Agricultural Research & Technology
Open Access Journal
ISSN: 2471-6774

Juniper Publishers
key to the Researchers

Research Article
Volume 23 Issue 5 - March 2020
DOI: 10.19080/ARTOAJ.2020.23.556250

Agri Res & Tech: Open Access J


# PEG-Induced Drought Stress During Germination: Effects on Soybean Germplasm

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**Submission**: February 17, 2020; **Published:** March 02, 2020

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## Abstract

Soybean is the one of the most important oilseed and protein sources worldwide. Among environmental stresses negatively affecting soybean, drought is considered as the most limiting factor in terms of yield losses encountered. In view of the need for improving drought tolerance, this study aimed at determining the seed germination and seedling growth potential under drought conditions as an approach to identify tolerant genotypes at early growth stages. The genetic material consisted of a set of commercial and pre-commercial varieties (Adonai, Neoplanta, Celina, Zora, PR92M22, P21T45, PR92M35, PR92B63, PR91M10). Drought stress was induced by different concentrations of polyethylene glycol (0, 5, 10 and 20 % PEG-6000). Genotype performance was assessed on the basis of Germination Percentage (GP), seed Water Uptake (WU), seedling Water Content (WC), root and shoot length and number of seedlings with abnormal phenotype. Stress substantially affected all traits associated to germination and seedling growth, with its effects though differing significantly among genotypes. Overall findings point to the superiority of varieties Neoplanta, Adonai, PR92M22 and Celina. Further, it was evidenced that GP as well as root and shoot length form suitable criteria for drought tolerance, while WU and WC did not allow the classification of genotypes in terms of drought tolerance. Our findings provide conclusive evidence that traits associated to germination and growth potential may be employed for revealing genetic variability related to drought tolerance, thus enabling their exploitation as screening criteria for drought tolerance. Upon confirmation of its validity, such short-cut selection approach may considerably upgrade the efficiency of breeding procedures targeted at the improvement of drought tolerance in soybean.

**Keywords:** Soybean; Drought stress; Polyethylene glycol; Drought tolerant genotypes; Early selection; Germination stage

## Introduction

Soybean [*Glycine max* (L.) Merr.] is an important annual crop worldwide, as it consists a major source of oil and protein for both human and animal food. Soybean is classified as a drought sensitive crop and under water scarcity conditions, most cultivars are incapable of sustaining their high productivity. Given the detrimental effects of drought on soybean, tremendous efforts have been placed on the genetic improvement of drought tolerance traits, with the focus being centered to retaining yield under drought [1,2]. Classical breeding approaches involve combination of desirable traits from soybean germplasms, via repeated crossing and selections processes, while selections routinely rely on estimates of yielding ability in water-deficient field environments [3]. 2014). The achievement of this breeding objective, however, poses major challenges due to the genetic complexity of drought tolerance traits, coupled to the significant G x E interactions, as well as the difficulties encountered in achieving uniformity in selective environments under field conditions [4]. The development of drought tolerant cultivars is further depended on the availability of optimized screening methodologies to robustly assess a large number of genotypes required for improving such complex traits [5].

Soybean is most susceptible to drought during germination and at reproductive stages, specifically during pod filling, when stress adversely affects yield due to reduction in pod number, seed number per pod as well as seed size and weight [6-12]. At germination, which is considered as a key stage in plant development and determinant for plant productivity, water deficit leads to various physiological and biochemical disturbances relating to water availability, mobilization of stored reserves, hormonal balance and protein structure. Such changes impact considerably seedling survival rate and vegetative growth, thus ultimately affecting yield and seed quality [13].

Given that drought affects seed germination and seedling growth, to an extent that is determined by the stress intensity

and the genetic background, it has been proposed that relative traits may be employed as accurate criteria for determining the genotypic response to drought at germination phase. In this line, an increasing number of studies employed various *in vitro* screening methods for the identification of drought tolerant germplasm in a series of plant species [14-20]. More importantly, previous studies provide evidence that drought tolerance during *in vitro* germination assays is well correlated to field conditions [21,22]. *In vitro* screening most commonly involves the induction of stress via osmotic agents such as polyethylene glycol (PEG-6000) which, due to its high molecular weight, is inert, non-ionic and cell impermeable, thus simulating drought without any toxic effects [23-25]. In this framework, this study aimed at determining the response of soybean germplasm to PEG-induced drought stress at germination stage. The effects of stress were evaluated on the basis of traits related to seed germination and seedling growth potential under different stress levels.

## Materials and methods

### Plant Material

The genetic material consisted of nine commercial and pre-commercial varieties: Adonai, Neoplanta, Celina, Zora, PR92M22, P21T45, PR92M35, PR92B63 and PR91M10.

### Drought stress treatments and experimental design

Drought stress was achieved via the osmotically active macromolecule Polyethylene Glycol (PEG-6000). Seeds were initially surface-sterilized, using 20% hypochlorite / $H_2O$ solution supplemented with Tween-20 under gentle agitation for 5min, and washed 4x with excess of sterile $H_2O$. Sterilized seeds were subsequently placed into plastic trays containing PEG solutions of different concentration: 0, 5, 10 and 20% PEG 6000. Trays were regularly monitored and, when necessary, $H_2O$ was added in order to retain a constant concentration of PEG. Plants were grown under controlled conditions (25˚C, 16h light/8h dark) for a period of 20 days. The experiment was performed using a completely random design with 4 replications, each consisting of 30 seeds. Each experimental plot consisted of 4 rows, of which the 2 middle provided the genetic material for the measurements.

