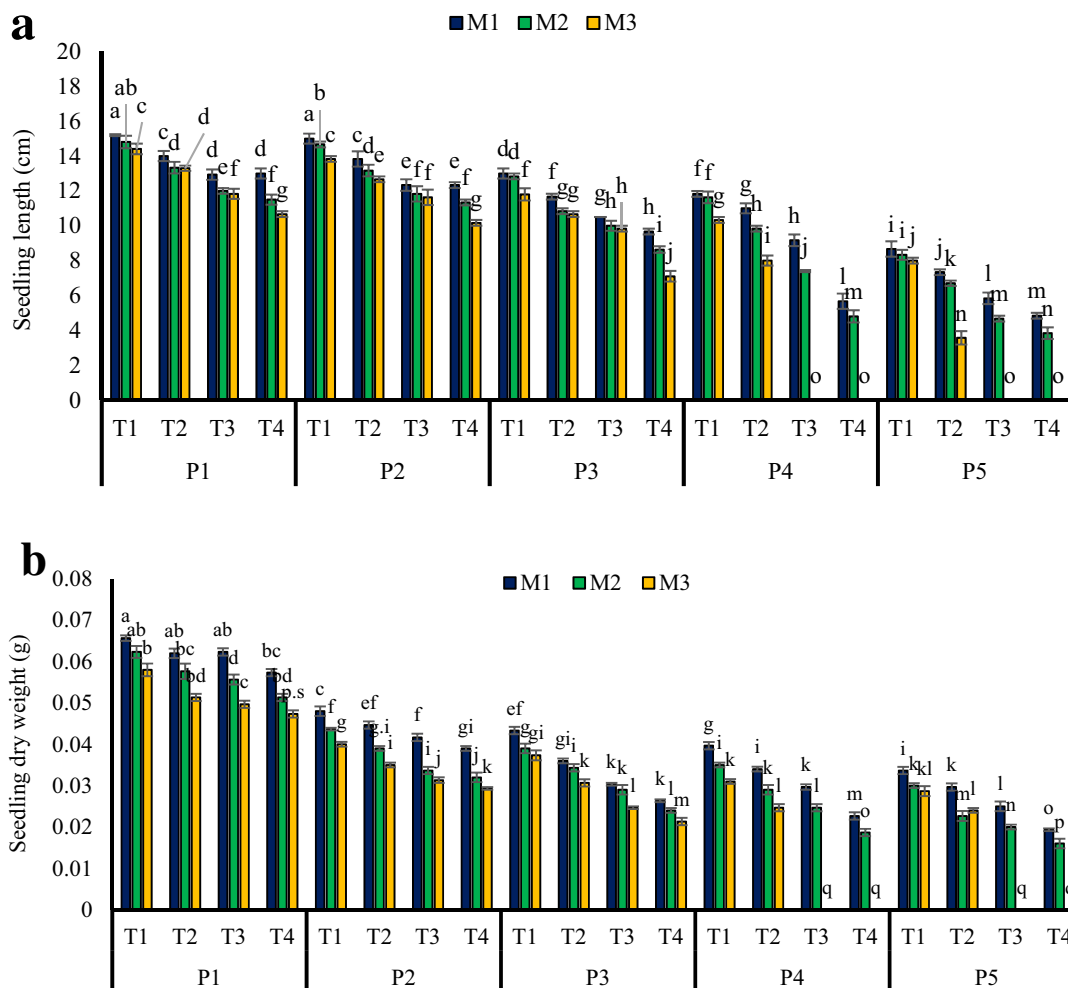
The statistical analysis showed that antioxidant activities decreased with rising seed moisture, temperature, and storage period and as a result of an interaction between three factors (Fig. 4). CAT activity (Fig. 4a) and APX activity (Fig. 4b) decreased with an increase in SMC of *L. iberica*. The highest and lowest activities of the antioxidant enzymes were found in *L. iberica* seeds stored at 5% and 15% SMC, respectively. The antioxidant enzymes activities were highest in seeds stored at lower temperature (15 °C), but decreased with increasing temperature of the storage environment. The results also showed that the enzymes activities strongly decreased from 60 to 120 days relative to shorter storage durations.

