

Article

Effect of Low Temperature on Germination, Growth, and Seed Yield of Four Soybean (*Glycine max* L.) Cultivars

Anna Szczerba ¹, Agnieszka Płazek ¹ , Jakub Pastuszek ¹, Przemysław Kopec ^{2,*} , Marta Hornyák ¹ and Franciszek Dubert ²

¹ Department of Breeding, Plant Physiology and Seed Science, University of Agriculture, Podłużna 3, 30-239 Kraków, Poland; anna.szczerba21@gmail.com (A.S.); rrplazek@cyf-kr.edu.pl (A.P.); jakub.pastuszek@urk.edu.pl (J.P.); marta.golebiewska1990@gmail.com (M.H.)

² Polish Academy of Sciences, The Franciszek Górski Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland; dubert@ifr-pan.edu.pl

* Correspondence: p.kopec@ifr-pan.edu.pl

Abstract: During germination at low temperatures, seeds rich in proteins may experience damage to their cytoplasmic membranes. The study aimed to investigate the influence of the germination temperature on growth, development, and yield of four cultivars of soybean, a typical thermophilic species. The seeds were germinated at 10, 15, and 25 °C in the dark. After 48 h, one part of the seeds was analyzed for their amylase and dehydrogenase activity, cell membrane permeability, and germination vigor. The other part was transferred into soil and cultivated up to yielding. Chlorophyll fluorescence, fresh (FW) and dry weight (DW) of shoots, pod and seed number, and seed DW were analyzed. The plants of cvs. ‘Abelina’, ‘Malaga’, and ‘Merlin’, germinating at low temperature, produced the highest number of seeds. Seed number negatively correlated with their DW and positively with the number of active reaction centers (RC/CS_m) in all cultivars. In cvs. ‘Abelina’ and ‘Malaga’, the number of seeds also positively correlated with the index performance of photosystem II (PSII), which was the highest in all plants germinating at low temperature. We suggest cultivating cv. ‘Abelina’ in cooler regions, while cvs. ‘Petrina’ and ‘Malaga’ in warmer areas.

Keywords: amylase; chlorophyll fluorescence; cold; dehydrogenases; ion leakage; seed germination; soybean cultivars



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1. Introduction

Legumes are an important economic crop group and play a crucial role in the nutrition of humans and animals [1]. *Glycine max* ranks high among *Fabaceae* plants because its seeds contain more protein (40%) than other legume species. Soybean is economically very important, as it meets the protein needs of modern populations. Soybean can be a useful substitute for meat and dairy products for humans. Its seeds contain almost all amino acids essential in the human diet [2,3]. Soybean occupies an important position on the list of plants sourced for vegetable oils. It is widely used as a feed product for farm animals [4]. Soybean plants live in symbiosis with *Bradyrhizobium japonicum* that assimilates free atmospheric nitrogen. This allows for less extensive nitrogen fertilization of the crop [5,6]. With abundant nodulation, symbiotic nitrogen bacteria can provide soybean plants with up to 100 kg N per 1 ha [7]. It is crucial to understand the physiology of the growth and yielding of various soybean cultivars, especially in the temperate climate. Soybean is a thermophilic plant that requires a temperature of soil above 10 °C—the optimal temperature is 25 °C—for germination. It is grown worldwide in a broad range of latitudes (50° N–35° S). Nevertheless, the subtropical region of South America is the largest soybean cropping area in the world [8–10]. Soybean requires a minimum of 400 mm of well-distributed rainfall during the vegetative growth period. However dry weather is necessary for ripening. Soybeans can grow on a wide range of soils, but the soil should

be well-drained, fertile, and rich in calcium with a pH range of 5.6 to 7.0. In temperate climates, the cultivation of soybean is limited by spring and autumn frosts and low ground temperatures. Soybean was found to be intolerant to low temperature. Robison et al. [11] investigated the possibility of acclimatization of eight *Glycine max* and six *Glycine soja* varieties to the cold. All soybean varieties significantly increased their cold tolerance after short acclimatization of seedlings, however, studied genotypes did not differ significantly in their tolerance. The authors concluded that domestication did not affect the overall ability of soybean to acclimatize to cold temperatures. Their study confirmed that wild species may not be used as an additional source of genetic diversity in cold tolerance. Soybean is a short-day species. Photoperiodic requirements and sensitivity to temperature limit the growing season and geographic distribution of soybean. It is, therefore, necessary to select cultivars with a shorter vegetation season, which is associated with an earlier sowing date. Farmers and breeders used to be faced with similar problems when growing maize in the temperate climate. Maize shows higher seed yields when sown earlier. However, the yield may be unstable [12]. In warm China regions, soybean yield did not change significantly from 2001 to 2017, while an upward trend was observed in the cold region [13]. According to Zhao et al. [13], the average seed yield was higher in warmer regions than in cold ones. So, the selection of cultivars with greater tolerance to cold can reduce the economic risk of soybean cultivation and convince farmers to increase the acreage allocated to this species [14]. In Poland, the most common soybean cultivar is 'Abelina'.

Seed germination is a three-phase process. The first phase is imbibition, consisting in the rapid uptake of water by the cell wall and colloidal compounds in the cytoplasm, mainly proteins and starch, and DNA repair (about 0–24 h after imbibition). In this phase, the hydrolysis of macromolecular compounds takes place. The second phase is the activation of ATP synthesis in the glycolysis, Krebs cycle, and the respiratory chain, and the translation of stored mRNA (24–48 h after imbibition), while the third phase occurs after 48–72 h and is characterized by the emergence of the radicle [15]. Water absorption by the seeds is a critical step during germination. The speed of imbibition involving the hydrophilic components of the cell wall, the cytoplasm, proteins, and carbohydrates depends on the temperature. The higher the temperature, the faster water absorption is. Coldwater not only slows the process down but also poses a threat to cell membranes not adapted to such thermal conditions. Legume seeds typically respond to cold with membrane rupture when the pressure of water is too high [16]. Under optimal conditions, the absorption process is fast. However, when it involves cold water in the soil, it is slowed down and, consequently, becomes dangerous for the embryos. Damage may occur when cell membranes do not adapt quickly to cold, cannot withstand the pressure of water, and break [16]. When water enters dry seeds, cell membranes are disrupted, which in turn leads to changes in membrane selectivity. There is an immediate response manifested in a rapid leakage of ions and metabolite solutions of low molecular weight [17]. Our earlier studies on the influence of low temperature on germination of narrow lupine seeds clearly indicated that temperature below 10 °C severely decreased their germination ability [18,19]. It should be noted that lupines are much less thermally demanding than soybean. It is important to know the factors influencing the germination capacity, such as water absorption at low temperatures. This information may help to improve seed germination vigor and yield [20]. There are methods that limit the damage caused by cold. One of them is seed osmoconditioning and seed moisturizing under higher temperature conditions [21]. The cell membrane consists mainly of phospholipids, proteins, and sterols. Its structure can be modified under the influence of various environmental factors. However, the key factor is temperature. Under the influence of cold (0 to 5 °C), during the so-called frost hardening, there is a quantitative and qualitative change in individual structural compounds [22]. Rapid temperature changes in nonhardened plants cause irreversible damages to membranes, which results in an uncontrolled outflow of ions [23,24]. In addition, gradual acclimation of seeds to cold conditions allows the damage to cell membranes to be minimized through changes in the composition of fatty acids. The seed brittleness is due to the content

of unsaturated fatty acids. The higher their content, the greater the flexibility of cell membranes is. Such a reorganization of cell membranes requires at least a few days, as they need time to rebuild [25].

