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EVALUATION OF SOYBEAN GENOTYPE TOLERANCE TO WATER STRESS DURING GERMINATION

Filipe Moreno Ruiz[1], Wagner Menechini[2] and Thiago Alberto Ortiz[1,2]

ABSTRACT

Water deficit is a major limiting factor for crop productivity and significantly impacts the viability and vigor of soybean seeds (*Glycine max*). This study aimed to evaluate the effects of water deficit on the germination-emergence phenological stage in soybean genotypes classified as either drought-tolerant or sensitive during flowering. The experiment followed a completely randomized design with four replications. A factorial scheme of 10 × 6 was used, comprising 10 soybean genotypes (genotype 1, genotype 2, genotype 3, genotype 4, genotype 5, genotype 6, genotype 7, genotype 8, genotype 9, and genotype 10) and six osmotic potentials (-0.0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa), achieved using polyethylene glycol 6000 (PEG 6000). The analyzed variables included first germination count and germination percentage (from the germination test), shoot and root length, shoot and root dry mass (from the seedling growth test), and vigor from the cold test. Data were fitted to regression models. The results showed that reducing the osmotic potential with PEG 6000, from -0.2 MPa onwards, negatively affected the germination and vigor of soybean seeds, regardless of whether the genotypes were drought-tolerant or sensitive during flowering. Soybean genotypes displayed varying responses to water deficit during the germination-emergence stage, indicating that drought tolerance during flowering does not necessarily predict their performance under water stress at earlier growth stages.
Additional Keywords: Drought stress, *Glycine max*, physiological quality of seeds, polyethylene glycol, viability

RESUMEN

Evaluación de la tolerancia de genotipos de soja al estrés hídrico durante la germinación

El déficit hídrico es un factor limitante importante para la productividad de los cultivos que afecta significativamente la viabilidad y el vigor de las semillas de soja (*Glycine max*). Este estudio tuvo como objetivo evaluar los efectos del déficit hídrico en la etapa fenológica de germinación-emergencia en genotipos de soja clasificados como tolerantes o sensibles a la sequía durante la floración. El experimento se realizó con un diseño completamente aleatorizado con cuatro repeticiones. Se utilizó un esquema factorial 10 × 6, que incluyó 10 genotipos de soja (genotipo 1, genotipo 2, genotipo 3, genotipo 4, genotipo 5, genotipo 6, genotipo 7, genotipo 8, genotipo 9 y genotipo 10) y seis potenciales osmóticos (-0.0, -0.2, -0.4, -0.6, -0.8 y -1.0 MPa), obtenidos mediante el uso de polietilenglicol 6000 (PEG 6000). Las variables analizadas incluyeron el primer conteo de germinación y el porcentaje de germinación (del test de germinación), la longitud y la masa seca de la parte aérea de la plántula y de la raíz (del test de crecimiento de plántulas) y el vigor de la prueba de frío. Los datos fueron ajustados a modelos de regresión. Los resultados mostraron que la reducción del potencial osmótico con PEG 6000, a partir de -0.2 MPa, afectó negativamente la germinación y el vigor de las semillas de soja, independientemente de si los genotipos eran tolerantes o sensibles a la sequía durante la floración. Los genotipos de soja mostraron respuestas variables al déficit hídrico durante la etapa de germinación-emergencia, lo que indica que la tolerancia a la sequía durante la floración no necesariamente predice su desempeño bajo estrés hídrico en etapas tempranas del crecimiento.
Palabras clave adicionales: Calidad fisiológica de las semillas, estrés hídrico, *Glycine max*, polietilenglicol, viabilidad

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INTRODUCTION

The soybean (*Glycine max* [L.] Merrill) is an Asian-origin crop, specifically from China, that has become a commodity and plays a fundamental role in the development of several Brazilian regions due to the constant advancement of technologies (Lazzarotto and Hirakuri, 2011). Brazil is the world's largest soybean producer, responsible for a production of 147.7 million tons in the 2023/24 crop season, with a cultivated area of 46.1 million hectares and a yield of 3,201 kg·ha^{-1}. For the 2024/25 crop season, a 12.4 % increase in production is expected, with a yield of

