

Influence of seed moisture content and storage period on germination and biochemical indices: *Lallemantia iberica* and *Lallemantia royleana*

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Article

Keywords: APX, CAT, Germination, H₂O₂, Seed deterioration

Posted Date: April 17th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4053248/v1>

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on February 6th, 2025.

See the published version at <https://doi.org/10.1038/s41598-025-88881-w>.

Abstract

The longevity of seeds varies greatly between species and seed viability reduction due to seed ageing is one of the major problems affecting agricultural productivity. To comprehend the mechanisms involved in the ageing, seeds of two plant species dragon head (*Lallemantia iberica*) and lady's mantle (*Lallemantia royleana*) and with 5, 15 and 25% seed moisture content (SMC) were tested for 24 and 48 hours storage period (SP) at 40°C. Increased SMC and SP significantly reduced germination percentage (GP), protein content, catalase (CAT) and ascorbate peroxidase (APX) enzyme activity. During storage, most significant deterioration was observed in *L. iberica* seeds showing lower GP, protein content, CAT and APX enzyme activities. As a result, the cell membrane of *L. iberica* seeds was damaged, resulting in an increase in electrical conductivity (EC), hydrogen peroxidase (H₂O₂) and malondialdehyde (MDA) contents compared to *L. royleana*. Overall, the lowest deterioration was obtained in stored seeds of both *Lallemantia* species by 5% SMC and 24 h SP; in contrast increasing of SMC and SP induced the faster deterioration of *Lallemantia* seeds. Furthermore, *L. iberica* deteriorates rapidly by rising of SMC and SP in comparison with *L. royleana*.

Introduction

Seeds that are not properly stored gets deteriorated. Seed deterioration during storage reduces viability, which is a major problem affecting the production and productivity of agricultural crops (Zhang et al. 2021). The seed deterioration process depends on seed moisture, temperature and oxygen level (Baladi and Balouchi 2016). Some researches indicated that viability and germination in seeds of *Lallemantia royleana* (Baladi and Balouchi 2016) and *Lallemantia iberica* (Paravar et al. 2018) at 25, 35, and 45 °C under different relative humidity with various moisture content decreased and concluded that deterioration went up at high temperature, relative humidity and moisture content. Storage conditions of seeds affect germination indices and seed vigor (Gerna et al. 2022). Success in germination depends on plant antioxidant mechanisms during germination (Paravar et al. 2018). The seed deterioration results in accumulation of reactive oxygen species (ROS) where mitochondria is the main source of ROS in the plant cells (Zhang et al. 2021). Changes in the process of oxidation and the level of oxidative radicals are considered as common events of environmental stress and ageing process (Ebone et al. 2019). Studies conducted in various species also reveal the increase in oxidative damage with seed age and increase in ageing free radicals by reducing the effectiveness of the cell antioxidant defense (Zhang et al. 2021)

Production of hydrogen peroxide by mitochondria is strongly controlled by enzymatic and non-enzymatic antioxidant systems (Paravar et al. 2018). It was reported that antioxidant enzymes play a significant role in controlling the plant hydrogen peroxidation (Hu et al. 2020). The main antioxidant enzymes in plants viz., catalase (CAT) and ascorbate peroxidase, are necessary to remove toxic hydrogen produced by hydrogen peroxidation (Ebone et al. 2020). Increased accumulation of hydrogen peroxide indirectly increases lipid peroxidation. Lipid peroxidation refers to the production of poly unsaturated fatty acids by free radicals in mitochondria (Hu et al. 2020).

Malondialdehyde is a product of lipid peroxidation index, which can react to eliminate or reduce the cell performance (Xia et al. 2017). Biotics (fungi, bacteria, protists, viruses) and non-biotics stress (climatic conditions and soil properties) increases the content of malondialdehyde and hydrogen peroxide in wet tissues causing severe damage to the functioning and structure of the mitochondrial membrane (Paravar et al. 2021b). Researchers found that seed deterioration under the conditions of high moisture and temperature reduces the seed life by increasing the membrane phospholipids (Zhang et al. 2021).

Lallemantia sp is a herb belonging to the *Lamiaceae* family with five species, the most important of which are *Lallemantia iberica* and *Lallemantia royleana* (Paravar et al. 2021b). The seeds of *Lallemantia* species contain mucilage and oil is being used in the treatment of neurological disorders, liver and kidney diseases, works as sexual stimulant and mucus is used in local Iranian medicines (Paravar et al. 2021a; Paravar et al. 2023). *Lallemantia iberica* is cultivated for oil extraction and contains > 30% of the dry weight of grain and > 68% of the oil is linolenic acid (Paravar et al. 2022b; Paravar et al. 2021b). High unsaturated fatty acids in *Lallemantia* seeds (Paravar et al. 2022a) may increase lipid peroxidation and causes seed deterioration (Paravar et al. 2018). On the other hand, there has been no specific literature on seed storage of both *Lallemantia* species. Therefore, we investigated for the first time the effects of moisture content and storage period of *Lallemantia iberica* and *Lallemantia* seeds on germination and biochemical indices.

Materials and methods

Plant material and treatment

Seed samples

Lallemantia iberica and *Lallemantia royleana* seeds (Table 1) were used in this study. The seeds were procured from Pakan Bazr Company, Iran in the harvest season of Jun 2021. The seeds (approximately 6% of moisture content) were stored at -5 °C for two months in tightly sealed containers prior to the experiments to avoid moisture variation.

Determination of initial seed moisture content (SMC)

The SMC was determined (on August 2021) in accordance with international seed testing association (ISTA) (ISTA 2013). Approximately 4.5 g of seeds were dried at 103 °C for 17 hours, then placed in desiccators at (25 °C) for 40 min.