The germination is controlled not only by external factors but also by internal ones, such as growth regulators, especially gibberellins (GA), and abscisic acid (ABA) that act antagonistically [16]. Gibberellins induce α -amylase to hydrolyze starch deposited in the endosperm to glucose, which initiates the oxygen respiration pathway and elongation of germ cells. Starch accumulates in the endosperm as two D-glucose homopolymers, including amylose with only linear α -1,4-glucan and amylopectin with α -1,6 glucan linked branches [26]. The dry seed rapidly resumes metabolic activity upon imbibition, which is needed for the degradation of macromolecules stored in the endosperm. Energy and nutrients are necessary for the development of cotyledons and radicles. Dehydrogenases play an important role in energy generation needed for the first metabolic processes during seed germination. These enzymes indirectly take part in oxidative phosphorylation, transferring hydrogen protons from the substrates to the coenzymes, which delivering protons to the oxidative chain [27]. Yang et al. [27] pointed out the role of glucose-6-phosphate dehydrogenase in plant response to environmental stresses.

In many studies, the condition of plants is assessed based on measurement of chlorophyll *a* fluorescence. Evaluation of chlorophyll *a* fluorescence is a fast, non-invasive, and very sensitive method of assessing the efficiency of photosystem II (PSII) [18]. It is commonly used to determine plant response to various environmental stresses [19,20]. Determination of chlorophyll fluorescence parameters provides information on the amount of energy used in photochemical processes, as well as energy lost as heat [21]. Chlorophyll fluorescence can also be used to predict yields of crop plants under various environmental conditions [20].

In recent years, soybean cultivation in temperate climates has increased significantly. However, farmers can only cultivate this species in regions characterized by specific microclimate with higher annual temperatures and longer vegetative season. In Poland, there is a large variation between regions in these parameters. Each year, the National Register of Crops' Cultivars (COBORU) recommends cultivars for individual agricultural areas. Moreover, in Poland, we do not have soybean cultivars that would guarantee the Polish farmers a stable seed yield. The main reason for this is too low spring and autumn temperatures.

We hypothesized that the cold occurring during the first days of soybean germination and early growth stages affects its later development and yield. The study aimed to investigate the effect of low temperature during seed germination of four soybean cultivars on physiological and biochemical properties as well as the yield of the species. The studied cultivars varied in terms of earliness and seed yield per hectare. We analyzed the seed vigor germination, cell membrane permeability, activity of α -amylase and dehydrogenases in the germinating seeds, fresh and dry weight of plants, the kinetics of chlorophyll fluorescence, number of pods and seeds, and their weight calculated per a single plant. Based on the outcomes, we were able to identify physiological markers of greater soybean tolerance to low temperature. The identification of such markers could help breeders select soybean cultivars suitable for colder regions.

2. Materials and Methods

2.1. Plant Material

This study involved four cultivars of *Glycine max* L. Cultivars 'Abelina', 'Malaga', and 'Merlin' were obtained from SaatBau Poland Sp. z o.o., while cv. 'Petrina' came from Danko Plant Breeding Sp. z o.o. in Chorynia (Kościan, Poland), a company belonging to the National Centre for Agricultural Support. Cultivars 'Abelina', 'Malaga', and 'Merlin' originated from Austria and cv. 'Petrina' from Canada'. All seeds were collected during the vegetation season of 2019. Cultivar Abelina (registered in Poland 2016), a medium-early cultivar, is distinguished by a very high yielding potential and exceptional stability over

the years. Cultivar Merlin' (registered in Common Catalog of Varieties of Agricultural Plant Species—CCA 2013) is early, characterized by very high yield and strong vigor. Cultivar Malaga (registered in CCA 2010) is the late cultivar of soybean, and it has a long vegetation period. The description of these three cultivars was described on the Website of SaatBau [28]. Cultivar Petrina (registered in Poland 2017) is very late and demonstrates a high yield of seeds rich in proteins [29].

2.2. Study Design

The study involved two experiments performed from May to September 2020 under controlled conditions (growth chamber) and in an open foil tunnel. Soybean seeds were put into Petri dishes lined with filter paper and wetted with distilled water. They were germinated in the dark at three different temperatures: 10, 15, and 25 °C. After 48 h, the percentage of non-germinating seeds and germination vigor were determined. Then, one batch of the seeds was used for the analysis of cell membrane permeability evaluated by electrolyte leakage and for assessing the activity of the pool of dehydrogenases and α -amylase. The other batch of the germinating seeds was transferred into pots located in an open foil tunnel. The plants were watered as needed and fertilized once a week. One week after sowing into the soil, the percentage of seedlings grown from seeds germinating at different temperatures was calculated. Seven weeks after sowing, the fresh and dry weight of shoots with leaves were analyzed. At the same time, measurements of the kinetics of chlorophyll *a* fluorescence in leaves were performed. Yield parameters were analyzed at the end of the vegetative season. We also determined the number of pods and seeds and their weight per single plant. Figure 1 shows the design of both experiments.

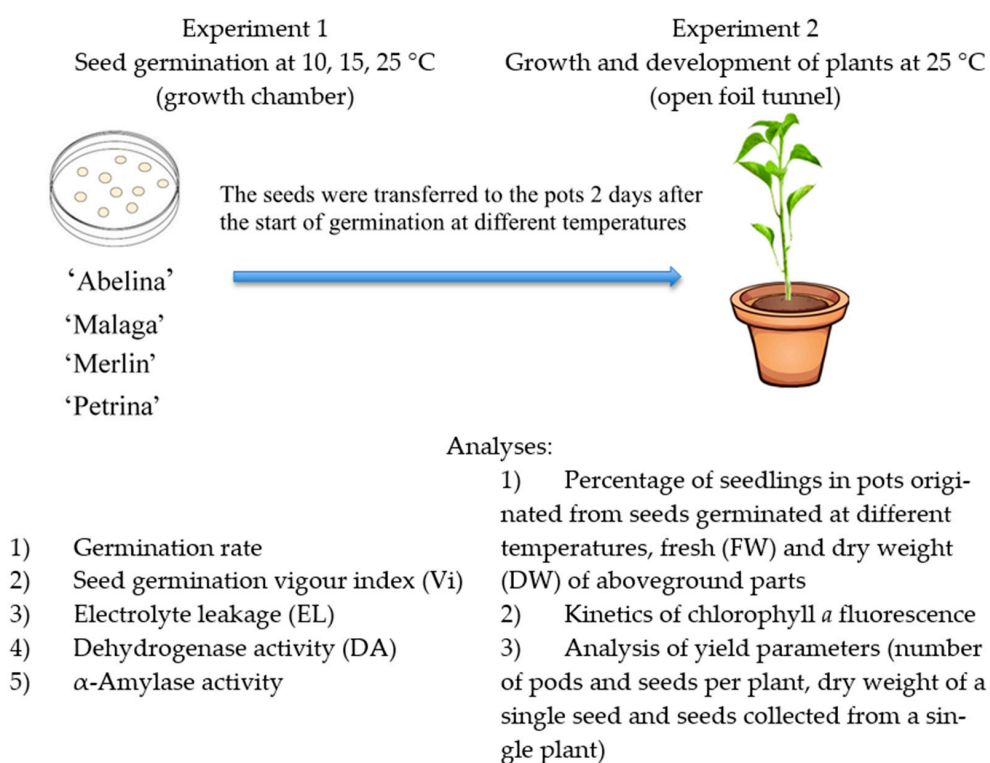


Figure 1. Design of the experiments carried out between May and September 2020.

