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Influence of temperature and variety on seed germination of soybean (*Glycine max* L. Merr) at different germination times

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SUMMARY

Seed germination is an important stage in crop development that affects plant performance, crop yield, and quality. Many factors influence seed germination, and one of the most important factors is temperature. The closer the temperature is to the optimum, the faster germination will occur. Temperature affects seed germination in various plants and varies depending on the variety. Therefore, the effect of temperature on seed germination is necessary to investigate, also for soybean varieties. Soybean is one of the world's most valuable oil-seed crops. Generally, proper seedling establishment and germination of soybean seeds are crucial processes in the survival and growth cycle of the crop. Thus, a study was done to investigate the influence of temperature and variety on soybean seed germination at different times after sowing. The experiment was carried out at the Institute of Agronomy, Hungarian University of Agriculture and Life Sciences (MATE), Crop Production Laboratory. Seeds of two soybean varieties were subjected to three different temperatures: 15, 25, and 35 °C, and two soybean varieties, Martina and Johanna were tested. There were four germination times based on the days after sowing: Days 3, 5, 7, and 9. This experiment was set up with a completely randomized block design and four replications. According to the findings of this study, the Martina variety showed a better germination rate as well as higher vigor and viability than the Johanna variety in the first 12 days after sowing. At temperatures of 15 °C and 25 °C, both varieties achieved comparable percentages of viability, but they were more vigorous at 25 °C due to better total seedling length. Thus, the information gained from this study will indirectly determine and confirm the proper temperature for the initial growth of the Martina and Johanna varieties.

Keywords: *Glycine max; seed germination; temperature; vigour; viability*

INTRODUCTION

Seeds are an essential component of crop production. The independence of the next generation of plants begins with the seed. The seed, which contains the embryo as the new plant in miniature, is physically and physiologically adapted for its function as a dispersion unit and is well supplied with food reserves to sustain the growing seedling until it establishes itself as a self-sufficient, autotrophic organism (Bewley, 1997). Germination of seeds is one of the most essential phases in crop production, and it is an important process affecting the subsequent performance of the plant, crop yield, and quality (Almansouri et al., 2001). Germination starts when a seed gets exposed to the appropriate conditions; water and oxygen are absorbed through the seed coat. The cells of the embryo begin to expand in size. The seed coat then breaks down, allowing a root or radicle to emerge first, followed by a shoot or plumule containing the leaves and stem. Germination is complete when the radicle emerges from the seed coating layers and the coleoptile protrudes (Miransari and Smith, 2014). Seeds stay dormant or inactive until the conditions for germination are achieved. Many factors influence seed germination, including environmental factors such as light, soil pH, soil salinity, and seed burial depth (Awan et al., 2014). However, one of the main and most important factors affecting the germination of seeds besides water and oxygen is temperature (Anonymous, 2023a).

Seeds need a certain temperature to germinate. The closer the temperature is to the optimum, the faster germination will happen (Jauron and Rindels, 2023). Seed germination patterns vary between plants, including legume crops such as soybeans. The soybean crop is one of the most valuable oil-seed crops in the world. Soybean can also be used as a meat and dairy alternative product for humans. Its seeds comprise almost all of the essential amino acids in the human diet (Dong et al., 2014; Vorobyev et al., 2019). In general, proper seedling establishment and germination of soybean seeds are crucial processes in the survival and growth cycle of the crop. Soybean planting should not begin until the soil temperature reaches at least 15 °C (Jagdish, 2020). Soybean is a thermophilic plant that requires soil temperatures over 10 °C, with 25 °C being the ideal temperature for germination. According to the report by Jagdish (2020), soybean seed germinates slowly at low-temperature ranges; however, at temperatures over 25 °C, seed germination becomes fast. Slow germination exposes seedlings to pathogens that thrive in temperatures below 13 °C. According to Szczerba et al. (2021), no soybean varieties evaluated indicated the ability to germinate at a low temperature (10 °C) after two days, and all tested varieties required additional time (six days) to germinate at this low temperature. However, one of the tested varieties had better germination at 10 °C, with nearly double the amount of germinated seeds than other varieties. For other legume plants, such as lupine, low temperatures (below 10 °C) during seed germination greatly reduced their germination ability (Płażek et al., 2018a, b).

