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EVALUATION OF OSMOTIC STRESS TOLERANCE DURING THE GERMINATION STAGE IN SOYBEAN GENOTYPES

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Abstract: *To evaluate the tolerance of soybean genotypes to osmotic stress, a controlled germination experiment was performed on six soybean lines: three obtained by gamma-ray-induced mutagenesis ($C15M_7200$, $B2M_8200$, $Z21M_{12}200$) and three by hybridization (L.4, L.8, L.86). Seeds were treated with three concentrations of PEG 6000 (0%, 10%, 15%) to simulate a water stress gradient. The analyzed parameters – germination rate and root length – were recorded 5 and 7 days after sowing. A significant reduction of both parameters was found with increasing PEG concentration, highlighting a different sensitivity between genotypes. Lines $C15M_7200$ and $B2M_8200$ showed better tolerance, while L.8 and $Z21M_{12}200$ were the most affected. The results highlight genetic variability and support the utility of PEG testing in the early stages of breeding programs for the selection of drought-tolerant genotypes.*

Keywords: drought tolerance, germination, PEG, soybean.

INTRODUCTION

Under current climate change conditions, drought and water stress represent one of the greatest threats to global food security, severely affecting agricultural production, especially in arid and semi-arid regions. Osmotic stress, caused by soil water deficit, profoundly disrupts plant physiological processes, reducing germination rate, root system development and, ultimately, crop productivity. These effects are accentuated by increasing average temperatures and unpredictable rainfall, factors that also contribute to soil salinization and reduced water availability for plants [6].

To better understand and evaluate plant responses to water stress, agricultural research often uses polyethylene glycol (PEG), a compound that mimics water deficit conditions by creating a negative osmotic potential [3]. The use of PEG in germination experiments allows for the simulation of a controlled environment of osmotic stress and facilitates the comparison of drought tolerance between different genotypes. These studies provide relevant data on drought tolerance in the early stages of development, contributing to the selection of varieties with high agronomic potential under stress conditions [4].

In this context, soybean (*Glycine max*), a legume of strategic importance for both human and animal nutrition, is becoming a species of major interest. Due to its high protein content and its ability to fix atmospheric nitrogen, soybean plays a key role in sustainable agricultural systems [1]. However, this crop is particularly sensitive to water stress, especially in the early stages of germination and emergence, which can compromise the stability of production under adverse climatic conditions [2].

The identification of soybean genotypes with increased tolerance to osmotic stress holds significant practical relevance in the context of modern agriculture, which is increasingly marked by the frequency and intensity of drought episodes. Germination tests based on polyethylene glycol (PEG) enable rapid and efficient selection of promising genetic material, contributing to the shortening of the breeding cycle and the development of cultivars adapted to water stress conditions. Moreover, the strong correlation between germination parameters (such as germination rate, radicle emergence, and root elongation) and root system development provides reliable criteria for the indirect selection of tolerant genotypes [5].

Genotypes that demonstrate resilience during the germination phase generally show superior field establishment, greater biomass accumulation, and better yield performance under limited water availability [4]. The implementation of these findings in breeding programs can accelerate the development of high-performing varieties, contributing both to enhanced food security and to the long-term sustainability of agricultural production in increasingly unpredictable climatic conditions.

In this context, the present study aims to evaluate the response of six soybean genotypes to osmotic stress induced by polyethylene glycol (PEG 6000) under controlled laboratory conditions. The goal is to identify genetically valuable and tolerant material that can be further used in breeding programs focused on adapting soybean cultivation to increasingly unpredictable climatic conditions.

MATERIALS AND METHODS

To evaluate osmotic stress tolerance, a germination experiment was performed under controlled conditions, using six soybean genotypes from the "Applied Genetics" laboratory of the Institute of Genetics, Physiology and Plant Protection (IGFPP), USM. Three of these ($C15M_7200$, $B2M_8200$, $Z21M_{12}200$) were obtained by experimental gamma-ray mutagenesis, and the other three (L.4, L.8, L.86) by conventional hybridization. For each genotype, 10 seeds per experimental variant were used, in three repetitions, covering the following treatments:

0% PRG (control) – distilled water, no stress

10% PEG – moderate stress
15% PEG – severe stress

The concentrations of polyethylene glycol (PEG 6000) were established according to the values proposed by Michel and Kaufmann [3], and the solutions were prepared in distilled water. Germination was performed in sterile Petri dishes, lined with filter paper, onto which 5 ml of each treatment solution was distributed. The seeds were previously disinfected with a 1% sodium hypochlorite solution for 5 minutes, followed by three successive rinses with sterile distilled water. The Petri dishes were placed in a climate chamber, under controlled conditions of temperature (25 ± 1°C) and relative humidity (70%). Germination assessments were performed 5 and 7 days after the initiation of the experiment. A seed was considered germinated if the radicle exceeded 2 mm in length. The experimental data were statistically analyzed using STATISTICA 7 software, and the graphs and presentation tables were processed in Microsoft Excel.

RESULTS AND DISCUSSION

The results obtained from germination tests performed at 5 and 7 days highlight a significant influence of osmotic stress on the germination capacity of the soybean genotypes analyzed. The data presented in Table 1 show that at the control level (0% PEG), most genotypes showed a high germination rate, over 80%, with the exception of genotypes L.8 and L.86, which recorded 70% and 60%, respectively. This indicates a generally good viability of the seeds, but also genetic differences in terms of initial vigor.

With the application of osmotic stress, all genotypes underwent significant reductions in germination. Lines C15$M_7$200 and B2$M_8$200 were noted for maintaining a relatively high germination percentage at 10% PEG (80–90%), but suffered a drastic decrease at 15% PEG, indicating moderate tolerance to moderate stress and increased sensitivity to severe stress. Line Z21M_{12}200 showed reduced tolerance, with only 5% germination at 5 days under 15% PEG conditions, but increased to 15% at 7 days, suggesting a delay in the germination process, possibly due to a protective strategy under unfavorable conditions. This delayed response may be associated with metabolic adjustments or activation of specific stress-related pathways, allowing certain seeds to overcome the initial inhibitory effects of PEG. The observation highlights the importance of evaluating germination dynamics over multiple time points, rather than relying solely on early measurements, which may underestimate the potential of some genotypes to adapt or recover under stress conditions. Among the hybrid lines, L.4 performed best under moderate stress (70–80%), but performed very poorly at 15% PEG.

Table 1. Effect of PEG on germination percentage at 5 and 7 days

Genotype	Treatment	Number of seeds	Germination in 5 days	Germination on in 5 days, %	Germination in 7 days	Germination in 5 days, %
$C15M_7200$	Control	30	27	90%	30	100%
$C15M_7200$	10% PEG	30	24	80%	27	90%
C15M7200	15% PEG	30	3	10%	4	15%
$B2M_8$ 200	Control	30	30	100%	30	100%
$B2M_8$ 200	10% PEG	30	24	80%	24	80%
$B2M_8$ 200	15% PEG	30	9	30%	9	30%
$Z21M_{12}$ 200	Control	30	25	85%	30	100%
$Z21M_{12}$ 200	10% PEG	30	19	65%	22	75%
$Z21M_{12}$ 200	15% PEG	30	1	5%	4	15%
L.4	Control	30	30	100%	30	100%
L.4	10% PEG	30	21	70%	24	80%
L.4	15% PEG	30	1	5%	1	5%
L.8	Control	30	21	70%	24	80%
L.8	10% PEG	30	15	50%	15	50%
L.8	15% PEG	30	0	0%	1	5%
L.86	Control	30	18	60%	21	70%
L.86	10% PEG	30	13	45%	16	55%
L.86	15% PEG	30	4	15%	6	20%

Figure 1 highlights the clear negative effect of PEG-induced water stress on germination percentage at 7 days. A progressive decrease in germination capacity is observed with increasing PEG concentration from 0% (control) to 15%, confirming the general sensitivity of soybean seeds to osmotic stress.