2.3. Experiment 1

2.3.1. Seed Germination Vigor Index (V_i)

Soybean seeds of four cultivars: Abelina, Malaga, Merlin, and Petrina, were put into Petri dishes lined with filter paper (10 seeds per dish; 5 dishes per cultivar/temperature combination), wetted with 10 cm³ of distilled water, and germinated in the dark at three

different temperatures: 10, 15 and 25 °C. The seeds germinated in the growth chambers ST 5C SMART (POL-EKO Aparatura, Wodzisław Śląski, Poland). Two days after sowing, the number of non-germinated seeds was counted and presented as the percentage of all sown seeds per combination.

Germination vigor on the base of hypocotyl length was estimated 48 h after sowing, as described by Płażek et al. [18]. The visual scale was used, where: 0—no germination; 1—hypocotyl length of 1 mm; 2—hypocotyl length of 2–3 mm; 3—hypocotyl length of 4–7 mm; and 4—hypocotyl length greater than 7 mm. The V_i index was calculated according to the formula:

$$V_i = \frac{n_0 \times 0 + \dots + n_4 \times 4}{N} \quad (1)$$

where: n_x is the number of seeds assigned to a given hypocotyl length; N is the total number of seeds in the dish. V_i was assessed in 20 replicates for each cultivar/temperature combination.

2.3.2. Electrolyte Leakage (EL)

The seeds collected 48 h after sowing were placed in test tubes containing 10 cm³ of redistilled water (one seed per tube) and shaken (100 rpm) at 20 °C for 24 h. After this time, electrical conductivity (E1) was measured with a conductometer (CI 317, Elmetron, Poland). Next, the samples were frozen for 24 h at −80 °C, then thawed and shaken again. Conductivity measurements were repeated, and the resulting values represented total ion content (E2). Membrane permeability was expressed as a percentage of total EL according to the formula:

$$EL = \frac{E1 \times 100}{E2} \quad (2)$$

All measurements were carried out in 10 replicates for each cultivar/temperature variant.

2.3.3. Dehydrogenase Activity

The activity of the dehydrogenase pool (DA) in seeds was determined according to Steponkus and Lanphear [30], with slight modifications. The seeds from each cultivar/temperature combination were collected and weighed. Then, they were placed into plastic tubes and poured over with a reaction mixture containing 1.5 cm³ of 0.4% (v: w) aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC; Sigma-Aldrich) and 1.5 cm³ of 0.1 M phosphate buffer (pH 7.5). The seeds were incubated for 3 h at 10 °C, 15 °C, and 25 °C in the dark. For the extraction of the TTC reduction product—triphenylformazan, the seeds were homogenized with 5 cm³ of 96% ethanol. Centrifugation of the extract lasted for 5 min at 16,000 × *g*. Absorbance was measured at 485 nm with an Ultrospec 2100 spectrophotometer (Amersham Biosciences, Little Chalfont, UK). Dehydrogenase activity was expressed as µg formazan per 1 g of protein. The concentration of protein in the seeds was determined spectrophotometrically at 595 nm, as described by Bradford [31]. Albumin of bovine serum (Sigma-Aldrich) was used as a standard. The analyses were performed in five biological replicates.

2.3.4. α-Amylase Activity Assay

Alpha-amylase activity in the seeds was analyzed as described earlier by Płażek et al. [19]. Determination of α-amylase was carried out by quantifying the reducing sugar (maltose equivalent) liberated from hydrolysis of starch by the enzyme. The reducing sugar content was determined using the modified dinitrosalicylic acid (DNS) method, according to Bernfeld [32]. The seeds collected one day after sowing were ground with 2 cm³ ice-cold 1 M sodium phosphate buffer (pH 7.0) and then centrifuged (20,000 × *g*) for 15 min. The supernatant was heated at 70 °C for 15 min in the presence of 1 mM CaCl₂. The heat-treated supernatant (125 µL) was added to 125 µL of 1% soluble starch in 100 mM Na-acetate buffer (pH = 4.5) containing 10 mM CaCl₂. The mixture was incubated for 15 min at 37 °C and the reaction was stopped by adding 250 µL of DNS reagent (96 mM 3,5-dinitrosalicylic acid, 2 M NaOH, 1 M K-Na tartrate). After that, the samples were heated in boiling water bath for

5 min and cooled on ice. Absorbance was measured at 540 nm. The reducing sugar formed by enzymatic activity was calculated using a standard curve for maltose. The results were expressed as U mg^{-1} protein. One unit (U) is defined as the amount of enzyme releasing 1 mg of maltose from starch in 15 min under the assay conditions. All measurements were performed in three replicates for each cultivar/temperature combination.

2.4. Experiment 2

2.4.1. Percentage of Seedlings Grown from Seeds Germinated at 10, 15, and 25 °C

The seeds germinated for 48 h at 10, 15, and 25 °C, as described in Experiment 1, were transferred into pots filled with a 1: 1: 1 (v: v: v) mixture of commercial soil (EKOziem, Poland, pH 6.0), sand, and perlite. Each pot harbored five seeds. Each combination included five pots per cultivar/germination temperature. Although the seeds subjected to 10 °C did not manage to germinate, although they were sown into the soil at the same time as the seeds from other temperatures. The plants were grown outdoors, in the open foil tunnel. One week after sowing the seeds, the percentage of seedlings was calculated. The plants were fertilized weekly with Hoagland medium [33] and grown until seed yielding.

2.4.2. Kinetics of Chlorophyll *a* Fluorescence

Chlorophyll *a* fluorescence was measured in seven-week-old plants in a fully developed third leaf after 30 min of dark adaptation. The photochemical efficiency of photosystem II (PSII) was measured using a plant fluorescence analyzer (PEA; Hansatech Ltd., King's Lynn, Norfolk, UK) with an excitation light intensity of $3 \mu\text{mol m}^{-2} \text{s}^{-1}$. The individual PSII performance parameters were calculated according to Strasser et al. [34]: ABS/CS_m —energy absorption by antennas; TR_0/CS_m —the amount of excitation energy trapped in PSII (energy transferred to the reaction center); ET_0/CS_m —the amount of energy used to transport electrons; DI_0/CS_m —energy dissipation from PSII (energy lost as heat), RC/CS_m —number of active reaction centers, where CS_m is the sample cross-section; PI—PSII performance index (PI) normalized for equal absorption. Measurements were done in 10 replicates for each combination of cultivar/temperature.

2.4.3. Fresh and Dry Weight

Seven-week-old plants were assessed for their fresh (FW) and dry weight (DW). The aboveground parts (shoots with leaves) were cut and weighed to determine the FW, then dried at 70 °C for 48 h to determine the DW. Analysis was done in 20 replicates for each combination of cultivar/temperature.