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[1]Universidade Estadual de Londrina (UEL), Departamento de Agronomia, Londrina, Paraná, Brasil. e-mail: filipe_ruiz99@hotmail.com; thiago.ortiz@hotmail.com (corresponding author)
[2]Universidade Paranaense (UNIPAR), Programa de Pós-graduação em Biotecnologia aplicada à Agricultura (Stricto Sensu), Umuarama, Paraná, Brasil. e-mail: wagner.menechini@edu.unipar.br

3,499 kg ha^{-1} (Conab, 2025). The crop cycle is annual, ranging from 70 days (early cultivars) to 200 days (late cultivars) (Sediyama *et al*., 2016). Due to advances in genetic improvement, the crop can be implemented in much of the national territory. However, the largest producing centers are the Midwest, Southeast, and South regions of Brazil, which favor the crop's development due to the favorable climate, with temperatures between 20 and 30 ºC, and satisfactory water availability (Embrapa, 2013).

Like other crops, soybean production is limited by biotic and abiotic stresses (Mohammadi *et al*., 2012). According to Poltronieri *et al*. (2011), stresses caused by adverse weather are the main limiting factors for growth and productivity. Among the limiting factors for soybean production, water deficit stands out, as the crop has high water requirements. Brazil, being a large country with considerable territorial and climatic variability, faces regions with extended drought periods. This factor interferes with the plant's physiological processes, leading to stresses that are often irreversible, causing financial losses to producers and, consequently, to both the national and international economy (Elliott *et al*., 2013). Losses due to abiotic factors, such as water scarcity, account for a significant portion of global losses, with water deficit considered a harmful factor in reaching the maximum productive potential of crops. Future projections of freshwater availability indicate that agricultural production will face a significant global impact, posing a threat to food security and sustainability (Biswas *et al*., 2025).

In soybean, to achieve maximum yield, water requirements throughout the cycle range from 450 to 800 mm, depending on the genetic material (Embrapa, 2013). According to Beutler *et al*. (2024), water availability plays a critical role in two main periods of soybean development: germination-emergence and flowering-grain/seed filling, depending on the climate, management practices, and cultivar.

Understanding how plants respond to drought conditions is crucial to assess the impacts on production. This has been a significant area of research in genetic improvement due to global climate changes (Shao *et al*., 2007). It is known that no technology currently exists that can transform non-resistant plants into drought-resistant plants after planting. Therefore, the goal is to develop cultivars with high drought tolerance.

For seeds, laboratory tests assessing germination and vigor under water stress conditions are widely used to evaluate their performance during the germination-emergence phenological stage. Studies using polyethylene glycol (PEG) at different osmotic potential levels are commonly carried out due to its ability to simulate water deficit conditions similar to soil water scarcity (Machado *et al*., 2016).

Thus, it is essential to evaluate the development of soybean genotypes at different phenological stages, subjected to various levels of water stress, to minimize potential damage in regions vulnerable to drought conditions. Therefore, this study aimed to evaluate the effect of water deficit on the germination-emergence phenological stage in soybean genotypes classified as drought-tolerant or sensitive during flowering.

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Seed Production Technology at the State University of Londrina (UEL), in a completely randomized experimental design with four replications, in a 10 × 6 factorial scheme (soybean genotypes × osmotic potentials).

Seeds from 10 soybean genotypes, provided by a plant genetics research company, were used. The seeds were harvested from different soybean-producing areas in Brazil during the 2021/2022 crop season. They were then artificially dried using the stationary drying method with forced air flow, relative humidity at 40 %, and air temperature around 40 °C. The seeds were dried to 11 % moisture content and stored in a cold chamber at 10 °C until the tests were set up, which took place 10 days later.

The materials were identified as genotype 1 through genotype 10 (seven tolerant and three sensitive). These genotypes were classified based on selection in the breeding program by the granting company, following agronomic evaluation criteria in the field. Drought tolerance assessments were conducted during the reproductive stage (flowering). The classification of the genotypes is presented in Table 1.