### Parameters for evaluation of drought tolerance

Genotypic evaluation for drought tolerance was performed on the basis of Germination Percentage (GP), seed Water Uptake (WU), seedling Water Content (WC), root and shoot length and number of seedlings with abnormal phenotype. Specifically, GP (%) was assessed at five time intervals ($3^{rd}$ until $7^{th}$ day), with seeds considered as germinated when the radicle had extended for at least 2mm. WU (%) was estimated at $5^{th}$ and $7^{th}$ day, according to the formula WU (%) = (W2-W1) / W1 × 100, with $W_1$ and $W_2$ referring to initial seed weight and seed weight after water absorbance [26]. For estimation of WU, the weight of twenty seeds (five per replication) was taken into account. Shoot and root length (cm) were estimated at $5^{th}$, $7^{th}$, $9^{th}$, $12^{th}$, $15^{th}$ day, taking into account five seedlings. WC (%) was determined at $7^{th}$, $12^{th}$ and $15^{th}$ day, based on the formula WC (%) = (FW - DW / FW) x 100, with FW and DW referring to fresh and dry weight respectively [27]. For WC, the weight of twenty seedlings (five per replication) was taken into account and DW was determined following incubation at 70°C for a period of 2 days. The number of seedlings with abnormal genotype was determined throughout the period of observation.

### Statistical Analysis

Data were analyzed by ANOVA according to the experimental design. Genotypic performance was comparatively assessed within stress levels applied at specified time intervals. Comparisons were further performed for genotypes across drought stress levels as well as for drought stress levels across genotypes. The significance of differences between pairs of means was assessed by the Student's LSD test ($p \leq 0.05$). Statistical analyses were performed using JMP statistical software v. 8.

## Results

Our findings indicate that drought stress adversely affected all traits related to seed germination and seedling growth, while the severity of effects was correlated to the stress level applied. All genotypes were affected, yet a considerable variation was noted in relation to their response to drought stress. Germination was significantly affected by the stress level as well as by the genotype (Figure 1), Supplementary Table 1. Germination commenced 3 days after sowing, while significant differences were noted among stress levels and genotypes at this timepoint. In the absence of stress, Adonai and PR92M22 exhibited the highest germination rate, followed by Neoplanta, PR92M35, Celina and Zora, whereas PR92B63, PR91M10 and P21T45 showed extremely low or zero germination. Upon stress at low and medium levels (5% and 10% PEG), Neoplanta and Celina showed increased rates compared to controls, while the germination of PR92M22 ranged at similar level with controls. Adonai presented a decreasing trend in germination rate, as the stress level increased, whereas PR92B63, PR92M35, PR91M10, Zora and P21T45 showed considerably lower rates. However, at high stress level (20% PEG), the germination potential of all genotypes was severely affected. Although at $4^{th}$ and $5^{th}$ day all genotypes showed similar to the abovementioned germination patterns, over time Neoplanta, Adonai and PR92M22 showed superior performance. Such superiority was evidenced by their mean response across stress levels as well as their final germination rates. In this line, PR92M22 and Adonai showed the highest germination rate at low and medium stress levels (PR92M22: 98% and 88%, Adonai: 91% and 90%, at 5% and 10% PEG respectively), while Neoplanta exhibited the best performance at high stress level (52% at 20% PEG). Overall findings classified Neoplanta, Adonai and PR92M22 as most tolerant genotypes and, at the same time, point to the ability of the former to retain its germination ability under conditions of severe drought stress.


How to cite this article: Ourania I P, Chrysanthi F, Georgia S, Georgia K, Asimo P, et al.PEG-Induced Drought Stress During Germination: Effects on Soybean Germplasm. Agri Res& Tech: Open Access J. 2020; 23(5): 556250. DOI: 10.19080/ARTOAJ.2020.23.556250