The dryness of the seeds occurs when they are subjected to high temperatures. This causes specific physiological changes in seeds, such as a decrease in

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viability, vigour, protein content, and protein molecular size. Higher temperatures influence the depletion of free and bound water within biomolecules, resulting in seed dryness and tighter packing of biochemicals. According to Ray et al. (2015), who did a study on temperature stress on soybean found that the viability and vigour of soybean seeds were increasingly reduced when seeds were exposed to temperature stress ranging from 40 °C to 60 °C. Similarly, high temperatures (35 °C) have lowered the percentage of viability and radicle length, which was very significant for some hybrid maize varieties when compared to maize seeds exposed to the optimal temperature (25 °C) (Khalid et al., 2021). A study on the effects of temperature on ryegrass seed by Javaid et al. (2022) found similar results. Therefore, for specific crops, a suitable and ideal temperature for seed germination is critical for improving plant growth at the next growth stage.

Although the seed has gone through the germination process under ideal conditions, including a perfect temperature, the germination period must also be considered. A study on the seed germination of maize seed varieties, including four parent varieties and three hybrid varieties, in a growth chamber at 23 °C revealed that the germination rates of some varieties reached 100% earlier and others achieved 100% later (Omar et al., 2022). The study also discovered that different maize varieties responded differently to seedling lengths, with some varieties showing a rapid increase in seedling length and others showing very gradual growth. Thus, a study was done to investigate the effect of temperature and variety on the seed germination of soybeans at different germination times. Currently, there is no information about seed germination for the soybean varieties used in this study, which is crucial to investigate because the physical characteristics of the seeds themselves vary in terms of seed size. The information gained from this study can also be used as a guideline for the next stage of planting.

MATERIALS AND METHODS

Experimental Location and Growth Conditions

The experiment was carried out in the Crop Production Laboratory of the Institute of Agronomy, Szent Istvan Campus, Hungarian University of Agriculture and Life Sciences (MATE), Gödöllő, Hungary (47º35’37” N, 19º21’55” E).

The germination test was conducted according to general laboratory standards. Two soybean varieties with different seed sizes were used in this study. Seeds of both varieties that were in good condition with a germination rate above 90% were selected and treated using a 5% hypo solution to prevent the formation of fungi during the germination test period. The seed treatment was done by soaking the seeds in the solution for 3 minutes and then rinsing them with distilled water. After being cleaned and rinsed, the seeds were placed in 13.5 cm Petri dishes. The Petri dishes were lined with Whatman filter paper (AOSA, 1992), which were moistened with 10 ml of distilled water. The Petri dishes were then sealed with parafilm to prevent water evaporation and exposed to different temperatures according to the treatment that represented suboptimal, optimal, and high temperatures in the plant growth chamber for 12 days.

Treatments and Experimental Design

Three factors that influence the germination of soybean seeds were tested in this study, namely temperature, variety, and germination time. Two soybean varieties (Martina and Johanna) were exposed to three different levels of temperatures, which were 15, 25, and 35 °C. There were four germination times based on the number of days after sowing, which were Days 3, 5, 7, and 9. This experiment was arranged according to a completely randomized block design with four replicates, in which each Petri dish contained six seeds and the total number of seeds per treatment was 24.

Data Collection

Observation and data collection were recorded for germination rate (%), total seedling length (cm), and viability (%). All these parameters were recorded on days 3, 5, 7, and 9 after sowing, except for the viability data (%), which was recorded on day 12. Seeds were considered germinated if radicles with a size of 1 mm or greater emerged from the seeds. Meanwhile, in this experiment, viability refers to the capability of the soybean seeds to germinate, survive, and produce healthy seedlings that were recorded when the germinated seeds produced a shoot (plumule), which was after 10 days of sowing.

Statistical Analysis

All the recorded data were subjected to a three-way analysis of variance (ANOVA) using IBM SPSS V.23 software (SPSS Inc., Chicago, IL, USA). The data presented are the mean values of the main effects of temperature, variety, and day, as well as the means of interaction effects. At a probability level of 0.05, the least significant difference (LSD) test was applied to compare treatment mean differences.