Biochemical indices

Statistical analysis by ANOVA indicated that EC, K^+/Na^+ , and MDA increased with rising seed moisture, temperature, and storage period and as a result of an interaction between three factors (Fig. 5). High SMC (10 and 15%) had the most significant effect on the increment of EC (Fig. 5a) and K^+/Na^+ (Fig. 5b) levels of the stored seeds of *L. iberica* compared with 5% SMC. Increases in EC and K^+/Na^+ were also observed in seeds stored at high temperatures. The highest and lowest EC and K^+/Na^+ values were recorded in seeds stored at 45 °C and 15 °C, respectively. By increasing storage period, EC and K^+/Na^+ were increased. These increases were greater in seeds stored for 60 to 120 days than those stored for shorter periods. With the lowest value recorded at 5% SMC, MDA concentration increased by increasing SMC to 10% and 15% (Fig. 5c). The MDA concentration was significantly lower at the lowest temperature (15 °C) than the other temperature treatments and highest under high temperatures of the storage environment. The MDA concentration significantly increased from 60 to 120 days, and the lowest and highest concentrations were observed in seeds stored for 0 and 120 days, respectively.

The correlation analysis, showed significant correlations of treatments. Across treatments, α -amyl showed the strongest positive correlations with total soluble sugar (TSS) ($r = 0.96$), as well as APX ($r = 0.96$), whereas β -amyl had the weakest negative correlation ($r = -0.50$) with MDA. Interestingly, GP was positively correlated with GR ($r = 0.95$), SL ($r = 0.90$), SDW ($r = 0.83$), VI ($r = 0.94$), α -amylase ($r = 0.85$), β -amylase ($r = 0.79$), TSS ($r = 0.83$), CAT ($r = 0.81$), and APX ($r = 0.87$). Moreover, K^+/Na^+ positively correlated with EC ($r = 0.84$), and MDA ($r = 0.65$) ($p < 0.01$). In contrast, EC, K^+/Na^+ and MDA exhibited a significant negative correlation with GP, GR, SL, SDW, VI, α -amyl, β -amyl, TSS, CAT, and APX ($p < 0.01$) (Fig. 6).