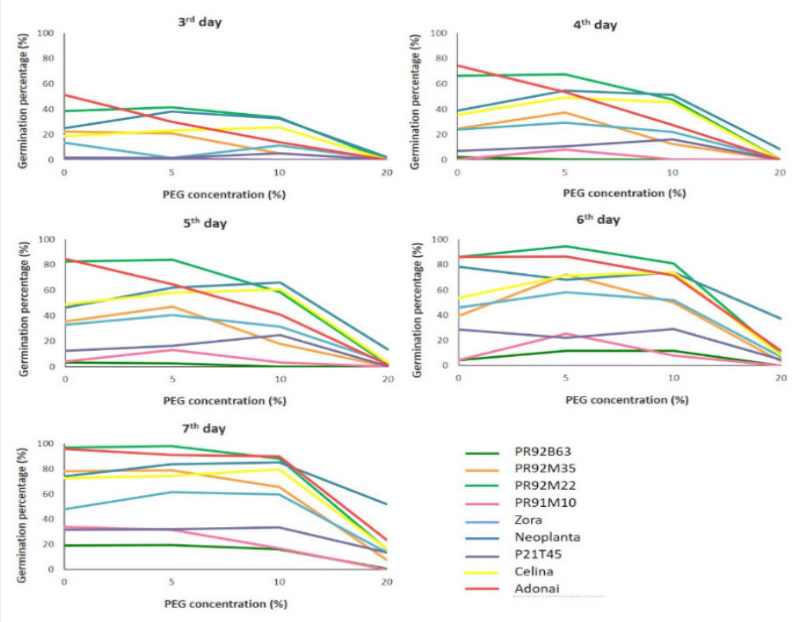


**Figure 1:** A Germination percentage (%) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

As expected, seed water uptake (WU%) showed a decreasing trend as stress intensity increased Table 1. At 5th day and at high stress level (20% PEG), the lowest decrease for WU was noted in Zora and PR92M22, whereas the highest decrease was recorded in Celina and P21T45. In contrast, at 7th day PR92B63 showed the lowest decrease, while all other genotypes were severely affected. As far as seedling water content (WC%) is concerned, the lowest and highest decrease was noted in PR92B63 and PR91M10, respectively Table 2. Despite differences observed, mainly at the mean response of different stress levels across genotypes, WU and WC did not allow for genotypic classification according to their tolerance.

**Supplementary Table 1:** Germination percentage (%) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

| Day | Genotype (G) | PEG Concentration (%) ( C ) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | |
| 3rd | | | | | | MEAN ( G ) |
| | PR92B63 | 0,00d | 0,00d | 0,00c | 0,00b | 0,00 e |
| | PR92M35 | 22,50b | 21,00c | 5,00c | 0,00b | 12,13 bc |
| | PR92M22 | 38,50a | 41,50a | 33,50a | 0,00b | 28,38 a |
| | PR91M10 | 0,00d | 1,00d | 0,00c | 0,00b | 0,25 e |
| | Zora | 13,50bc | 1,50d | 11,50bc | 0,00b | 6,63 cd |
| | Neoplanta | 25,00b | 38,00ab | 32,50a | 2,00a | 24,38 a |
| | P21T45 | 1,67cd | 1,50d | 5,00c | 0,00b | 2,04 de |
| | Celina | 18,67b | 23,00c | 25,50ab | 0,00b | 16,79 b |
| | Adonai | 51,33a | 30,00bc | 14,00bc | 0,00b | 23,83 a |
| | SED | 3,98 | 3,91 | 4,91 | 0,54 | SED ( G ) = 1,80 |
| | Mean ( C ) | 19,01a | 17,5a | 14,11b | 0,22c | SED ( C ) = 1,20 |
| 4th | | | | | | MEAN ( G ) |
| | PR92B63 | 2,00d | 0,50d | 0,00d | 0,00b | 0,63 e |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | PR92M35 | 24,50c | 37,50bc | 12,50cd | 0,00b | 18,62 c |
| | PR92M22 | 66,50a | 67,50a | 47,50a | 0,50b | 45,5 a |
| | PR91M10 | 0,50d | 8,00d | 0,50d | 0,00b | 2,25 de |
| | Zora | 24,00c | 29,50c | 22,00bcd | 0,50b | 19 c |
| | Neoplanta | 39,00b | 54,50ab | 51,50a | 8,50a | 38,37 ab |
| | P21T45 | 7,00d | 10,50d | 16,00cd | 0,00b | 8,37 d |
| | Celina | 35,50bc | 49,00b | 45,50ab | 0,00b | 32,50 b |