RESULTS

Germination rate (%)

According to the ANOVA table (*Table 1*) below, there were significant effects of the day after sowing, temperature, and variety on the germination rate of soybeans at $p < 0.05$. However, the two main factors, which were temperature and variety, showed a significant ($p < 0.05$) interaction effect. Meanwhile, there was no significant interaction between day x temperature, day x variety, and day x temperature x variety on the germination rate.

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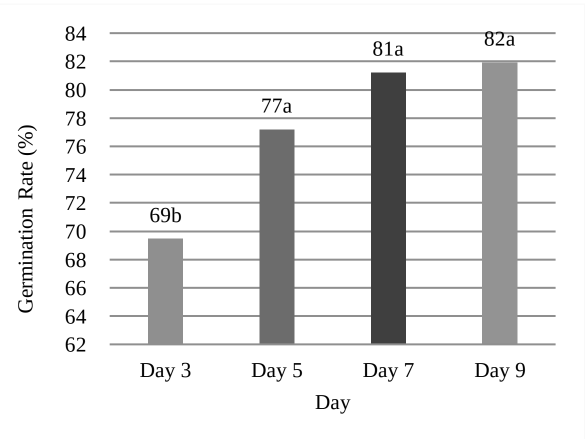
Table 1. **Analysis of variance (ANOVA) for germination rate of soybean as affected by day, temperature and variety**

Source	Sum of Squares	df	Mean Square	F	Sig.
Day (D)	2352.54	3	784.18	7.58	0.00
Temperature (T)	15568.00	2	7784.00	75.28	0.00
Variety (V)	6112.04	1	6112.04	59.11	0.00
D x T	343.08	6	57.18	0.55	0.77
D x V	691.38	3	230.46	2.23	0.09
T x V	2465.33	2	1232.67	11.92	0.00
D x T x V	890.25	6	148.38	1.44	0.21
Error	7445.00	72	103.40		
Total	**35867.63**	**95**			

df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

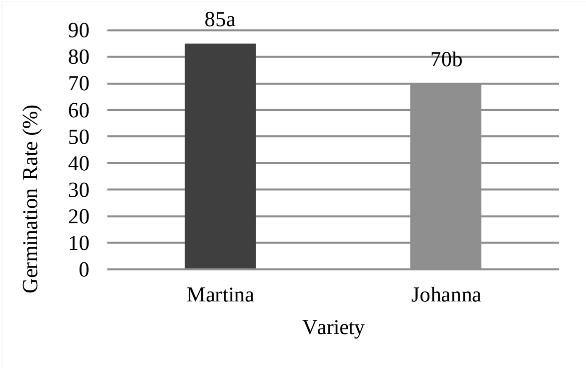
The effect of day on the germination rate is shown in *Figure 1*. Both varieties showed an increase in germination rate from Day 3 (69%) to Day 9 (82%). The germination rate on Day 3 was the lowest and significantly differed from Days 5, 7, and 9. However, the rates on Days 5, 7, and 9 were not significantly different from each other. The Martina variety had a higher germination rate (85%) compared to the Johanna variety (70%) (*Figure 2*). The varieties interacted with temperatures and had a significant effect on the germination rate (*Figure 3*). The Martina reached 100% germination at a temperature of 15 °C and 96% at a temperature of 25 °C. Meanwhile, the Johanna variety showed a much lower germination percentage than Martina, which was 74% at 15 °C and 76% at 25 °C. The germination dramatically decreased when both varieties were exposed to high temperatures (35 °C), and the germination only achieved 61% for Martina and 58% for Johanna.

Figure 1. **Effect of day on germination rate of soybean**



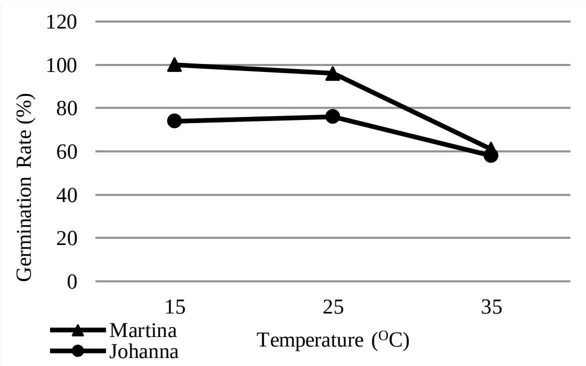
Note: Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Figure 2. **Effect of variety on germination rate of soybean**



Note: Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Figure 3. **Interaction effect of temperature and variety on germination rate of soybean**



Total seedling length (cm)

The analysis of variance (ANOVA) on the total seedling length showed that all main and interaction effects gave significant results at $p < 0.05$ (*Table 2*). Therefore, the results were only shown and discussed for the interaction effect between three factors (day, temperature, and variety).

