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Impact of thermal stress on the germination and early growth of soybean seedlings

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Abstract

Soybean is cultivated over a large area in various countries, being subject to different environmental conditions where abiotic stresses such as drought and high temperature are recurrent. Therefore, this research aimed to evaluate the physiological response of soybean seeds with contrasting vigor, subjected to high-temperature stress. The study was conducted in two experiments, both in a completely randomized design and factorial arrangement, with four replications. Initially, the response of high and low vigor seeds was assessed when subjected to thermal stress for different periods, evaluating this response with variables related to germination. Subsequently, an experiment was conducted to evaluate the physiological response of high and low vigor seeds. Seeds classified as high vigor were used, with part of the lot artificially aged in the laboratory to reduce vigor for the experiments. Thermal stress was imposed in an oven at 38 °C, applied after radicle protrusion. Analysis of variance confirmed the significant influence of vigor, both in isolation and in interaction with stress and time, on the seedling characteristics. These results demonstrated that high vigor seeds showed better performance in variables such as normal seedlings, axis dry mass, seedling length, and reserve mobilization, even under stress conditions and over time in the physiological response experiment. This fact reinforces the need for using high vigor seeds to optimize the growth and development of soybean crops, particularly in environments subject to thermal stress.

Introduction

Soybean is one of the most relevant crops in the world and has played a central role in global food security and in supplying raw materials for various industrial sectors. Its cultivation has expanded rapidly in recent decades, driven by the growing demand for plant-based protein sources, edible oils, and biofuels. This legume, in addition to being highly adaptable to different edaphoclimatic conditions, has the ability to fix atmospheric nitrogen, contributing to the sustainability of agricultural systems and reducing the need for synthetic fertilizers (Bewley et al., 2013).

Despite its broad adaptability, soybean cultivation faces significant challenges in tropical and subtropical regions, especially in the context of global climate change. The increasing frequency and intensity of extreme weather events (such as heat waves, prolonged droughts, and irregular rainfall distribution) have threatened agricultural production stability and global food security (Viana et al., 2021). Among the abiotic factors most critical to soybean development, thermal stress stands out for directly affecting vital physiological processes of the plant (Wang et al., 2020).

High temperatures interfere with germination, emergence, and initial seedling growth, potentially compromising the entire production cycle. During the vegetative phase, excessive heat alters the photosynthetic rate, causes water imbalance, and leads to the excessive production of reactive oxygen species (ROS), which cause oxidative damage to cell membranes and photosynthetic structures (Gerganova et al., 2016). In the reproductive phase, exposure to heat can reduce pollen viability, cause floral abortion, and impair grain filling, resulting in significant yield losses (Qaseem et al., 2018).

Seed germination is particularly sensitive to temperature, as this physiological process depends on suitable environmental conditions of moisture, oxygen, and heat. When exposed to temperatures above the optimal range (generally between 25 and 30 °C) soybean seeds may exhibit delayed germination, abnormal seedling formation, and reduced initial vigor (Hetfield & Egli, 1974). At lower temperatures, germination tends to be slower, which increases susceptibility to pathogen attack and reduces stand uniformity (Carneiro et al., 1999). Thus, thermal control and the selection of seeds with high physiological quality are key aspects for crop success.

Seed physiological quality is one of the main determinants of field performance, as it directly influences initial establishment and final yield (Marcos Filho, 2013). This concept encompasses attributes such as viability, germination, and vigor, which reflect the metabolic state and structural integrity of the seed. High-vigor seeds have greater capacity to withstand adverse conditions, producing more robust and uniform seedlings with better adaptation to environmental variations (Bewley et al., 2013). In contrast, low-vigor seeds are more susceptible to thermal and water stress, which can lead to stand failures and reduced productivity.

In this context, understanding the physiological responses of seeds with different vigor levels under thermal stress is essential for developing mitigation strategies and improving seed production practices (Wang et al., 2020). Studies that evaluate germinative behavior under high-temperature conditions enable the identification of more tolerant genotypes and guide breeding programs focused on heat resistance (Viana et al., 2021). Furthermore, such investigations support agronomic decisions related to planting management, sowing time selection, and storage techniques, contributing to greater resilience of production systems in the face of climate change.

Given the challenges posed by thermal stress and the importance of seed vigor for initial crop establishment, this study aimed to evaluate the physiological response of soybean seeds with different vigor levels when subjected to high-temperature conditions.

Materials and Methods

Seeds Used

The research was conducted in 2018, using soybean seeds of the BS2606 IPRO® cultivar, produced in the 2017/18 season. These seeds were previously characterized with a germination rate above 97% (Brasil, 2009) and vigor of 89% according to the accelerated aging (AA) test, at 41°C for 48 hours (Marcos Filho 2013). Subsequently, a significant portion of the seed sample was subjected to artificial aging using an AA chamber to reduce vigor and obtain contrasting batches of the same cultivar. Thus, the initial batch seeds were placed in a uniform layer on a stainless-steel screen inside gerbox® containers containing 40 mL of distilled water (41°C for 48 hours). The moisture content of these seeds was then standardized to ±13% in an oven with air circulation at 35°C. Following this, a new germination and vigor test was conducted on these seeds as previously described. As a result, the experiments

started with two batches of seeds: i) High vigor seeds with 97% germination and 89% vigor; ii) Low vigor seeds with 89% germination and 50% vigor.

