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ARTICLE

Effects of Static Magnetic Field and Cold Stratification on Germination and Starch-Related Biochemical Traits in Blackberry (*Rubus fruticosus* L.) Seeds

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ABSTRACT: Seed germination in blackberry (*Rubus fruticosus* L.) is frequently constrained by deep physiological dormancy, limiting its use in breeding and germplasm studies. This study evaluated the combined effects of cold stratification (CS) and static magnetic field (SMF; 80 mT) exposure on germination performance and associated biochemical changes related to starch metabolism. Seeds of the commercial cultivar 'Loch Ness' were subjected to different stratification periods and magnetic treatments prior to germination under laboratory conditions. Some SMF + CS combinations showed higher germination than the untreated control, although overall germination remained low. Higher germination values were associated with increased α- and β-amylase activities and reduced residual starch content, indicating enhanced reserve mobilization during early germination. Correlation and exploratory modelling analyses suggested that starch content was one of the biochemical variables most consistently associated with germination performance within the present dataset. These results suggest that SMF may contribute to germination-associated metabolic activation, particularly through changes related to starch mobilization, once dormancy has been partially alleviated by cold stratification. However, overall germination remained low under the tested conditions, and the study should be interpreted as an exploratory physiological evaluation rather than as an optimized germination protocol.

KEYWORDS: *Rubus fruticosus*; seed dormancy; cold stratification; static magnetic field; starch mobilization; amylase activity; seed physiology

1 Introduction

Blackberry belongs to the genus *Rubus*, a taxonomically complex group comprising numerous wild and cultivated species of horticultural interest [1,2]. Among them, *Rubus fruticosus* L. is widely cultivated in temperate regions, mainly for fresh consumption and processing. Although commercial blackberry propagation is usually achieved vegetatively, seed germination remains relevant for breeding programs, germplasm conservation, and studies on dormancy and early seed physiology. However, germination in blackberry is often slow, irregular, and markedly low because seeds exhibit deep dormancy, which strongly limits their practical use and complicates the establishment of standardized protocols [3–5].

Seed dormancy in *Rubus* has been associated with both physical and physiological components. In many species, seed coverings restrict imbibition, but this barrier alone does not fully explain the poor germination response. Several studies have shown that even after scarification, seeds often require prolonged cold stratification to complete dormancy release, indicating that physiological constraints remain active within the embryo or surrounding tissues [6,7]. In this context, cold stratification is one of the most widely used methods to promote germination in temperate species, yet the biochemical mechanisms underlying its effects in blackberry seeds remain poorly understood.

The mobilization of storage reserves is a central process during seed germination. In particular, starch degradation provides soluble carbohydrates required for respiration and embryo growth and largely depends on the coordinated activity of hydrolytic enzymes such as α-amylase and β-amylase [8,9]. Recent studies have shown that physical seed treatments such as magneto-priming can enhance hydrolytic enzyme activity and modulate key physiological processes, including ion transport and reactive oxygen species (ROS) balance, thereby promoting reserve mobilization and improving germination performance [10]. Reserve mobilization during germination is also closely linked to hormonal regulation, particularly the balance between abscisic acid (ABA) and gibberellins (GA), which influences embryo growth potential and hydrolytic enzyme activation. Changes in these biochemical traits have been linked to dormancy release and seed viability in several species. For example, β-amylase activity has been associated with starch breakdown and successful germination in recalcitrant Chinese chestnut seeds [11]. In contrast, information on these processes in *Rubus* is still scarce, despite the recognized difficulties of germination in this genus and the recent preliminary evidence available for blackberry seeds [12,13].

Besides conventional dormancy-breaking methods, physical treatments such as static magnetic field (SMF) exposure have attracted increasing attention as environmentally friendly tools to stimulate seed metabolism and improve germination performance. Positive effects of SMF have been reported in several crops, including sunflower, maize, soybean, and *Calotropis procera*, where magnetic exposure enhanced germination performance and, in some cases, was associated with early metabolic activation [14–18]. In addition, recent work has shown that magnetic field treatments may also affect dormancy release and internal reserve transformation in tree seeds [19]. These effects are associated with the stimulation of metabolic activity and enzymatic systems involved in germination, although the underlying mechanisms remain only partially understood and may vary depending on species, treatment intensity, and seed physiological status [20]. However, the physiological basis of these responses remains incompletely resolved, particularly in blackberry seeds.

Although germination is commonly analysed using conventional linear approaches, the relationship between germination and biochemical traits such as starch content, total soluble sugars, and amylase activity may not be strictly linear, especially in species with low and highly variable germination. Exploring these relationships may therefore help identify the most informative biochemical predictors of germination response.

Accordingly, the aim of this study was to evaluate the effects of cold stratification and static magnetic field exposure on cumulative germination, starch and total soluble sugar contents, and α- and β-amylase activity in seeds of the blackberry cultivar ‘Loch Ness’. In addition, the relationships among these variables were examined to identify the biochemical traits most closely associated with germination performance. We hypothesized that static magnetic field exposure could stimulate starch mobilization during germination, thereby contributing to germination once dormancy had been partially alleviated by cold stratification.

2 Materials and Methods

2.1 Plant Material

The seeds used in this study were obtained from fruits of the open-pollinated blackberry cultivar ‘Loch Ness’ [21], collected from plants located at the experimental field of La Rábida Campus, University of Huelva (southwestern Spain), during summer 2023. The 'Loch Ness' cultivar was selected due to its commercial importance and its relevance for studies on blackberry germination.