Table 2. **Analysis of variance (ANOVA) for total seedling length of soybean as affected by day, temperature and variety**

Source	Sum of Squares	df	Mean Square	F	Sig.
Day (D)	223.88	3	74.63	1093.00	0.00
Temperature (T)	239.44	2	119.72	1753.00	0.00
Variety (V)	47.46	1	47.46	694.90	0.00
D x T	72.22	6	12.04	176.24	0.00
D x V	5.41	3	1.80	26.39	0.00
T x V	16.10	2	8.05	117.90	0.00
D x T x V	4.45	6	0.74	10.86	0.00
Error	4.92	72	0.07		
Total	**613.87**	**95**			

Note: df: Degree of freedom; Sig.: Significance; Significance level = $p < 0.05$

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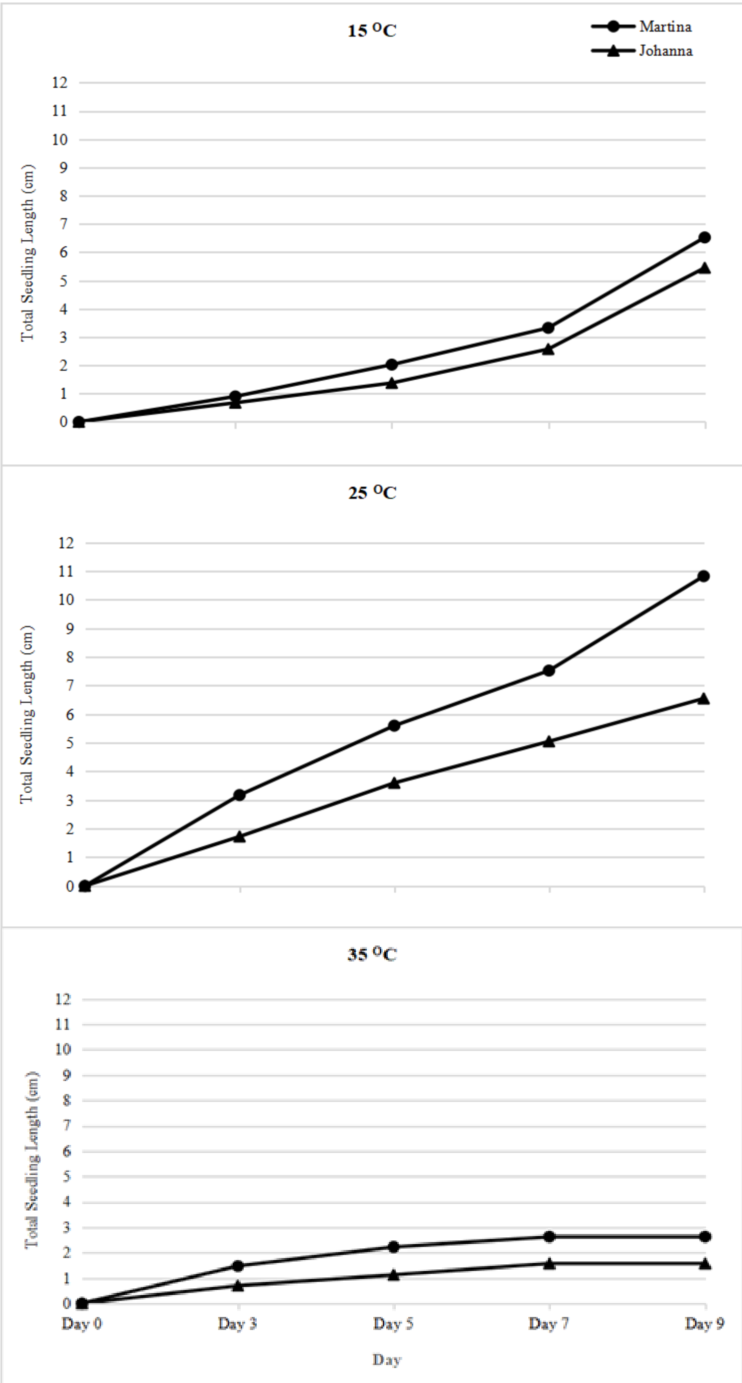
The interaction graph between day, temperature, and variety on total seedling length is shown in *Figure 4*. The total seedling length increased until Day 9 for both varieties when exposed to temperatures of 15 °C and 25 °C. The longest total seedling length was detected at a temperature of 25 °C, which was 10.83 cm (Martina) and 6.55 cm (Johanna). However, the total seedling length increased only until Day 7 for both varieties when exposed to a high temperature of 35 °C. The total seedling length at a temperature of 35 °C was also the shortest for both varieties compared to the length at temperatures of 15 °C and 25 °C. On Day 9, the total seedling length at the temperature of 35 °C was only 2.63 cm for the Martina and 1.58 cm for the Johanna. Overall, the Martina variety showed a longer total seedling length on varying days (3, 5, 7, 9) and at all temperatures compared to the Johanna variety. The difference in total seedling length between both varieties on Day 9 was 1.08 cm, 4.28 cm and 1.05 cm, respectively at temperatures of 15, 25, and 35 °C.