Germination of Seeds Subjected to Thermal Stress

This experiment was conducted in a completely randomized design with a factorial arrangement. The controlled factors included two levels of seed vigor (high and low) and five stress durations following radicle protrusion (0, 10, 12, 14, and 16 hours). Four replications of 50 seeds each were used to measure the variables: normal seedlings (%), abnormal seedlings (%), and dead seeds (%), as described in the Seed Analysis Manual (Brazil, 2009). Temperature stress was simulated in an air-circulated oven at 38°C, based on Hetfield and Egli (1974). Chronologically, the seeds were placed on moistened germitest® paper (2.5 times its dry weight) and conditioned in a germinator (25°C) until they reached stage III of the germination process (50% + 1 seed with radicle protrusion) to standardize the physiological stage. After this period (24 hours for high vigor and 33 hours for low vigor), the seeds were transferred to an air-circulated oven at 38°C for periods of 0, 10, 12, 14, and 16 hours. Subsequently, the seeds were returned to the germinator at 25°C with moisture restored (2.5 times dry weight) to assess the response to stress, considering the response variables. Counts were performed on the 7th day (Vanzolini et al. 2007).

These data were subjected to a fixed effects test, considering the Poisson distribution, using the GLIMMIX procedure with SAS® OnDemand for Academics software. This procedure adjusts a generalized function to the data, considering the specified distribution (Poisson). The statistical model considered all factors as fixed (vigor, stress, vigor x stress). The underlying hypotheses test (H_0: $\mu 1 \neq \mu 2 \neq \mu n$) was performed using the F test, considering a 5% probability of error. If the interaction or the quantitative factor (stress) was significant, it was decomposed into orthogonal contrasts to adjust the polynomial, as described by Narula (1979), considering the irregularly spaced stress intervals.

Physiological Response to Thermal Stress

Similar to the previous experiment, this trial was conducted concurrently in a completely randomized design. The experimental factors included seed vigor levels (high and low), thermal stress with four durations (10, 12, 14, and 16 hours), and time with four levels (1: radicle protrusion (time 0); end of stress; 24 hours post-stress; 48 hours post-stress). The variables evaluated were seedling length (cm), residual dry mass in the cotyledon (mg), axis dry mass (mg), and reserve mobilization (%). To determine these variables, subsamples of 20 seeds were used, placed along two lines drawn in the upper third of germitest® paper, moistened with distilled and deionized water at 2.5 times the dry weight. The rolls were kept vertical in a germinator (25°C) until they reached stage III of the germination process (radicle protrusion). At this stage, the variables were measured for the zero time point of the "time" factor. The rolls were then subjected to thermal stress, and the variables were measured again upon exiting the stress, and successively after returning the rolls to the germinator at 24 hours post-stress and 48 hours post-stress.

The variables were measured as follows (Nakagawa 1994): *i)* **Seedling length:** measured with a caliper, determining the length (mm) of 10 randomly selected seedlings; *ii)* **Residual dry mass in the cotyledons:** determined from the previously used seedlings, using aluminum capsules dried at 80°C for 24 hours; *iii)* **Axis dry mass:** measured with the growth axes of the previously used seedlings, dried in aluminum capsules at 80°C for 24 hours; *iv)* **Reserve mobilization rate:** determined as described by Andrade et al. (2019), expressed as a percentage TMR (%) = (axis dry mass × seed dry mass^{-1}) × 100, where the seed dry mass was determined by drying the seeds in an oven at 105°C for 24 hours.

These data were subjected to the assumptions of analysis of variance (normality of residues: Shapiro-Wilk test; homogeneity of variances: Levene; additivity: Tukey). Once these assumptions were met, the analyses were performed following the mathematical model for a completely randomized design: $Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijk}$, where: $\mathbf{Y_{ijk}}$**:** represents the observation of the response variable in the experimental unit i, j, k, which received the treatment corresponding to the i-th level of the vigor factor (α), the j-th level of the stress factor (β), and the k-th level of the time factor (γ); μ: represents the overall mean; α_i: represents the fixed effect of the i-th level of the vigor factor (α); β_j: represents the fixed effect of the j-th level of the stress factor (β); γ_k: represents the fixed effect of the k-th level of the time factor (γ); $(\alpha\beta)_{ij}$: represents the interaction effect between the i-th level of the vigor factor (α) and the j-th level of the stress factor (β); $(\alpha\gamma)_{ik}$: represents the interaction effect between the i-th level of the vigor factor (α) and the k-th level of the time factor (γ); $(\beta\gamma)_{jk}$: represents the interaction effect between the j-th level of the stress factor (β) and the k-th level of the time factor (γ); $(\alpha\beta\gamma)_{ijk}$: represents the interaction effect between the i-th level of the vigor factor (α), the j-th level of the stress factor (β), and the k-th level of the time factor (γ); ε_{ijk}: represents the random error effect in the experimental unit i, j, k, with a normal distribution, zero mean, and constant variance.