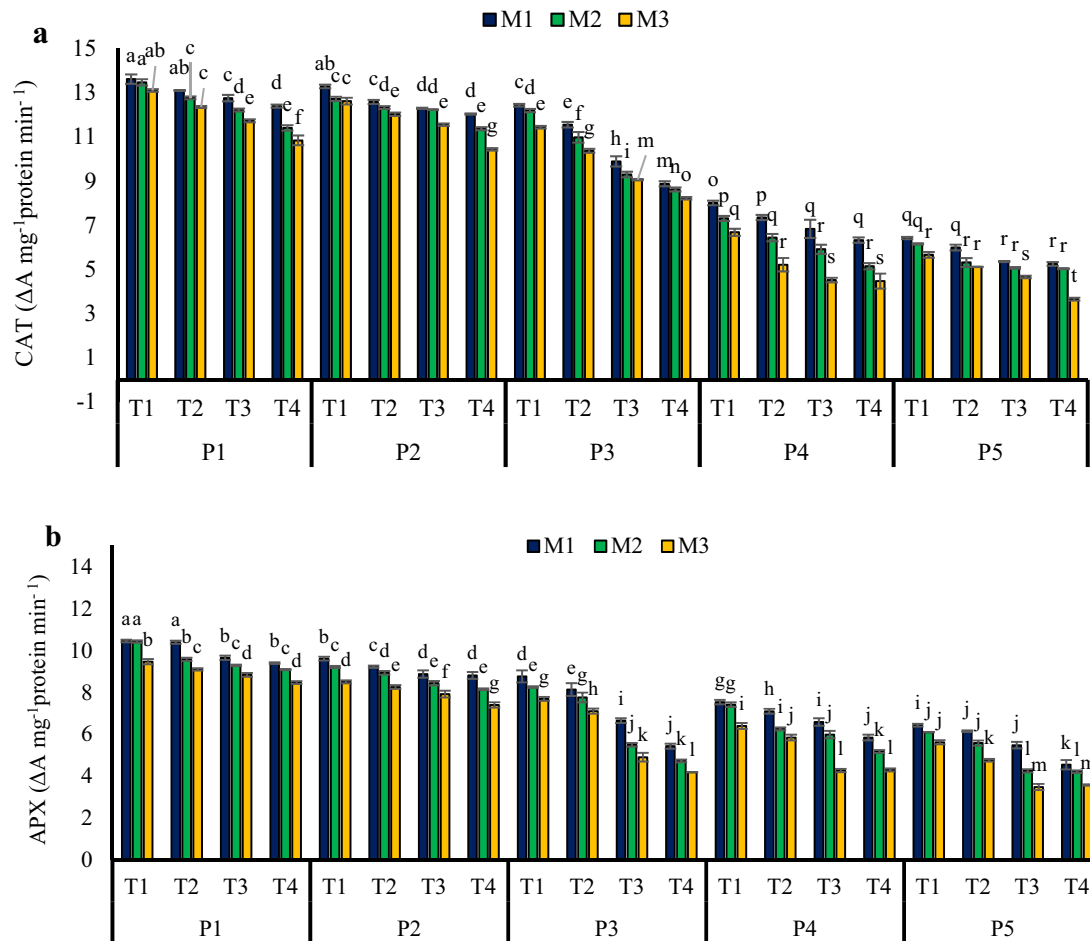
The correlation of storage treatments with changes in physiological and biochemical parameters in stored seeds of *L. iberica* is shown in Fig. 7. The PC-1 and PC-2 contributed 85.2% and 4.65%, respectively of the total variance, respectively. As shown, storage temperature (T) and storage period (SP) are positively correlated, while SMC shows no correlation with SP and a low correlation with T. Increasing temperature causes increase in K^+/Na^+ and EC, and MDA increased with lengthening storage period. Increasing SP and T had a negative influence on GP, β -amylase, VI, α -amylase and CAT, as higher temperatures and longer storage periods decrease the GP, β -amylase, VI, α -amylase and CAT contents.



Source of variation	Mean squares	
	SL	SDW
Seed moisture (SM)	94.19**	0.002**
Storage period (SP)	423**	0.007**
Temperature (T)	198.2**	0.002**
SM × SP	10.79**	0.0001**
SM × T	6.08**	0.0001**
SP × T	8.8**	0.0001**
SM × SP × T	2.71**	0.00003**

Fig. 3 Effects of different storage treatments on seedling length (a) and seedling dry weight (b) of *L. iberica*. The bars indicate means ± SEM of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan’s multiple range test. Seed moisture contents (M1: 5%, M2: 10%, and M3: 15%); Temperatures (T1: 15 °C, T2: 25 °C, T3: 35 °C, and T4: 45 °C); Storage periods (P1: 0,

P2: 30 days, P3: 60 days, P4: 90 days, and P5: 120 days). The sources of variance for SL and SDW were as follows: seed moisture, storage period, temperature and and interaction between three factors. ns: no significant difference; **significantly different at 0.01 probability level. *Significantly different at 0.05 probability level. *SL* seedling length, *SDW* seedling dry weight



Source of variation	Mean squares	
	CAT	APX
Seed moisture (SM)	19.25**	22.94**
Storage period (SP)	400.81**	122.45**
Temperature (T)	35.06**	33.31**
SM × SP	1**	0.18**
SM × T	0.24**	0.24**
SP × T	1.58**	2.23**
SM × SP × T	0.15**	0.13**