Seed ageing treatments

Seed moisture content (SMC) was set at its initial value (approximately 5%). For higher moisture contents (15 and 25%), water was added to the seeds (ISTA 2013). The seed moisture content (SMC) was calculated using the formula: $W_2 = \frac{(A-B)}{100-A} \times W_1$ where A is the desired, B is the primary moisture content, W_1 is the initial mass of seed (g), and W_2 is the required distilled water (μl). Approximately 25 g

of seeds with different SMC (5, 15 and 25%) were sealed in aluminum foil bags and were kept at 10 °C to achieve moisture equilibration. After that, bags of seeds were kept in oven (40 °C) for 24 and 48 hours.

Germination assays

Germination tests were performed with 50 seeds in three replications in Seed Science Laboratory of Shahed University. Seeds were placed in 9 cm diameter petri dishes on Whatman No. 1 filter paper and 10 ml distilled water was added to each plate. Germination test was performed at 10°C, 16/8 h (light/darkness), and 85% RH for 14 days (Paravar *et al.* 2018). Seeds were considered germinated when radicals were at least 2 mm in length (Paravar *et al.* 2018).

Electrical conductivity assays (EC)

To determine EC, 50 seeds were weighed and then soaked in 520 mL deionized water at 20 °C under dark conditions for 24 hours. Results were expressed as μS per one g of dry weight (DW) (ISTA 2013).

Hydrogen peroxide (H_2O_2) content

The dry seed (0.07 g) was extracted with 4 mL trichloroacetic acid 0.1% (w/v) (TCA) containing 10 mM EDTA. The homogenate was centrifuged at 11,000 rpm for 15 min at 4 °C, and the clear supernatant was then mixed with 0.2 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of KI (1 M). The samples were placed in dark for one hour, then the total volume of supernatant was analyzed at 390 nm (Loreto and Velikova 2001).

Lipid peroxidation (MDA) content

The content of MDA was measured using thiobarbituric acid (TBA)-reactive substance (Heath and Packer 1968). The samples of seed (1 mg) were homogenized in TBA (4 ml of 0.5% w/v) and 20% (w/v) trichloroacetic acid and heated at 100°C for 35 min, then quickly cooled and centrifuged at 11,000 rpm for 15 min. Finally, the absorbance of samples was measured at 532 nm and 600 nm

Enzyme extraction and assay

For extraction of enzyme and assay, dry seeds (2 mg) were ground in 5 mL solution of 50 mM phosphate buffer (pH 7.0), 2 mM α -dithiothreitol, 0.1 mM EDTA. The samples were centrifuged at 15,000 rpm for 20 min at 4 °C (Bailly *et al.* 1998). For measuring the activity of catalase (CAT, EC 1.11.1.6), 10 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0) and 200 μL of enzyme extract were used. The reaction was measured at 240 nm (Nakano and Asada 1981). For measuring ascorbate peroxidase (APX, EC 1.11.1.11) activity, the reaction buffer solution included 50 mM phosphate buffer (pH 7.0), 0.5 mmol/L ascorbic acid, 0.1 mM H_2O_2 in a final volume of 0.5 ml. The reaction was read at 265 nm every 15 sec (Nakano and Asada 1981).

Soluble protein content

For extraction of soluble protein content, 250 mg seeds were powdered in 5 ml solution of 50 mM phosphate buffer (pH 7.0). Supernatant were centrifuged at 15,000 rpm for 15 min at 4 °C. In order to prepare the protein reagent, 100 mg coomassie brilliant blue G-250 was dissolved in 50 mL ethanol (95%) and 100 mL phosphoric acid (85%). The protein reagent (5 mL) was mixed with 0.2 mL protein extract. Then the reaction was measured at 595 nm. Bovine serum albumin was used as a protein standard (Aluko and Yada 1997).

Experimental design and statistical analysis

The experiment was carried out in a completely randomized design in a factorial scheme with three replications. The number of germinated seeds at the end of the germination period (14 days) was used in the analysis of variance by transforming with the function $y = \arcsin p/100$, where p is the germination percentage. Parameters related to germination, such as GP, was measured by the software package of Seed Calculator V3.0 (Plant Research International, Wageningen, The Netherlands). Analysis of variance (ANOVA) was determined by SAS for Windows Ver. 9.2. Duncan's multiple range test (0.05%) was used for mean difference comparison. The correlation charts and heat map between traits were generated using JMP Pro 15.

The study is in accordance with relevant institutional, national, and international guidelines and legislation.

Results

Germination percentage

The results of analysis of variance in *L. iberica* (Table 2) and *L. royleana* (Table 3) showed significant interaction of SP and SMC on germination percentage. Increasing SP and SMC reduced germination percentage in both species of *Lallemantia*. The reduction in germination percentage was very high with 25% SMC and 48 h SP (Fig. 1). Across all levels of SP and SMC treatments, the reduction in germination percentage in *L. iberica* (Fig. 1a) was higher compared to *L. royleana* (Fig. 1b).

Electrical conductivity

The analysis of variance in both species of *Lallemantia* showed that the two-way interaction of SP and SMC had a significant effect on electrical conductivity (Tables 2, 3). Electrical conductivity in both species of *Lallemantia* increased in response to increasing SMC, SP and highest electrical conductivity was observed in both species aged at 25% SMC and 48 h SP. Electrical conductivity in seeds of *L. iberica* (Fig. 1c) was more than that in *L. royleana* seeds (Fig. 1d) across all levels of SP and SMC.

Hydrogen peroxide and Lipid peroxidation content

The effects of storage period and seed moisture content were significant on H₂O₂ and MDA contents of both *Lallemantia* species (Table 2, 3). Contents of H₂O₂ (Fig. 2a, 2b) and MDA (Fig. 2c, 2d) in both

Lallemantia species increased along with the increase in SMC and SP. Indeed, H₂O₂ and MDA contents reached the highest at 25% SMC and 48 h SP (Fig. 2). Between the two species, H₂O₂ (Fig. 2a, 2b) and MDA (Fig. 2c, 2d) were fairly low in *L. royleana* seeds than that in *L. iberica* seeds under storage conditions.

