PLANT SCIENCE TODAY
Vol 12(sp4): 1-8
https://doi.org/10.14719/pst.8321

eISSN 2348-1900

RESEARCH ARTICLE

In vitro response of maize (*Zea mays* L.) hybrids to polyethylene glycol induced drought using the slanting plate technique

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Received: 17 March 2025; Accepted: 21 May 2025; Available online: Version 1.0: 28 October 2025

Cite this article: Kavya AS, Naidu GK, Bhat JS, Kachapur RM, Talekar SC, Ummigatti UV, Pawar KN. *In vitro* response of maize (*Zea mays* L.) hybrids to polyethylene glycol induced drought using the slanting plate technique. Plant Science Today. 2025; 12(sp4): 1-8. https://doi.org/10.14719/pst.8321

Abstract

Maize is an important cereal crop of the world and in India, it is grown in the rainy and post-rainy seasons. In the rainy season, among other stresses affecting the maize productivity, drought is significant under a changed climatic scenario. Although maize is sensitive to drought at all growth stages, drought at the seedling stage results in a poor crop stand and establishment, significantly impacting productivity. Identifying moisture-stress tolerant maize inbred lines for seedling stage drought situations and using those in developing drought-tolerant hybrids play a pivotal role in increasing maize yield. In this study, a set of hybrids derived from tolerant and susceptible inbreds was studied for their response under *in vitro* seedling drought induced by polyethylene glycol, employing the slanting plate technique. Substantial variability among different polyethylene glycol levels (0, 10 and 20 %), maize hybrids and interaction between polyethylene glycol levels and maize hybrids was observed for germination and seedling traits, indicating differential response of the maize hybrids. Higher genetic advance with higher heritability was observed for root and shoot length, indicating the preponderance of additive gene action governing these traits. It was interesting to note that the germination ability of seeds was significantly reduced in all hybrids at 20 % polyethylene glycol, following poor seedling vigour that was reflected in diminished expression of seedling traits. The hybrids, GPM 114 × CML 451 and GPM 114 × CAL 1426-2, involving seedling drought-tolerant parents, showed lesser reduction for root length, suggesting these drought-tolerant lines can be used as donors for the development of seedling drought-tolerant hybrids suitable for cultivation in rainfed eco-systems. It is evident from the present study that screening maize genotypes at 20 % polyethylene glycol would help in identifying reliable genotypes for seedling drought tolerance.

Keywords: maize; PEG; seedling drought; tolerant

Introduction

Maize, an important cereal crop, is grown both during the rainy and post-rainy seasons in India. Maize, a member of the Poaceae family, has terminal male (tassel) and lateral female (ear) inflorescences, producing caryopsis, a fruit (1). On a global scale, maize is grown in an area of 203 million ha producing 1163 million tonnes with a productivity of 5.73 tons per ha (2). In India, the area under maize is 9.95 million ha, producing 33.72 million tons with an average productivity of 3.38 tons per ha. Nearly 30.0 % of the global land area experiences moderate to severe drought (3). In India, 68.0 % of the total cultivable area is vulnerable to drought (4). More than 70 % of the rainy season crop is grown under rain fed conditions, with the prevalence of many biotic and abiotic stresses. This has resulted in lower productivity during the rainy season (5) compared to post-rainy season productivity (4436 $kgha^{-1}$), which is predominantly grown under an irrigation ecosystem (6). During the rainy season, drought is one of the most important abiotic stresses, which is exacerbated by the climate change scenario, leading to increased intensity and frequency of droughts that may differ from location to location (7).

Maize is highly sensitive to drought and affected by low moisture availability at every stage of crop growth, development and flowering (8-10). The occurrence of the drought at the seedling stage leads to poor plant stand and, in extreme cases, may result in complete failure of seedling establishment (11).

Germination is initiated by seed imbibition driven by the osmotic gradient created by the seed and soil. A highly negative osmotic potential would affect water uptake by the seeds, making germination impossible (8). Reduction of osmotic potential or a delay in initial germination and a reduction in the rate and total germination are the most common responses observed in different crops (12,13). Drought tolerance at the seedling stage needs to be assessed to predict the crop stand at maturity (14). Polyethylene glycol (PEG) is widely used to induce uniform drought stress at early germination and seedling growth stages in different crops (15-20). PEG was utilized to develop a rapid and efficient system for evaluating drought tolerance in maize aimed at selection and breeding of drought-tolerant resources (21). Maize is more susceptible to osmotic stress than other cereals, such as sorghum, with a drastic reduction in germination (22). Significant reduction in germination percent, germination rate, root length, shoot length, seedling length and seedling vigour (19,23-25) and physiological indices was noted in maize genotypes at higher osmotic potential induced by PEG 6000. Water use efficiency is a key adaptive trait that enables plants in general and maize in particular to maintain productivity and survive under drought conditions (26, 27).