These analyses were performed using SAS® OnDemand for Academics software with the GLM procedure (general linear model). After the analysis of variance, if a significant triple interaction (5% probability of error) was found, a response surface model was adjusted using multiple linear regression. This model is particularly suitable when there are multiple quantitative factors of equal importance, as in this experiment. The response surface was adjusted using R software, and the respective graphs were generated with the same software (R Core Team 2023).

Results

Understanding the importance of seed vigor for successful agricultural production has significant practical implications. Producing high-vigor seeds through appropriate agronomic techniques and proper storage is crucial for ensuring good crop establishment and maximizing productivity. The results presented below contribute to these aspects.

Initially, the hydration curve for high-vigor and low-vigor seed lots (Fig. 1) demonstrates the three-phase behavior of the hydration process. The radicle protrusion time for high-vigor seeds was 24 hours,

reaching 50% plus one seed with a primary root protrusion of more than 2mm. For the low-vigor lot, this time was 36 hours, indicating a slower hydrolysis of reserve components, which are transformed into energy and structural cellular components until the plant reaches autotrophy. This stage was crucial for determining the appropriate time to subject the seeds to thermal stress.

The results of the analysis of variance (ANOVA) for the fixed effects of vigor, stress, and the interaction between vigor and stress on normal, abnormal, and dead seedlings are presented in Table 1. For normal seedlings, vigor had a significant effect (P>0.0001), indicating that this source of variation influences seedling development. Stress also had a significant effect (P>0.0312), suggesting that different levels of stress affect the number of normal seedlings. Furthermore, the interaction between vigor and stress was significant (P>0.0227), highlighting the importance of this combination in the response of normal seedlings. These results emphasize that both vigor, individually and in interaction with stress, are crucial determinants for the proper development of seedlings.

For abnormal seedlings, vigor had a highly significant effect (P>0.0001), showing that vigor also influences the quantity of seedlings that develop abnormally. However, stress did not have a significant effect (P>0.7142), indicating that different stress levels did not affect this characteristic. The interaction between vigor and stress was also not significant (P>0.2796) for abnormal seedlings. These results suggest that, for abnormal seedlings, vigor is the main determining factor, while stress and the interaction between vigor and stress have little influence.

Similarly, for dead seeds, vigor again had a large effect (P>0.0001), indicating that variations in vigor influence seedling mortality. Like abnormal seedlings, stress did not have a significant effect on dead seedlings (P>0.2704), and the interaction between vigor and stress was also not significant for seedling mortality (P>0.4331). These results indicate that, similar to abnormal seedlings, vigor is the predominant factor, while stress and its interaction with vigor are not relevant.

Thus, vigor has a significant impact on seed germination characteristics (normal, abnormal, and dead seedlings). Additionally, the results provide information that stress affects only normal seedlings, also resulting in a significant interaction between vigor and stress. This is important for agricultural management strategies aimed at optimizing germination and seedling performance, minimizing the reduction of genetic potential when subjected to thermal stress.

Orthogonal polynomial contrasts indicated that the relationships between stress duration and the percentage of normal seedlings are best described by linear equations for both vigor levels (high and low). For high-vigor seeds, the linear regression equation is y=−0.1237x+96.887, with a coefficient of determination R^2=0.75. This adjusted equation suggests that, with increased stress duration, the percentage of normal seedlings did not decrease, maintaining relatively high levels of normal seedlings over time, above the 80% required by the Ministry of Agriculture, Livestock, and Supply (MAPA) for soybean cultivation. For low-vigor seeds, the linear regression equation is y=−2.7229x+94.119 with a coefficient of determination R^2=0.97.

This equation shows a more pronounced decrease in the percentage of normal seedlings with increased stress duration, indicating higher sensitivity to stress in low-vigor seeds. These results confirm the superiority of high-vigor seeds in withstanding high-temperature stress, maintaining a consistent percentage of normal seedlings over increasing periods of stress, compared to low-vigor seeds, which exhibit a rapid decrease in seedling viability with extended stress duration. According to Marcos Filho (2013), hysiological potential encompasses information inherent to seed germination and vigor, and its assessment allows for identifying lots with a higher probability of achieving the desired performance under field conditions. In this sense, germination remained consistent in the high-vigor lot despite increased stress hours, likely due to the adequate degradation of reserve components in the cotyledons and subsequent allocation during initial growth.