Soluble protein content

The analysis of variance in both species of *Lallemantia* indicated that the two-way interaction of SP and SMC had a significant effect on soluble protein content (Table 2, 3). Increasing SP and SMC decreased the content of soluble protein in both species of *Lallemantia* significantly. The aged seeds in both species had the least soluble protein content across 25% SMC and 48 h SP (Fig. 3). Compared to *L. iberica* (Fig. 3a), the soluble protein content was more in *L. royleana* under deterioration conditions (Fig. 3b).

CAT and APX enzymes activity

Storage period and seed moisture content had significant effects on CAT and APX enzyme activities in both *Lallemantia* species (Table 2, 3). The non-aged seeds of both *Lallemantia* species had the highest CAT and APX enzyme activities compared to aged seeds. Indeed, significant reduction in CAT and APX enzyme activities were found in *L. iberica* (Fig. 4a) and *L. royleana* (Fig. 4b) with increasing SP and SMC. The CAT and APX enzyme activities for seeds of both *Lallemantia* species were lowest with 25% SMC than 5 and 15% with seed ageing for 24 and 48 h SP. (Fig. 4). Compared to *L. iberica*, the highest CAT and APX activities was observed in *L. royleana* under deterioration conditions (Fig. 4).

Correlations among the studied traits

The Pearson correlation showed significant correlation for most of the studied traits in both species of *lallemantia*. In *L. iberica* the highest and positive correlation was demonstrated between MDA and H₂O₂ ($r = 0.98^{**}$), while the lowest correlation was obtained between soluble protein content and GP. In *L. royleana*, significantly high positive correlation was observed between EC and H₂O₂ ($r = 0.97^{**}$) and, lowest correlation between soluble protein, and CAT. Interestingly, GP of *L. iberica* was positively associated with CAT, APX and soluble protein ($r = 0.94^{**}$, 0.94^{**} , 0.80^{**} , respectively), and that of *L. royleana* also showed positive correlation of GP with CAT, APX, and soluble protein protein ($r = 0.97^{**}$, 0.93^{**} , 0.93^{**} , respectively) (Fig. 5a, b).

Cluster analysis

Cluster analysis was performed visualizing a heat map to understand the exact relation between MDA, H₂O₂, EC, soluble protein, CAT, APX, GP traits of *L. iberica* and *L. royleana*. The result showed clustering of these traits into two main groups. Group I with CAT, APX, soluble protein and GP and Group II with EC, MDA, and H₂O₂. In this plot, positive and negative correlations were indicated by red and blue colors, respectively (Fig. 5c, 5d).

Discussion

The SMC and SP are vital factors that not only affect seed deterioration, but also can influence seed viability and longevity (Baladi and Balouchi 2016; Shvachko and Khlestkina 2020). A reduction in germination of both *Lallemantia* species was obtained in response to increasing SMS and SP. According to the results, decline in germination reported in *Avena sativa* L. (Xia et al. 2017), *Glycine max* L. (Ebene et al. 2020) and *Lallemantia royleana* L. (Baladi and Balouchi 2016) due to the production of ROS under unfavorable storage condition. Indeed, ROS during storage can be due to oxidative stress on lipids, proteins and DNA, ultimately leading to a loss of viability (Zhang et al. 2021). The SMC and SP are vital factors that not only affect seed deterioration, but also can influence seed viability and longevity (Shvachko and Khlestkina 2020). Higher germination in seeds of *L. royleana* than *L. iberica* seeds is probably due to the lower oil content and fatty acid components (Paravar et al. 2022b). It is well documented that higher oil and fatty acid components are the main reason for seed deterioration and reduction of germination (Ludwig et al. 2021). Membrane lipids are primarily composed of saturated and unsaturated fatty acids, which promote the integrity and function of cell membranes (Thyagaraju et al. 2022). Thus, any increase in saturated fatty acids and decrease in unsaturated fatty acids during seed storage causes reduction in the lipid fluidity of cell membranes (Ebene et al. 2020). In addition, further germination in *L. royleana* than *L. iberica* can be related to the increase in antioxidant enzymes activities and decline in MDA and H₂O₂ (Xu et al. 2022).

The electrical conductivity of seeds is one of the parameters used to determine the rate of seed deterioration (Shvachko and Khlestkina 2020). An increase in electrical conductivity in stored seeds of both *Lallemantia* species at different levels of SP and SMC could be attributed to cell membranes damage (Mehrabi Kooshki et al. 2018). Indeed, increasing SP or SMS during storage causes phospholipids in the cell membrane to change and develop a cone shape (Ebene et al. 2019).

These morphological changes cause the membranes of cells to lose their selective permeability (Zhang et al. 2021). Therefore, cations, anions, and sugar in cells of seed leach into intercellular spaces (Ebene et al. 2020). The highest EC observed in *L. iberica* seeds than that of *L. royleana* was probably due to the most significant destruction in the cell membrane of *L. iberica* seeds (Paravar et al. 2018). It has been widely reported that seeds with a high oil content are prone to decay faster during storage (Shvachko and Khlestkina 2020).