The use of digestive biochar could be an alternative option that helps in reducing the impact of drought up to 15 % by retaining more water and enhancing soil structure (28). However, this may not be a sustainable approach due to its unfeasibility in application to the fields and it increases the production cost of the maize crop.

In this context, understanding and selecting for drought tolerance at the seedling stage is not only critical for improving plant resilience but also essential from an environmental and economic view point. Early vigor under stress minimizes the need for replanting, conserves water through more efficient use and reduces input costs, aligning with goals of sustainable intensification. Thus, seedling-stage drought screening helps to breed climate-resilient maize hybrids that support both ecological balance and farm-level profitability. In the present study, a set of maize hybrids derived using inbred lines differing for their response to seedling drought stress were assessed for their response to seedling drought induced *in vitro* by using PEG. A slanting plate technique, earlier used in cotton, was employed to allow unrestricted root and shoot growth, offering a more accurate assessment than the conventionally used petri plate technique.

Materials and Methods

The material for the study consisted of 23 maize hybrids. Among these, 21 hybrids were generated using tolerant and susceptible maize inbreds identified in an *in vitro* study using PEG 6000, while 2 were released drought-tolerant hybrids. The details of these hybrids are provided in Table 1. The study was carried out in a factorial design with 3 replications. The first factor was osmotic concentrations (0 %, 10 % and 20 % induced by PEG-6000) and the second factor was maize genotypes.

PEG solution with 3 concentrations, 0 %, 10 % and 20 % was prepared to induce osmotic stress levels of 0 bars, -1.48 bars and -3.49 bars, respectively. Distilled water was used as a control (0 %). 10 % and 20 % PEG solution was prepared by dissolving 10 g and 20 g of PEG 6000, respectively, in 100 mL of distilled water. The concentration of PEG-6000 required to achieve these osmotic potentials was determined by using the following equation (30).

$$\Psi_s = -(1.18 \times 10^{-2})\,C - (1.18 \times 10^{-4})\,C^2 + (2.67 \times 10^{-4})\,CT + (8.39 \times 10^{-7})\,C^2T$$

Where,

Ψ_s = Osmotic potential (MPa)

C = Concentration (grams per liter of PEG in water)

T = Temperature (°C)

1.18, 2.67 and 8.39 were constants

The slanting plate technique was employed to study the response of maize hybrids to seedling drought stress. Glass plates measuring 3 mm in thickness, with a length of 25 cm and a width of 30 cm, were used. The dimensions of the glass plates were selected based on the number of seeds used in the laboratory germination study. Each glass plate was covered from bottom to top with 560 × 570 mm blotting paper. Uniformly sized good-quality seeds were selected from each of the 23 different maize hybrids. This method is very simple and does not involve much cost, making it an effective way to screen maize hybrids for their seedling drought tolerance.

Table 1. List of maize hybrids, checks and their parental reaction to drought under field conditions

SN	Hybrid	Reaction of parental inbreds
1	IMIC 2024 × GPM 114	Tolerant x Tolerant
2	IMIC 2024 × CML 451	Tolerant x Susceptible
3	IMIC 2024 × PDM 4641	Tolerant x Susceptible
4	IMIC 2024 × CML 582	Tolerant x Susceptible
5	IMIC 2024 × CAL 1426-2	Tolerant x Tolerant
6	IMIC 2024 × IMIC 2030	Tolerant x Susceptible
7	GPM 114 × CML 451	Tolerant x Susceptible
8	GPM 114 × PDM 4641	Tolerant x Susceptible
9	GPM 114 × CML 582	Tolerant x Susceptible
10	GPM 114 × CAL 1426-2	Tolerant x Tolerant
11	GPM 114 × IMIC 2030	Tolerant x Susceptible
12	CML 451 × PDM 4641	Susceptible x Susceptible
13	CML 451 × CML 582	Susceptible x Susceptible
14	CML 451 × CAL 1426-2	Susceptible x Tolerant
15	CML 451 × IMIC 2030	Susceptible x Susceptible
16	PDM 4641 × CML 582	Susceptible x Susceptible
17	PDM 4641 × CAL1 426-2	Susceptible x Tolerant
18	PDM 4641 × IMIC 2030	Susceptible x Susceptible
19	CML 582 × CAL 14262	Susceptible x Tolerant
20	CML 582 × IMIC 2030	Susceptible x Susceptible
21	CAL 1426-2 × IMIC 2030	Tolerant x Susceptible
22	P 3550	Private drought tolerant hybrid
23	GH 150125	Public drought tolerant hybrid