The analysis of variance for the variables seedling length (SL), residual dry mass in the cotyledon (RDMC), axis dry mass (ADM), and reserve mobilization (RM) is presented in Table 2. Considering the sources of variation vigor, stress, and time, along with their interactions, the significances were assessed using the F test at a 5% probability level. For the variable seedling length (SL), all sources of variation were statistically significant. The variability explained by these sources of variation was considerably greater than the residual variability, as indicated by the mean square values compared to the residual value. The sources of variation vigor and time accounted for a significant portion of the variability; however, due to the significance of the triple interaction, it is essential to study it further.

For residual dry mass in the cotyledon (RDMC), only the sources of variation vigor and time were statistically significant. The other sources of variation were not significant, indicating that these interactions did not have a substantial impact on this variable. For axis dry mass (ADM), the highest-order interaction (vigor × stress × time) showed statistical significance, indicating the influence of these factors on the dry mass of the seedling's axis, including roots and other organs (radicle, hypocotyl, and primary leaves). The variability explained by this and other significant sources of variation for ADM was substantially higher than the residual. For reserve mobilization (RM), the sources of variation vigor, time, vigor × stress, vigor × time, and vigor × stress × time showed statistical significance. This suggests that vigor and time, as well as their interactions, play a crucial role in reserve mobilization, unlike stress alone and its interaction with time. Given that the residual values for all variables were relatively low compared to the mean squares of the controlled sources of variation, indicating a good association between the controlled model variables and the measured variables in the experiment. This resulted in coefficients of variation ranging from 13% to 26%, reflecting the relative dispersion of the data around their overall means. These results highlight the importance of vigor and time, as well as their interactions, in determining the growth and development characteristics of the studied seedlings.

The statistical significance of these sources of variation provides a solid basis for inferences about the factors influencing these variables under experimental conditions. In this sense, the response surface was applied to the two quantitative variables for each specific vigor level to explain the significant triple interaction observed in the variables axis dry mass, seedling length, and reserve mobilization. The response surface allows visualization and understanding of complex interactions between multiple

quantitative experimental factors, providing crucial information about the relationships among different factors (Fig. 3).

The upper figures (Fig. 3) illustrate the response surface for seedling length (SL) under low vigor (a) and high vigor (b) conditions. In low-vigor seedlings, SL increases gradually over time, with little influence from stress, ranging from approximately 10 cm to 35 cm. In high-vigor seedlings, the values are more pronounced, with SL ranging from about 10 cm to 110 cm. The response surface for seedling length indicates that increased time favors growth in length, which is relatively obvious; however, in addition to the better performance of high-vigor seeds, there is a greater reduction in performance, which may be related to this better intrinsic performance.

The middle figures (Fig. 3) show the response surface for axis dry mass (ADM) under low vigor (c) and high vigor (d) conditions. It is observed that ADM increases with time and reduced stress at both vigor levels. However, the magnitude of the response is significantly greater in high-vigor seedlings. Under low vigor conditions, ADM ranged from approximately 0.03 g to 0.08 g, while under high vigor conditions, it ranged from about 0.03 g to 0.15 g. This suggests that seedlings from higher vigor seeds are more efficient in responding to the axis dry mass variable, especially under lower stress conditions and longer times.

The lower figures (Fig. 3) show the response surface for reserve mobilization (RM) under low vigor (e) and high vigor (f) conditions. In low-vigor seedlings, RM ranges from about 3.0% to 7.5% of cotyledon mass, with increasing time and reduced stress. In high-vigor seedlings, RM ranges from approximately 3.0% to 14.0% of seed dry mass, indicating a greater capacity for reserve mobilization in seedlings derived from high-vigor seeds. Thus, seedlings from high-vigor seeds demonstrate more efficient reserve mobilization, contributing to their development under thermal stress conditions.

These results presented in the response surfaces highlight the significant influence of vigor, time, and stress on the measured physiological quality variables. High-vigor seedlings demonstrate superior performance in terms of ADM, SL, and RM compared to low-vigor seedlings. This performance is even more pronounced with reduced stress and increased time, demonstrating adequate physiological development in seedlings derived from these seeds. The observed differences between high and low vigor seedlings reinforce the importance of selecting high-vigor seeds to maximize seedling growth and efficiency, especially in environments where thermal stress is expected, favoring the proper establishment of the plant stand.

Discussion

Phenotypic Response According to Seed Vigor Level

The present research aimed to investigate the physiological response of soybean seeds with different vigor levels to thermal stress. Through various experiments, the goal was to examine how seed vigor influences the physiological development of seedlings, assessing traits such as germination, seedling

performance, and reserve mobilization. High-vigor seeds successfully overcame thermal stress, demonstrating consistent germination rates of over 80%, even under prolonged stress conditions. In contrast, low-vigor seeds succumbed to stress, showing dramatic decreases in germination and highlighting their fragility.