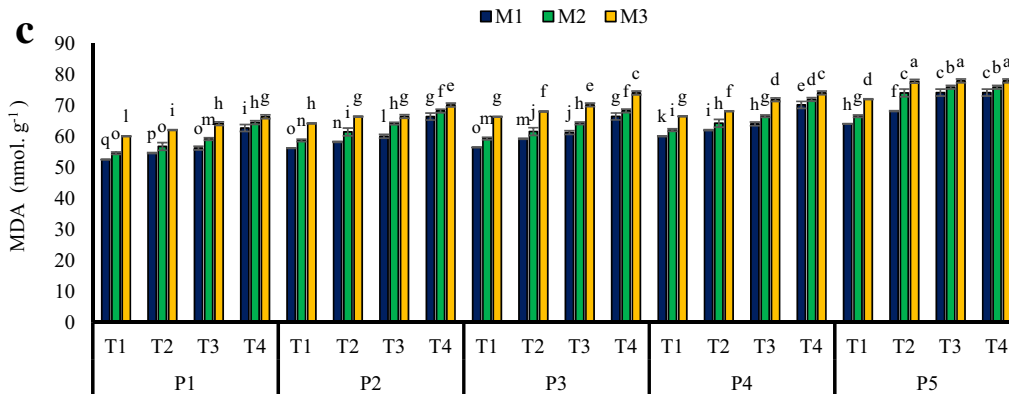
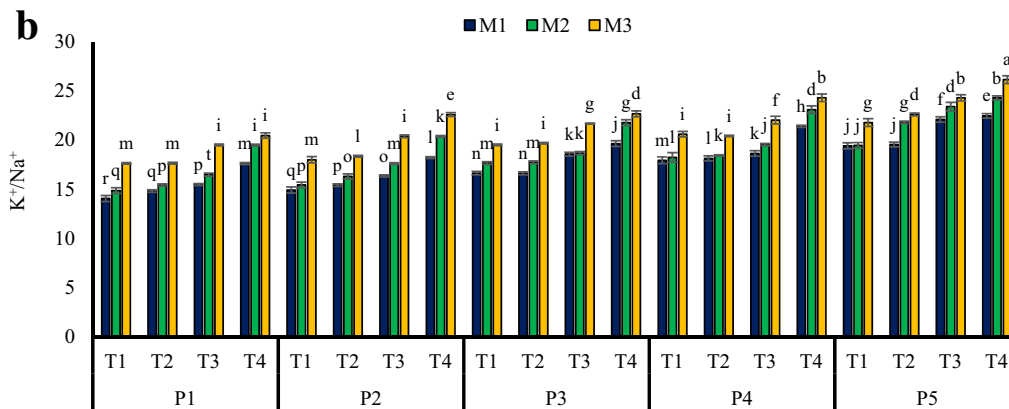
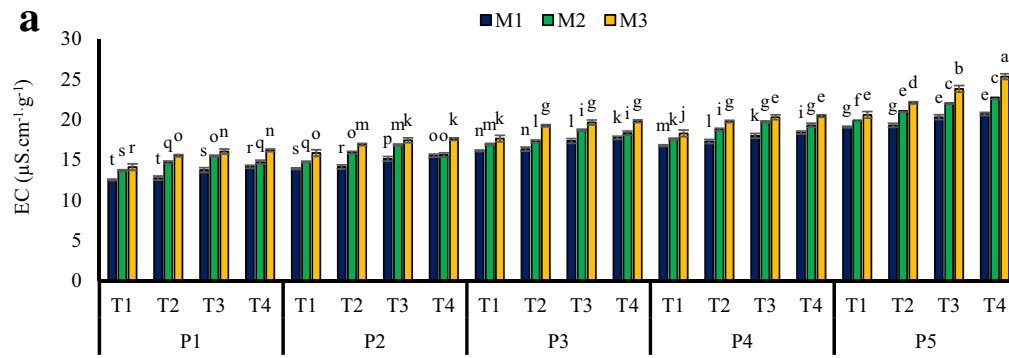
Fig. 4 Effects of different storage treatments on CAT (a) and APX (b) activities of *L. iberica*. The bars indicate means \pm SEM of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Seed moisture contents (M1: 5%, M2: 10%, and M3: 15%); Temperatures (T1: 15 °C, T2: 25 °C, T3: 35 °C, and T4: 45 °C); Storage period (P1: 0, P2: 30 days, P3: 60 days,