Fruits were mashed and blended for 1 min using a blender with plastic-covered blades. After resting for approximately 2 min to allow seed deposition, the pulp was separated by inverting the container. The juice was discarded and the remaining solids were rinsed through a fine-mesh sieve. Seeds were subsequently cleaned by immersion in a 0.5% commercial sodium hypochlorite solution for 5 min and then dried in the shade for approximately 24 h. Seed viability was assessed using the tetrazolium test according to Peters [22]. No scarification treatment was applied before static magnetic field exposure or cold stratification. This decision was made to isolate the effects of these treatments on germination and associated biochemical responses, although it may have contributed to the low absolute germination percentages observed.

2.2 Static Magnetic Field and Cold Stratification Treatments

A laboratory experiment was conducted at the Plant and Crop Science Laboratory of La Rábida Campus (37°12′32.3980″ N, 6°55′09.9948″ W), University of Huelva, to evaluate the germination response of 'Loch Ness' blackberry seeds. Seeds were exposed or not exposed to an 80 mT static magnetic field (SMF) for 24 h, based on exposure durations previously reported as effective in seed stimulation studies [23], and subsequently subjected to cold stratification (CS) for 0, 15, 30, 45, 60, or 75 days.

Blackberry seeds were exposed to the SMF emitted by a homemade electromagnetic field generator previously described by Dueñas et al. [24], which allows a controllable magnetic intensity between 7 and 200 mT. Seeds were placed in a plastic container between the N–S poles of the electromagnet and subjected to the magnetic treatment for the required duration. The magnetic field intensity was verified using a digital teslameter.

After SMF treatment, seeds were transferred to labelled Petri dishes and subjected to cold stratification at 5–7°C for the corresponding CS periods.

Following each stratification period, seeds were incubated for germination at 20 ± 4°C under natural laboratory light conditions. Relative humidity during the experimental period was approximately 36 ± 6%. Photoperiod and light intensity were not instrumentally controlled and should therefore be considered a limitation of the study.

For biochemical analyses, seeds were placed on moistened filter paper in sterile 90 mm Petri dishes, sealed with parafilm to minimize water loss, and incubated for 15 days under the conditions described above to stimulate metabolic activation. Immediately after pre-incubation, seeds were stored at −20°C until biochemical analyses.

For germination assessment, the remaining seeds were incubated on moistened filter paper in sterile Petri dishes for 10 weeks under the same temperature and light conditions described above. Petri dishes were kept closed during incubation to minimize moisture loss, but no periodic rehydration was applied. Germination was evaluated twice per week, and seeds were considered germinated when radicle emergence reached at least 1 mm.

Due to the persistent dormancy of *Rubus* seeds, morphological parameters such as shoot length, root length, or chlorophyll content could not be consistently measured. Therefore, cumulative germination and internal biochemical markers were used as the main indicators of dormancy release.

2.3 Experimental Design

Seeds of the 'Loch Ness' cultivar were subjected to 12 treatment combinations arranged in a 2 × 6 factorial design, consisting of two SMF levels (treated and untreated) and six cold stratification periods (0, 15, 30, 45, 60, or 75 days). The experiment followed a completely randomized design with six replications per treatment. Each replicate included 30 seeds. The entire experiment was repeated twice.

2.4 Determination of Starch and Total Soluble Sugar Contents

Starch content was determined following the method of Novelo-Cen and Betancur-Ancona [25], with minor modifications [12]. Seeds (five per replication) were dried at 45°C for 24 h to obtain dry weight. Samples were homogenized in 1 mL of distilled water using a polytron homogenizer (PT 2500 E, Kinematica AG, Switzerland). The homogenate was filtered through a 100-mesh filter and centrifuged at 7000× *g* for 15 min at 4°C. The supernatant was removed and the sediment was resuspended in 1 mL of distilled water and centrifuged again under the same conditions. This step was repeated until the supernatant became transparent. The final sediment was dried at 45°C for 24 h and weighed using a precision balance to estimate starch content (mg g^{-1} DW, where DW indicates dry weight).

Total soluble sugars were quantified using the phenol–sulfuric acid method described by DuBois et al. [26], with slight modifications [12]. Seeds were dried at 45°C for 24 h and homogenized in 1 mL of 80% ethanol. The suspension was vortexed, transferred to an Eppendorf tube and heated in a water bath at 75°C for 10 min. The extract was centrifuged at 5000× *g* for 10 min at 4°C. A 250 µL aliquot of the supernatant was mixed with 750 µL of concentrated sulfuric acid and 5 µL of 5% aqueous phenol. After standing for 30 min, absorbance was measured at 490 nm using a spectrophotometer (PG1800, Lan Optics; Labolan, Spain). Quantification was based on the following calibration curve:

$$\mathrm{Y} = 0.0596\mathrm{X} - 0.0676$$

where Y represents absorbance and X the glucose concentration (mg mL^{-1}).

Five seeds per replicate were used for biochemical analyses because of the small seed size, the destructive nature of the assays, and the limited amount of material available for each treatment combination. This sampling approach was adopted for an exploratory evaluation of early metabolic changes, although it should be regarded as a limitation due to the expected biological variability among seeds.

2.5 Determination of α- and β-Amylase Activities

Amylase activity was determined using the classical colorimetric method described by Bernfeld [27], adapted to independently estimate α-amylase and β-amylase activities. Seeds were dried at 45°C for 24 h, homogenized using a polytron homogenizer and centrifuged at 14,000× *g* for 30 min at 4°C to obtain a crude enzyme extract.

For α-amylase activity, 750 µL of 0.1% (w/v) soluble starch and 750 µL of enzyme extract were incubated at 30°C for 60 min. The reaction was stopped by adding 0.5 mL of color reagent prepared with 1 g of 3,5-dinitrosalicylic acid, 20 mL of 2 mol L^{-1} NaOH, and 30 g of potassium sodium tartrate in 100 mL of distilled water. The mixture was heated at 50°C for 5 min and absorbance was measured at 546 nm.