Despite its simplicity, these results demonstrate that seed vigor acts as a protective factor against thermal stress. Numerous studies evaluate the impact of thermal stress on soybean seed germination and seedling development, generally focusing on cold stress, with high-vigor seeds showing better performance (Staniak et al. 2021; Zhang et al. 2017; Basalari et al. 2021). Furthermore, it was observed that seedlings from high-vigor seeds exhibited greater development, with increased length and dry mass of the axis, even under adverse conditions. On the other hand, low-vigor seedlings experienced the harsh effects of stress, showing stunted growth and reduced dry mass. High-vigor seeds also excelled in reserve mobilization, efficiently extracting components from cotyledons and translocating them to the seedling's growing axis, even under stress conditions. In contrast, the reserves mobilized by low-vigor seedlings were limited, reflecting their reduced ability to adapt to high-temperature stress.

Practical Implications

The results of this research highlight the critical importance of seed vigor for the success of soybean cultivation, particularly in challenging scenarios marked by thermal stress. The recommendation to use high-vigor seeds emerges as a powerful tool for optimizing germination, seedling development, and the final productivity of the crop. The relevance of seed vigor is supported by various studies across different crops (Abati et al. 2017; Cover et al. 2019; Krzyzanowski et al. 2018; Mathias and Coelho, 2021). Using vigorous seeds ensures the adequate establishment of the desired plant population in the field, even if the response in terms of productivity may not be significant (Marcos Filho 2013).

Choosing high-vigor seeds is crucial for soybean cultivation, especially in the corn-soybean rotation system found in Brazil's cerrado region and the wheat-soybean system in southern Brazil, where high soil temperatures at planting can compromise productivity (Oliveira et al. 2015). Considering current legislation, there are numerous gaps; for example, Normative Instruction No. 45/2013 from the Ministry of Agriculture establishes a minimum germination standard of 80% for soybean seeds but does not specify a minimum vigor level (Brasil 2013; Mathias et al. 2020). This gap can lead to the sale of low-vigor seed lots, negatively impacting field establishment. Therefore, research and collaborative actions with the public sector are necessary to ensure the commercialization of high-quality physiological seed lots. This is particularly relevant for standardizing the vigor level of seeds to be sold, which could contribute to the safety and quality of soybean production in Brazil.

Considering the realistic simulation of high and low vigor seed lots, the methodology used in this research, which simulates seed deterioration under laboratory conditions, accurately reflects the reality of seed production, where vigor variability is common (Vergara et al. 2019). However, antagonistic responses are observed in soybean seedlings regarding thermal and water stresses (Prasad et al. 2017; Feller 2016). Further studies are needed to uncover the underlying mechanisms of thermal stress

tolerance and develop strategies for managing the crop to minimize these damages (Elferjani and Soolanayakanahally 2018). Understanding the physiological mechanisms of stress tolerance may also lead to the development of more resilient soybean cultivars.

In the physiological and morphological adaptation of plants in their quest to adapt to adversities, they undergo physio-morphological modifications (Elferjani and Soolanayakanahally 2018). Seedlings originating from low-vigor seeds, due to their inferiority in membrane integrity and cell repair potential, may be more susceptible to damage and exhibit limited metabolism under thermal stress. Thus, this research significantly contributes to understanding the relationship between seed vigor and thermal stress response in soybeans.

Deepening Knowledge

The path to uncovering the secrets behind vigorous soybean seeds is further enriched with each published work in this area. Building on the contributions of various studies that shed light on the importance of seed vigor and its influence on soybean response to different stresses, this research reveals several insights. For instance, regarding thermal stress, Basalari et al. (2021) conducted a study that supports our findings by investigating the effect of seed vigor on germination and early seedling vigor under different temperatures. Their results also highlight the superiority of vigorous seeds under adverse conditions, reinforcing the importance of recommending high-quality seeds for subsequent success in cultivation.

Advancing the discussion, it is well-established that seed vigor significantly impacts the physiological performance of soybean seedlings, including parameters such as growth, photosynthesis, and dry matter accumulation (Fernandes et al., 2020). This research underscores the influence of seed vigor throughout the plant's development cycle, from germination to maturity. In a comprehensive study, some authors investigate the physiological mechanisms underlying thermal stress tolerance in soybean seeds with different vigor levels. Their results provide valuable information for developing more efficient management strategies, such as breeding new soybean varieties resistant to thermal stress (Zhang et al. 2017).

Exploring the relationship between seed vigor and salt stress tolerance in soybeans, it has been shown that vigorous seeds exhibit a higher capacity for adaptation to saline conditions (Akram et al. 2019). This finding further corroborates the importance of vigor in challenging scenarios, demonstrating that higher vigor seeds can ensure crop productivity even in high-salinity environments. Conversely, regarding the antagonistic aspect (water stress), some authors have analyzed the effect of seed vigor on soybean productivity under various levels of water stress, showing that vigorous seeds provide higher productivity even under stress conditions (Luo et al. 2018).