To evaluate the influence of water potential on

the physiological quality of seeds, germitest paper was used as a substrate in both the germination and vigor tests, which was soaked with polyethylene glycol (PEG) solutions (PEG 6000) at the following osmotic potential levels: 0.0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa, calculated according to the standards by Braccini *et al*. (1996) and Teixeira *et al*. (2008) (Table 2). The 0.0 MPa level served as the control, where only distilled water was used to moisten the substrates.

Table 1. Classification of genotypes as tolerant or sensitive to water deficit during flowering, as carried out by the granting company

Material	Water deficit tolerance
Genotype 1	Tolerant
Genotype 2	Tolerant
Genotype 3	Tolerant
Genotype 4	Sensitive
Genotype 5	Sensitive
Genotype 6	Tolerant
Genotype 7	Tolerant
Genotype 8	Sensitive
Genotype 9	Tolerant
Genotype 10	Tolerant

Table 2 - PEG concentration (in grams per liter of water) to obtain osmotic potentials

Osmotic potential (MPa)	PEG concentration ($g \cdot L^{-1}$)
0.0	0.0
-0.2	119.571
-0.4	178.343
-0.6	223.664
-0.8	261.948
-1.0	296.713

To evaluate the physiological quality of the seeds, the following tests were performed:

Germination test: Four repetitions of 50 seeds were placed on two sheets of germitest paper and covered with another sheet of germitest paper, forming germination rolls. The paper sheets were previously moistened with 2.5 times the dry mass of the substrate with PEG solutions, according to each treatment (osmotic potentials), or with distilled water (0.0 MPa). The rolls containing the seeds were kept in a germinator at 25 °C. Evaluations were carried out on the fifth day (first germination count) and the eighth day (final count) after the test installation, with results expressed as the percentage of normal seedlings according to Brasil (2009). The analyzed variables were first germination count (vigor) and germination (first germination count + final count = viability).

Seedling growth test: Four replications of 20 seeds were used for each genotype. Two lines were drawn in the upper third of the germitest paper, in the longitudinal direction, where the seeds were arranged (Krzyzanowski, 2021). The papers were moistened with distilled water (control) and PEG solutions (osmotic potentials) equivalent to 2.5 times the dry mass of the paper. The soybean seeds were positioned so that the micropyle was facing the bottom of the paper. The rolls were placed vertically in the germinator for seven days at 25 °C. At the end of this period, the lengths of the emerged normal seedlings (shoot and root) were measured using a millimeter ruler, and the results were expressed in centimeters (cm). The analyzed variables were shoot and root length. Subsequently, the seedlings whose length was measured were sectioned into shoots and roots, excluding the cotyledons, and placed in separately identified paper bags. Each experimental unit was then placed in an oven with forced air circulation, maintained at 80 °C for 24 h (Krzyzanowski, 2021). After drying, the experimental units were weighed on a precision

scale (0.001 g), with the mass of the paper bags deducted, and the results were expressed in milligrams (mg). The analyzed variables were shoot and root dry mass.

Cold test: The cold test was conducted according to the methodology described in the germination test (Brasil, 2009), but the rolls containing the seeds were wrapped in plastic bags, sealed with masking tape, and placed vertically in a BOD oven at 10 °C for five days. After this period, the rolls were removed from the plastic bags and placed in a germinator at 25 °C for four days (Krzyzanowski *et al*., 2021). After the incubation, the percentage of vigor was assessed by counting the number of normal seedlings. The analyzed variable was vigor from the cold test.

The data for the variables first germination count, germination, shoot and root length, shoot and root dry mass, and vigor from the cold test were analyzed using regression models, with the Sisvar software version 5.6.

RESULTS AND DISCUSSION

The statistical analysis revealed a significant interaction between the soybean genotypes and osmotic potentials for the variables: first germination count, germination, shoot and root length, shoot and root dry mass, and vigor from the cold test (Table 3).