Seeds of all the maize hybrids taken from the previous season harvest were disinfected with 0.1 % $HgCl_2$. Eight seeds were placed on the upper portion of the filter paper on each glass plate, spaced 4 cm a part. A small strip of filter paper was used to cover the seeds. A supporting wooden block was positioned to prevent the seeds from falling when placed in a slanting position. Initially, a small quantity (5 mL) of prepared PEG solution (0 %, 10 % and 20 %) was added onto the small strip of filter paper, which helps in the adsorption of seeds onto the filter paper firmly. A Glass plate was inserted in a polythene cover. 25 mL of corresponding concentrations of PEG osmotic solutions were added separately into the respective polyethylene covers carrying separate genotype seed, in a slanting plate. PEG solution moved upward and reached the seeds by capillary movement through the filter paper. Seedlings were allowed to germinate and grow at room temperature. A fresh PEG-6000 solution was added equally to all respective polythene covers at regular intervals of three days to maintain the level of PEG solution.

Observations on germination percentage, root length and shoot length (primary data) were recorded on the 7^{th} day and 12^{th} day after keeping for germination. The seedlings that emerged from the PEG solution were considered germinated and expressed as a percentage (%) at the 7^{th} day after incubation and the 12^{th} day after incubation. Germination velocity index, root vigour index, shoot vigour index, seedling vigour index and root to shoot ratio were calculated using the primary data. The Germination Velocity Index (GVI) was calculated on the 12^{th} day after incubation as the mean of the ratio of the number of seeds germinated over the days after incubation, using the formula given below (31). Germination was taken regularly at two-day intervals up to the 12^{th} day for calculation of GVI.

$$GVI = \sum(G1/(V1) + G2/V2 + ... + \frac{Gn}{Vn}$$

Where,

G - Number of seeds germinated on the day of observation

V - Number of days for germination

Five seedlings were carefully removed without damaging their roots and root length was measured from the collar region to the tip of the longest root on the 7^{th} and 12^{th} day after incubation to assess root length in centimeters (cm). Similarly, the shoot length was measured from the collar region to the tip of the shoot on the 7^{th} and 12^{th} day after incubation and also expressed in cm. The ratio of mean root length to the shoot length was calculated for five seedlings on the 7^{th} and 12^{th} day after incubation.

Root Vigour Index (RVI) was calculated at the 7^{th} and 12^{th} day after incubation by multiplying the germination per cent on the corresponding day with the mean root length of 5 seedlings.

RVI = (Mean Root Length × Germination %)

Shoot Vigour Index (SVI) was calculated on the 7^{th} and 12^{th} day after incubation by multiplying the germination per centon the corresponding day with the mean shoot length of 5 seedlings.

SVI = (Mean Shoot Length × Germination %)

Seedling Vigour Index (SdVI) was calculated at the 7^{th} and 12^{th} day after incubation by multiplying the germination per centwith the mean seedling length of 5 seedlings. Mean seedling length was the sum of the mean shoot length and root length of 5 seedlings.

SdVI = [(Mean Shoot Length+ Root Length) × Germination %]

The data recorded on various seedling traits was statistically analyzed for genetic variability (ANOVA) based on the model proposed by using R software (version 4.2.1) (32).

Results and Discussion

Analysis of variance revealed significant differences among PEG levels and maize genotypes for germination percentage, germination velocity index, root length, root vigour index, shoot length, shoot vigour index, root to shoot ratio and seedling vigour index (Table 2), indicating substantial genetic variability and response to osmotic stress. The significant interaction between PEG concentration (0 %, 10 % and 20 %) and genotypes suggested differential behavior of maize hybrids to different levels of osmotic stress, indicating the scope for selecting seedling drought-tolerant hybrids (23-24).