2.4.4. Analysis of Yield Parameters

Ripe seeds were collected successively from plants. The number of pods, seeds, and seed dry weight was measured per plant. The seeds were placed in paper bags and slowly dried in a greenhouse until a constant weight was obtained. The analysis involved 10 plants for each combination of cultivar/temperature.

2.4.5. Statistical Analysis

The experiments were arranged in a fully randomized design. Two-way ANOVA was performed using the statistical package STATISTICA 12.0 (Stat-Soft, Inc., Tulsa, OK, USA). Normal distribution data were analyzed using the Shapiro–Wilk test. Linear correlation coefficients (Pearson's) were assumed significant at $p < 0.05$. All data were presented as means $\pm$ SE (standard error).

3. Results

3.1. Experiment 1

3.1.1. Seed Germination

No studied cultivar demonstrated the ability to germinate at 10 °C after 48 h (Table 1). All the cultivars needed more time (six days) to germinate at this temperature (data not

shown). An increase in temperature improved the germination rate of all the cultivars. However, at 15 °C, it still was very low, with the exception of Petrina showing an almost two-fold greater number of germinated seeds than the other cultivars. At 25 °C, all cultivars demonstrated a very high ability to germinate.

Table 1. Percentage (%) of seeds of four soybean cultivars germinated after 48 h at different temperatures.

Temperature/Cultivar	10 °C	15 °C	25 °C
Abelina	0 cA	26.7 ± 3.7 bB	100 ± 0 aA
Malaga	0 cA	16.7 ± 2.8 bC	100 ± 0 aA
Merlin	0 cA	19.9 ± 2.4 bC	97.5 ± 1.3 aA
Petrina	0 cA	52.5 ± 5.8 bA	98.5 ± 1.2 aA

Values represent means ($n = 50$) ± SE. The values within rows followed by the same lowercase and within columns followed by the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

At 10 °C, Vi amounted to zero for all studied cultivars due to no germination ability for 48 h (Table 2). At 15 °C, cv. Petrina showed the highest Vi, while Vi values for the remaining cultivars were similar. Germination vigor of all cultivars at 25 °C was higher than at 15 °C, with the highest Vi for Abelina and Merlin as compared with Malaga and Petrina.

Table 2. Temperature effect on germination vigour index (Vi) of seeds of four soybean cultivars evaluated after 48 h.

Temperature/Cultivar	15 °C	25 °C
Abelina	0.35 ± 0.08 bB	3.30 ± 0.28 aA
Malaga	0.35 ± 0.1 bB	2.88 ± 0.22 aB
Merlin	0.28 ± 0.08 bB	3.13 ± 0.54 aA
Petrina	1.05 ± 0.10 bA	2.80 ± 0.39 aB

Values represent means ($n = 20$) ± SE. The values within rows followed by the same lowercase and within columns followed by the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.1.2. Electrolyte Leakage

The increase in temperature reduced electrolyte leakage from germinating seeds of all studied cultivars (Table 3). The highest cell membrane permeability at 10 °C and 15 °C was observed in cv. Malaga, and the lowest at 10 °C in cv. Abelina. The latter showed similar EL at 10 °C and 15 °C but lower at 25 °C. The other cultivars showed a gradual decrease in EL with increasing temperature. At 25 °C, the lowest cell membrane permeability in germinating seeds was recorded for cv. Petrina.

Table 3. Electrolyte leakage (%) from seeds of four soybean cultivars germinated for 48 h at different temperatures.

Temperature/Cultivar	10 °C	15 °C	25 °C
Abelina	26.72 ± 2.70 aD	26.15 ± 2.82 aB	21.60 ± 2.01 bB
Malaga	52.22 ± 1.13 aA	35.98 ± 0.70 bA	26.12 ± 1.42 cA
Merlin	32.09 ± 2.49 aC	26.68 ± 2.94 bB	21.26 ± 2.94 cB
Petrina	37.25 ± 1.94 aB	26.74 ± 4.64 bB	17.34 ± 1.93 cC

Values represent means ($n = 10$) ± SE. The values within rows followed by the same lowercase and within columns followed by the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.1.3. Activity of Dehydrogenase (DA) Pool

The temperature influenced DA activity in the germinating seeds of all studied plants. Each cultivar showed a considerable increase in DA activity with increasing temperature (Figure 2). At 10 °C, the highest DA activity was seen in the seeds of cv. Petrina, and the lowest in the seeds of cv. Abelina. At 15 °C, the activity of these enzymes increased four

times in cv. Abelina seeds, and 2.6, 3, and 1.6 times in cvs. Malaga, Merlin, and Petrina, respectively. The highest DA activity at 25 °C was noted in cv. Merlin seeds, and the lowest in cv. Petrina seeds. The latter showed the smallest changes in DA activity under the increasing temperature.

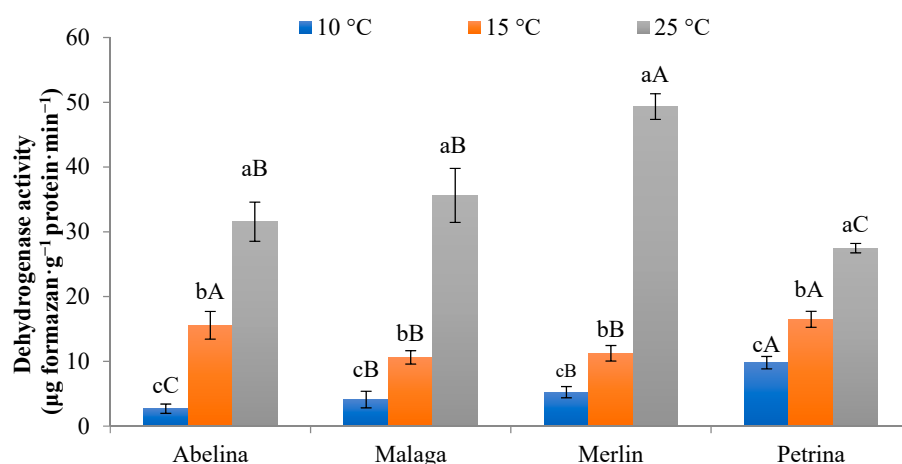


Figure 2. Dehydrogenase activity ($\mu\text{g formazan g}^{-1} \text{ protein min}^{-1}$) in the seeds of four soybean cultivars germinated at different temperatures. Values represent means ($n = 5$) $\pm$ SE. The values for each cultivar followed by the same lowercase letter do not differ significantly, while means for the same temperature marked with the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.1.4. Activity of α -Amylase

Changes in the activity of α -amylase in the seeds of Abelina, Malaga, Merlin, and Petrina followed a similar pattern (Figure 3). In the case of cvs. Abelina and Malaga, activity increased at 15 °C compared to that at 10 °C, and it did not change at 25 °C. In cultivars Merlin and Petrina, α -amylase activity increased at 25 °C. The weakest response to temperature increases in terms of α -amylase activity was observed in cv. Merlin seeds, and the strongest in cv. Petrina ones, where at 25 °C, the activity of this enzyme increased by 2.2 times compared to that at 10 °C.