P4: 90 days, and P5: 120 days). CAT: catalase, APX: ascorbate peroxidase. The sources of variance for CAT and APX enzyme activity were as follows: seed moisture, storage period, temperature and interaction between three factors. *ns* no significant difference, **Significantly different at 0.01 probability level. *Significantly different at 0.05 probability level.. CAT catalase, APX ascorbate peroxidase

Discussion

Favorable storage conditions, including temperature, moisture content, and storage period are ideal for slowing down

the decline in seed quality (Adetunji et al. 2021b; Tian et al. 2019). In contrast, under inopportune seed storage conditions, physiological and biochemical changes may happen that may be reflected in quantitative or qualitative losses



Source of variation	Mean squares		
	EC	K ⁺ /Na ⁺	MDA
Seed moisture (SM)	67.16**	141.18**	220.47**
Storage period (SP)	263.75**	157.09**	922.37**
Temperature (T)	36.76**	143.16**	573.42**
SM × SP	1.54**	2.07**	20.34**
SM × T	4.11**	1.32**	108.95**
SP × T	0.84**	1.36**	11.36**
SM × SP × T	0.84**	0.75**	7.84**

Fig. 5 Effects of different storage treatments on EC (a), K+/Na+ (b), and MDA (c) in *L. iberica* seeds. The bars indicate means \pm SEM of three replicates. Different letters indicate significant differences ($P < 0.05$) by Duncan's multiple range test. Seed moisture contents (M1: 5%, M2: 10%, and M3: 15%); Temperatures (T1: 15 °C, T2: 25 °C, T3: 35 °C, and T4: 45 °C); Storage periods (P1: 0, P2: 30 days, P3: 60 days, P4: 90 days, and P5: 120 days). The sources of variance for EC, K+/Na+, and MDA enzyme activity were as follows: seed moisture, storage period, temperature and interaction between three factors. *ns* no significant difference, **significantly different at 0.01 probability level. *Significantly different at 0.05 probability level. *EC* electrical conductivity; *MDA* malondialdehyde

(Ziegler et al. 2021). Quantitative loss can cause changes in the metabolism of seeds and result in a decrease in dry matter. Qualitative losses, on the other hand, can result in chemical and enzymatic reactions. Generally, these alterations cause the loss of nutritional quality and germinability (Demito et al. 2019). The present study revealed highest loss of germinability in stored *L. iberica* seeds with highest SMC (15%), temperature (45 °C) and storage period (90 to 120 days) (Fig. 1a). The significantly reduced germination percentage, germination rate, and vigor index (Fig. 1a - c) were due to the heightened storage treatments (SMC, temperature, and storage period) as previously observed in *Glycine max* L. (Ebene et al. 2020) and *Pisum sativum* L. (Fatokun et al. 2022). These increases cause modifications in seed metabolism during storage (Adetunji et al. 2020).

Additionally, the decrease in germination indices may be related to the reduction of food reserves such as protein, starch, oil, carbohydrates (Sudhakaran 2020; Tian et al. 2019), as well as adverse changes at cellular level (Gerna et al. 2022). It has been reported that mitochondria are not only the center for the synthesis of cellular energy and material metabolism but also the first organelles that are injured by unfavourable storage conditions (Zhang et al. 2021). In contrast, the higher germination, germination rate, and vigor index in stored *L. iberica* seeds with 5% SMC for 0 to 30 days under a 15 °C storage environment may be related to the higher activities of antioxidant enzymes (CAT and APX) (Sudhakaran 2020) and starch hydrolyzing enzymes (α -amylase and β -amylase) (Silva et al. 2020).

The decrease in seedling length and seedling dry weight (Fig. 2a and b) of stored *L. iberica* seeds as a result of increasing temperature, SMC, and storage period may be related to the accelerated reactions of key biochemicals (lipid peroxidation and protein glycosylation) in seeds ageing (Adetunji et al. 2021c; Murthy et al. 2003). The increase in oxidative processes imposed by ROS accumulation under storage conditions influences the cellular and metabolic integrity and causes a decrease in germination vigor (Ebene et al. 2019). Furthermore, destroying the metabolic system of stored seeds inhibits seedling germination, development, and establishment (De Vitis et al. 2020).