Table 3 - Analysis of variance for the variables: first germination count (%), germination (%), shoot length (cm), root length (cm), shoot dry mass (mg), root dry mass (mg), and vigor from the cold test (%) of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

First germination count	***p*-value**	**Germination**	***p*-value**
Genotype (G)	0.0000*	Genotype (G)	0.0000*
Osmotic potential (OP)	0.0000*	Osmotic potential (OP)	0.0000*
G × OP	0.0000*	G × OP	0.0002*
CV (%)	15.03	CV (%)	22.12
Shoot length	***p*-value**	**Root length**	***p*-value**
Genotype (G)	0.0000*	Genotype (G)	0.0000*
Osmotic potential (OP)	0.0000*	Osmotic potential (OP)	0.0000*
G × OP	0.0000*	G × OP	0.0000*
CV (%)	25.07	CV (%)	19.72
Shoot dry mass	***p*-value**	**Root dry mass**	***p*-value**
Genotype (G)	0.0000*	Genotype (G)	0.0000*
Osmotic potential (OP)	0.0000*	Osmotic potential (OP)	0.0000*
G × OP	0.0000*	G × OP	0.0000*
CV (%)	1.63	CV (%)	1.15
Vigor from cold test	***p*-value**		
Genotype (G)	0.0000*		
Osmotic potential (OP)	0.0000*		
G × OP	0.0000*		
CV (%)	10.33		

NS Non-significant, * Significant p≤0.05.

As shown in Figure 1, all genotypes exhibited a decreasing trend in the first germination count as the osmotic potential decreased. Interestingly, genotypes 4 and 5, which are classified as sensitive to water deficit during the flowering phase (Table 1), showed the greatest increase in the first germination count, rising from 0.0 to -0.2 MPa. However, genotypes 7 and 8 (classified as tolerant and sensitive to drought, respectively) showed the largest reductions in first germination count, with reductions of 82 % and 89 %, respectively, from -0.2 MPa to -0.4 MPa.

Similarly, the germination variable (Figure 2) demonstrated a decrease in germination percentage as osmotic potential decreased, confirming previous findings by Machado *et al*.

(2016), who also noted the impact of water stress on germination in soybean genotypes. The reduced germination observed at -0.8 and -1.0 MPa for most genotypes suggests that severe water stress conditions critically impair seedling establishment. Furthermore, Dantas *et al*. (2017) highlighted the importance of the germination variable in selecting soybean genotypes for drought tolerance in the germination-emergence stage. Since several genotypes showed low or no germination at more negative osmotic potentials, they were excluded from the seedling growth and cold test analyses.