| | Adonai | 74,50a | 53,50ab | 27,50abc | 0,00b | 38,87 ab |
| | SED | 3,61 | 5,13 | 7,25 | 1,73 | SED ( G ) = 2,44 |
| | Mean ( C ) | 30,39a | 34,5a | 24,77b | 1055c | SED ( C ) = 1,63 |
| 5th | | | | | | MEAN ( G ) |
| | PR92B63 | 3,50e | 2,50d | 0,00e | 0,00b | 1,50 e |
| | PR92M35 | 35,50cd | 47,00bc | 18,00cde | 0,00b | 25,13 c |
| | PR92M22 | 82,50a | 84,00a | 58,50ab | 2,00b | 56,75 a |
| | PR91M10 | 4,00e | 13,00d | 3,50de | 0,00b | 5,13 de |
| | Zora | 33,00d | 40,50c | 31,50bcd | 3,50b | 27,12 c |
| | Neoplanta | 46,50bc | 62,00b | 66,00a | 13,50a | 47 b |
| | P21T45 | 12,50e | 16,50d | 25,00cde | 0,50b | 13,62 d |
| | Celina | 49,00b | 58,00bc | 60,50ab | 3,50b | 42,75 b |
| | Adonai | 84,50a | 64,50b | 41,00abc | 1,50b | 47,87 ab |
| | SED | 3,83 | 5,54 | 9,17 | 2,43 | SED ( G ) = 2,90 |
| | Mean ( C ) | 39a | 43,11a | 33,77b | 2,72c | SED ( C ) = 1,94 |
| 6th | | | | | | MEAN ( G ) |
| | PR92B63 | 4,25d | 11,50d | 11,50d | 0,00b | 6,82 e |
| | PR92M35 | 39,25bc | 72,00bc | 50,00bc | 3,00b | 41,06 c |
| | PR92M22 | 86,25a | 94,50a | 81,00a | 8,50b | 67,56 a |
| | PR91M10 | 4,17d | 25,50d | 8,00d | 0,00b | 9,42 e |
| | Zora | 46,00bc | 58,00c | 52,00bc | 7,00b | 40,75 c |
| | Neoplanta | 78,50a | 68,00bc | 74,00ab | 37,00a | 64,38 a |
| | P21T45 | 28,67c | 22,00d | 29,00cd | 4,50b | 21,05 d |
| | Celina | 53,67b | 71,50bc | 74,00ab | 8,00b | 51,79 b |
| | Adonai | 86,17a | 86,50ab | 71,50ab | 11,50b | 63,92 a |
| | SED | 6,51 | 6,02 | 7,34 | 3,62 | SED ( G ) = 3,02 |
| | Mean ( C ) | 47,43b | 56,61a | 50,11b | 8,83c | SED ( C ) = 2,01 |
| 7th | | | | | | MEAN ( G ) |
| | PR92B63 | 19,25d | 19,50e | 16,00e | 0,50c | 13,81 e |
| | PR92M35 | 78,00b | 79,00bc | 65,50bc | 7,50bc | 57,50 b |
| | PR92M22 | 97,00a | 98,00a | 88,00a | 16,00bc | 74,75 a |
| | PR91M10 | 33,75cd | 31,50e | 17,00e | 0,00c | 20,56 de |
| | Zora | 48,00c | 61,50d | 59,50c | 13,50bc | 45,62 c |
| | Neoplanta | 74,00b | 83,50abc | 85,00a | 52,00a | 73,62 a |
| | P21T45 | 31,50cd | 32,00e | 33,50d | 13,50bc | 27,62 d |
| | Celina | 72,50b | 74,50cd | 79,50ab | 16,50bc | 60,75 b |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | Adonai | 96,00a | 91,00ab | 90,00a | 23,50b | 75,12 a |
| | SED | 4,91 | 4,49 | 4,19 | 5,59 | SED ( G ) = 2,41 |
| | Mean ( C ) | 61,11a | 63,39a | 59,33a | 15,89b | SED ( C ) = 1,61 |