In seed formation, a study reveals that heat tolerance in soybean genotypes is related to their metabolic composition, particularly the abundance of antioxidants in plant tissues (Chebrolu et al. 2016). Compared to heat-sensitive genotypes, heat-tolerant soybeans exhibit less drastic metabolic changes in

response to thermal stress. Moreover, higher concentrations of flavonoids, ascorbate precursors, and tocopherols (antioxidant molecules) are observed in the tolerant genotype. This enhanced ability to combat free radicals and protect cells from oxidative damage contributes to preserving seed integrity and optimizing production in thermal stress environments, potentially aiding in the development of new strategies for producing seeds with better physiological quality under such conditions.

Heat stress is an increasing concern for global agricultural production, as it significantly impacts plant growth, development, and productivity. The severity of this impact varies according to climatic zones, the frequency, and the duration of heat waves. In response to thermal stress, plants accumulate metabolites such as antioxidants, protectors, and heat shock proteins, which activate various crucial metabolic pathways for tolerance (Hasanuzzaman et al. 2013). Thus, physiological and molecular studies are essential to understand the mechanisms underlying thermotolerance. Understanding signaling cascades and specific genes expressed in response to heat can facilitate the development of varieties more resistant to thermal stress. This research highlights the importance of seed vigor for the economic viability of soybean cultivation, showing that using high-quality seeds provides a better return on investment, especially under high-temperature conditions. However, to complement the laboratory results, field studies are needed to account for thermal stress conditions and plant development in these scenarios, aiming to supplement the present research.

Conclusion

The research evaluated the physiological response of soybean seeds with different vigor levels subjected to high-temperature conditions, highlighting the importance of seed vigor. The high-vigor seedlings exhibited superior performance regarding physiological quality variables, including normal seedlings, seedling length, dry mass of the axis, and reserve mobilization, even under greater thermal stress. The response surface demonstrated the complex interactions between vigor, time, and stress, suggesting that high-vigor seeds are crucial for resilience against the adverse effects of thermal stress.

Declarations

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Authors' contribution

Luan Tiago dos Santos Carbonari: Conceptualization, methodology, data curation, formal analysis, writing – original draft. Cileide Maria Medeiros Coelho: Supervision, project administration, writing – review and editing. Jefferson Luís Meirelles Coimbra: Data collection, investigation, statistical analysis.

Matheus Santin Padilha: Methodology, writing – review and editing. Clovis Arruda de Sousa: Supervision, validation, writing – review and editing.

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Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

On behalf of all authors, the corresponding author states that there are no competing interests.

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Tables

Table 1 Analysis of variance for the fixed effects of vigor, stress, and vigor x stress related to the characteristics of normal seedlings, abnormal seedlings, and dead seedlings with Poisson distribution

Characteristic	Effect	NDF[/1]	DDF[/2]	Valor F	*P* > F
Normal Seedlings	Vigor	1	30	135,06	0,0001
	Stress	4	30	3,49	0,0312
	Vigor x Stress	4	30	3,82	0,0227
	Vigor	1	30	214,66	0,0001
Abnormal Seedlings	Stress	4	30	0,46	0,7142
	Vigor x Stress	4	30	1,36	0,2796
	Vigor	1	30	86,76	0,0001
Dead Seedlings	Stress	4	30	1,39	0,2704
	Vigor x Stress	4	30	0,95	0,4331

[1/]Numerator degrees of freedom; [2/]Denominator degrees of freedom

Table 2 Analysis of variance with the respective sources of variation and their corresponding mean square values for the variables seedling length (SL), remaining cotyledon dry mass (RCDM), axis dry mass (ADM), and reserve mobilization (RM)

Source of Variation	DF	Mean square			
		SL	RCDM	ADM	RM
Vigor	1	29896.23*	0.0621*	0.0273*	230.58*
Stress	3	167.24*	0.0103	0.0001	0.94
Time	3	22714.34*	0.0871*	0.0286*	246.38*
Vigor x Stress	3	157.63*	0.0240	0.0016*	14.66*
Vigor x Time	3	6018.91*	0.0082	0.0086*	75.00*
Stress x Time	9	330.10*	0.0115	0.0005*	4.44
Vigor x Stress x Time	9	400.70*	0.0148	0.0009*	8.45*
Residual	96	44.39	0.0147	0.0002	2.50
Overall Mean		35.91	0.92	0.06	5.89
Coefficient of Variation (%)		18.55	13.18	26.79	26.87

*/Significant at 5% by the F-test with H_0: $\mu_1 \neq \mu_2 \neq \mu_n$; DF/Degrees of Freedom