At 12 days after sowing (DAS), low (< 7 %) to moderate (10

Table 2. Mean sum of squares for various traits in maize under PEG-induced drought from different sources of variation

Source of variation / Trait	Days after sowing	PEG Levels	Genotypes	PEG levels × Genotypes	Error	Total
df		2	29	58	180	269
Germination percentage	7^{th} day	756.94*	55.79**	28.97**	10.99	852.7
	12^{th} day	376.74**	38.73**	16.94**	9.26	441.67
GVI		1.604**	0.039**	0.012**	0.003	1.658
Root length	7^{th} day	1248.79**	40.65**	9.33**	0.28	1299.05
	12^{th} day	1506.43***	60.43***	11.81***	0.54	1579.21
Root vigour index	7^{th} day	13879093.06**	406734.23**	95078.14**	3789.02	13879093.06**
	12^{th} day	17518185.69***	593020.27**	110173.62**	9706.74	17518185.69***
Shoot length	7^{th} day	2752.14**	13.71***	4.57**	0.26	2770.68
	12^{th} day	11052.87**	61.99***	23.89***	0.33	11139.1
Shoot vigour index	7^{th} day	27515165.96**	140794.81**	47171.53**	2661.25	27515165.96**
	12^{th} day	112558300.00**	570731.80**	237127.10**	5103	112558300.00**
Root: shoot ratio	7^{th} day	2039.71***	84.11**	99.54**	1.97	2225.36
	12^{th} day	1056.22**	518.71***	519.68**	2.02	2096.64
Seedling vigour index	7^{th} day	2039.71***	84.11**	99.54**	1.97	2225.36
	12^{th} day	1056.22**	518.71***	519.68**	2.02	2096.64

*, ** and *** - Significant at 5 %, 1 % and 0.1 % level of probability, respectively.

df- degrees of freedom, GVI- Germination Velocity Index

- 20 %) phenotypic (PCV) and genotypic coefficient of variation (GCV) were observed for root length and shoot length under control and 10 % PEG treatment. Moderate (10 - 20 %) to high (> 20 %) PCV and GCV were observed at 7 DAS (Table 3), indicating greater variability at earlier stages. In case of 20 % PEG, all the seedling traits except germination per centand germination velocity index exhibited higher PCV and GCV both at 7 and 12 DAS, with higher magnitude of PCV and GCV for root length and shoot length at 7 DAS (Table 3). This suggests that 20 % PEG can be effectively used in assessing the response of maize genotypes for seedling-level moisture stress. Consistently higher PCV and GCV for root length and shoot length, while lower values for germination per cent and germination velocity index were noted in maize and sorghum under drought conditions (33-34). Across PEG treatments, all seedling traits showed higher heritability (> 66.7 %) except germination percentage (< 50 %). A higher magnitude of GAM with higher heritability was observed for root and shoot length at 7 DAS compared to 12[th] DAS (Table 3), indicating the preponderance of additive gene action. This suggests effective phenotypic selection for these traits under seedling drought conditions. Similarly high heritability with high GAM was noted for shoot length and root length in sorghum genotypes, reinforcing the reliability of these traits for seedling-level drought screening (35).

Response of maize hybrids to PEG-induced drought stress

There was a progressive decline in germination and seedling traits (Fig. 1) across all the studied maize hybrids with an increase in PEG concentration from 0 to 20 % and the highest decline occurred under 20 % PEG. This indicates that 20 % PEG is an effective concentration for studying drought tolerance of different maize hybrids at the seedling level (24, 35-37). Among the 23 maize hybrids, only 14 showed 100 % germination at 20 % PEG. Germination velocity index across genotypes was also low in the case of 20 % PEG, which is evident due to the reduction in germination among the studied maize hybrids at 20 % PEG. Previous studies have showed that seed vigour index is most sensitive to drought and can better reflect the drought tolerance and germination characteristics (38). The hybrids PDM 4641 × CAL 1426-2 (1.79), IMIC 2024 × PDM 4641(1.71) had higher germination velocity index when compared to PDM 4641× IMIC 2030 (1.52) involving susceptible × susceptible combination. In these hybrids, PDM 4641 is reported as susceptible and CAL 1426-2 and IMIC 2024 are reported as tolerant to seedling drought (29).

Mean root length and shoot length across maize hybrids were higher at 0 % and 10 % PEG concentration and decreased with higher PEG (20 %) concentration, especially at 12 DAS. There was a decrease in root length and shoot length as the concentration of PEG increased (9,19, 22). Under moisture stress, maize seedlings tend to allocate more resources to root growth than shoot growth. At higher levels of water potential, continuous growth of the maize root will occur, inhibiting growth of the aerial part. The adaptive strategy of enhanced root growth is likely to be driven by increased cell wall elasticity. Earlier, observed 32 % inhibition of aerial part as compared to root (20 %) in the case of maize inbreds (39). The hybrids, GPM 114 × CAL 1426-2 and GPM 114 × CML 451, exhibited higher root length and shoot length at 20 % PEG (Table 4; Plate 1). In the former hybrid, both parents are tolerant, while in the latter, one parent is tolerant and the other is susceptible