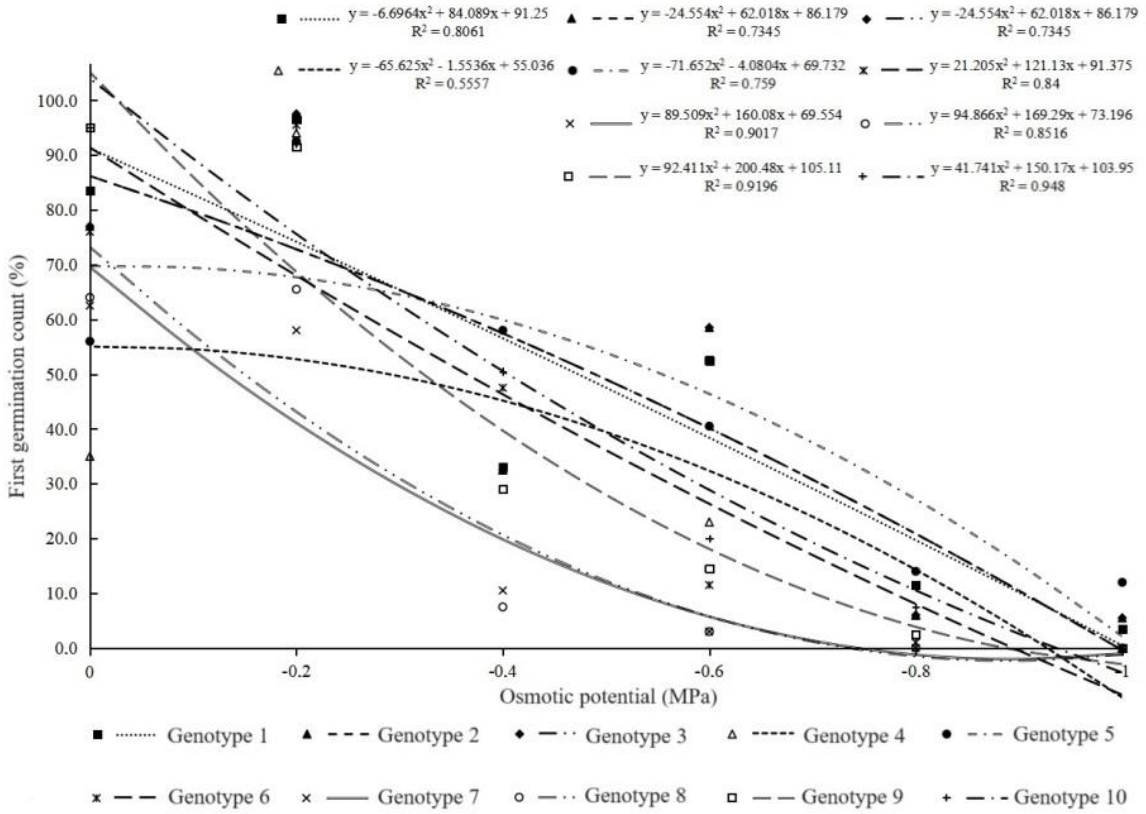

Figure 1. First germination count of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

Shoot and root lengths were significantly affected by osmotic potential reductions (Figures 3 and 4). As osmotic potential decreased from 0.0 to -0.2 MPa, a marked reduction in shoot length occurred for all genotypes, with genotype 5 (sensitive) showing the largest decrease. At -0.6 MPa, only genotypes 1 and 8 maintained shoot lengths greater than 1 cm. Similarly, root length decreased for all genotypes as the osmotic potential became more negative, with reductions becoming more pronounced as the osmotic potential approached increasingly negative values. These results align with the findings of Texeira *et al*. (2008) and Vieira *et al*. (2013), who also observed reductions in seedling growth under water stress. This effect is attributed to the sensitivity of growth processes, such as cell elongation and cell wall synthesis, to water deficit, which ultimately limits cell expansion and negatively affects seedling development.

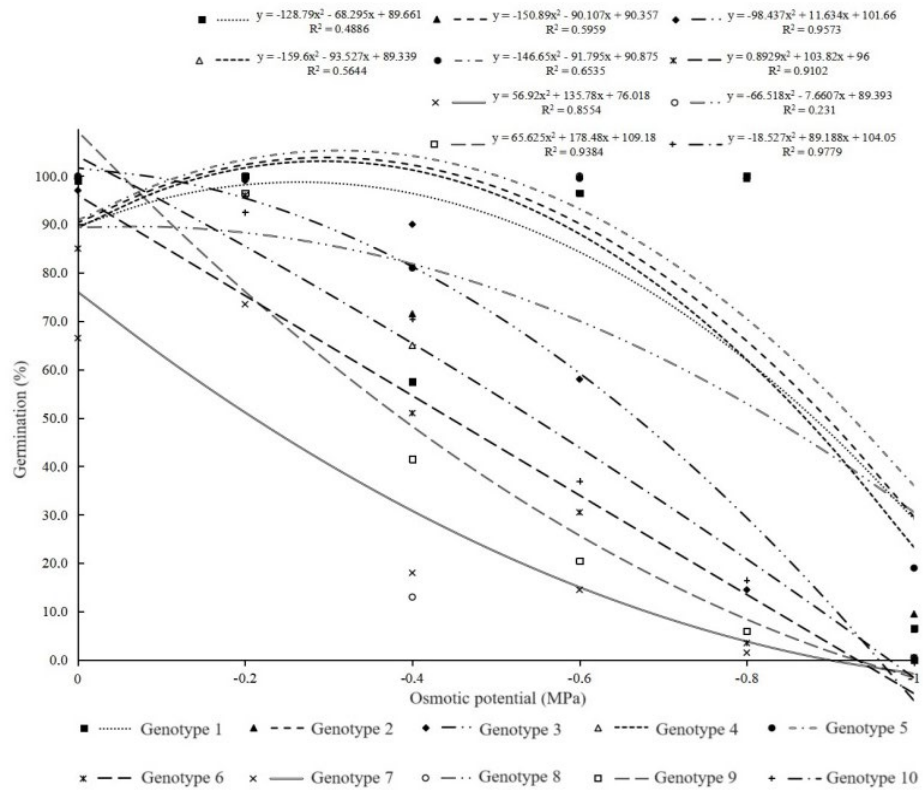

Figure 2. Germination of soybean genotypes subjected to different osmotic potentials induced by PEG 6000.