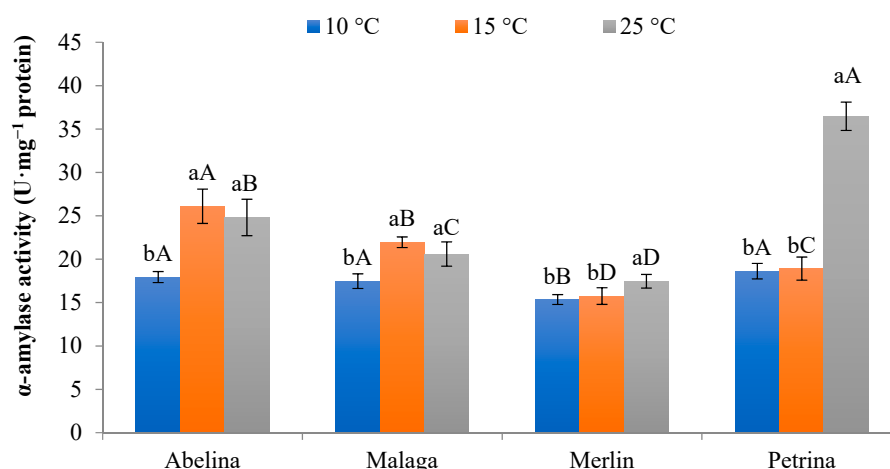


Figure 3. Activity of α -amylase ($\text{U mg}^{-1} \text{ protein}$) in the seeds of four soybean cultivars germinated at different temperatures. Values represent means ($n = 3$) $\pm$ SE. The values for each cultivar followed by the same lowercase letter do not differ significantly, while means for the same temperature marked with the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.2. Experiment 2

3.2.1. Percentage of Seedlings

Among the seeds germinating at 10 °C, the highest number of seedlings was obtained for cv. Abelina, and the lowest for cv. Petrina (Figure 4). The temperature of 15 °C applied during germination significantly increased the percentage of seedlings, especially for cvs. Malaga and Petrina. One hundred percent of Abelina seeds produced seedlings at 25 °C, while in the other cultivars, this percentage was significantly lower, with the poorest results for cv. Petrina.

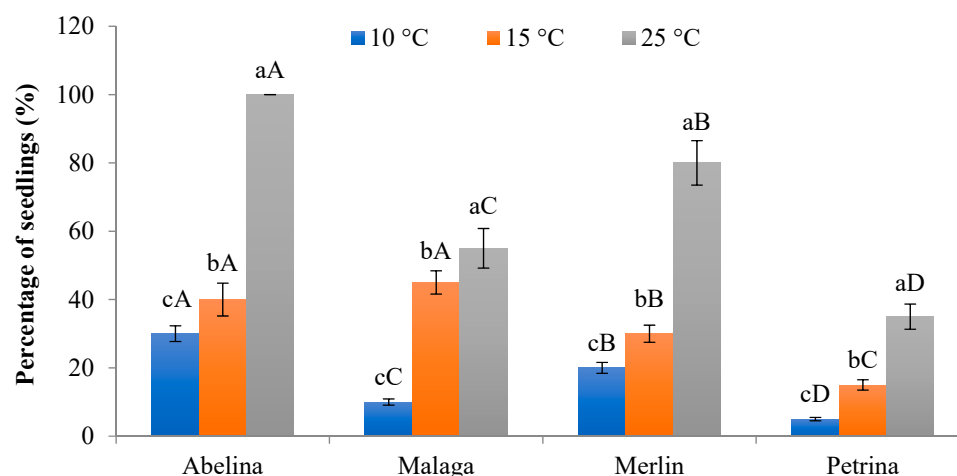


Figure 4. Percentage of seedlings grown from the entire pool of seeds germinated for 48 h at different temperatures. The percentage of seedlings was calculated one week after the seed transfer into pots filled with soil and cultivated at 25 °C. Values represent means ($n = 25$) $\pm$ SE. The values for each cultivar followed by the same lowercase letter do not differ significantly, while means for the same temperature marked with the same uppercase do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.2.2. Kinetics of Chlorophyll *a* Fluorescence (Chlf)

Chlf analysis showed that the amount of energy absorbed by the antennas (ABS/CS_m) and excitation energy trapped in PSII (TR_o/CS_m) did not depend on the germination temperature in any studied cultivar except for cv. Petrina, where both parameters were significantly lower in the plants grown from seeds germinating at 10 and 15 °C than in those germinating at 25 °C (Table 4). ET_o/CS_m , the amount of energy used for electron transport, was higher only in plants of cv. Abelina germinated at 25 °C and cv. Petrina germinated at 15 and 25 °C. Energy dissipation from PSII (DI_o/CS_m) was significantly higher only in the case of cvs. Merlin and Petrina plants germinated at 10 °C as compared to those germinated at 15 and 25 °C. The lower germination temperatures (10 and 15 °C) decreased the number of reaction centers in all the cultivars. The most surprising result was that the photochemical efficiency of the photosynthetic apparatus (PI) was higher in the plants of cv. Malaga and Petrina germinating at 10 °C and 15 °C than at 25 °C, while in the case of cv. Abelina, PI was higher only in plants germinating at 10 °C. The temperature of seed germination did not affect PI of cv. Merlin plants.

Table 4. Kinetics of chlorophyll *a* fluorescence in four soybean cultivars grown from seeds germinated for 48 h at different temperatures.

Cultivar	Temp. (°C)	ABS/CS _m	TR _o /CS _m	ET _o /CS _m	DI _o /CS _m	RC/CS _m	PI _{ABS}
Abelina	10	312 ± 19 ^{aA}	259 ± 13 ^{aA}	109 ± 3 ^{bB}	57 ± 6 ^{aB}	793 ± 61 ^{cB}	2.54 ± 0.24 ^{aAB}
	15	312 ± 18 ^{aA}	259 ± 14 ^{aA}	111 ± 8 ^{bB}	53 ± 4 ^{aA}	922 ± 83 ^{bB}	1.88 ± 0.26 ^{bB}
	25	324 ± 21 ^{aB}	266 ± 18 ^{aB}	120 ± 2 ^{aB}	52 ± 3 ^{aB}	1057 ± 129 ^{aB}	1.42 ± 0.13 ^{cD}
Malaga	10	320 ± 18 ^{aA}	264 ± 13 ^{aA}	114 ± 7 ^{aAB}	60 ± 7 ^{aB}	954 ± 73 ^{bA}	2.43 ± 0.25 ^{aB}
	15	327 ± 21 ^{aA}	270 ± 18 ^{aA}	118 ± 9 ^{aAB}	57 ± 4 ^{aA}	996 ± 78 ^{bA}	2.19 ± 0.08 ^{abAB}
	25	336 ± 28 ^{aB}	276 ± 14 ^{aB}	125 ± 11 ^{aB}	55 ± 5 ^{aA}	1178 ± 110 ^{aA}	2.11 ± 0.1 ^{bB}
Merlin	10	297 ± 15 ^{aA}	250 ± 15 ^{aA}	121 ± 6 ^{aA}	52 ± 2 ^{aC}	971 ± 77 ^{bA}	2.68 ± 0.22 ^{aA}
	15	309 ± 24 ^{aA}	257 ± 19 ^{aA}	122 ± 7 ^{aAB}	50 ± 2 ^{abB}	1050 ± 84 ^{aA}	2.92 ± 0.38 ^{aA}
	25	308 ± 24 ^{aB}	257 ± 17 ^{aB}	126 ± 9 ^{aB}	46 ± 3 ^{bC}	1063 ± 83 ^{aB}	2.62 ± 0.05 ^{aA}
Petrina	10	311 ± 21 ^{bA}	256 ± 12 ^{bA}	118 ± 8 ^{bAB}	89 ± 4 ^{aA}	891 ± 71 ^{bAB}	2.74 ± 0.18 ^{aA}
	15	323 ± 22 ^{bA}	267 ± 16 ^{bA}	131 ± 10 ^{aA}	55 ± 6 ^{bA}	957 ± 83 ^{abAB}	2.96 ± 0.17 ^{aA}
	25	382 ± 30 ^{aA}	292 ± 12 ^{aA}	135 ± 12 ^{aA}	55 ± 5 ^{bA}	1111 ± 88 ^{aA}	1.82 ± 0.08 ^{bC}