Calibration curves were generated using increasing concentrations of D–(+) maltose monohydrate as standard. The calibration equation for α-amylase activity was:

$$\mathrm{Y} = 0.0616\mathrm{X} + 0.0129 \ (\mathrm{R}^2 = 0.87)$$

where Y is absorbance and X is maltose concentration (mg mL^{-1}).

For β-amylase activity, the same procedure was followed, except that 0.1% (w/v) soluble amylopectin was used instead of soluble starch as substrate. After incubation and color development, absorbance was measured at 540 nm. The corresponding calibration curve was:

$$Y = 0.0677X + 0.0128 \ (R^2 = 0.87)$$

where Y is absorbance and X is maltose concentration (mg mL^{-1}).

Amylase activity was finally expressed as μmol of hydrolysed maltose equivalents g^{-1} DW.

2.6 Data Analyses and Software

Cumulative germination, starch content, total soluble sugars, and α- and β-amylase activity were analysed to test the effects of SMF and CS. Germination percentages were arcsine-transformed prior to analysis to ensure normality and homoscedasticity. Experiment and replication nested within experiment were treated as random effects, whereas SMF, CS, and their interaction were treated as fixed effects. When significant effects were detected in the mixed-effects models, mean comparisons among treatments were performed using Tukey's HSD test ($p \leq 0.05$).

Pearson's correlation coefficients were calculated to evaluate relationships among biochemical variables and germination. Linear regression models were constructed using stepwise selection procedures.

To explore potential nonlinear relationships within the present dataset, 40 machine learning regression algorithms were screened as exploratory approaches (Table S1). The dataset was randomly divided into training (70%) and test (30%) subsets. During the initial screening of algorithms, model performance was evaluated primarily using the coefficient of determination (R^2) and the root mean square error (RMSE). For the final comparison of the selected models presented in the main text (Table 1), performance was summarized using R^2, RMSE, and mean absolute error (MAE) [28,29].

Table 1: Predictive performance of linear and nonlinear models for cumulative germination in seeds of the blackberry cultivar 'Loch Ness'.

Method	Predictor Variables*	R^2	RMSE	MAE
Univariate (linear)	α-amylase	0.09	5.79	4.49
Multivariate (linear)	α-amylase + starch + β-amylase	0.12	5.68	4.25
Regression tree (machine learning)	starch + α-amylase + β-amylase + total soluble sugars	0.32	4.77	3.51

*Starch and total soluble sugar contents are expressed as mg g^{-1} DW, and α- and β-amylase activities as μmol g^{-1} DW.

Mixed-effects models were fitted in R using the lme4 package, and inference for fixed effects was obtained through Type III tests with Satterthwaite's approximation of denominator degrees of freedom. Post hoc comparisons among treatment means were performed using estimated marginal means with Tukey adjustment.

Mixed-effects models, correlation analyses, and linear regressions were conducted in R using the packages lme4, PerformanceAnalytics, and MASS [30–32]. Machine learning analyses were performed in Python using the LazyPredict library.

3 Results

3.1 Germination Response

Fresh seed viability exceeded 95% according to the tetrazolium test. Despite this high viability, seeds exhibited a low mean germination percentage of 4.4 ± 1.8% after 10 weeks under laboratory conditions (20 ± 4°C, natural laboratory light) (Figs. 1 and 2a).

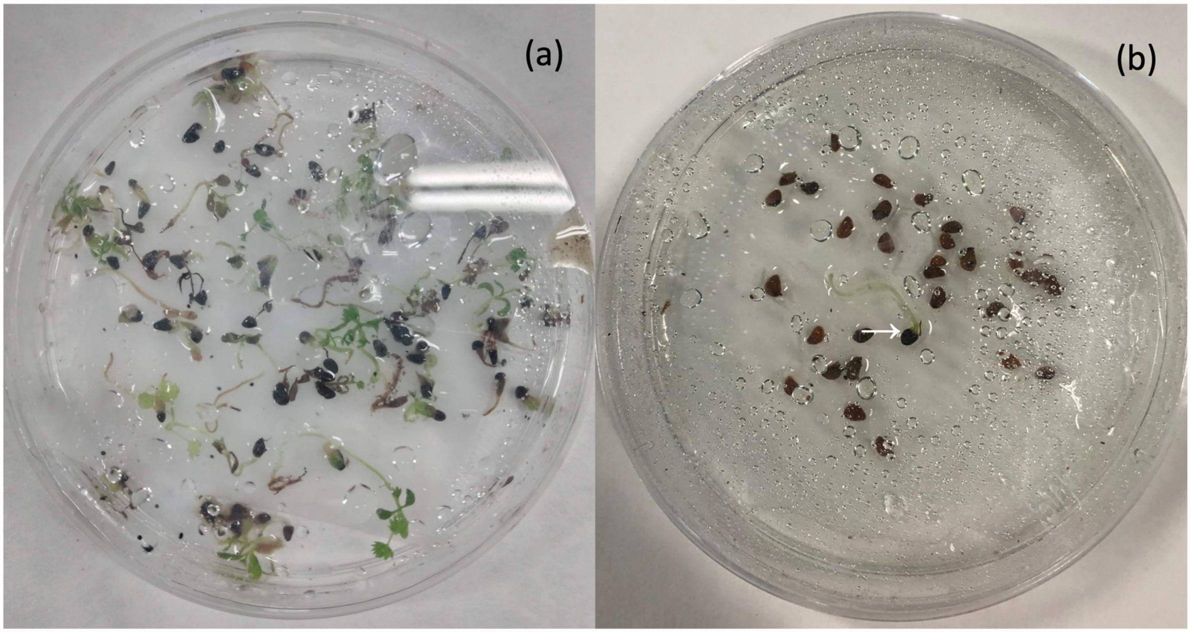

Figure 1: Representative Petri dishes containing 30 *Rubus fruticosus* seeds after 10 weeks of incubation on moistened filter paper under laboratory conditions (20 ± 4°C, natural laboratory light). (**a**) Germinated seeds showing visible radicle emergence (≥1 mm). (**b**) Non-germinated seeds, defined as seeds with no visible radicle emergence at the end of the incubation period (white arrow). Petri dishes were kept closed during incubation to minimize moisture loss; therefore, some condensation was visible on the inner surface of the lid.