At each time interval (days), values followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05).

**Supplementary Table 2:** Root length (cm) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

| Day | Genotype (G) | PEG Concentration (%) ( C ) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 20 | |
| 5th | | | | | | MEAN ( G ) |
| | PR92B63 | 0,17d | 0,02d | 0,00c | 0,00b | 0,046 e |
| | PR92M35 | 0,32d | 0,29cd | 0,15c | 0,00b | 0,19 de |
| | PR92M22 | 1,91a | 1,22ab | 1,19a | 0,11ab | 1,11 a |
| | PR91M10 | 0,15d | 0,15d | 0,05c | 0,02ab | 0,091 e |
| | Zora | 0,78c | 0,75bc | 0,20c | 0,00b | 0,43 c |
| | Neoplanta | 1,32b | 1,26a | 0,92ab | 0,21a | 0,93 b |
| | P21T45 | 0,90c | 0,31cd | 0,20c | 0,02ab | 0,36 cd |
| | Celina | 1,82a | 1,09ab | 0,82ab | 0,06ab | 0,95 ab |
| | Adonai | 2,07a | 1,39a | 0,65b | 0,08ab | 1,045 ab |
| | SED | 0,1 | 0,14 | 0,11 | 0,06 | SED ( G ) = 0,05 |
| | Mean ( C ) | 1,05a | 0,72b | 0,46c | 0,054d | SED ( C ) = 0,04 |
| 7th | | | | | | MEAN ( G ) |
| | PR92B63 | 0,11f | 0,08d | 0,50d | 0,00c | 0,17 e |
| | PR92M35 | 1,44d | 1,17c | 0,34d | 0,12bc | 0,77 d |
| | PR92M22 | 2,84a | 2,84a | 1,36a | 0,21abc | 1,81 a |
| | PR91M10 | 0,79e | 0,32d | 0,38d | 0,00c | 0,37 e |
| | Zora | 1,46d | 1,21c | 0,78bcd | 0,17abc | 0,90 d |
| | Neoplanta | 2,11bc | 1,84bc | 1,34ab | 0,58a | 1,46 bc |
| | P21T45 | 1,64cd | 1,28c | 0,73cd | 0,06bc | 0,92 d |
| | Celina | 2,69a | 1,49bc | 1,19abc | 0,17abc | 1,38 c |
| | Adonai | 2,61ab | 2,00b | 1,71a | 0,46ab | 1,69 ab |
| | SED | 0,15 | 0,29 | 0,17 | 0,12 | SED ( G ) = 0,08 |
| | Mean ( C ) | 1,74a | 1,36b | 0,92c | 0,2d | SED ( C ) = 0,05 |
| 9th | | | | | | MEAN ( G ) |
| | PR92B63 | 0,32f | 0,68f | 0,50f | 0,12de | 0,37 d |
| | PR92M35 | 1,86d | 1,72cde | 1,61c | 0,48cde | 1,41 c |
| | PR92M22 | 3,16ab | 3,08a | 2,96a | 0,82bc | 2,50 a |
| | PR91M10 | 1,02ef | 0,73f | 0,28ef | 0,04e | 0,51 d |
| | Zora | 1,59de | 1,57de | 0,91de | 0,49cde | 1,14 c |
| | Neoplanta | 2,00cd | 2,30bc | 1,95bc | 1,21ab | 1,86 b |
| | P21T45 | 1,85d | 1,36ef | 1,54cd | 0,40cde | 1,28 c |
| | Celina | 2,67bc | 2,11cd | 1,84bc | 0,69bcd | 1,82 b |
| | Adonai | 3,67a | 2,88ab | 2,42ab | 1,58a | 2,63 a |
| | SED | 0,23 | 0,22 | 0,21 | 0,18 | SED ( G ) = 0,10 |
| | Mean ( C ) | 2,04a | 1,82b | 1,51c | 0,64d | SED ( C ) = 0,07 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| 12th | | | | | | MEAN ( G ) |
| | PR92B63 | 0,32d | 0,66e | 0,78e | 0,35de | 0,52 d |
| | PR92M35 | 1,80c | 1,93cd | 1,68bc | 0,79cd | 1,60 c |
| | PR92M22 | 3,90a | 3,82a | 3,61a | 1,32bc | 3,16 a |
| | PR91M10 | 1,15c | 1,34de | 1,03de | 0,11e | 0,90 d |
| | Zora | 1,54c | 1,80cd | 1,69bc | 1,18bc | 1,55 c |
| | Neoplanta | 2,00c | 2,56bc | 3,09a | 1,47ab | 2,23 b |
| | P21T45 | 1,86c | 1,41de | 1,53cd | 0,90bcd | 1,42 c |
| | Celina | 2,68b | 2,22cd | 2,29b | 1,44ab | 2,15 b |
| | Adonai | 4,60a | 3,48ab | 3,69a | 1,95a | 3,42 a |
| | SED | 0,24 | 0,33 | 0,19 | 0,18 | SED ( G ) = 0,12 |
| | Mean ( C ) | 2,19a | 2,16a | 2,15a | 1,056b | SED ( C ) = 0,08 |
| 15th | | | | | | MEAN ( G ) |
| | PR92B63 | 0,47e | 0,76e | 0,81d | 0,66cd | 0,67 h |
| | PR92M35 | 2,64bc | 2,70bcd | 1,90c | 1,06bcd | 2,07 de |
| | PR92M22 | 3,79ab | 5,19a | 3,94a | 1,60bc | 3,62 b |
| | PR91M10 | 1,15de | 1,31de | 1,37cd | 0,11d | 0,98 gh |
| | Zora | 1,66cde | 2,15bcde | 1,80c | 1,41bc | 1,75 ef |
| | Neoplanta | 2,71bc | 3,18b | 3,05b | 2,05ab | 2,74 c |
| | P21T45 | 1,86cd | 1,41cde | 1,53cd | 0,90cd | 1,42 fg |
| | Celina | 2,81bc | 2,94bc | 2,76b | 1,60bc | 2,52 cd |
| | Adonai | 4,96a | 4,97a | 3,96a | 2,96a | 4,21 a |
| | SED | 0,36 | 0,46 | 0,21 | 0,3 | SED ( G ) = 0,17 |
| | Mean ( C ) | 2,45ab | 2.73a | 2.34b | 1,37c | SED ( C ) = 0,11 |