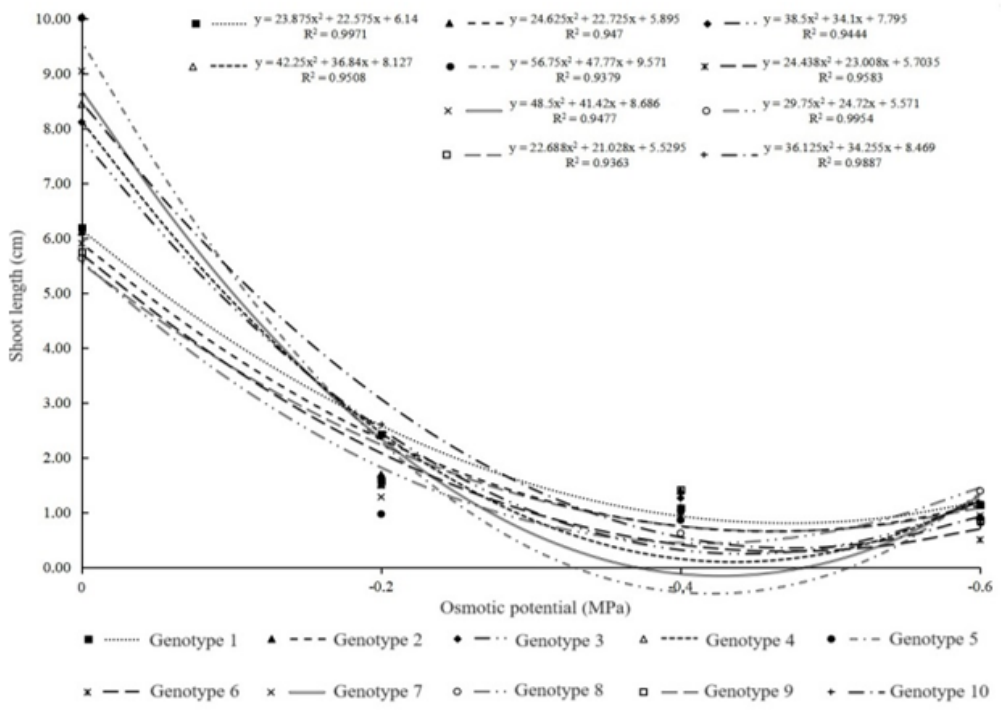

Figure 3. Shoot length of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

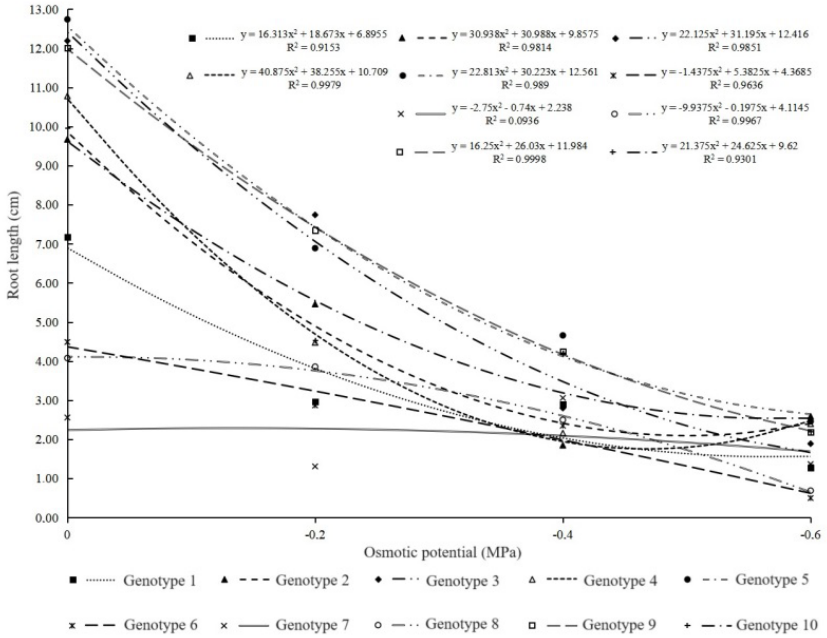

Figure 4. Root length of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

The reduction in shoot dry mass (Figure 5) and root dry mass (Figure 6) with decreasing osmotic potential further confirms the detrimental effects of water stress. A sharp reduction in shoot dry mass was noted at -0.2 MPa, impacting all genotypes. According to Vieira *et al*. (2013), dry mass accumulation is a key indicator of seedling development, and reduced dry mass suggests hindered metabolic activities during seedling growth under water stress.