Cold stratification (CS) significantly affected cumulative germination (Table 2). Mean germination was 6.0 ± 1.4% without CS, decreased to 0.0% after 15 days of stratification, and slightly increased to 1.5 ± 0.3% after 30 days. Germination increased again with longer stratification periods, reaching 6.0 ± 1.4%, 6.8 ± 1.3%, and 6.0 ± 1.5% after 45, 60, and 75 days of CS, respectively (Fig. 2a). The transient reduction in germination observed after 15 days of stratification may reflect incomplete and heterogeneous dormancy release during the early chilling phase. Under these conditions, short-term chilling may have initiated partial physiological changes without being sufficient to support consistent radicle emergence.

Type III tests also revealed significant effects of SMF and the SMF × CS interaction (Table 2). Except for the 15- and 30-day CS treatments, SMF-treated seeds consistently showed higher germination values than untreated seeds, with differences approaching twofold in several stratification treatments ($p \leq 0.05$), irrespective of stratification duration (Fig. 2a).

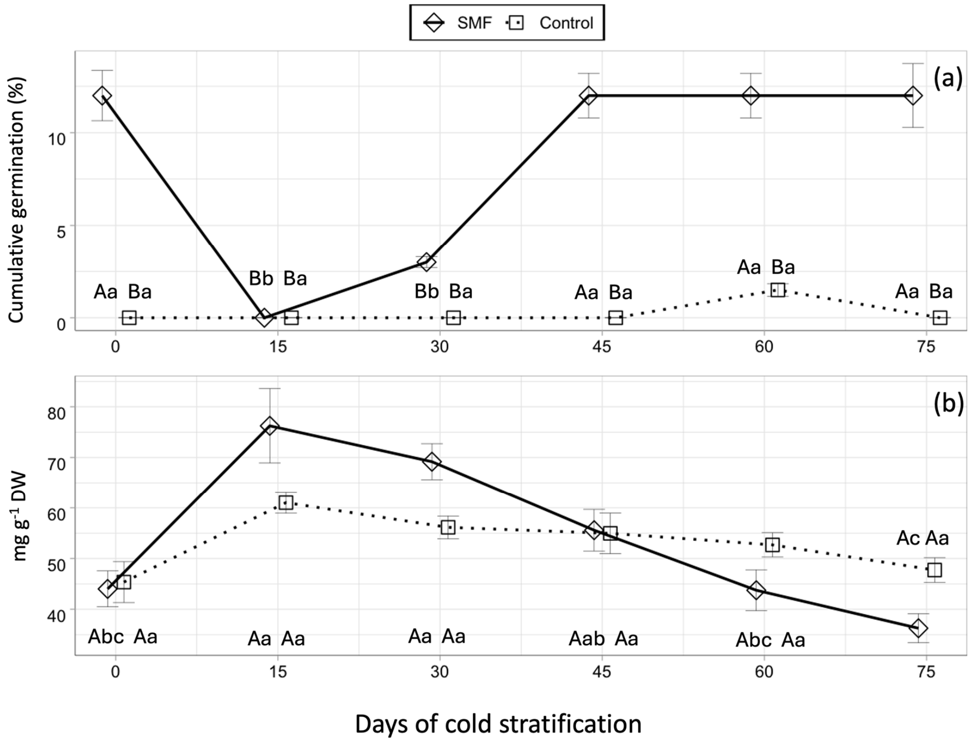

Figure 2: Effects of static magnetic field (SMF) exposure and cold stratification (CS) on (**a**) cumulative germination and (**b**) starch content in seeds of the blackberry cultivar 'Loch Ness'. For cumulative germination, points represent means from two independent experiments, each comprising six replicates of 30 seeds per treatment. For starch content, values represent means ± SE from two independent experiments with six replicates of five seeds per treatment. Different uppercase letters indicate significant differences between SMF treatments within each CS level, whereas different lowercase letters indicate significant differences among CS levels within each SMF treatment according to Tukey's HSD test ($p \leq 0.05$).

3.2 Starch Content

Starch content was significantly influenced by CS and the CS × SMF interaction (Table 2).

Table 2: Type III tests of fixed effects from the mixed-effects model analysis for cumulative germination, starch content, total soluble sugars, and α- and β-amylase activity in *Rubus fruticosus* seeds.

Effect/Parameter	**Static Magnetic Field (SMF) (*dfnum* = 1)**			**Cold Stratification (CS) (*dfnum* = 5)**			**SMF × CS (*dfnum* = 5)**		
	dfDen	**F-Value**	***p*-Value**	***dfDen***	**F-Value**	***p*-Value**	***dfDen***	**F-Value**	***p*-Value**
Cumulative germination (%)	131	434.2	<0.001***	131	34.8	<0.001***	131	30.4	<0.001***
Starch (mg g^{-1} DW)	126	0.3	0.57	126	16.6	<0.001***	126	4.6	0.001**
Total soluble sugars (mg g^{-1} DW)	131	1.1	0.30	131	54.9	<0.001***	131	0.9	0.42
α-amylase (μmol g^{-1} DW)	132	9.1	0.003**	132	22.1	<0.001***	132	2.0	0.08
β-amylase (μmol g^{-1} DW)	132	17.5	<0.001***	132	1.9	0.09	132	0.1	0.98

dfnum indicates degrees of freedom for the numerator and *dfDen* indicates degrees of freedom for the denominator. *p*-values for all fixed effects were computed using the Satterthwaite approximation for degrees of freedom. Significance codes: $^{***}p \leq 0.001$; $^{**}p \leq 0.01$.