An increase in seed moisture content from 5–25% and long-term storage from 24 to 48 h decreased H₂O₂, and MDA contents which was probably related to the decline in the glassy state of the cytoplasm (Nagel et al. 2019). as the glassy state promotes the maintenance of raffinose family oligosaccharides (RFOs), antioxidant enzymes, heat shock proteins, and LEA proteins (Gerna et al. 2022). It is well documented that increasing seed moisture content or long-term storage causes the glassy state to be transformed to a fluid state, resulting in the enhancement of respiration in cells of aged seed (Matilla 2021). The increase in respiration indicates metabolic activities and the production of ROS in stored seed (Ebene et al. 2020). Accumulation of ROS in seed cells contributes to membrane plasma

destruction by breaking down long-chain fatty acids into smaller components (Ebene et al. 2020; Gerna et al. 2022). Compared to *L. royleana*, higher H₂O₂ and MDA content in *L. iberica* seeds may be due to the existence of unsaturated fatty acids and phospholipids in *L. iberica* seeds (Al-Snafi 2019a, b; Paravar et al. 2022a; Zlatanov et al. 2012). Thus, increase in seed moisture content during storage can cause the production of ROS which can oxidize unsaturated bonds and goes up the lipid peroxidation (Thyagaraju et al. 2022).

Our results indicated that the soluble protein content, and enzyme activities of CAT and APX in seeds of both *Lallemantia* species decreased as SMC and SP increased (Fig. 3a, b). The soluble protein content, CAT and APX enzyme activities in *Zea mays* L. (Gomes et al. 2021), *Triticum aestivum* L. (Sohan et al. 2021) and *Avena sativa* (Xia et al. 2015) were reduced due to increase in H₂O₂ and MDA content. According to some reports, antioxidant enzymes are responsible for protecting the structure of the mitochondria, because the dysfunction of the mitochondria plays a central role in cell death (Ebene et al. 2020). The decrease in soluble protein content, CAT and APX enzyme activities were most likely caused by the accumulation of superoxide anion radical (O₂^{•-}), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH[•]) during storage (Zhang et al. 2021). Our results showed that the soluble protein content, CAT and APX enzyme activities in seeds of *L. iberica* were less compared to *L. royleana* seeds, which might be due to the accumulation of H₂O₂ and MDA (Gerna et al. 2022). It has been reported that decline in antioxidant enzymes activities was probably due to higher lipid peroxidation which damages to the defense system of antioxidant enzymes (Xu et al. 2022).

A negative correlation between germination percentage and electrical conductivity in both *Lallemantia* species under storage is likely to be due to lipid membrane damage (Shvachko and Khlestkina 2020). Relation between the decline in germinability, soluble protein content, CAT and APX activity with electrical conductivity, H₂O₂ and MDA accumulation in both species of *Lallemantia* under storage conditions is probably due to the damage of lipid membranes (Shvachko and Khlestkina 2020) causing the reduction of energy into seed cells. Thus, energy reduction is associated with the loss of seed viability (Zhang et al. 2019). In addition, it has been found that the accumulation of H₂O₂ leads to turning the cytoplasmic phase from a glassy to fluid state, thus, damages mitochondrial structure, causing a decline in germination (Xia et al. 2015).

Conclusions

Since seed viability and seed quality are important for agriculture and food industries. As a general rule, seeds must be stored in suitable conditions to maintain seed viability and quality. There was a decline in germination, soluble protein content, CAT and APX enzyme activities in *L. iberica* than *L. royleana* seeds than that in seeds by increasing SMC and SP. In stark contrast, an increase in EC, MDA, and H₂O₂ content was observed in *L. iberica* in comparison with *L. royleana*. Overall, higher germination and antioxidant enzymes activities in seeds aged of *L. royleana* by increasing SMC and SP was probably due to the lesser damages in lipid structures of membrane and production of H₂O₂ content which induced the reduction

of EC and MDA content. Eventually, this study illustrated that an increase in SMC from 5–25% SP from 24 h to 48 h during storage can cause the ROS production, seed deterioration, and germination reduction.

Declarations

Conflict of interest

This manuscript has not been published or presented elsewhere in part or entirety and is not considered by another journal. All the authors have approved the manuscript and agree with the submission. The authors declare no conflicts of interest. The study is in accordance with relevant institutional, national, and international guidelines and legislation.

Contributors' statement

Saeideh Maleki Farahani designed the experiments. Arezoo Paravar performed experiments and wrote the manuscript. Arezoo Paravar, Saeideh Maleki Farahani and Alireza Rezazadeh revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This research project was supported by Shahed University, Tehran, Iran

Author Contribution

Conceptualization, SMF (0000-0002-1678-2743) (; Methodology, Writing and figurespreparing SMF and AP (0000-0002-0388-4935).; Software, Arezoo Paravar; Validation, Saeideh Maleki Farahani., Arezoo Paravar. and Alireza Rezazadeh (0000-0002-6104-1717).; Formal Analysis, Saeideh Maleki Farahani., Arezoo Paravar. And Alireza Rezazadeh; Investigation, Saeideh Maleki Farahani. and Arezoo Paravar; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, Saeideh Maleki Farahani., Arezoo Paravar. And Alireza Rezazadeh; Writing – Review & Editing, Radan Edit English.; Visualization, Arezoo Paravar.; Supervision, Saedieh Maleki Farahani and Alireza Rezazadeh.; Project Administration, Saeideh Maleki Farahani. andAlireza Rezazadeh.; Funding Acquisition, Saeideh Maleki Farahani. All authors reviewed the manuscript.