At each time interval (days), values followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05).

In relation to post-germination growth of seedlings, the findings revealed that the intensity of stress considerably affects both root and shoot length in the entire set of genotypes under study. The analyses showed statistically significant differences in root and shoot length and, as expected, the effects of drought were most profound as PEG concentration increased. In relation to root length, the best performance was recorded in Adonai, Neoplanta and PR92M22, followed by Celina (Figure 2, Supplementary Table 2). The superiority of the abovementioned genotypes was reflected at their mean response across stress levels but also their root length at high stress level (Adonai: 2,96cm, Neoplanta: 2,05cm, PR92M22: 1,60cm, Celina: 1,60cm at 15$^{th}$ day of drought stress). For shoot length, the most drastic effects of stress were observed in PR92M22 and Adonai throughout the period of observation (Figure 3, Supplementary Table 3). Although certain genotypes, namely PR92B63, PR91M10 and P21T45, exhibited low decrease in shoot length, such observations are not indicative of a superior performance as they reflect their low shoot elongation potential *per se*. Indicatively, it is noted that at 0% and 20% PEG their shoot length ranged at 0,47 and 0,04cm, while the respective values for Adonai were 6,59 and 0,61cm.

**Supplementary Table 3:** Shoot length (cm) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

| Day | Genotype (G) | PEG Concentration (%) (C) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | | 20 | |
| 5th | | | | | | | MEAN ( G ) |
| | | | | | | | |
| | PR92B63 | 0,00b | 0,00e | 0,00c | c | 0,00a | 0,00 d |
| | PR92M35 | 0,24b | 1,16cd | 0,33c | c | 0,03a | 0,43 bcd |
| | PR92M22 | 4,75a | 3,06a | 1,55ab | ab | 0,11a | 2,36 a |
| | PR91M10 | 0,00b | 0,00e | 0,04c | c | 0,00 | 0,008 d |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Zora | 0,80b | 0,78cde | 0,11c | c | 0,02a | 0,42 bcd |
| | Neoplanta | 1,11b | 1,60bc | 1,10b | b | 0,33a | 1,033 b |
| | P21T45 | 0,31b | 0,33de | 0,48c | c | 0,00a | 0,276 cd |
| | Celina | 1,19b | 0,98cde | 1,18ab | ab | 0,06a | 0,84 bc |
| | Adonai | 4,06a | 2,46ab | 1,73a | a | 0,33a | 2,14 a |
| | SED | 0,67 | 0,34 | 0,18 | | 0,14 | SED ( G ) = 0,20 |
| | Mean ( C ) | 1,38a | 1,15a | 0,72b | b | 0,1c | SED ( C ) = 0,13 |
| 7th | | | | | | | MEAN ( G ) |
| | PR92B63 | 0,04c | 0,01c | 0,22d | d | 0,00b | 0,066 e |
| | PR92M35 | 1,89b | 1,51bc | 2,54abc | abc | 0,04ab | 1,49 bc |
| | PR92M22 | 4,88a | 4,53a | 3,85a | a | 0,14ab | 3,34 a |
| | PR91M10 | 0,47bc | 0,69c | 0,15d | d | 0,01ab | 0,33 de |
| | Zora | 1,30bc | 0,79c | 0,12d | d | 0,03ab | 0,55 de |
| | Neoplanta | 1,18bc | 3,03ab | 3,06ab | ab | 0,14ab | 1,84 b |
| | P21T45 | 0,85bc | 0,77c | 0,46cd | cd | 0,01ab | 0,52 de |
| | Celina | 1,04bc | 1,08c | 1,56bcd | bcd | 0,03ab | 0,92 cd |
| | Adonai | 5,91a | 3,78a | 2,43abc | abc | 0,27a | 3,09 a |
| | SED | 0,48 | 0,54 | 0,62 | | 0,08 | SED ( G ) = 0,24 |
| | Mean ( C ) | 1,94a | 1,79a | 1,59a | a | 0,07b | SED ( C ) = 0,16 |
| 9th | | | | | | | MEAN ( G ) |
| | PR92B63 | 0,03d | 0,03d | 0,24d | d | 0,02b | 0,079 e |
| | PR92M35 | 2,75b | 2,24bc | 2,54abc | abc | 0,10b | 1,84 cd |
| | PR92M22 | 5,38a | 4,78a | 4,05a | a | 0,07b | 3,56 a |
| | PR91M10 | 0,47cd | 1,54cd | 0,51cd | cd | 0,05b | 0,59 e |
| | Zora | 1,56bcd | 1,06cd | 0,36d | d | 0,07b | 0,76 e |
| | Neoplanta | 2,48b | 3,53ab | 3,73ab | ab | 0,14ab | 2,46 bc |
| | P21T45 | 0,98bcd | 0,96cd | 0,60cd | cd | 0,04b | 0,64 e |
| | Celina | 2,08bc | 1,78c | 2,06bcd | bcd | 0,09b | 1,50 d |
| | Adonai | 6,00a | 3,84ab | 2,34abc | abc | 0,44a | 3,15 ab |
| | SED | 0,54 | 0,49 | 0,55 | | 0,1 | SED ( G ) = 0,23 |
| | Mean ( C ) | 2,39a | 2,19ab | 1,79b | b | 0,11c | SED ( C ) = 0,15 |
| 12th | | | | | | | MEAN ( G ) |
| | PR92B63 | 0,29e | 0,12e | 0,97d | d | 0,05b | 0,35 d |
| | PR92M35 | 3,84bc | 4,15bc | 3,38ab | ab | 0,12b | 2,87 b |
| | PR92M22 | 5,70a | 6,18a | 4,93a | a | 0,32ab | 4,27 a |
| | PR91M10 | 0,97de | 1,54d | 0,93d | d | 0,05b | 0,79 d |
| | Zora | 3,16c | 3,14c | 1,36cd | cd | 0,24ab | 1,97 c |
| | Neoplanta | 3,84bc | 4,42b | 3,81ab | ab | 0,16b | 3,05 b |
| | P21T45 | 1,61 | 0,94de | 0,85d | d | 0,08b | 0,86 d |
| | Celina | 3,98b | 3,34c | 3,04bc | bc | 0,75a | 2,77 b |
| | Adonai | 6,36a | 5,03b | 3,16ab | ab | 0,56ab | 3,77 a |
| | SED | 0,23 | 0,31 | 0,53 | | 0,17 | SED ( G ) = 0,17 |
| | Mean ( C ) | 3,3a | 3,17a | 2,49b | b | 0,25c | SED ( C ) = 0,11 |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15th | | | | | | | MEAN ( G ) |
| | PR92B63 | 0,47d | 0,76d | 0,95c | c | 0,04b | 0,55 e |
| | PR92M35 | 3,93b | 4,06bc | 3,73ab | ab | 0,13b | 2,96 b |
| | PR92M22 | 5,79a | 6,13a | 5,12a | a | 0,34b | 4,34 a |
| | PR91M10 | 0,84cd | 1,73d | 1,19c | c | 0,05 | 0,95 de |
| | Zora | 3,16b | 3,10c | 1,62c | c | 0,25b | 2,03 c |
| | Neoplanta | 3,95b | 4,81b | 4,31ab | ab | 0,29b | 3,33 b |
| | P21T45 | 1,71c | 1,43d | 1,60c | c | 0,09b | 1,20 d |
| | Celina | 3,90b | 4,13bc | 3,06b | b | 1,08a | 3,04 b |
| | Adonai | 6,59a | 4,98ab | 3,26b | b | 0,61ab | 3,85 a |
| | SED | 0,28 | 0,37 | 0,42 | | 0,2 | SED ( G ) = 0,16 |
| | Mean ( C ) | 3,37a | 3,45a | 2,76b | b | 0,31c | SED ( C ) = 0,11 |

At each time interval (days), values followed by the same letter, within each factor, are not significantly different according to LSD (p≤0.05).