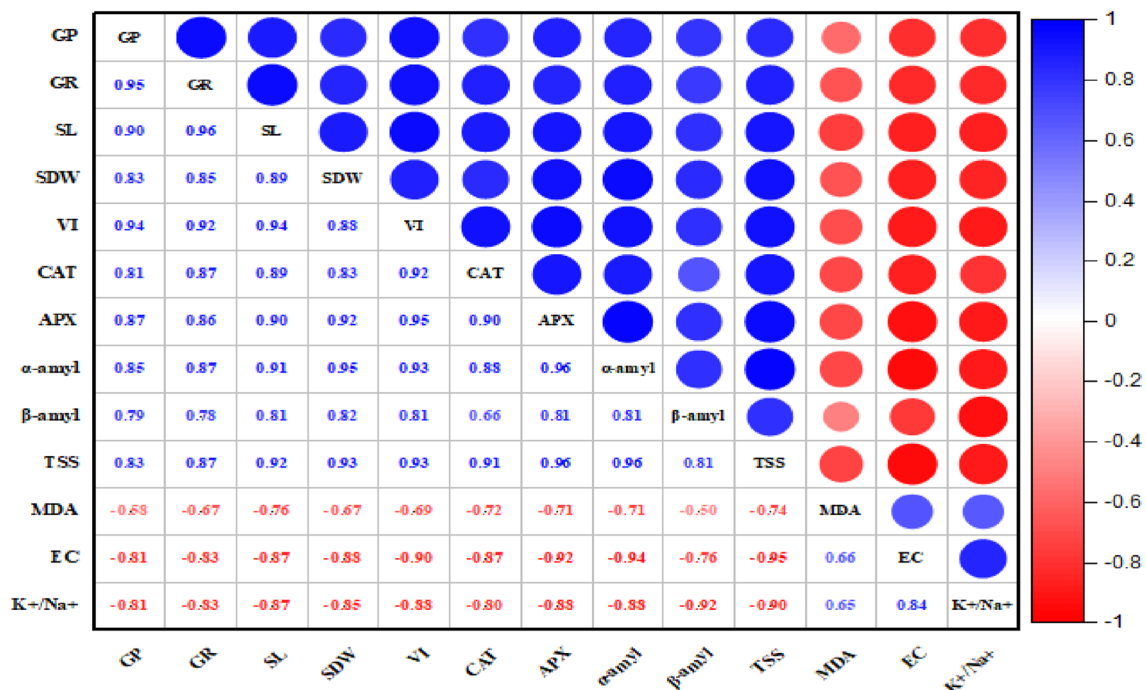


Fig. 6 Correlation analysis for evaluated traits. *GP* germination percentage, *GR* germination rate, *SL* seedling length, *SDW* seedling dry weight, *VI* vigor index, α -amyl α -amylase, β -amyl β -amylase, *CAT*

catalase, *APX* ascorbate peroxidase, *MDA* malondialdehyde, *EC* electrical conductivity, *TSS* total soluble sugar

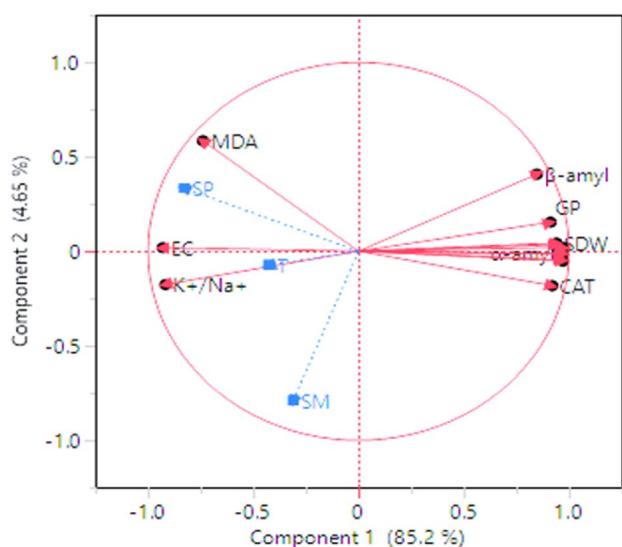


Fig. 7 Principle component analysis for studied traits. *SP* storage period, *T* temperature, *SM* seed moisture, *GP* germination percentage, *SDW* seedling dry weight, *α-amyl* α-amylase, *β-amyl* β-amylase, *CAT* catalase, *MDA* malondialdehyde, *EC* electrical conductivity