Acknowledgment

Shahed University of Tehran for the research support

Data Availability

All data generated or analyzed during this study are included in this published article. All data supporting the findings of this study are available within the paper and its Supplementary Information

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Tables

Table 1
Characteristics of the seeds tested in this research

Plant species	Seed viability (%)	1000-Seed weight (g)	Oil content (%)	LNA (%)	LA (%)	OA (%)
<i>L.iberica</i>	98	3.9	32.56	67.28	13.45	19.23
<i>L.royleana</i>	98	1.5	20.43	48.45	10.21	11.30
LNA:; linolenic acid; LA: linoleic acid; OA: oleic acid						

Table 2 Analysis of variance of SMC and SP on physiological and biochemical indices of *Lallemantia iberica*

S.V	d.f	GP	EC	H ₂ O ₂	MDA	Soluble protein content	CAT	APX
SP	2	7873.45**	1256.8**	7841.9**	16446.4**	23.19**	13.04**	0.523**
SMC	2	130.18**	73.66**	545.8**	261.7**	2.06**	0.989**	0.025**
SP×SMC	4	39.68**	24.9**	153.4**	78.7**	1.13**	0.66 ³ **	0.012**
Experimental error	18	3.22	1.2	8.03	10.6	0.01	0.026	0.0004
C.V (%)		2.85	4.1	1.7	3.2	1.52	7.7	2.9

ns non-significant, * and ** Significant at 5% and 1% respectively. S.V: source of variation; d.f: degrees of freedom; C.V: coefficient of variation; SMC: Seed moisture content, SP: Storage period; GP: Germination percentage; EC: Electrical conductivity; H₂O₂: Hydrogen peroxide; MDA: Malondialdehyde; CAT: Catalase enzyme activity; APX: Ascorbate peroxidase enzyme activity.

Table 3 Analysis of variance of SMC and SP on physiological and biochemical indices of *Lallemantia royleana*

S.V	d.f	GP	EC	H ₂ O ₂	MDA	Protein content	CAT	APX
SP	2	803.87**	733.0**	24830.0**	2994.6**	81.7**	18.0**	1.08**
SMC	2	87.98**	43.2**	982.7**	314.7**	5.1**	1.3**	0.663**
SP×SMC	4	29.93**	12.6**	277.3**	81.1**	1.4**	0.528**	0.019**
Experimental error	18	1.83	0.91	11.8	0.99	0.086	0.025	0.0007
C.V(%)		1.73	4.6	2.8	1.8	2.5	3.28	2.7

ns, * and ** non-significant, Significant at 5% and 1% respectively. S.V: source of variation; d.f: degrees of freedom; C.V: coefficient of variation; SMC: Seed moisture content; SP: Storage period; GP: Germination percentage; EC: Electrical conductivity; H₂O₂: Hydrogen peroxide; MDA: Malondialdehyde; CAT: Catalase enzyme activity; APX: Ascorbate peroxidase enzyme activity.

Figures

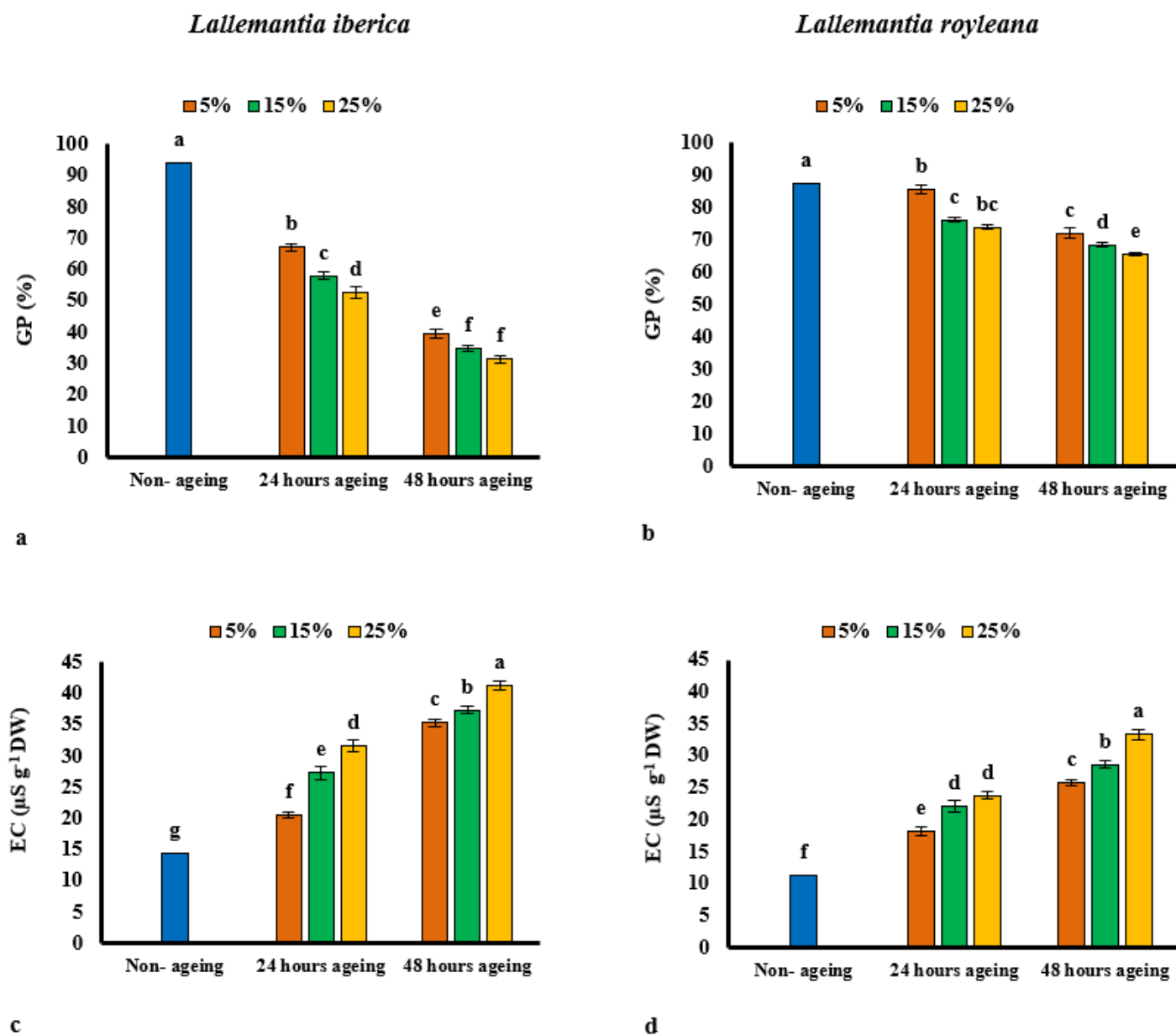


Figure 1

The effect of SMC and SP on GP (a, b) and EC (c, d) of *L. iberica* and *L. royleana*, respectively. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The bars indicate the standard error of the means ($\pm$ SE). SMC: Seed moisture content, SP: Storage period; GP: Germination percentage; EC: Electrical conductivity.