Table 3. Genetic variability parameters for different traits under PEG-induced drought in maize

Sl. No.	Trait	DAS	Range			Mean			PCV			GCV			H			GAM		
			0 %	10 %	20 %	0 %	10 %	20 %	0 %	10 %	20 %	0 %	10 %	20 %	0 %	10 %	20 %	0 %	10 %	20 %
1	G (%)	7	87.5 - 100.0	87.5 - 100.0	75.0 - 100.0	99.7	99.2	94.4	1.9	3.2	7.2	1.3	2.2	4.8	50.0	47.6	44.3	1.9	3.1	6.6
		12	87.5 - 100.0	87.5- 100.0	87.5 - 100.0	99.7	99.6	96.1	1.9	2.3	6.1	1.3	1.3	3.5	50.0	32.9	33.3	1.9	1.5	4.2
2	GVI	-	1.6 - 1.9	1.6 - 1.9	1.4 - 1.8	1.8	1.8	1.6	4.4	5.0	7.1	3.8	4.1	5.4	75.8	66.7	58.6	6.9	6.9	8.7
3	RL (mm)	7	11.6 - 22.1	6.0 - 18.6	3.9 - 13.8	16.1	13.3	8.7	11.8	20.2	35.6	11.5	19.6	35.1	95.4	94.3	97.1	23.1	39.2	71.3
		12	21.2 - 30.8	16.2 - 28.8	7.0 - 25.1	25.9	22.9	17.9	9.9	11.8	21.8	9.6	11.4	21.2	95.1	92.7	95.1	19.3	22.6	42.6
4	RVI	7	11600 - 2210.0	602.0 - 1862.0	372.7 - 1378.0	1600.1	1318.8	824.4	12.1	20.5	37.7	11.7	19.8	36.8	94.4	93.4	95.1	23.4	39.4	73.8
		12	2118.0 - 3080.0	1622.0 - 2880.0	702.0 - 2512.0	2588.3	2282.9	1718.6	9.9	11.8	22.7	9.4	11.2	21.6	89.4	89.9	90.5	18.2	21.8	42.3
5	SL (mm)	7	6.8 - 15.9	1.2 - 8.8	0.0 - 2.1	11.6	5.1	0.6	18.5	34.8	99.3	17.5	32.9	98.5	89.6	90.1	98.3	34.2	64.5	201.1
		12	21.2- 35.6	15.8 - 29.8	0.1 - 15.5	29.0	23.4	7.6	12.1	14.7	47.5	11.8	14.5	47.4	94.9	96.9	99.8	23.7	29.3	97.5
6	SVI	7	680.0 - 1592.0	116.0 - 884.0	0.0 - 206.0	1158.3	507.8	58.5	18.9	35.5	99.7	17.9	33.7	98.4	90.2	90.1	97.3	35.1	65.9	199.9
		12	2118.0 - 3560.0	1580.0 - 2950.0	8.8 - 1546.0	2893.8	2323.3	735.6	12.2	14.1	48.4	11.8	13.7	48.2	94.1	93.5	99.1	23.6	27.2	98.8
7	R:S ratio	7	0.9 - 2.2	1.3 - 9.7	0.0 - 42.5	1.4	2.9	10.3	19.5	47.1	94.9	18.1	44.9	92.3	85.6	90.8	94.6	34.4	88.1	184.9
		12	0.7 - 1.1	0.7 - 1.4	1.3 - 137.2	0.9	1.0	6.9	10.4	14.9	332.4	9.8	14.3	330.5	88.6	93.2	98.8	18.9	28.5	676.8
8	SdVI	7	2062.0 - 3542.0	938.0 - 2588.0	372.8 - 1528.0	2758.3	1826.6	882.4	12.3	21.7	40.5	11.9	20.9	39.6	93.6	93.7	95.8	23.8	41.8	79.8
		12	4482.0 - 6480.0	3610.0 - 5550.0	1116.0 - 4058.0	5485.9	4606.2	2454.3	9.8	10.9	26.9	9.6	10.4	26.3	95.7	95.4	95.4	19.4	20.5	52.9

DAS- Days After Sowing, PCV- Phenotypic Coefficient of Variation, GCV- Genotypic Coefficient of Variation, H- Heritability, GAM- Genetic Advance as per cent of Mean, G % - Germination Percentage, GVI- Germination Velocity Index, RL- Root Length, RVI- Root Vigour Index, SL- Shoot Length, SVI- Shoot Vigour Index, R: S ratio- Root to Shoot ratio and SdVI- Seedling Vigour Index