Figure 4. **Interaction effect of day, temperature and variety on total seedling length of soybean**



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Viability (%)

The viability results revealed that the main effect of temperature and variety had a significant value at $p < 0.05$ (*Table 3*). Meanwhile, there was no significant interaction between temperature and variety.

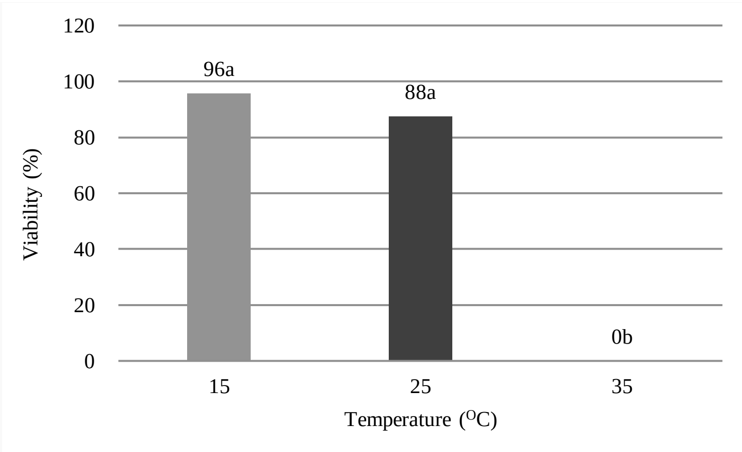
The effect of temperature on the viability of soybeans is shown in *Figure 5*. At temperatures of 15 °C and 25 °C, the percentage of viability for both varieties was not significantly different. However, the percentage of viability at those two temperatures was significant with the percentage of viability under a temperature of 35 °C (0%). In addition, variety also had a significant effect on viability. The Martina variety performed better and was more viable than the Johanna variety (*Figure 6*). The difference in the viability percentage of the Martina and Johanna varieties was 8%.

Table 3. **Analysis of variance (ANOVA) for viability of soybean as affected by temperature and variety**

Source	Sum of Squares	df	Mean Square	F	Sig.
Temperature (T)	45046.33	2	22523.17	324.20	0.00
Variety (V)	416.67	1	416.67	5.99	0.03
T x V	272.33	2	136.17	1.96	0.17
Error	1250.50	18	69.47		
Total	**46985.83**	**23**			