Values represent means ($n = 10$) ± SE. The values of each parameter for each cultivar/temperature combination followed by the same lowercase letter and values of all cultivars at the same temperature followed by the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.2.3. Fresh and Dry Weight of Aboveground Parts

Plants of cv. Abelina showed changes in FW and DW resulting from germination at different temperatures (Figures 5 and 6). Cultivar Malaga plants grown from the seeds germinating at 10 °C and 15 °C had the same FW and DW, while germination at 25 °C significantly increased both parameters. In the case of cv. Merlin, germination temperature did not affect FW and DW of the aboveground parts. Cultivar Petrina plants showed similar FW following germination at all temperatures, but their DW was greater when seeds germinated at 15 and 25 °C than at 10 °C.

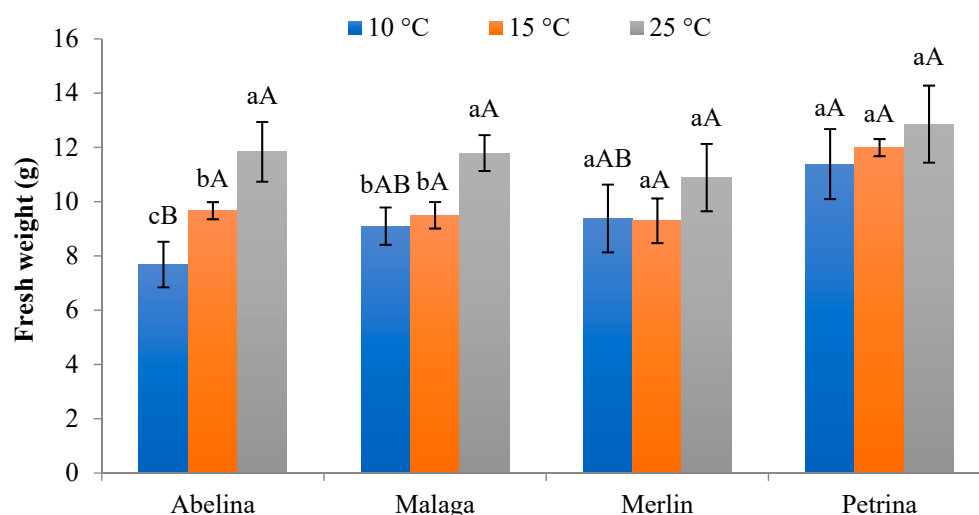


Figure 5. Fresh weight of aboveground parts in four soybean cultivars grown for the first seven weeks from the seeds germinated for 48 h at different temperatures. Values represent means ($n = 20$) ± SE. The values for each cultivar followed by the same lowercase letter do not differ significantly, while means for the same temperature marked with the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

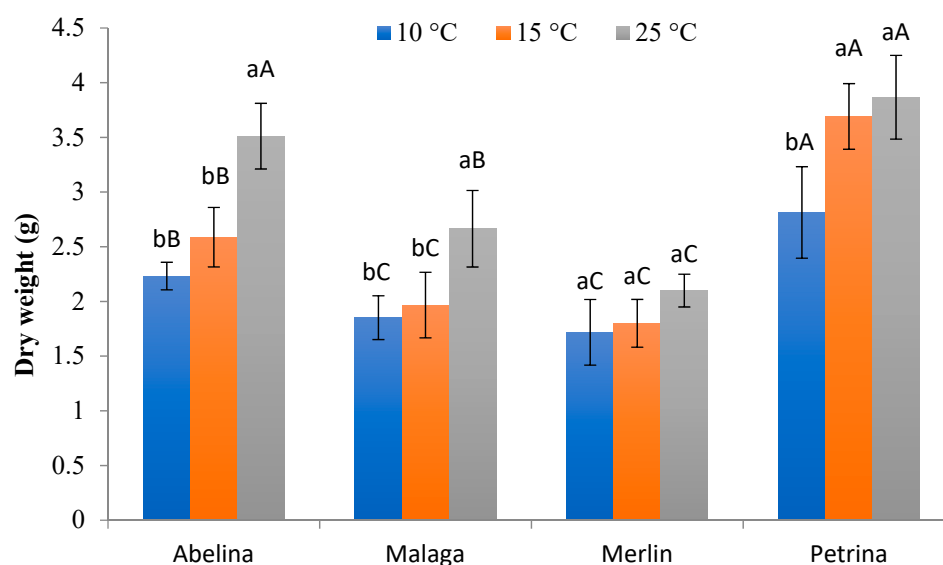


Figure 6. Dry weight of aboveground parts in four soybean cultivars grown for the first seven weeks from the seeds germinated for 48 h at different temperatures. Values represent means ($n = 20$) $\pm$ SE. The values for each cultivar followed by the same lowercase letter do not differ significantly, while means for the same temperature marked with the same uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

3.2.4. Yield Parameters

All plants grown from the seeds germinated at 10 °C produced fewer pods than those germinated at 15 and 25 °C (Table 5). The increase in the germination temperature positively affected the pod number, especially in cv. Petrina. Among the plants grown from the seeds germinating at 10 °C, cvs. Merlin and Petrina produced more pods than cvs. Abelina and Malaga (Table 5).

Table 5. Average number of pods, seeds, dry weight of seeds, and dry weight of a single seed in plants of four soybean cultivars germinating for 48 h at different temperatures.

