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EVALUATION OF ROOT MORPHOLOGICAL RESPONSES TO PEG-SIMULATED DROUGHT IN MAIZE INBREDS FOR EARLY-STAGE TOLERANCE

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ABSTRACT

The productivity of maize (*Zea mays* L.) is seriously threatened by drought stress, especially in the early phases of seedling development, as it hinders root growth and plant establishment. The objective of this study was to determine tolerant genotypes appropriate for breeding programs by assessing the root morphological responses of 100 genetically heterogeneous maize inbred lines under drought stress generated by polyethylene glycol (PEG). Three osmotic stress regimes (0%, 5%, and 10% PEG-6000) were applied to the seedlings, and after seven days of growth, the primary root length, number of seminal and lateral roots, and root biomass were measured. Significant genotypic diversity and stress-induced reduction in all root characteristics were found in the data, with some inbreds exhibiting resilience under 10% PEG. Interestingly, under stress, genotypes including IMR-619, IMR-620, IMR-8, and IMR-235 showed better biomass retention, branching, and root elongation. While correlation analysis suggested possible trade-offs between elongation and biomass accumulation, principal component analysis (PCA) revealed the main factors influencing genotypic variability. These results demonstrate how well PEG-based screening works to uncover drought-tolerant maize lines and serve as a foundation for choosing strong parental lines to create cultivars that are climate-resilient.

Keywords **:** maize inbred lines, drought stress, PEG-6000, root morphology, seedling tolerance, PCA, root biomass.

Introduction

Maize (*Zea mays* L.) is a major grain crop globally, contributing significantly to food and feed security. However, its productivity is highly susceptible to abiotic stresses, particularly drought, which is a major constraint in rainfed and arid regions (Farooq *et al.*, 2009; Campos *et al.*, 2004; Lobell *et al.*, 2014; Daryanto *et al.*, 2016). Drought at the seedling stage can severely impair root development, water uptake, and early plant establishment, compromising final yield potential (Klein *et al.*, 2020; Comas *et al.*, 2013).

Polyethylene glycol (PEG), specifically PEG-6000, is commonly used in laboratory settings to simulate osmotic drought stress as it reduces water availability without being toxic to plant tissues (Michel & Kaufmann, 1973; Verslues *et al.*, 2006; Islam *et al.*, 2024). PEG-induced osmotic stress causes physiological and morphological changes, particularly in the root system, including reduced elongation, branching, and biomass (Luo, 2010; Anjum *et al.*, 2011; Zheng *et al.*, 2022).

Root traits such as primary root length, number of seminal and lateral roots, and total root biomass are recognized as key indicators of early vigour and drought adaptation (Sharp *et al*., 2004; Wasson *et al*., 2012; Ali *et al*., 2021; Kadam *et al*., 2015). Genotypes that exhibit adaptive traits such as enhanced lateral root formation or biomass retention under PEG stress are considered promising candidates for breeding drought-resilient cultivars (Gao & Lynch, 2016; Raziuddin *et al*., 2010; Yue *et al*., 2019).

Considerable genetic variability in root architecture exists among maize inbred lines, reflecting their diverse evolutionary backgrounds and adaptation mechanisms (Blum, 2011; Li *et al*., 2015; Song *et al.,* 2020). This variability provides a foundation for identifying genotypes that perform well under early-stage drought and for improving breeding programs through selection of resilient parental lines (Lynch, 2013; Comas *et al.,* 2013; Zhan *et al.,* 2015).

This study evaluates 100 maize inbred lines under controlled PEG-induced stress to assess variation in root traits. The objectives were to quantify primary root length, seminal and lateral root number, and root biomass across treatments, and to identify genotypes that exhibit adaptive responses. Findings from such early-stage phenotyping can enhance selection efficiency in drought breeding programs (Trachsel *et al.,* 2011; Ren *et al.,* 2022; Smith & De Smet, 2012).

Materials and Methods

The experiment was conducted at the Dryland Agricultural Research Station (DARS), Rangreth, under the Division of Genetics and Plant Breeding, SKUAST-Kashmir, with the purpose of evaluating maize inbreds' drought sensitivity to polyethylene glycol (PEG)-induced osmotic stress.

In this study, we used 100 inbred maize lines (*Zea mays* L.). Polyethylene glycol-6000 (PEG-6000) was obtained from HIMEDIA and administered at three levels: 0% (control), 5%, and 10% (w/v) to simulate increasing degrees of water stress.

Five seeds from each inbred line were surface-disinfected with 0.5% sodium hypochlorite (NaOCl) for one minute. The seeds were washed repeatedly with distilled water. After washing, the seeds were placed on filter paper-lined Petri dishes that had been steeped in proper PEG solutions. These plates were placed in a germination chamber for seven days at a constant temperature of 25°C, 75% relative humidity, and total darkness. The PEG solution was changed daily to maintain a steady osmotic pressure. At the end of the incubation period, the following root parameters were recorded:

Primary root length (cm): was measured as the length from the root–shoot junction to the tip of the primary root using a ruler.

Fresh root biomass (mg): Roots were gently separated, blotted to remove surface moisture, and weighed to determine fresh weight.