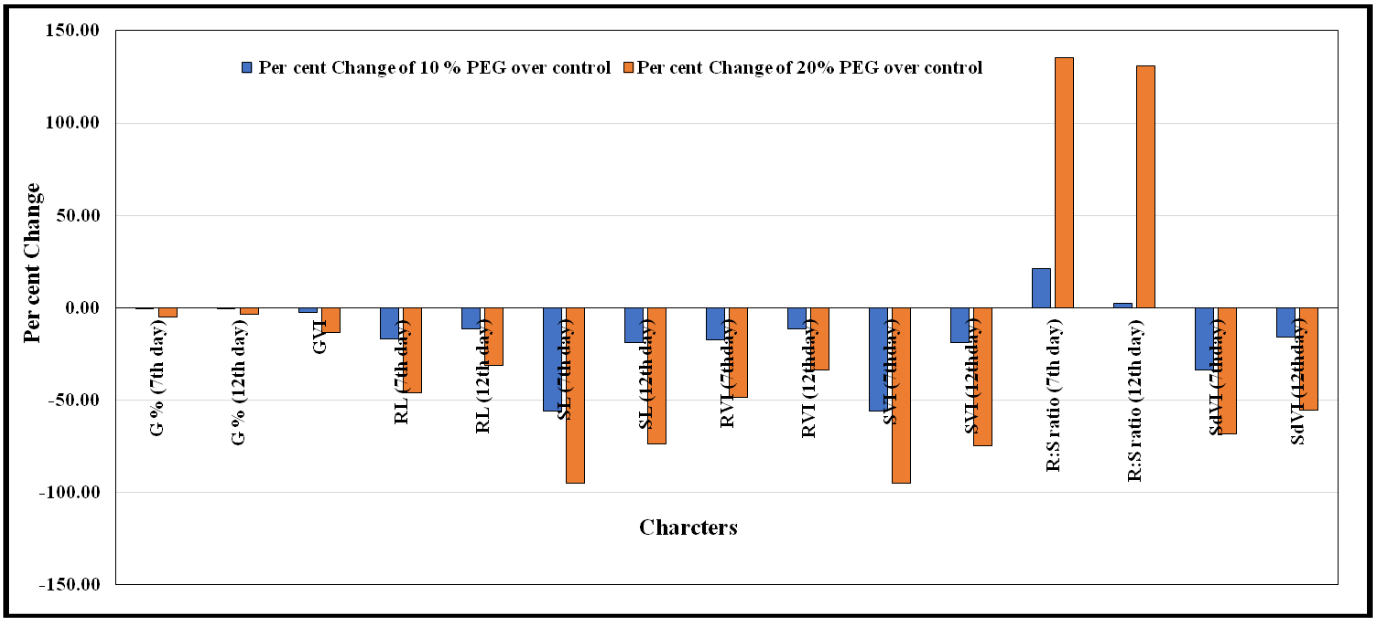

Fig. 1. Percentage change across genotypes at 10 % and 20 % PEG over control (0 % PEG).