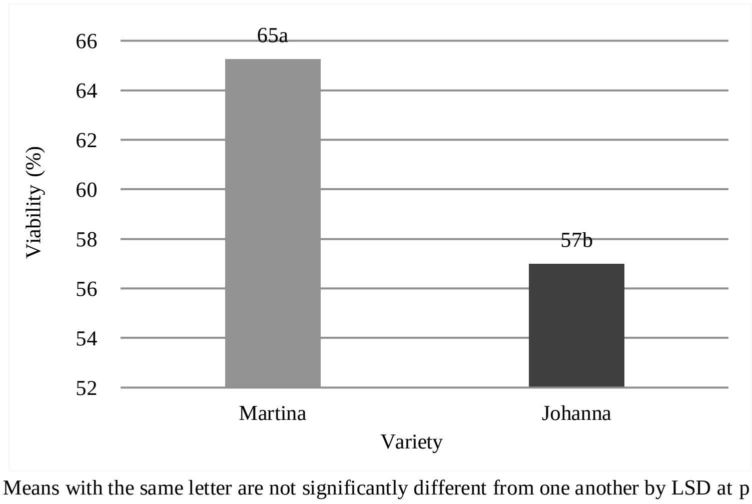
Note: df: Degree of freedom; Sig.: Significance; Significance level = p < *0.05*

Figure 5. **Effect of temperature on viability of soybean**



Note: Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

Figure 6. **Effect of variety on viability of soybean**



Note: Means with the same letter are not significantly different from one another by LSD at $p < 0.05$

DISCUSSION

Germination time affected germination rate

According to the findings of our study, the percentage of germination rate peaked at 82% on Day 9, but there was no significant difference between the germination rate on Day 5 and Day 7. This means that seeds from both varieties completed germination as early as the fifth day. Other research on soybean seeds discovered that the number of germinated soybeans

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increased as the number of days increased (Ray et al., 2015). A study involving several maize varieties revealed that all varieties began germination on the third day. All the varieties reach 100% germination on the 12th day, except one variety that germinated on the 9th and one variety on the 7th day (Omar et al., 2022).

The percentage of germination that increases with time is typical in the initial stages of plant growth. However, the increase will vary depending on the type of plant and its variety. Gloria and Osborne (2014) and Wainwright et al. (2012) reported that species that germinate earlier than other species can benefit from early access to some resources, space, and reduced competition during the establishment of the early stage. Apart from that, information on germination percentage is important not only to understand the early growth stages of soybeans but also to be used in the determination of seed rate for field or next crop planting. If the seed has an 80% germination rate, 70 to 80 kg of seed per hectare is required (Jagdish, 2020).

Temperature and variety interacted on germination rate

The study also discovered that temperature and variety interacted strongly in affecting the germination rate. The Martina variety had a higher germination rate not only at optimal temperatures (25 °C) but also at low temperatures (15 °C). Szczerba et al. (2021) similarly found an interaction between temperature and variety in the germination of four varieties of Polish soybean seeds. Their result found that at 15 °C, only one variety germinated more than 50%, and the other three varieties germinated between 19.9% and 26.7%. At 25 °C, the seeds of the two varieties germinated 100%, and the seeds of the other two varieties germinated 98%. Their findings revealed that the responses of soybean seeds were highly different when influenced by low and optimal temperatures, but there was also a variety responding well to low temperatures.

Studies on maize varieties also discovered a significant interaction between temperature and variety on germination percentage (Ali, 2018). The study found that at a temperature of 10 °C, all 20 tested varieties were able to germinate by 93–100%. On the other hand, at temperatures of 8.6 °C and 7.2 °C, the difference in germination percentage in most varieties was very significant, with some varieties reaching only 7% germination and others reaching up to 85% germination. Liu et al. (2021) also found a significant impact on the interaction between temperature and variety on the germination percentage of common grass.

For the effect of high temperatures on seed germination, previous studies reported that most seed germination performances dropped and were lower at high temperatures. Likewise, our research observed that the germination rate decreased and was lowest at a temperature of 35 °C. This finding was supported by a study conducted by Ray et al. (2015) on the effects of temperature stress on the germination of soybean seeds. They discovered that the number of seedlings that emerged at high temperatures of 40, 50, and 60 °C was lower than the control. The range of germinated seedlings was between 60 and 40 seeds per 100 seeds at those high temperatures.

Most research, including ours, found that temperatures influenced most of the germination of plant varieties. When exposed to a certain temperature, the seed may react in the formation of the chemical composition, subsequently affecting germination. Most varieties have their own particular characteristics and differ from one another in terms of physical or chemical composition. In oil-seed plants, such as soybean varieties that contain low lipids, the germination process is slow. This was reported by Miquel and Browse (1994), who conducted a study on two *Arabidopsis thaliana* varieties and discovered that cultivars with low lipid content germinated slower at temperatures of 10 °C and 6 °C. This confirmed our findings that the Johanna variety showed a low percentage of germination at all tested temperatures (15, 25, and 35 °C), which is one of the causes probably due to the fact that the lipid content of the Johanna variety was lower than the Martina varieties (data not included).