Figures

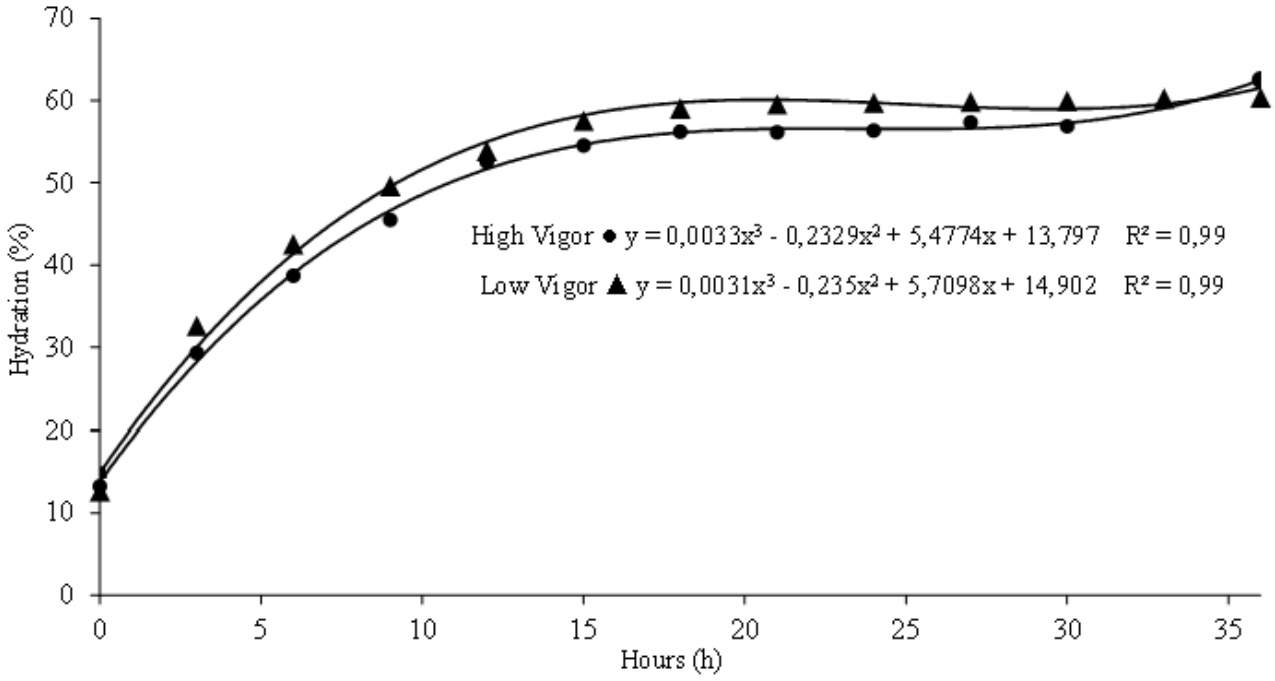

Figure 1

Soybean seed hydration curve for high and low vigor seeds, with the respective percentage moisture as a function of time