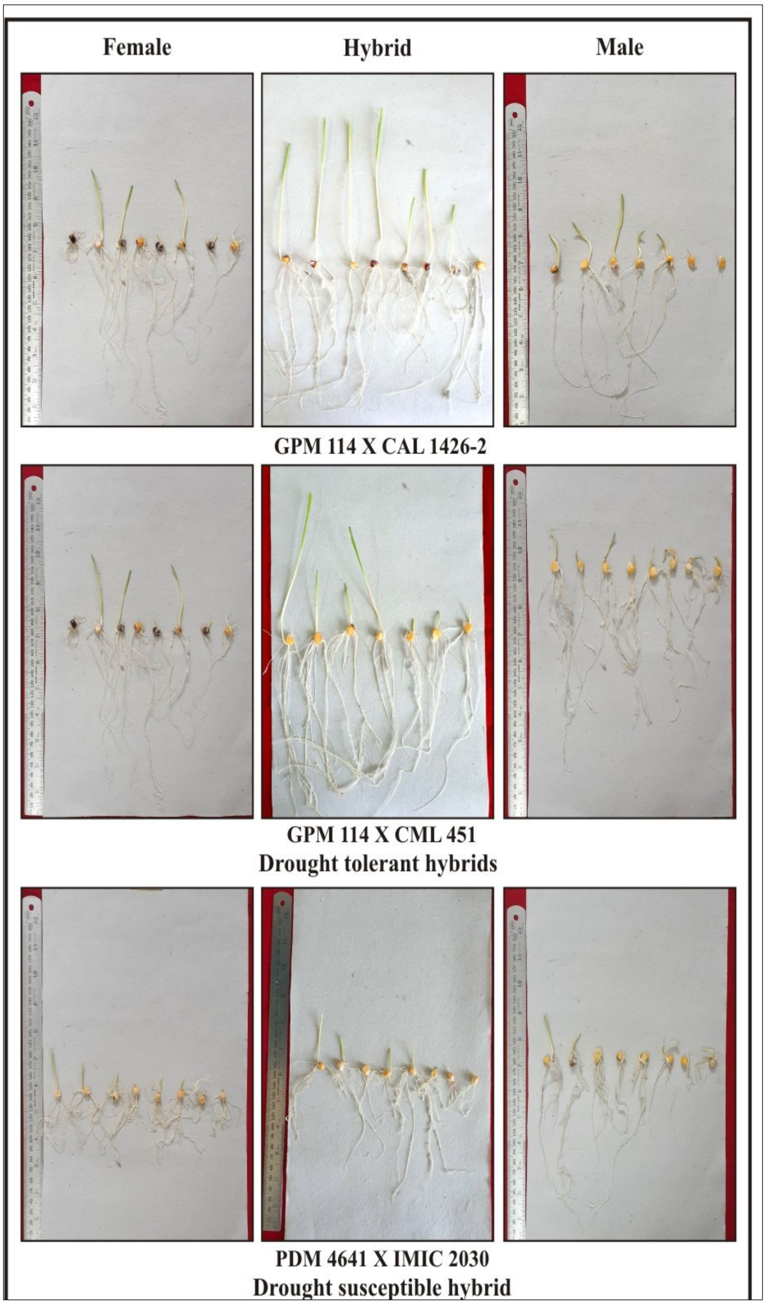

Plate 1. Seedling drought-tolerant and susceptible corn hybrids with their parental inbreds under induced moisture stress (20 % PEG).

Table 4. Superior maize hybrids for various seedling traits under different PEG concentrations

Sl. No.	Characters	DAS	Genotypes		
			0 % PEG	**10 % PEG**	**20 % PEG**
1	**Germination per cent**	7th	23genotypes (> 91 %)	21 genotypes (> 91 %)	11 genotypes (100 %)
		12th	23 genotypes (> 91 %)	21 genotypes (> 91 %)	14 genotypes (100 %)
2	**Germination velocity index**	-	PDM 4641 × CML 582 (1.92), GH 150125 (1.92)	IMIC 2024 × PDM 4641 (1.95)	PDM 4641 × CAL 1426-2 (1.79), IMIC 2024 × PDM 4641(1.71)
3	**Root length (cm)**	7th	-	CAL 1426-2 × IMIC 2030 (17.76 cm)	IMIC 2024 × CAL 1426-2 (13.19 cm), GPM 114 × PDM 4641 (12.97 cm)
		12th	GPM 114 × IMIC 2030 (30.43 cm), GPM 114 × PDM 4641 (30.27 cm)	GPM 114 × IMIC 2030(28.43 cm)	GPM 114 × CAL 1426-2 (24.24 cm), GPM 114 × CML 451 (23.61 cm)
4	**Root vigour index**	7th	GPM 114 (2135.33)	GPM 114 (1797.33), CAL 1426-2 × IMIC 2030 (1776)	GPM 114 × IMIC 2030 (1284.67)
		12th	GPM 114 × IMIC 2030 (3043.33), GPM 114 × PDM 4641 (3026.67)	GPM 114 × IMIC 2030 (2284)	GPM 114 × CAL 1426-2 (2424)
5	**Shoot length (cm)**	7th	IMIC 2024 × CAL 1426-2 (15.59 cm)	PDM 4641 × CAL 1426-2 (8.53 cm)	IMIC 2024 × CAL 1426-2(1.95 cm)
		12th	GPM 114 × CML 582 (35.30 cm)	CAL 1426-2 × IMIC 2030 (28.91 cm), GPM 114 × CML 582 (28.63 cm)	GPM 114 × CAL 1426-2 (15.32 cm)
6	**Shoot vigour index**	7th	IMIC 2024 × CAL 1426-2 (1558.67)	PDM 4641 × CAL 1426-2 (853.33)	IMIC 2024 × CAL 1426-2 (186.83)
		12th	GPM 114 × CML 582 (3530)	CAL 1426-2 × IMIC 2030 (2890.67)	GPM 114 × CAL 1426-2 (1532)
7	**Root: Shoot ratio**	7th	CML 451 × CML 582 (2.03)	CML 451 × PDM 4641 (8.74)	CAL 1426-2 × IMIC 2030 (37.74)
		12th	IMIC 2024 × IMIC 2030 (1.11), GPM 114 × CAL 1426-2 (1.01)	IMIC 2024 × CML 451 (1.29)	IMIC 2030 (127.33)
8	**Seedling vigour index**	7th	CAL 1426-2 × IMIC 2030 (3374)	CAL 1426-2 × IMIC 2030 (2492.67)	IMIC 2024 × CAL 1426-2 (1450.83)
		12th	GPM 114 × CML 582 (6406.67)	CAL 1426-2 × IMIC 2030 (5437.33), GPM 114 × CAL 1426-2 (5408)	GPM 114 × CAL 1426-2 (3956)