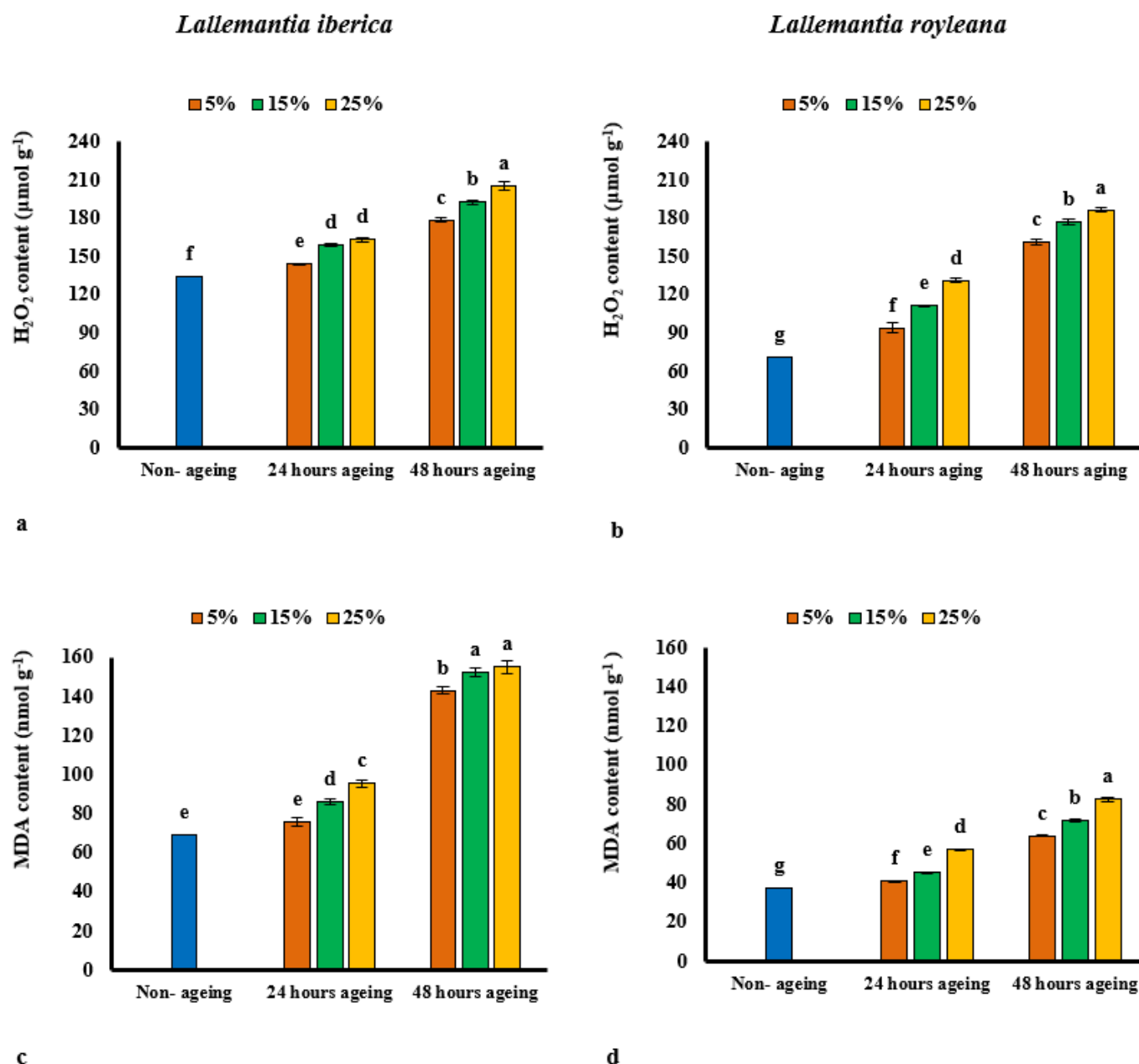


Figure 2

The effect of SMC and SP on H₂O₂ content (a, b) and MDA (c, d) of *L. iberica* and *L. royleana*, respectively. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The bars indicate the standard error of the means (±SE). SMC: Seed moisture content, SP: Storage period; H₂O₂: Hydrogen peroxide; MDA: [Malondialdehyde](#).