Cultivar	Temp. (°C)	Number of Pods Per Plant	Number of Seeds Per Plant	DW of Seeds Per Plant (g)	DW of a Single Seed (g)
Abelina	10	1.8 $\pm$ 0.2 ^{bB}	15.7 $\pm$ 1.7 ^{aB}	0.23 $\pm$ 0.04 ^{aB}	0.019 $\pm$ 0.002 ^{cC}
	15	2.3 $\pm$ 0.3 ^{aB}	5.0 $\pm$ 1.1 ^{bB}	0.21 $\pm$ 0.02 ^{aC}	0.033 $\pm$ 0.003 ^{bD}
	25	2.1 $\pm$ 0.1 ^{aB}	5.6 $\pm$ 1.4 ^{bC}	0.25 $\pm$ 0.04 ^{aC}	0.047 $\pm$ 0.005 ^{aB}
Malaga	10	1.4 $\pm$ 0.2 ^{bC}	25.7 $\pm$ 2.5 ^{aA}	0.15 $\pm$ 0.03 ^{cC}	0.007 $\pm$ 0.001 ^{bD}
	15	2.4 $\pm$ 0.5 ^{abB}	4.8 $\pm$ 0.6 ^{cB}	0.19 $\pm$ 0.02 ^{bD}	0.039 $\pm$ 0.004 ^{aC}
	25	2.6 $\pm$ 0.5 ^{aB}	9.0 $\pm$ 0.9 ^{bB}	0.32 $\pm$ 0.03 ^{aB}	0.036 $\pm$ 0.004 ^{aC}
Merlin	10	2.1 $\pm$ 0.4 ^{bA}	13.8 $\pm$ 1.4 ^{aB}	0.37 $\pm$ 0.05 ^{aA}	0.024 $\pm$ 0.002 ^{bA}
	15	2.6 $\pm$ 0.2 ^{aB}	11.1 $\pm$ 1.3 ^{aA}	0.36 $\pm$ 0.04 ^{aB}	0.051 $\pm$ 0.005 ^{aA}
	25	2.4 $\pm$ 0.1 ^{abB}	6.7 $\pm$ 1.4 ^{bC}	0.35 $\pm$ 0.05 ^{aB}	0.054 $\pm$ 0.005 ^{aA}
Petrina	10	2.0 $\pm$ 0.3 ^{cA}	4.0 $\pm$ 0.5 ^{bC}	0.08 $\pm$ 0.02 ^{bD}	0.021 $\pm$ 0.002 ^{cA}
	15	5.9 $\pm$ 0.2 ^{aA}	5.6 $\pm$ 1.4 ^{bB}	0.57 $\pm$ 0.07 ^{aA}	0.041 $\pm$ 0.004 ^{aB}
	25	5.0 $\pm$ 0.5 ^{bA}	12.8 $\pm$ 1.4 ^{aA}	0.56 $\pm$ 0.08 ^{aA}	0.045 $\pm$ 0.005 ^{aB}

Values represent means ($n = 10$) $\pm$ SE. The values within columns for each cultivar followed by the same lowercase letter do not differ significantly, while values within columns for each combination cultivar/germination temperature followed by an uppercase letter do not differ significantly according to Duncan's multiple range test ($p < 0.05$).

An interesting effect of cold applied during germination was the highest number of seeds produced by a single plant (Table 5). Plants of all studied cultivars, with the exception of cv. Petrina grown from the seeds germinated at 10 °C produced the highest number of seeds despite a small number of pods. Their seed number was two to four

times greater than in the plants germinated at other temperatures. The most abundant seed production at this germination temperature was noted in cv. Malaga which produced 5.4 times more seeds than the plants germinating at 15 °C, and 2.8 times more seeds than the plants germinating at 25 °C. Contrary to that, in cv. Petrina, the number of seeds produced by plants germinating at 10 °C was three times and at 15 °C, two times lower than at 25 °C.

However, the number of seeds did not correlate with their DW. The dry weight of Abelina and Merlin seeds was similar irrespective of the germination temperature (Table 5). Seed DW in cv. Malaga increased gradually with rising germination temperature, while cv. Petrina seeds produced by plants germinating at 15 °C and 25 °C had a DW seven times greater than those produced by plants germinating at 10 °C. In cvs. Abelina, Malaga, and Merlin, the number of seeds correlated negatively with the weight of a single seed ($r = -0.88$; -0.90 ; -0.64 ; $p < 0.05$, respectively). Contrary to the DW of a single seed, the DW of the seeds collected from a single plant of cvs. Abelina and Merlin did not depend on the germination temperature. This result confirmed our observations that higher production of seeds per plant resulted in lower weight of a single seed, i.e., the seeds were smaller.

The number of seeds correlated with the number of active centers (RC/CS_m) in cvs. Abelina and Malaga and amounted to $r = 0.80$ ($p < 0.05$), and $r = 0.70$ ($p < 0.05$), respectively. Another positive correlation ($r = 0.89$; $p < 0.05$) was found between the number of seeds and PI in cvs. Abelina, Malaga, and Merlin.

4. Discussion

4.1. Consequence of Low Temperature on Seed Germination Process

The outcomes of our experiment concerning the effect of temperature on the germination of soybean seeds are in line with other studies. Hatfield and Egli [35] found that at 10 °C, soybean hypocotyl elongation was extremely slow and reached its maximum at 30 °C. Alm et al. [36] showed that an increase in temperature from 10 to 25 °C enhanced the elongation of maize and soybean seedlings. In our experiment, low temperature (10 °C) completely inhibited seed germination in all studied cultivars. As the temperature increased, so did the number of germinated seeds. At 10 °C, the seeds needed more time to germinate, and in our experiment, it was six days.

Cell membranes are the first structures responding to temperature stress. Their structural integrity and stability of their functions under stress can be measured by means of ion leakage [37,38]. Cell membranes are complex and dynamic structures made of lipids and proteins. Stress factors, i.e., salinity, high temperature, low temperature, and drought, may damage the cell membranes and cause electrolyte leakage that can be easily assessed [38,39]. The ion leakage from seeds of studied soybean cultivars germinating at 10 °C significantly differentiated their tolerance to low temperature. In our study, a decrease in the EL with increasing temperature was observed in all cultivars except for cv. Abelina, which showed a similar EL at all the temperatures. It seems this cultivar may be recommended for areas characterized by lower spring temperatures. In cv. Malaga, the differences in EL at different temperatures were greater—even by two times than in the other cultivars. Cultivar ‘Malaga’ is more sensitive to cold in the first phase of germination, and it can be risky to grow in areas with frequent low temperatures and ground frosts. We suggest that the degree of membrane permeability of soybean seeds germinating at 10 °C can be considered a physiological marker of tolerance to low temperature.

The temperature affected the activity of seed dehydrogenases in all studied cultivars. In general, increasing the germination temperature resulted in enhanced DA activity. In all cultivars, the activity was similar at 10 °C and 15 °C, but it increased significantly at 25 °C. Our results were similar to Duke et al. [40], who analyzed the activity of various dehydrogenases isolated from mitochondria of germinating soybean seeds for 48 h at 10 and 23 °C. At 10 °C, the activity of glutamate dehydrogenase (GDH), alcohol dehydrogenase (ADH), glucose-6-phosphate dehydrogenase (G6P-DH), and NADP-isocitrate dehydrogenase (NADP-ICDH) decreased during the first 6 h, while at 23 °C their activity increased. Our previous study on narrow-leaved lupine showed significant differences

in DA activity at 7 and 13 °C depending on the cultivar [18,19]. In the study by Płażek et al. [19], the cultivar more resistant to cold showed the same DA activity in the seeds at both temperatures, while in the cultivar sensitive to cold, DA activity was significantly higher at 13 °C than at 7 °C. In the present study, we measured the dehydrogenase pool. As the analyses were performed in germinating seeds, we mainly studied dehydrogenases involved in glycolysis and the Krebs cycle. They are the first to be induced to generate energy for the growth of hypocotyls, cotyledons, and embryonic roots. Airaki et al. [41] observed an increase in NADP-hydrogenase activity in thermophilic pepper seedlings exposed to cold. Similar results were obtained by Saruyama and Tanida [42] in pea seedlings and van Heerden et al. [43] in soybean. These findings may suggest a specific response of seed and leaf dehydrogenases to low temperature. In our experiment, DA activity did not differentiate soybean cultivars.