DAS- Days after sowing PEG- polyethylene glycol

(29). The root to shoot ratio increased both at 10 % and 20 % PEG, which is evident due to higher root growth and reduced shoot growth. In addition to early root elongation as seen in the present study, controlled stomatal opening, proline or sugar accumulation, higher relative water content and antioxidant enzyme activity in maize seedlings are other mechanisms for drought tolerance (40). The use of Artificial Intelligence, particularly in image-based phenotyping and machine learning algorithms, can help rapidly quantify growth patterns and stress responses. Additionally, predictive models utilizing genomic and physiological data can aid in identifying genotypes with superior drought resilience (41).

The most pronounced reduction in germination and seedling growth across all hybrids was observed at 7 DAS under 20 % PEG treatment, indicating its importance in identifying moisture stress-tolerant hybrids (Table 3). A decrease in the growth rate of the seedlings under 20 % PEG was due to a reduction in primary cellular growth parameters like wall extensibility and cell turgor and also due to a decrease in water uptake by the seedlings. Similarly, a previous study also attributed poor seedling emergence under PEG to some metabolic disorders (23). They also reported that increasing levels of moisture stress delayed seedling emergence due to reduced cell division and suppressed plant growth metabolism. These physiological responses during seedling drought stress also highlight the importance of 20 % PEG as a reliable screening tool for seedling drought tolerance in maize.

Among the studied hybrids, GPM 114 × CML 451 (14.44%) and GPM 114 × CAL 1426-2 (18.02 %) showed less per cent reduction for root length at 20 % PEG, indicating superior seedling drought tolerance. In contrast, hybrids PDM 4641 × CML 582 and PDM 4641 × IMIC 2030 showed an over 49 % reduction in root length and up to 85 % reduction in shoot length, signalling their drought susceptibility. The hybrid GPM 114 × CAL 1426-2 (Plate 1) also showed the least percentage reduction in shoot length (47.89 %) at 20 % PEG. Further supporting its resilience under moisture stress. Earlier studies also identified the maize hybrids *viz*., A 6659 and D57VP51 as seedling drought tolerant based on less reduction in shoot and root parameters (42). It is observed that the hybrids with at least one drought-tolerant parent have exhibited improved performance under PEG-induced drought. This suggests that parental drought tolerance is a strong predictor of hybrid resilience during early seedling growth. The hybrids, GPM 114 × CML 451 and GPM 114 × CAL 1426-2, need to be studied for their drought tolerance under actual field drought conditions, besides testing their yield potential under drought. Additionally, the seedling drought-tolerant parents involved in deriving these hybrids can be used as donors in the breeding program aimed at early-stage drought tolerance.

Conclusion

The study identified GPM 114 × CAL 1426-2 and GPM 114 × CML 451 as promising seedling drought-tolerant maize hybrids, as evidenced by lower reduction in root and shoot length under PEG-induced drought. The results highlight the importance of using at least drought-tolerant varieties in the hybrid to increase their seedling drought tolerance. These hybrids, along with their parental lines, serve as valuable genetic resources in the breeding programs aimed at drought tolerance. Future validation under field conditions, along with the integration of AI-driven phenotyping and selection tools, can further accelerate the development of drought-resilient maize cultivars.

Acknowledgements

The authors acknowledge the help of AICRP on Maize, UAS, Dharwad and IARI RRC, Dharwad for their help in sharing the maize inbred lines for the study.

Authors' contributions

KAS executed the experiment and conducted the data analysis. GKN conceptualized the study and wrote the manuscript. JSB, RMK, SCT and UVM facilitated the conduct of the study. KNP monitored the study in the laboratory. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors of the manuscript declare no competing interests concerneing the work carried out in the present study.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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