In this study, total soluble sugar presented obvious decreasing tendencies with increasing SMC, temperature, and storage period (Fig. 3a). A decline in total soluble sugar might be due to the changes in the glassy state of cytoplasm in mitochondria (Boniecka et al. 2019). It has been shown that increased moisture content or temperature during seed storage can lead to the transition of glassy state of cytoplasm to fluid state by hydrolysis of soluble sugars, mainly the raffinose family oligosaccharides (RFOs) such as sucrose, raffinose, stachyose, and verbascose (Gianella et al. 2022). Increasing of oligosaccharides hydrolysis leads to the formation of reducing sugars like fructose, glucose and galactose (Mathias et al. 2019). Accordingly, the reducing sugars provide the driving force for the Maillard reactions, which initiate non-enzymatic protein degradation during seed ageing (Chen et al. 2022). The high total soluble sugar in *L. iberica* seeds stored with 15% SMC at 45 °C on day 120 may be due to glassy states of cytoplasm, which decreases respiration rate, mitochondrial activity, and other biochemical reactions that could bring about seed deterioration (Zhang et al. 2021). Soluble sugars can play an important role in seeds via stabilizing proteins during storage by helping seeds protect their physiological function (Nagel et al. 2019). It appears that soluble sugars contribute to maintaining seed cell membrane integrity from damage caused by increases in moisture content by permitting lipids to be in a fluid state during the desiccation phase when the seed is in the glassy state. Eventually, soluble sugars provide the high viscosity which may slow down deleterious reactions, protecting the cells against deterioration (Matilla 2021).

The decline in α-amylase and β-amylase activities in stored seeds by increasing SMC, temperature, and storage period (Fig. 3a, b) might be due to the inhibition of Krebs' cycle in mitochondria for the production of ATP (Suzuki et al. 2022). This cycle produces two essential electron carriers such as NADH and FADH, and also succinate for the transport of electrons (Kang et al. 2021). With the transportation of electrons, ATP is produced by pumping hydrogen protons or ions into the inter-membrane space of mitochondria (Gasmi et al. 2021). It has been revealed that ATP is the main source of energy for the activation of enzyme activities during seed germination (Farooq et al. 2018). Thus, the reduction of ATP in the mitochondria during storage indicate the seeds' deterioration (Klupczyńska et al. 2022). Further, α-amylase and β-amylase activities in stored *L. iberica* seeds with 5% SMC at 15 °C on day 0 is probably related to lower ROS accumulation and higher mitochondria ATP (Xin et al. 2014).

In this study, increased SMC, temperature, and storage period suppressed CAT and APX activities (Fig. 4a and b). Nevertheless, the greatest antioxidant enzyme activities were observed in stored *L. iberica* seeds with 5% SMC for 0 to 30 days at 15 °C storage environment. The reduction of CAT and APX activities under heightened seed storage treatments may be ascribed to increased ROS production, leading to oxidative stress (Lin et al. 2022). Previous studies have shown that a decrease in antioxidant enzyme activities can be caused by mitochondria's vulnerability to oxidative stress (Lima et al. 2021; Sun et al. 2022). Accordingly, the reduction of mitochondrial integrity results in the leakage of H₂O₂ from mitochondria to the cytosol during storage (Małocka et al. 2021). Therefore, excessive production of ROS and impaired antioxidant system during seed ageing cause the physiological imbalance in the seeds, resulting in lipid peroxidation, protein carbonylation, damage of genetic, and loss of viability (Zhang et al. 2021). The report by Tian et al. (2019) indicated that increasing antioxidant enzyme activities prevented the cellular ageing of *Triticum aestivum* seeds by inhibiting the formation of O₂^{•-} and H₂O₂ and the breakdown of harmful toxins (Tian et al. 2019). Besides, antioxidant enzymes play an important role in regulating the stability and integrity of cell membranes (Klupczyńska et al. 2022). Moreover, similar results have been reported for *Nicotiana tabacum* (Li et al. 2018) and *Brassica napus* (Boniecka et al. 2019), indicating that seed storage with lower SMC under lower temperature for a short storage period can be effective on seed viability, which is likely related to the minimum accumulation of ROS and relatively increased antioxidant enzyme activities (Zhang et al. 2021).