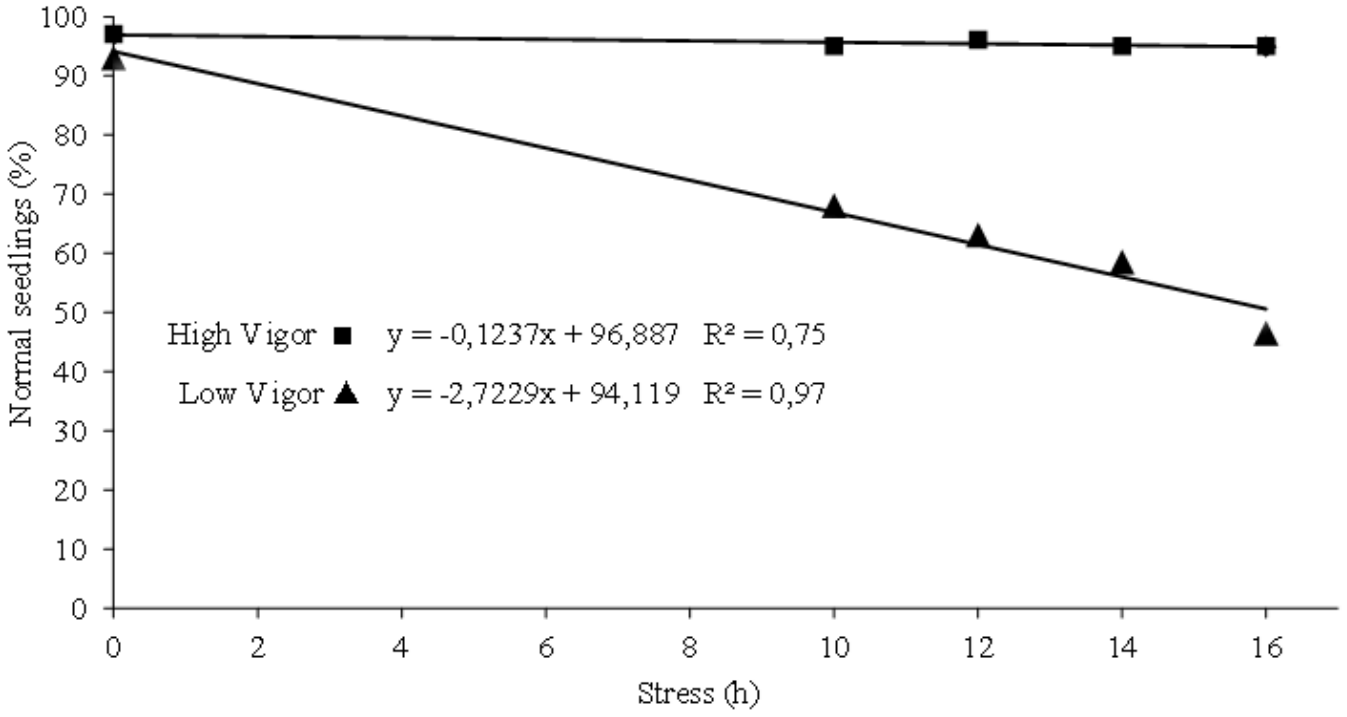

Figure 2

Effect of thermal stress duration in hours (0, 10, 12, 14, and 16 h) on the percentage of normal seedlings under High and Low vigor conditions

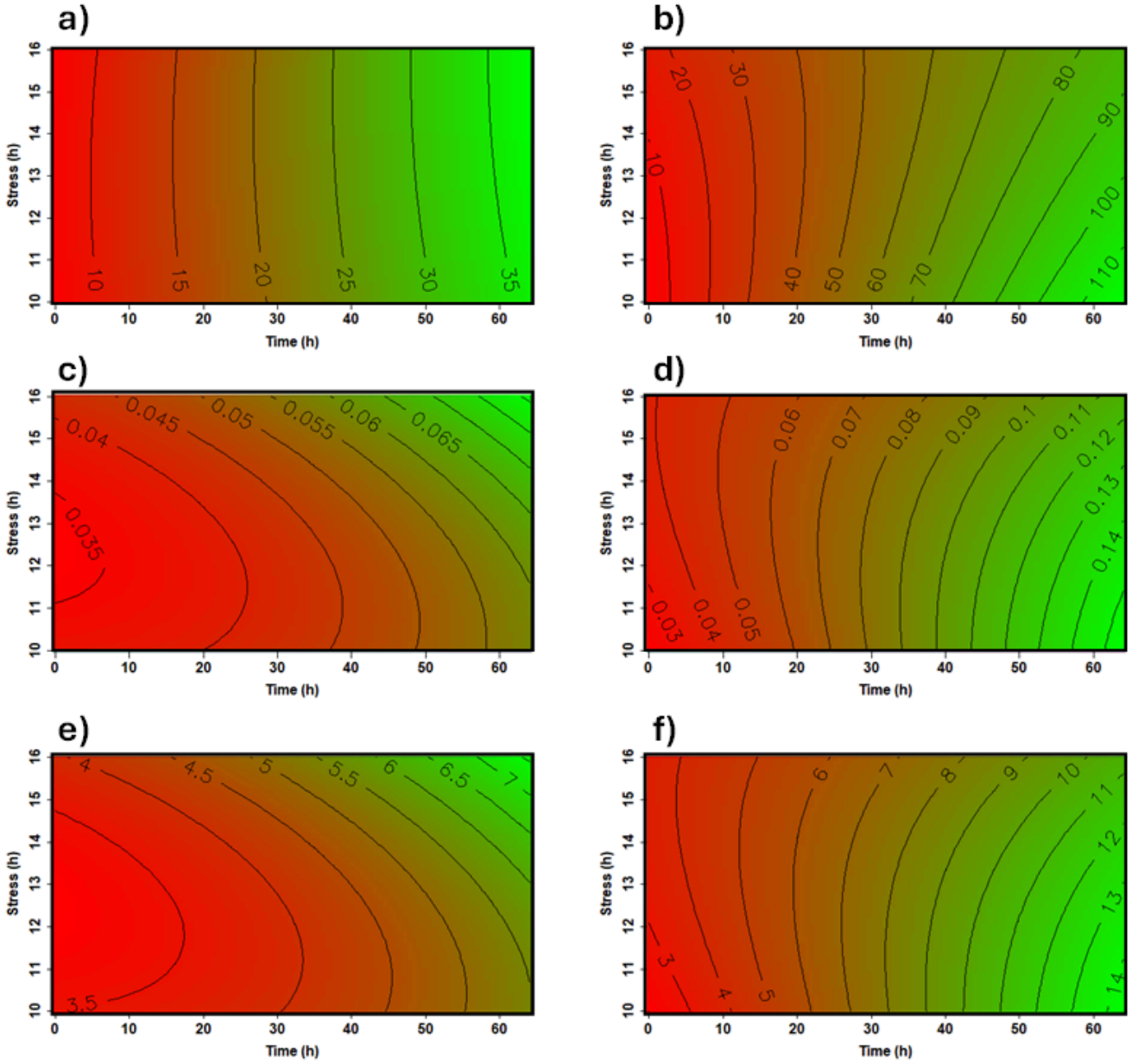

Figure 3

Response surface of the variables seedling length (a: low vigor, b: high vigor), axis dry mass (c: low vigor, d: high vigor), and reserve mobilization (e: low vigor, f: high vigor) as a function of evaluation time (h) and stress (h) for seeds