Seeds subjected to stratification periods associated with higher germination showed a marked reduction in starch reserves. In SMF-treated seeds, the CS treatments with the highest germination values (0-, 45-, 60-, and 75-day CS) showed approximately twofold lower starch content compared with treatments with the lowest germination (15- and 30-day CS) (Fig. 2b).

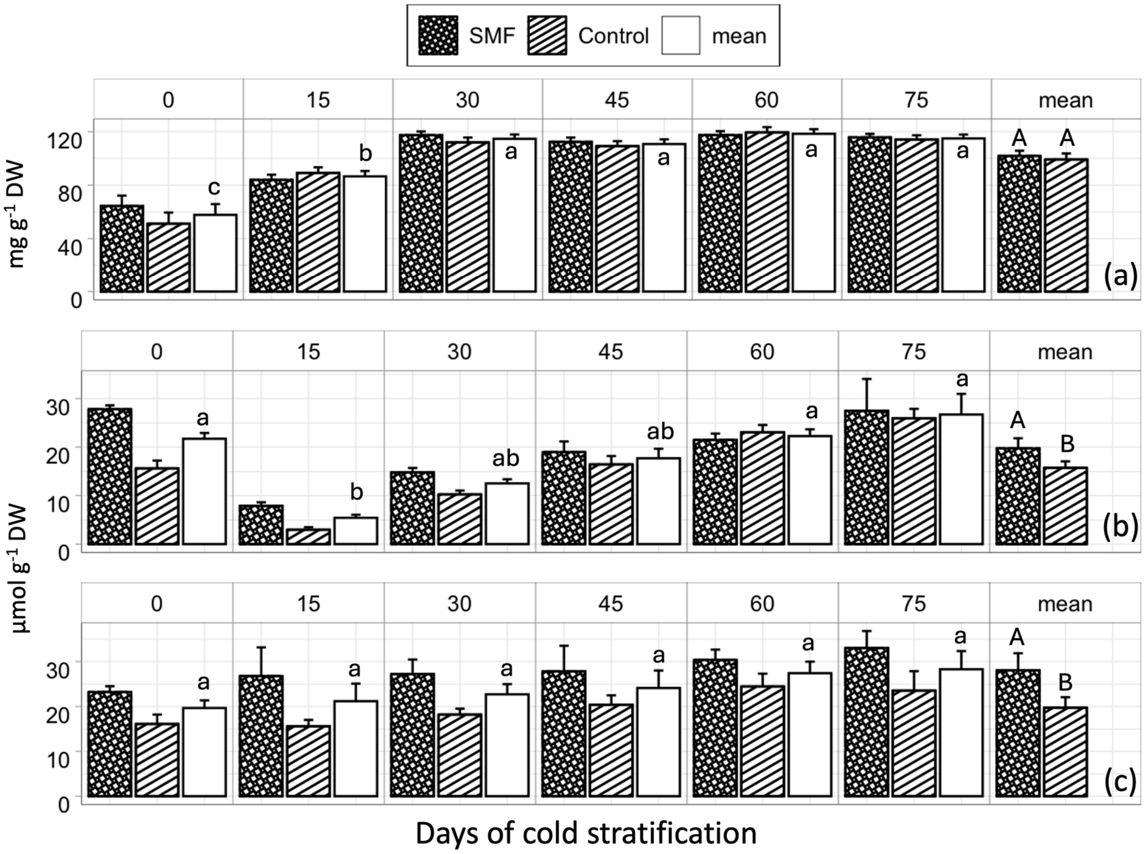

Figure 3: Effects of static magnetic field (SMF) exposure and cold stratification (CS) on (**a**) total soluble sugar content, (**b**) α-amylase activity, and (**c**) β-amylase activity in seeds of the blackberry cultivar 'Loch Ness'. Values represent means ± SE from two independent experiments with six replicates of five seeds per treatment. Different uppercase letters indicate significant differences between SMF treatments within each CS level, whereas different lowercase letters indicate significant differences among CS levels within each SMF treatment according to Tukey's HSD test ($p \leq 0.05$).

In contrast, when seeds were not exposed to SMF and germination remained minimal, starch levels did not differ significantly among CS treatments.

3.3 Total Soluble Sugar Content

Cold stratification significantly affected total soluble sugar levels independently of SMF treatment (Table 2).

Soluble sugars increased markedly from 0 to 15 days of CS (mean increase of 49.8%) and from 15 to 30 days (32.4%). After 30 days of stratification, sugar levels remained relatively stable until 75 days of CS, with no significant differences detected among later stratification periods (Fig. 3a).

3.4 α- and β-Amylase Activities

Enzymatic activity of both α- and β-amylase was significantly influenced by SMF treatment (Table 2). On average, SMF-treated seeds exhibited significantly higher α-amylase and β-amylase activities than untreated seeds, with increases of 15.2% and 42.6%, respectively (Fig. 3b,c).

These results suggest that SMF exposure was associated with higher enzymatic activity related to starch hydrolysis during germination.

3.5 Relationships between Germination and Biochemical Variables

Correlation analysis revealed moderate relationships between cumulative germination and several biochemical variables (Fig. 4).