From our results, the highest EC, K⁺/Na⁺, and MDA levels were observed in *L. iberica* seeds stored with 15% SMC at 45 °C for 120 days (Fig. 5a–c). Similar results were previously reported in *Triticum aestivum* L by Tian et al. (2019) and

on *Dipteryx alata* L. (Silva et al. 2020). The increase in EC, K^+/Na^+ , and MDA levels in stored *L. iberica* seeds might be related to peroxidative stress and impairments (Li et al. 2022). Lipid peroxidation might cause the membrane integrity compromise and ionic homeostatic loss (Ebone et al. 2019). In addition, increased accumulation of ions and increased EC in the stored *L. iberica* seeds under the harsh storage conditions can indicate the destruction of membrane structure, dysfunction of mitochondria and programmed cell death (Xia et al. 2020). It seems that lower EC, K^+/Na^+ , and MDA concentrations in the stored seed with 5% SMC at 15 °C for 0 to 30 days may be due to the maintenance of ROS balance and cellular homeostasis by antioxidant enzyme activities (Kurek et al. 2019). Lower accumulation of ROS in mitochondria allows for a reduction in membrane dysfunction as well as a decrease in oxidative damage to the mitochondria protein and DNA (Kluczyńska et al. 2022).

High germination and vigor were strongly connected with increased antioxidant enzymes and amylase activities, as well as low solute leakage and lipid peroxidation, as indicated by the correlation analysis. Nevertheless, the negative correlation of EC, K^+/Na^+ , and MDA with germination indices suggests that *L. iberica* seed deterioration may be linked with the destruction of mitochondria by ROS accumulation as well as membrane impairments due to lipid peroxidation. Higher antioxidant enzyme activities helps to scavenge ROS accumulated in mitochondria and protect the mitochondrial membrane integrity (Kurek et al. 2019). Overall, antioxidants enzyme provides energy supply for seed germination by maintaining mitochondrial structure (Li et al. 2022; Małeczka et al. 2021). In principal component analysis (PCA), on the other hand, MDA concentration, EC and K^+/Na^+ were more significantly correlated with storage period and temperature compared with seed moisture content (Fig. 6).

Conclusion

The overall value of seed for planting is mainly reflected in its quality, which is thus regarded as a fundamentally significant agronomic characteristic. High seed quality is required for crop production to be cost-effective and sustainable in all farming settings, from resource-constrained to industrialized agriculture. This study examined the effects of seed moisture content, temperature, and storage period on various physiological and biochemical parameters in *L. iberica*. The study highlighted the significance of appropriate storage conditions for preserving the vigor and viability of *L. iberica* seeds. The findings suggest that increasing seed moisture, temperature, and storage period significantly reduces total soluble sugar and amylase enzyme activity in *L. iberica* seeds. The accumulation of MDA, EC, and K^+/Na^+ during the

storage promote membrane impairment, cell aging, and reduce seed vigor, germination and seedling growth in *L. iberica*. However, the shelf life of *L. iberica* seeds can be prolonged when stored at low seed moisture content (5%) and low temperature (15 °C), accompanied by increasing antioxidant defense activity, particularly CAT and APX, and decreasing lipid peroxidation. Thus, while the seed quality can be maintained under these conditions, higher levels of these storage factors facilitate deterioration during storage.

Author contributions statement AP and SMF designed the concept study. AP and RP: carried and executed the experimental work. AP and AEA: jointly wrote the manuscript. AP, MO and RP: contributed in planning, statistical analysis. All the authors have approved the manuscript and agree with the submission.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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