Alpha-amylase is a catalytic factor in the degradation of starch. In seeds with high protein content, the amount of starch may be so low that the activity of amylases is insignificant. Our results indicated that amylases, in contrast to dehydrogenases, required higher operational temperatures. The activity of seed α -amylase at 10 °C was similar in all studied cultivars, while at 15 and 25 °C, it rose significantly, especially in cv. Petrina, where it basically doubled at 25 °C vs. 10 and 15 °C. We found similar relationships in the study on amylolytic activity in germinating narrow-leaf lupine seeds [18]. In the case of soybean, no correlation between the vigor of seed germination (Vi) and the activity of both studied enzymes was found. This result is different from those obtained for low-temperature germination of narrow-leaf lupine seeds [18], where a significant correlation between amylolytic activity and the vigor of seed germination was determined. Our results indicate that the studied cultivars did not differ significantly in the activity of this enzyme in particular temperatures, and it cannot be a physiological marker of greater tolerance to low temperature. It can be concluded that in protein-rich legume seeds, the analysis of protease activity might be more helpful.

4.2. Long-Effect of Low Temperature on Soybean Yielding

According to Revilla et al. [44], cold tolerance in thermophilic plants should be evaluated on the basis of their ability to germinate at low temperatures and the percentage of obtained seedlings, as well as their vigor and ability to further normal development. Environmental factors, such as low temperature, have a significant impact on plant growth, as they affect photosynthesis, water consumption, and nutrient availability. The production and quality of many crops of great economic importance, such as cotton, corn, pepper, rice, or soybeans, significantly decrease due to low temperatures [45]. In our experiment, all studied soybean cultivars showed a positive effect of higher germination temperature on the number of developed seedlings. The results of numerous publications confirmed that a higher temperature of 25 °C, also used in our experiment, is the most beneficial for the germination of thermophilic plant species, such as soybean [35,46], pea, bean [47], or maize [48]. Despite differences in seed germination conditions, we did not observe any differences in further plant development, and the plants entered the flowering period at the same time.

Measurements of chlorophyll *a* fluorescence reflect the effects of stress on plants [49]. Thermal stress changes the reduction–oxidation properties of PSII acceptors, and this reduces the efficiency of photosynthetic electron transport in both photosystems [50]. The reduced speed of photosynthesis induces the production of reactive oxygen species (ROS), which disrupt the photochemical processes in thylakoids and cause photoinhibition of PSII [51]. In most of the studied cultivars, the germination temperature did not affect the fluorescence parameters, except for the number of active reaction centers (RC/CSm) and performance index of photochemical efficiency of PSII (PI). However, at 25 °C, we observed a significant increase in all studied parameters in cv. Petrina. In this case, the amount of energy dissipated (DIO/CSm) grew at 10 °C, which may indicate that this temperature could disturb electron transport. Higher PI values at lower temperatures visible in cvs.

Abelina, Malaga, and Petrina is an effect observed mainly in spring cereals yielding better when the temperature during germination and in the seedling phase is around 5–10 °C. Such a treatment is required to induce the generative phase in winter crops [52]. In our experiment, the number of seeds per plant positively correlated only with RC/CSm and PI. However, these parameters did not correlate with seed DW. These results are complaint with earlier findings by Bolhar-Nordenkamp and Oquist [53]; Kalaji et al. [51] that some parameters of chlorophyll fluorescence can be used to predict the yield and indicate the condition of plants under stress. Hence, we suggest that the number of active reaction centers and performance index of photochemical effectiveness of PSII may be physiological markers of soybean ability to yielding after seed treatment with low temperature.

The optimal germination temperature is an important factor that influences further plant development. The rise in the germination temperature from 15 to 25 °C almost doubled the FW and DW of soybean plants, which was in line with the results reported by Wuebker et al. [54]. In cvs. Abelina and Malaga, the increase in the germination temperature positively affected the fresh and dry weight of shoots. This effect was not observed in cv. Merlin, while in cv. Petrina, it was only perceptible for dry weight of the aboveground part. In previous studies, we showed long-term effects of cold occurring during seed germination on the first weeks of growth and development of narrow-leaf lupine plants, which were inhibited at several development stages [19].

Cold occurring during plant growth can significantly reduce soybean yield. Kurosaki and Yumoto [55] studied the effects of cold (18 °C/13 °C day/night) during soybean flowering on seed yield in two cultivars differing in cold tolerance. The cultivar sensitive to cold demonstrated a significantly lower number of pods and reduced seed yield, while in the tolerant one, these parameters differed only slightly from those of the control. Cold can also negatively affect seed filling [13]. Zhao et al. [13] reported that increased day temperatures enhanced soybean seed yield and determined the degree of seed filling. Moreover, according to these authors, higher day temperatures reduced losses due to sudden frosts occurring in late summer and improved growth conditions of soybean plants. The present study showed that plants grown from the seeds germinating at low temperatures gave the highest yield in terms of the number of seeds. However, the seeds were small, and their weight did not differ from that of seeds harvested from plants germinating at higher temperatures. The reason for this might be so-called hormesis. Hormesis is a phenomenon of stimulation of plant and other organism growth under various environmental parameters occurring at low intensity [56]. We observed this phenomenon in our earlier study on durum wheat grown under cadmium soil pollution [57]. This effect was not observed in cv. Petrina which produced considerably more pods and seeds at higher temperatures. This cultivar should be recommended for cultivation in warmer regions. The seeds of cvs. Merlin and Petrina had the highest DW among the seeds collected from plants germinating at 10 °C. Different results were obtained in our study on narrow-leaf lupine plants. Lupine responded to cold with slower development and smaller fresh and dry mass of shoots but then produced a similar number of pods and seeds to plants grown at a higher temperature [19].

5. Conclusions

When planning the cultivation of soybean, it is important to analyze the properties of the available cultivars in terms of not only the length of the vegetation season but also the ability to germinate at a lower temperature. This would allow for earlier sowing, which would, in consequence, guarantee earlier harvesting of seeds. Our research shows that although cv. Merlin had the highest seed yield, it also featured exceptionally high permeability of cytoplasmic membranes during germination at low temperature, which may indicate the risk of membrane rupture. Cultivar Abelina demonstrated lower sensitivity to cold during germination, and, as it also had a high seed yield, it may be recommended for cooler regions, where the growing season may be shorter due to earlier autumn frosts. This cultivar could be used in breeding programs to obtain soybean better adapted to temperate climates. Finally, cvs. Petrina and Malaga should be cultivated mainly in warmer

regions. Based on the obtained results, we suggest that cell membrane permeability of soybean seeds germinating at low temperature is a physiological marker of germination ability in cooler soil, while the number of active reaction centers and performance index of photochemical effectiveness of PSII may be physiological markers of soybean yielding after exposure to a low temperature.

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Abbreviations

ABS/CS_m—energy absorption by antennas; Chlf—chlorophyll fluorescence; CS_m—excited cross section of a leaf; DA—activity of dehydrogenase pool; DI_o/CS_m—energy dissipation from PSII; DW—dry weight; EL—electrolyte leakage; EL1—initial electrolyte leakage; EL2—final conductivity; ET_o/CS_m—quantum yield of photosynthetic electron transport chain beyond Q_A per CS; FW—fresh weight; PI—performance index of PSII photochemistry; PSII—photosystem II; Q_A—the first stable electron quinone acceptor in PSII; RC/CS_m—number of active reaction centres; TR_o/CS_m—excitation energy trapped in PSII, Vi—seed germination index.

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