Germination time, temperature and variety interacted on total seedling length

Total seedling length, which includes root and shoot length, is one of the indicators used to determine seedling vigour (Redoña and Mackill, 1996). Seedling vigour is defined as a seed's ability to emerge rapidly from soil or water, mainly referring to early seedling growth (Huang et al., 2004). In our study, total seedling length increased with increasing time up to Day 9, when seeds were exposed to low (15 °C) and optimal (25 °C) temperatures. Therefore, both of the tested soybean varieties (Martina and Johanna) showed good seedling vigour because they can survive at a critical stage of early plant growth when exposed to low and optimal temperatures. However, seedlings for both varieties were more vigorous at 25 °C than at 15 °C because they had a longer seedling length at all germination periods (Day 3, 5, 7, and 9) under the temperature of 25 °C. This finding stands in line with previous research on soybean germination, which found that the optimal temperature for germination for most varieties of soybeans was 25 °C. Although soybeans can also easily germinate at soil temperatures as low as 10 °C, but germination is slow (Jagdish, 2020; Anonymous, 2023b). The Martina variety was more vigorous than the Johanna variety because it emerged faster and had a longer total seedling length at each temperature and germination time.

When seeds were exposed to high temperatures (35 °C), the total seedling length increased until Day 7, and the length was also the shortest at this temperature. According to a study conducted on temperature stress (40, 50, and 60 °C) in soybeans by Ray et al. (2015), there was a significant decrease in vigour when temperature and time (the number of days) were increased. This is most likely due to the drying of seedlings, which may be caused by conformational changes and hence the molecular deterioration of

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biochemicals within seeds under high temperatures and varying times.

Temperature and variety affected on percentage of seed viability

Our findings also revealed that the main factor of temperature affected the seed viability of soybeans. The percentage of viability at low temperatures was not much different from the percentage of viability at optimal temperatures. However, at high temperatures, no seeds were viable, although some seeds germinated, the germinated seeds only survived until Day 7 and then died. Also, our investigation found that the more viable variety was Martina, with a different viability percentage from the Johanna variety of 8%. Viability refers to whether a seed is alive or not, and the percentage of viable seeds is not necessarily the same as the percentage of germinated seeds (Basara et al., 2002; Anonymous, 2023c). This difference is probably because some seeds are immature or dormant.

According to the findings of Szczerba et al. (2021) on the percentage of germinated soybean seeds exposed to temperatures of 10, 15, and 25 °C, the percentage of germinated seeds from four soybean varieties was highest at 25 °C, followed by 15 °C and 10 °C. They discovered that seeds from the Abelina variety were the most viable when compared to the other three varieties, with the Petrina variety producing the lowest percentage of seedlings, which was less than 40%. The results of numerous publications confirmed that a temperature of 25 °C, also used in our experiment, is the most beneficial for the germination of thermophilic plant species such as soybeans (Hatfield and Egli, 1974; Ladror et al., 2022), peas, beans (Zaiter et al., 1994), and maize (Wang et al., 2018). The ideal germination temperature is an important factor that influences further plant development, and seed viability is one of the indications used to evaluate good further plant growth.

CONCLUSIONS

There is a need in soybean cultivation to understand the germination of each variety used because it is significantly influenced by many factors, such as temperature. As previously stated, certain varieties had slightly different germination patterns, which will affect the subsequent planting stage. Based on the germination experiments conducted on the soybean varieties Martina and Johanna, it can be concluded that the Martina variety has a higher germination rate as well as greater vigour and viability than the Johanna variety in the first 12 days after sowing. Both varieties achieved comparable percentages of viability at temperatures of 15 °C and 25 °C, but they were more vigorous at 25 °C due to greater total seedling length compared to total seedling length at 15 °C or 35 °C. Thus, the information obtained will indirectly contribute to the determination of the appropriate temperature for early growth for the planting of Martina and Johanna varieties while also providing specific information for the development of new planting technologies for these varieties in a field or in a controlled environment.

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