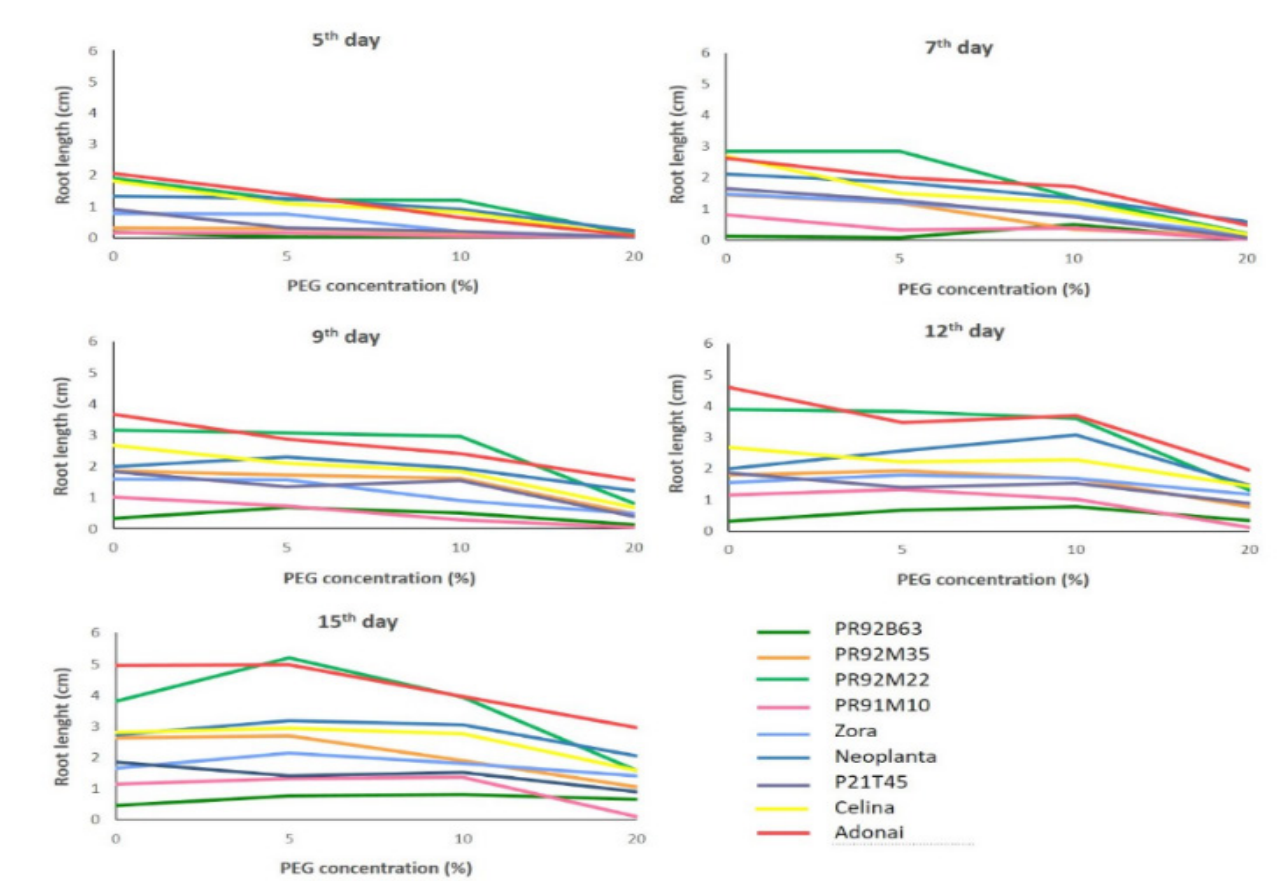


**Figure 2:** A Root length (cm) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

## Discussion

Drought is undoubtedly one of the most stressful environmental factors to a plethora of crops, including soybean, with agronomic importance, leading to considerable yield reductions and subsequent economic losses. In agricultural terms, drought is defined as a condition in which water availability through rainfall and/or irrigation is insufficient to meet the crop's transpiration needs. Minimization of yield losses under water deficit conditions


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mainly relies on the use of tolerant germplasm, therefore placing primary emphasis on the improvement of relevant traits. Given the bottlenecks arising from assessing the performance of a large number of genotypes under water-deficient field environments, screening for drought tolerance during germination has been attempted as an alternative short-cut approach in a variety of plant species [14-20]. In this study, the seed germination and seedling growth potential under drought stress conditions have been exploited as parameters for revealing the genetic variability related to drought tolerance and for identifying most tolerant soybean genotypes. Our findings revealed the adverse effects of drought during germination phase, with the severity of effects being in most cases correlated to the intensity of stress. These results are in agreement with previous studies and provide further evidence for the suitability of PEG as a molecule to simulate drought at *in vitro* conditions [15,28-30].