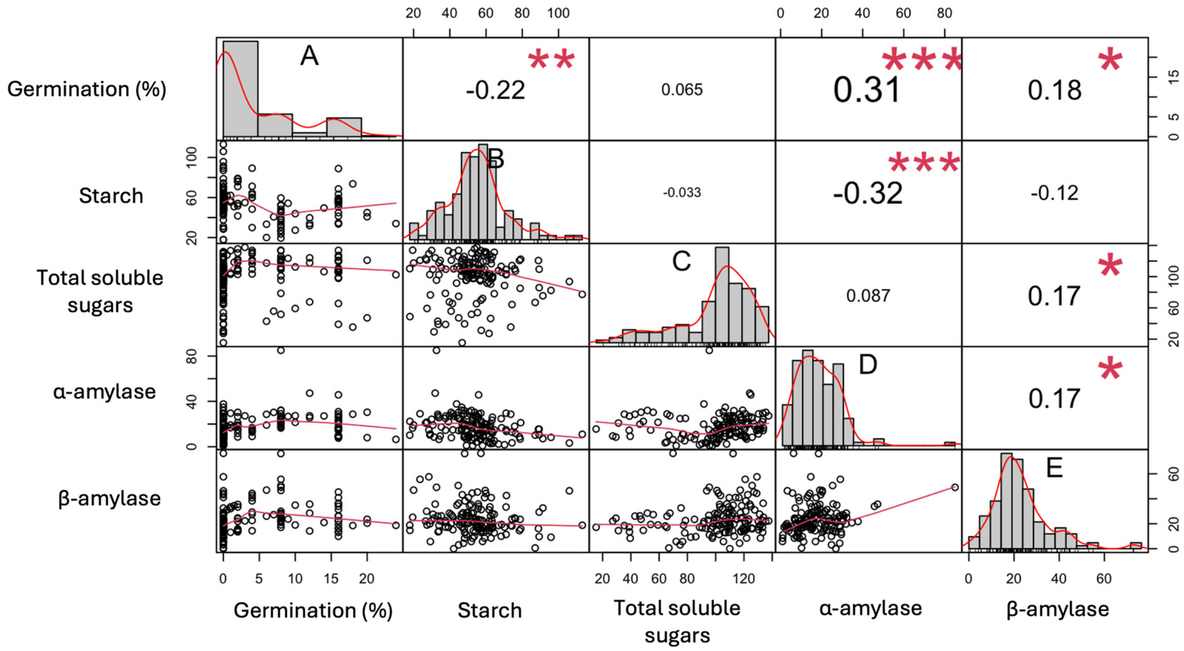

Figure 4: Correlogram showing the relationships between cumulative germination and biochemical variables in seeds of the blackberry cultivar ‘Loch Ness’: (**A**) cumulative germination (%), (**B**) starch content (mg g^{-1} DW), (**C**) total soluble sugar content (mg g^{-1} DW), (**D**) α-amylase activity (μmol g^{-1} DW), and (**E**) β-amylase activity (μmol g^{-1} DW). The lower panels show scatter plots with fitted curves (red line), the diagonal panels show the distribution of each variable, and the upper panels present Pearson correlation coefficients. Asterisks indicate significant correlations ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$).

Germination showed a significant negative correlation with starch content and significant positive correlations with α- and β-amylase activities ($|r| = 0.18–0.31$; $p \leq 0.05$), whereas the relationship with total soluble sugars was weak and not significant ($r = 0.065$; $p > 0.05$).

Linear regression analysis indicated that α-amylase activity alone explained a small proportion of germination variability ($R^2 = 0.09$). A multivariate regression model incorporating starch content and β-amylase activity slightly improved predictive performance ($R^2 = 0.12$) (Table 1).

Among the exploratory nonlinear approaches tested (Table S1), regression tree analysis provided the best, albeit still limited, predictive performance within the present dataset. The model achieved $R^2 = 0.40$ for the training dataset, whereas the test dataset yielded $R^2 = 0.32$ (Table 1). Under these conditions, starch content emerged as the first splitting variable in the model, with a threshold value of 46 mg g^{-1} DW (Fig. 5).