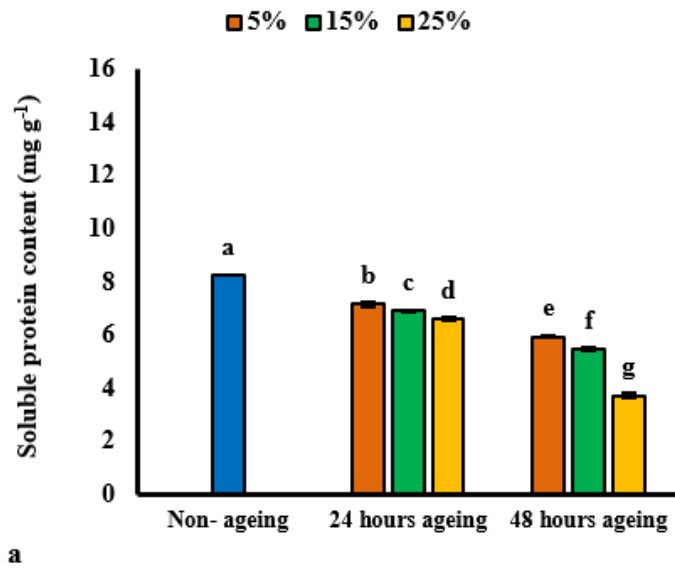
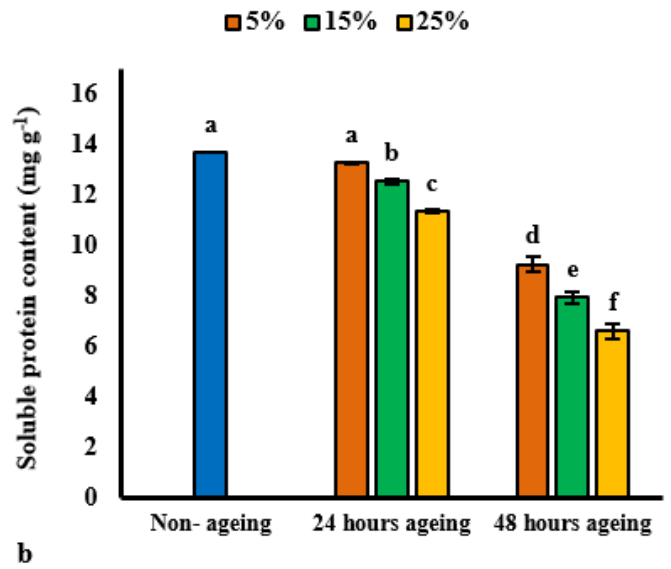
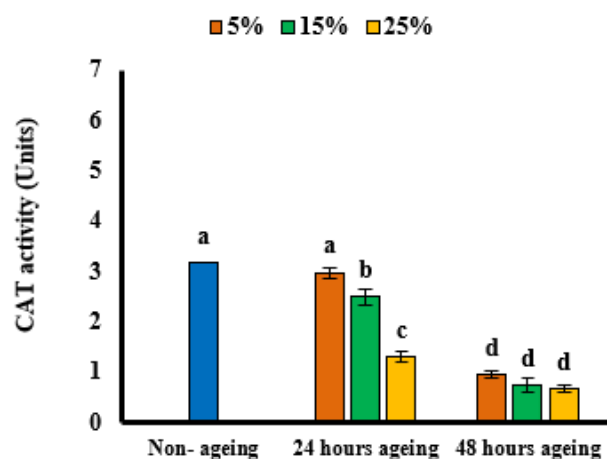
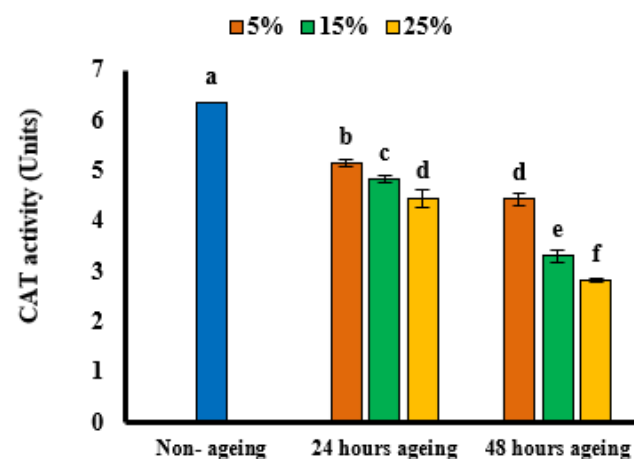
Lallemantia iberica*Lallemantia royleana*

Figure 3

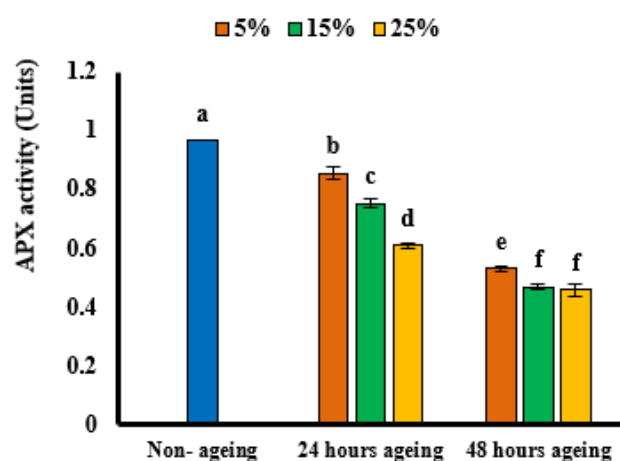
The effect of SMC and SP on the protein content (a, b) of *L. iberica* and *L. royleana*, respectively. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The bars indicate the standard error of the means (±SE). SMC: Seed moisture content, SP: Storage period.