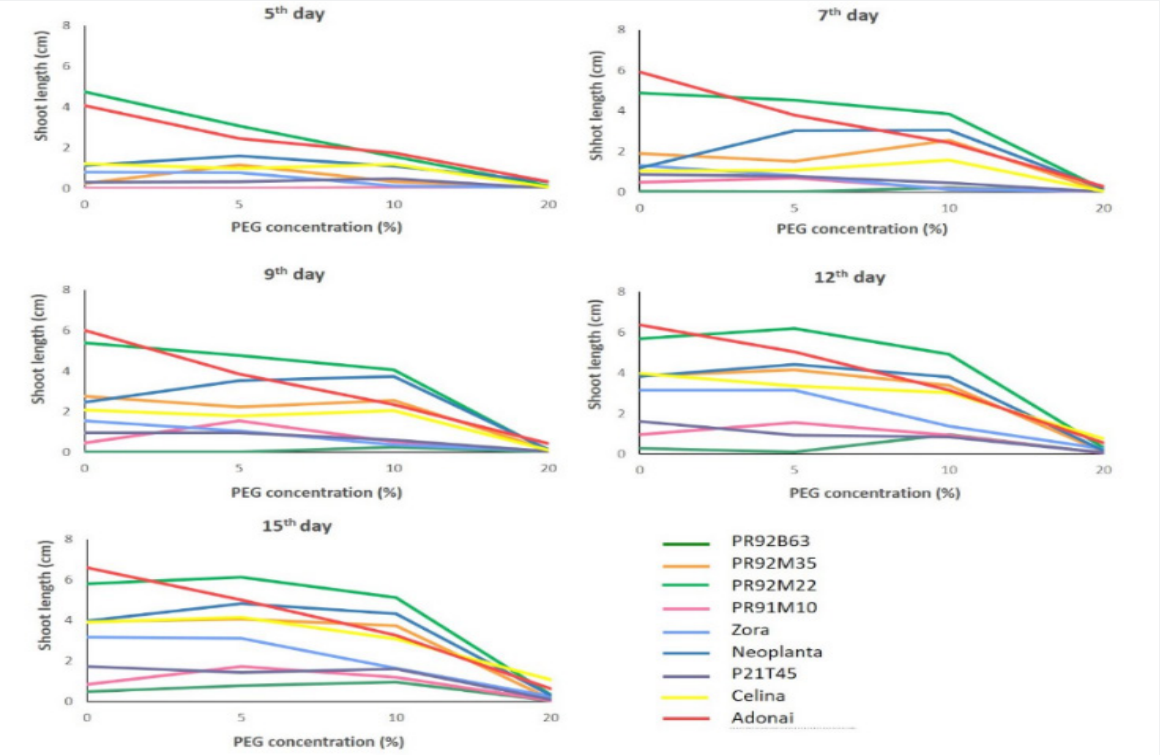


**Figure 3:** A Shoot length (cm) of nine soybean varieties at four different levels of PEG-induced drought stress and five-time intervals.

Although stress negatively affected the entire germplasm under study, the germination potential was substantially affected by the stress level as well as by the genotype. Such observations are in agreement with previous findings, thus underlining that the response to drought is under strong genotypic dependency both at species and genotype level [31-33]. In relation to germination potential, genotypes Neoplanta, Adonai and R92M22 were classified as most tolerant, with the former exhibiting a superior performance at high stress level thus highlighting its ability of sustaining satisfactory germination even under extreme water deficiency. Seed water uptake is a trait directly related to germination as water imbibition triggers the activation of metabolic processes relating to synthesis of hydrolytic enzymes, hydrolysis of food reserves, radicle protrusion and tissue elongation [13,34-35]. In our study, both WU and WC showed a decreasing trend as PEG concentration increased. Although certain differences were observed both at the genotype and stress level, these traits did not allow for a robust classification of genotypes according to their response to drought, thus indicating that WU and WC do not form robust screening criteria for drought tolerance in soybean germplasm. These findings contrast previous observations that the WU data allow for the selection of drought tolerant lentil genotypes when screening is conducted under conditions of high stress level (20% PEG) [19].

Following initiation of germination, the post-germinative elongation of root and shoot tissues is regarded as an essential parameter for evaluating drought tolerance at early growth stages [30]. To this respect, stress substantially affected the growth pattern of all genotypes, while its increasing intensity was accompanied by a progressive inhibition of seedling growth


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as evidenced by a reduced root and shoot length. At high stress level, where most profound repression was noted, Adonai, Neoplanta, PR92M22 and Celina, were classified as most tolerant genotypes. It is worth noting however that a different response for shoot and root tissues was noted, with the former presenting more drastic inhibition in response to drought, especially at high stress levels. Such observations are in total agreement with earlier studies where shoot and root length decreased up to 80% and 21% respectively in soybean genotypes subjected to drought at 15% PEG stress level [36]. Total findings underline the superior performance of Adonai and Neoplanta, followed by PR92M22, in terms of seed germination and seedling growth potential under drought stress. It is worth noting that Neoplanta proved as most capable of tolerating drought at high stress level, thus suggesting the possibility of its exploitation either for cultivation under severe water deficiency or for use as valuable germplasm material in breeding programs targeted at the improvement of drought tolerance traits.

## Conclusions

This study offers important information for traits associated with soybean drought tolerance at germination stage and further, provides evidence for the possibility of exploiting this screening approach for identifying drought tolerant soybean germplasm at early growth stages. Although field investigation under water-deficient environments is warranted in order to assess the relevance of data from *in vitro* assays, it is suggested that this approach is of great use for revealing the genetic variability for traits associated with drought tolerance to be exploited in relative breeding programs.

## Declaration of interest statement

The authors declare that they have no conflict of interest.

## Funding information

This paper did not receive support from funding organizations and/or grants.

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DOI:10.19080/ARTOAJ.2020.23.556250




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