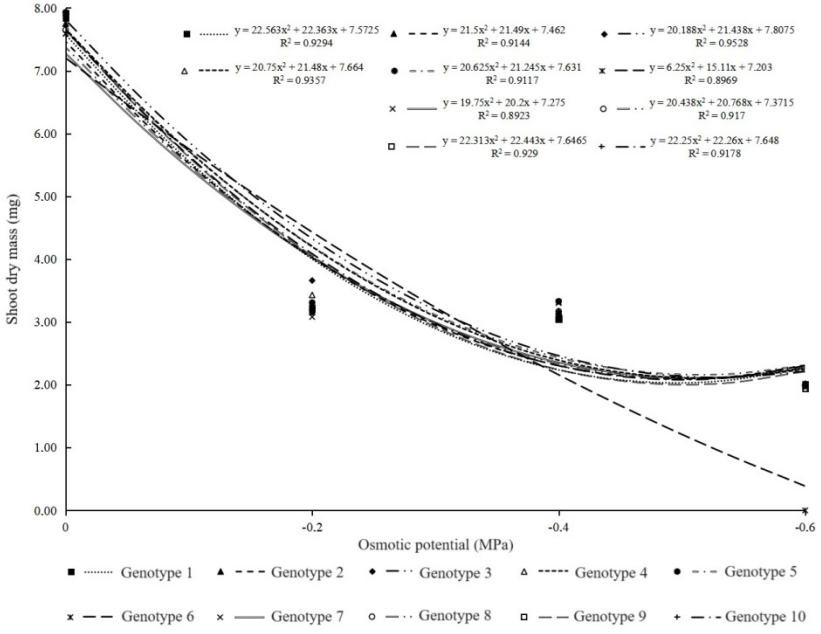

Figure 5. Shoot dry mass of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

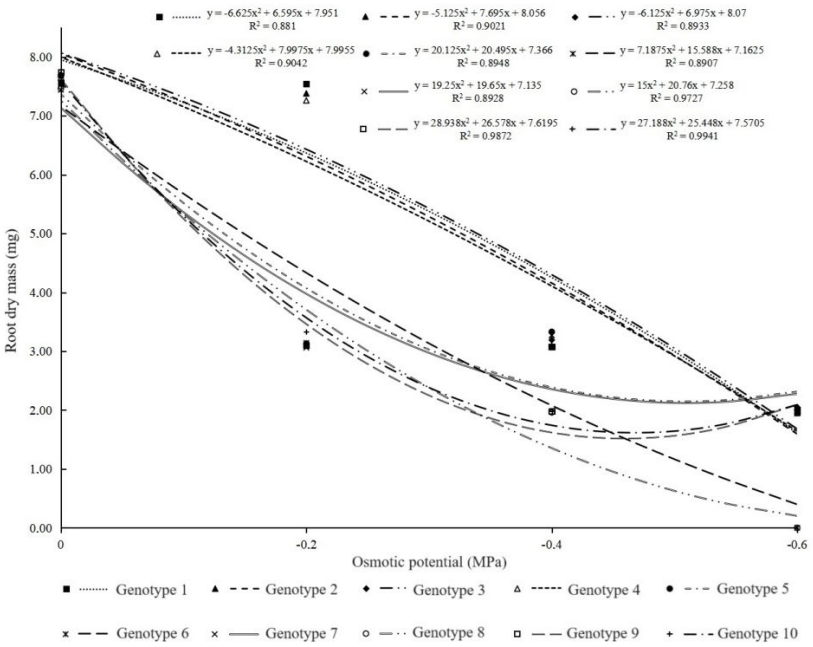

Figure 6. Root dry mass of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

However, for vigor from the cold test (Figure 7), the results indicated a less pronounced impact of water stress compared to other variables. All genotypes, except for genotype 9, showed a slight increase in vigor from 0.0 to -0.2 MPa, with genotypes 6 (tolerant), 7 (tolerant), and 8 (sensitive) exhibiting the greatest improvements. On the other hand, genotypes 7 and 8 experienced the most drastic reductions at -0.4 MPa. All genotypes were strongly negatively impacted at -0.6 MPa, except for genotype 10. These findings suggest that cold treatment prior to germination may mitigate some of the effects of water stress.

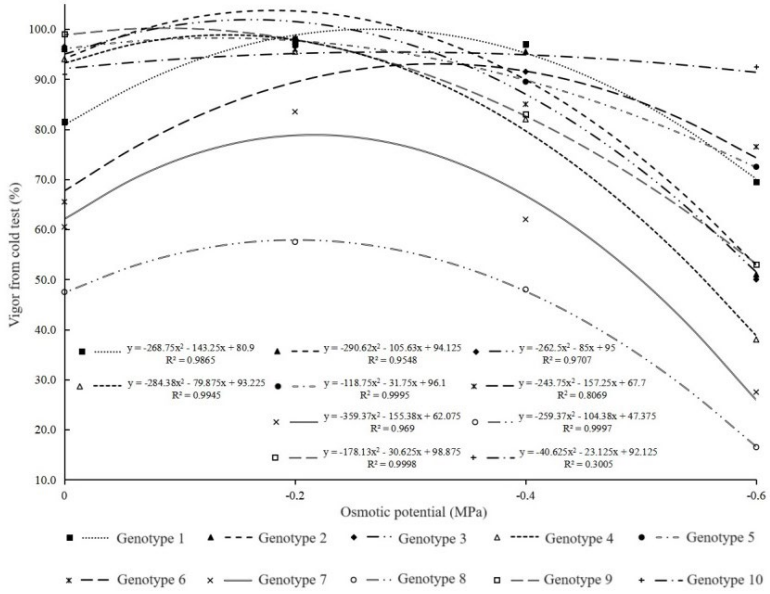

Figure 7. Vigor from the cold test of soybean genotypes subjected to different osmotic potentials induced by PEG 6000

These results demonstrate that soybean genotypes display varying responses to water stress during different phenological stages. Even genotypes known to be tolerant to drought during the flowering phase showed divergent behaviors when subjected to water stress during the germination-emergence stage. This highlights the complexity of drought resistance in soybean, where different stages of development may require distinct mechanisms of tolerance.

Given the importance of water availability in seed metabolism, particularly in the germination-emergence stage, it is evident that water stress negatively impacts the early development of soybean seedlings. Each genotype responds differently to water deficit, underscoring the necessity for further studies to evaluate genotypic performance at various growth stages. Understanding these differential responses is crucial for breeding programs aimed at developing soybean genotypes with improved drought tolerance across multiple phenological stages.

In line with the importance of drought tolerance assessment, Oliveira *et al*. (2023) proposed using machine learning algorithm, specifically 'Random Forest', to develop a classification model for selecting drought-tolerant soybean genotypes. The model successfully classified over 73% of genotypic patterns, and its decision tree structure could serve as a useful tool for genotype selection, even for non-experts. This approach provides a promising avenue for enhancing soybean breeding programs and facilitating decision-making in the selection of drought-tolerant genotypes.

CONCLUSIONS

The reduction of osmotic potential using PEG 6000, starting from -0.2 MPa, negatively impacted the germination and vigor of soybean seeds, regardless of the genotypes' tolerance or sensitivity to water deficit during flowering. These findings highlight that soybean genotypes exhibit varied responses to water deficit during the germination-emergence phase, demonstrating that drought tolerance during flowering does not necessarily predict performance under water stress in earlier stages of growth.

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