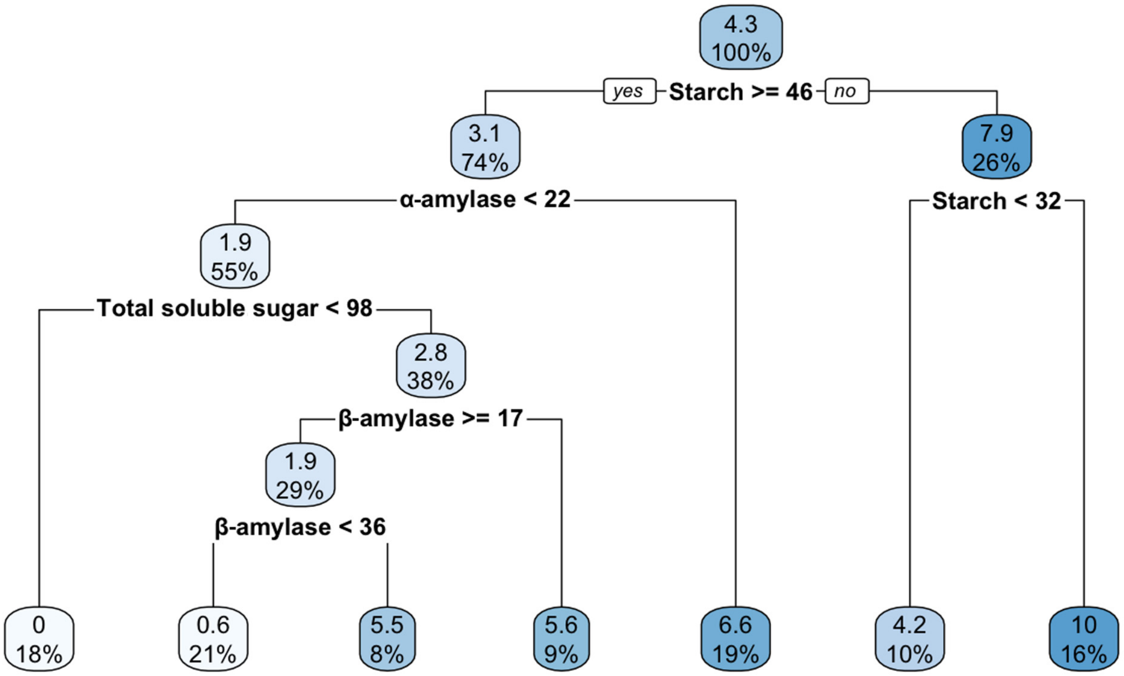

Figure 5: Regression tree predicting cumulative germination (%) of *Rubus fruticosus* seeds (n = 144) from starch content, total soluble sugar content (mg g^{-1} DW), and α- and β-amylase activities (μmol g^{-1} DW). Each terminal node shows the predicted mean germination and the proportion of observations assigned to that node.

Model validation using the test dataset yielded $R^2 = 0.32$, with RMSE = 4.77 and MAE = 3.51, indicating only moderate predictive ability under the present experimental conditions. Therefore, these results should be interpreted cautiously and viewed as exploratory evidence of possible associations rather than as a robust predictive framework.

4 Discussion

This study examined the physiological and biochemical responses of blackberry seeds to cold stratification and static magnetic field exposure under conditions of incomplete dormancy release. The results confirm the strong dormancy of this material and suggest that some treatment combinations were associated with modest changes in germination and reserve mobilization.

4.1 Germination Response and Dormancy Mechanisms

Seed germination remained low after 10 weeks, which is consistent with previous reports describing slow and irregular germination in *Rubus* species [4,5,33]. This behaviour reflects the presence of deep dormancy, typically involving both physical and physiological components. In particular, dormancy in *Rubus* seeds has been associated with seed coat impermeability and internal physiological constraints linked to embryo growth [3,6,7].

The absence of germination after 15 days of cold stratification suggests that early chilling exposure was insufficient to produce a stable transition from dormancy to germination. Rather than indicating a simple linear response to stratification, this result may reflect heterogeneous dormancy status within the seed lot, partial metabolic activation, or an intermediate physiological state in which some dormancy-related processes had already started but radicle protrusion was still prevented. This interpretation is consistent with the strong and complex dormancy previously reported in *Rubus* seeds.

The persistence of low germination even after stratification supports the idea that physiological dormancy remains only partially released under the tested conditions. In this context, the limited mobilization of seed reserves observed in low-germinating treatments may represent a key constraint, as insufficient metabolic activation can delay or prevent radicle emergence. These findings reinforce the complexity of dormancy regulation in *Rubus* and highlight the need to consider both structural and biochemical limitations simultaneously.

Although germination increased in some treatment combinations, the maximum values observed in this study remained low. This is consistent with the strong dormancy commonly reported in *Rubus* seeds, but it also suggests that the conditions tested were not sufficient to fully overcome dormancy in the 'Loch Ness' material. Several factors may have contributed to this outcome, including the absence of scarification, the possibility that the stratification periods were still insufficient, and the fact that germination conditions were not fully optimized. Therefore, the present study should be interpreted as an exploratory physiological evaluation of germination-associated processes rather than as an optimized propagation protocol.

4.2 Effects of SMF and CS on Germination

Cold stratification significantly influenced germination, confirming its well-established role as a dormancy-breaking factor in temperate species [6]. However, the effect of CS alone was limited, suggesting that stratification duration was not sufficient to fully overcome dormancy in all cases.

In contrast, SMF-treated seeds generally showed higher germination than untreated seeds in several stratification treatments. However, this pattern was not observed uniformly across all CS durations, and the absolute germination values remained low. Similar effects of magnetic fields on seed germination have been reported in several crops, including maize, sunflower, and soybean [15–17]. These effects have been associated with increased membrane permeability, improved ion transport, and enhanced enzymatic activity, thereby contributing to faster metabolic activation during germination [10,20].

Importantly, SMF alone did not replace the role of stratification, but was associated with higher germination in several stratification treatments, suggesting a complementary interaction between both factors. This pattern is consistent with previous findings in tree species such as *Tilia miqueliana*, where magnetic treatments accelerated dormancy release when combined with stratification [19].

The interaction between SMF and cold stratification may reflect the dependence of magnetic stimulation on the physiological state of the seed, as suggested by recent work indicating that magnetic responses depend on treatment intensity, exposure duration, and seed physiological status [10,19]. Under strong dormancy, SMF alone may not be sufficient to promote consistent germination, whereas after partial dormancy alleviation through chilling, its effect may become more evident through enhanced metabolic responsiveness. Similar context-dependent effects of magnetic treatments on seed physiology have been reported in other species, where responses varied according to treatment intensity, exposure duration, and the pre-existing dormancy or activation status of the seeds. A comparable response was reported in *Calotropis procera*, where static magnetic field exposure promoted faster germination and increased germination rate, together with biochemical changes consistent with stimulated cellular metabolism [18].

Overall, these results suggest that SMF may have contributed to metabolic activation associated with germination once dormancy had been partially alleviated, rather than acting as a direct dormancy-breaking factor.

4.3 Starch Mobilization during Germination

Treatments showing higher germination generally exhibited lower residual starch content, suggesting that reserve mobilization is associated with germination progression.

Starch is the main energy reserve in seeds and is hydrolyzed into soluble sugars that support respiration and embryo growth during germination [8,9]. Therefore, the reduction in starch content observed in this study likely reflects the metabolic transition from dormancy to active growth.