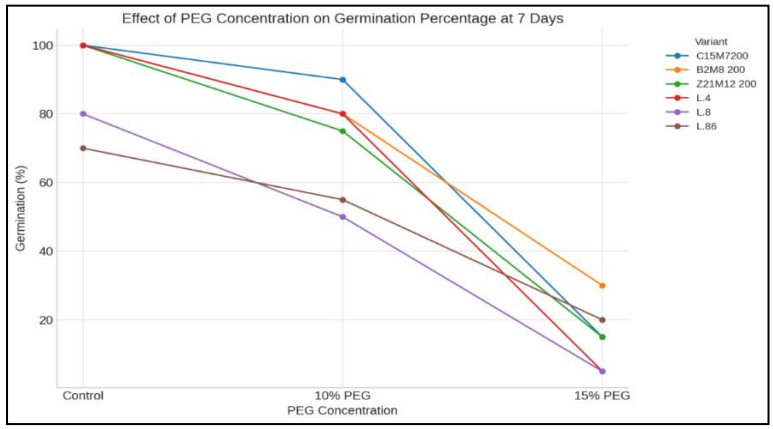


Figure 1. Germination percentage at 7 days as a function of PEG concentration for six soybean genotypes

Analyzing the variation of root length in the soybean genotypes studied, we

can mention that osmotic stress had a significant negative impact on the development of the root system. As the PEG concentration increased, the root length decreased considerably in all genotypes (fig.2).

The C15M$_7$200 and B2M$_8$200 genotypes were distinguished by the largest root lengths under control conditions, frequently exceeding 8–10 cm, but also by a better capacity to maintain root growth at 10% PEG. This behavior indicates a superior tolerance to moderate stress and a possible adaptation strategy by more efficient exploration of the substrate in search of water.

In contrast, the L.8 and Z21M$_{12}$200 genotypes showed a drastic reduction in root length, with very low average values at 10% and 15% PEG concentrations, suggesting a severe inhibition of root growth under osmotic stress.

Also, genotypes L.4 and L.86 showed intermediate resilience, maintaining some root growth at 10% PEG, but with a sharp decrease at 15%.

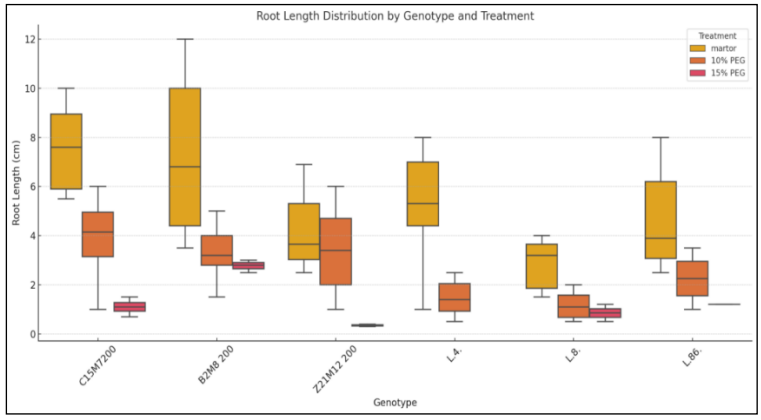


Figure 2. Effect of PEG-induced osmotic stress (0%, 10%, 15%) on root length in different soybean genotypes, evaluated 7 days after germination

Correlating these data with the germination percentage (figure 1), it is observed that root length is a good indicator of tolerance to water stress in the early development phase, and genotypes that maintained better root growth under stress generally also recorded higher germination rates.

CONCLUSIONS

The results obtained in this study highlight the significant impact of osmotic stress, induced by the use of polyethylene glycol (PEG 6000), on germination and root development of six soybean genotypes. Both the germination percentage and root length decreased progressively with increasing PEG concentration, confirming the sensitivity of soybean to water deficit in the early stages of development.

At the same time, the differences between genotypes demonstrated the existence of significant genetic variability in the response to water stress, an essential aspect for breeding programs. The genotypes C15$M_7$200 and B2$M_8$200 stood out for their superior performances in both germination and root system development, especially under moderate stress (10% PEG), suggesting a high adaptive capacity and valuable potential for selection.

On the other hand, genotypes L.8, L.86 and Z21M_{12}200 showed increased sensitivity, with drastic decreases in germination and root growth, especially under severe stress conditions (15% PEG), indicating reduced tolerance to water deficit.

Testing for osmotic stress tolerance during the germination phase, using PEG as a simulant, proved to be an effective method for differentiating soybean genotypes and identifying promising genetic sources. The tolerant genotypes identified in this study represent valuable material for use in breeding programs aimed at improving drought resistance. Their inclusion can support the development of more stable and resilient soybean varieties, better adapted to the challenges posed by climate change.

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