*Lallemantia iberica**Lallemantia royleana*

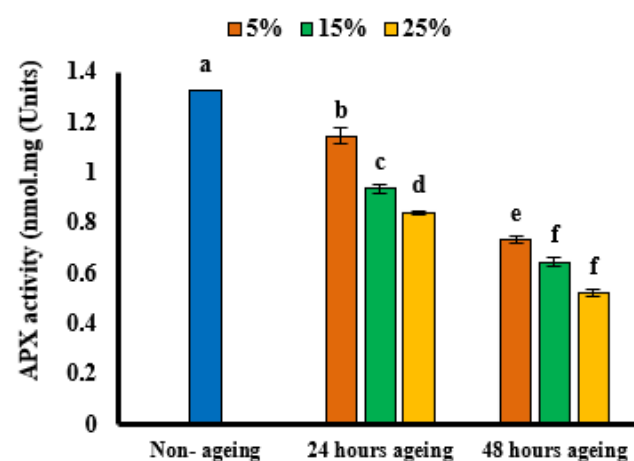
a



b



c

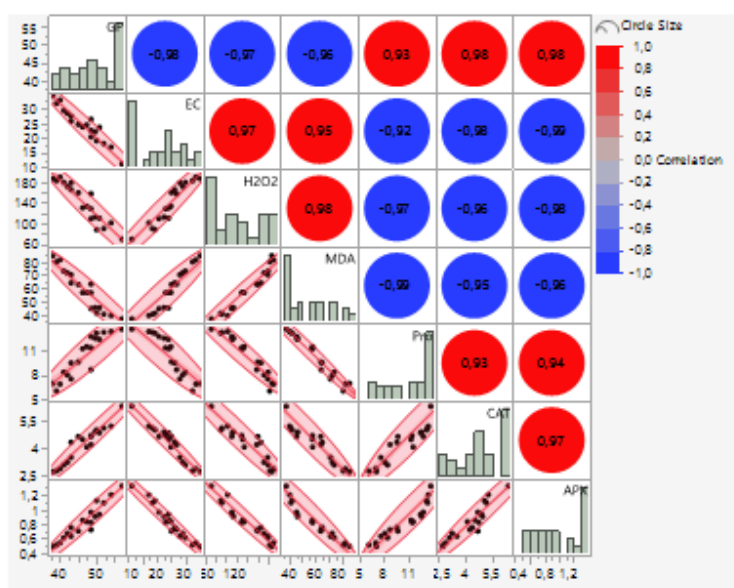


d

Figure 4

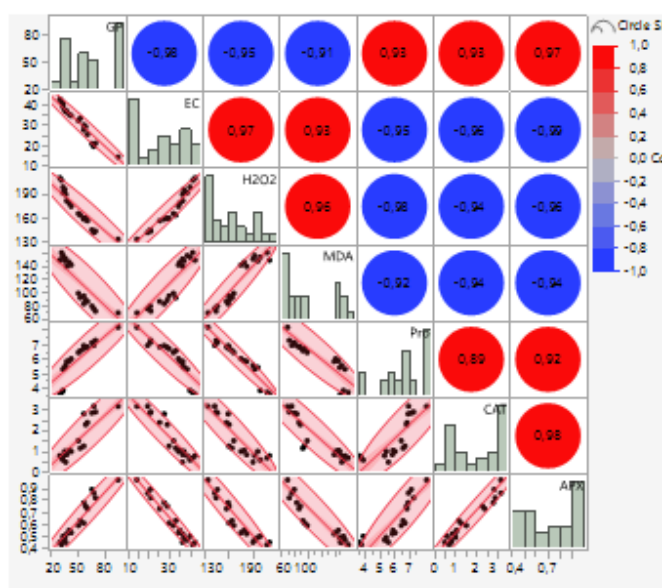
The effect of SMC and SP on CAT (a, b) activity and APX (c, d) of *L. iberica* and *L. royleana*. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The bars indicate the standard error of the means ($\pm$ SE). SMC: Seed moisture content, SP: Storage period; CAT: Catalase enzyme activity; APX: Ascorbate peroxidase enzyme activity.

Lallemantia iberica

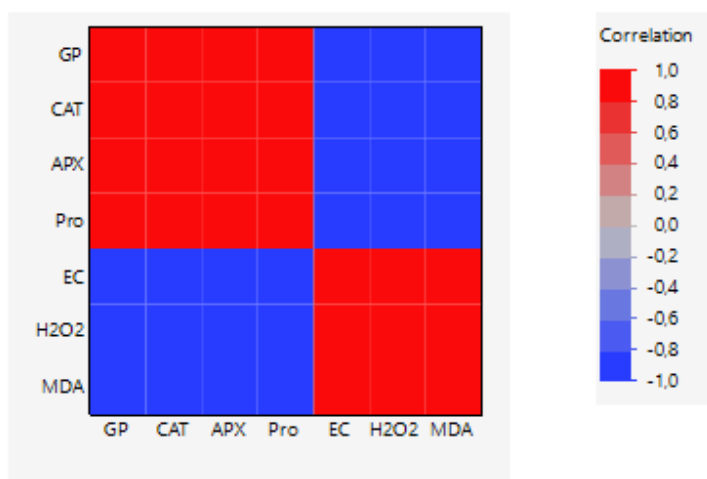


a

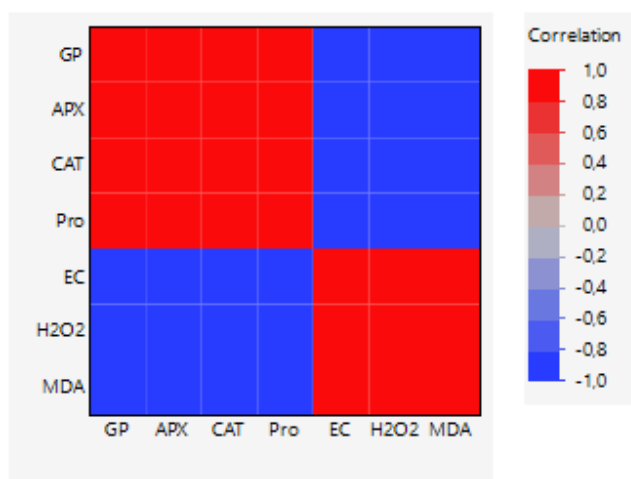
Lallemantia royleana



b



c



d

Figure 5

Pearson correlation between physiological traits (a, b) and cluster analysis of physiological traits (c, d) in *L. iberica* and *L. royleana* through heatmap plot. GP: Germination percentage; H₂O₂: Hydrogen peroxide; CAT: Catalase enzyme activity; APX: Ascorbate peroxidase enzyme activity; Pro: Soluble protein content; EC: Electrical conductivity; MDA: Malondialdehyde.

Supplementary Files

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