Similar patterns have been reported in other species, where the initiation of germination is closely linked to the degradation of stored reserves [11]. In *Rubus*, where dormancy is particularly strong, limited starch mobilization may be one of the factors contributing to the low germination percentages observed in untreated or poorly responsive treatments.

Although total soluble sugars increased during the early stratification periods, their weak relationship with final germination suggests that sugar accumulation alone is not a reliable indicator of successful dormancy release. Under the present conditions, this increase may reflect partial reserve mobilization, transient accumulation of soluble carbohydrates, or incomplete metabolic activation during the early stages of chilling. It is also possible that sugars accumulated before being effectively used to support embryo growth, particularly in seeds that remained dormant or only partially activated.

4.4 Role of Amylase Activity

The increase in α- and β-amylase activity observed in SMF-treated seeds indicates that magnetic exposure enhanced enzymatic starch hydrolysis. Amylases play a central role in germination by converting starch into soluble sugars that can be used for metabolic processes [9].

Previous studies have shown that β-amylase activity is particularly important in facilitating reserve mobilization and is often associated with successful germination [11]. In this study, the higher β-amylase activity in SMF-treated seeds is compatible with the hypothesis that enhanced enzymatic activity may contribute to the modest germination differences observed among treatments.

Furthermore, the association between low enzymatic activity and poor germination reinforces the role of metabolic activation as a limiting factor in *Rubus* seeds. In this context, the higher amylase activity observed in SMF-treated seeds is compatible with a possible association between SMF exposure and enhanced carbohydrate metabolism during germination. However, because the present study did not directly evaluate the underlying regulatory pathways, a direct mechanistic effect of SMF on enzymatic activation cannot be confirmed. Therefore, these findings should be interpreted cautiously as exploratory physiological evidence, in line with previous studies on magneto-priming and seed physiological quality [10,20].

Reserve mobilization during germination is closely linked to hormonal regulation, particularly the antagonistic balance between abscisic acid (ABA) and gibberellins (GA). In general, ABA promotes dormancy maintenance and restricts embryo growth, whereas GA promotes hydrolytic enzyme synthesis and the mobilization of storage reserves. In this context, the higher amylase activity observed in SMF-treated seeds may be physiologically compatible with a shift toward a more germination-prone metabolic state. However, because ABA, GA, or related signalling pathways were not measured in this study, this interpretation should be regarded as hypothetical and requires direct confirmation in future studies.

4.5 Predictive Value of Biochemical Markers

The statistical analyses suggested that starch-related traits were among the biochemical variables most closely associated with germination performance in the present dataset. Both linear and nonlinear approaches pointed in this direction, although overall predictive accuracy remained limited.

Linear regression models showed low explanatory power, which is not surprising given the complexity of germination responses and the expected nonlinearity among biochemical variables. The regression tree approach slightly improved model performance, probably because it was able to capture threshold-like relationships and interactions among variables. Similar advantages of nonlinear models have been reported in seed science and crop prediction studies [34,35]. However, this improvement remained modest.

These analyses should not be interpreted as robust predictive models, but rather as exploratory tools to identify candidate biochemical variables potentially associated with germination under the present conditions. The relatively low R^2 values indicate that germination in blackberry seeds is influenced by additional factors not captured in this study, including seed-to-seed variability and potentially relevant physiological processes such as hormonal regulation.

4.6 Implications and Future Research

The results of this study suggest that SMF deserves further evaluation as a complementary pre-sowing treatment in species with strong dormancy, such as *Rubus*. However, its effectiveness appears to depend on the physiological state of the seed and the degree of dormancy release achieved through stratification.

Future research should focus on evaluating SMF effects across different cultivars and species, integrating hormonal and molecular analyses to better understand underlying mechanisms, and testing the scalability of magnetic treatments under practical conditions. Additionally, combining biochemical markers with advanced modelling approaches may improve the prediction of germination behaviour in complex systems.

5 Conclusions

Under the conditions tested, the combined application of static magnetic field (SMF) exposure and cold stratification (CS) was associated with higher germination percentages than the untreated control in several treatment combinations, although absolute germination remained low because of the strong dormancy of the blackberry seed material. The highest germination values were observed in SMF-treated seeds, particularly in combination with 45–75 days of cold stratification, although differences among the best-performing treatments were small.

Germination responses were associated with increased α- and β-amylase activities and lower residual starch content, supporting the view that reserve mobilization is linked to germination progression. Among the variables analysed, starch content was the biochemical trait most consistently associated with germination performance in the present dataset, although its predictive value should be interpreted cautiously given the limited dataset and the biological variability expected in this species.

Overall, the results suggest that SMF may contribute to metabolic activation associated with germination once dormancy has been partially alleviated, rather than acting as a direct dormancy-breaking factor. Therefore, this study should be interpreted as an exploratory physiological evaluation rather than as an optimized germination protocol.

The main limitations of the study include the low overall germination percentages, the absence of scarification, the lack of full control over light conditions, the reduced sample size used for biochemical analyses, and the absence of direct hormonal or molecular measurements. Future studies should evaluate SMF under more optimized dormancy-breaking conditions, including scarification and refined stratification protocols, to better assess its practical value as a complementary pre-sowing treatment.

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Availability of Data and Materials: The data that support the findings of this study are openly available in Dryad at: https://datadryad.org/stash/share/LAnLtOYTS51zG6LgPToNJq8XijE0JMfKiG2DoTgtGE8.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at https://www.techscience.com/doi/10.32604/phyton.2026.082736/s1. Table S1: Performance metrics of different machine learning algorithms evaluated to predict cumulative germination of seeds of the blackberry cultivar 'Loch Ness' using starch content, total soluble sugars, and α- and β-amylase activities as predictors.

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