Number of seminal roots: Roots that emerged from the scutellar node were counted manually (excluding the primary root).

Number of lateral roots: The primary and seminal roots were counted using a stereomicroscope or visually, according to the root size.

A Factorial completely randomized design (CRD) was used in the experiment with four replicates per treatment. The collected data were statistically analyzed by using R Studio Software to establish the relevance of genotype and treatment effects on the measured attributes.

Results

A study of 100 inbred maize lines exposed to PEG-induced drought stress revealed significant variation in root properties, indicating a diverse response across genotypes. Several inbred lines outperformed in terms of root elongation, branching, and biomass accumulation when exposed to a maximum stress level of 20% PEG(table 1). Notably, IMR-620 exhibited the longest main root length (13.26 cm) in 20% PEG, significant lateral root growth (9.66) and a large number of seminal roots (5.14). IMR-610 also performed brilliantly, with a root length of 12.69 cm and balanced root system architecture, including a lateral root count of 4.23. (Figure 1)

With a notable root length of 12.98 cm and a high number of laterals (16.72) and root biomass (0.48 g), IMR-619 stood out among the top performers. Likewise, IMR-8 exhibited a maximum of 6.76 seminal roots, a lateral root count of 19.82, and a main root length of 12.09 cm.

IMR-602 (0.41 g), IMR-611 (0.35 g), and IMR-592 (0.32 g) were the greatest donors of root biomass, demonstrating their capacity to grow in the presence of osmotic stress. Both IMR-577 and IMR-235 showed strong branching and root length under stress (11.23 and 12.82 cm, respectively). (Figure 1)

These inbred lines showed improved root formation and seedling vigor even in extreme water deficiency situations, indicating their potential use in breeding initiatives to increase drought resilience.

Primary Root Length: IMR-620, IMR-235, and IMR-8 had the longest root lengths. IMR-8 had highest number of seminal roots, followed by IMR-620 and IMR-611. Lateral Root Count: IMR-8 and IMR-619 exhibited remarkable lateral root growth. Root Biomass: The highest root biomass was observed in IMR-619, IMR-602, and IMR-611. (Figure 1)

PCA and Correlation Analysis Discussion

Principal Component Analysis (PCA)

The PCA analysis under 20% PEG-induced drought stress revealed significant variability among the nine top-performing maize inbred lines based on root traits. The first two principal components (PC1 and PC2) accounted for 69.20% of the total variance, with PC1 explaining 42.26% and PC2 26.94%. Genotype IMR-8 showed the highest positive loading on PC1, indicating strong performance in lateral and seminal root development, while IMR-620, with high scores on both PC1 and PC2, exhibited a balanced root architecture combining elongation, branching, and biomass making it the most promising all-round performer. IMR-610 stood out for its high PC2 score, reflecting superior root elongation and biomass retention despite moderate branching, whereas IMR-577 showed moderate but stable performance across all traits. Genotypes such as IMR-235 and IMR-619 clustered near the origin, suggesting average performance without extreme trait expression, although IMR-619 was notable for its lateral root growth. In contrast, IMR-602, IMR-611, and IMR-592 showed negative loadings on both PCs, highlighting their relatively poor root development and adaptability under osmotic stress. These findings underscore the utility of PCA in distinguishing genotypes based on root trait contributions under stress and facilitate the identification of promising parental lines like IMR-620, IMR-8, and IMR-610 for breeding drought-resilient maize cultivars. (Figure 2, Table 3 and Table 4)

The number of seminal roots and lateral roots were positively correlated (r = 0.31), indicating that genotypes with more seminal roots tended to produce more lateral roots. It is interesting to note that primary root length and root biomass had a negative correlation (r = -0.35), which may suggest that under osmotic stress, elongation and mass accumulation are traded off. Under extreme PEG stress, root biomass had weak or negative associations with other parameters, indicating that it may be independently or more influenced by genotype-specific variables. (Figure 3, table 2)

Table 1: *In vitro* response of maize (*Zea mays* L.) inbreds to different levels of PEG6000

	Primary Root Length(mm)			**No. of Seminals**			**No. of Laterals**			**Biomass(g)**		
Genotypes	**control**	**10%**	**20%**	**control**	**10%**	**20%**	**control**	**10%**	**20%**	**control**	**10%**	**20%**
IMR A	18.06	26.3	14.19	7.40	7.39	4.90	24.76	16.57	9.93	0.94	0.40	0.30
IMR B	11.2	12.64	7.5	5.63	6.05	1.53	45.13	34.65	19.40	0.75	0.34	0.16
IMR C	14.49	21.79	10.43	3.90	2.40	1.91	31.90	22.54	12.23	1.21	0.46	0.46
IMR-1	14.52	9.04	7.79	6.03	3.05	2.34	25.95	12.14	7.34	0.46	0.17	0.06
IMR-100	9.74	10.8	2.9	5.15	3.31	2.51	23.85	12.99	5.87	0.41	0.14	0.05
IMR-104	12.12	12.97	6.73	2.06	1.72	3.83	14.25	10.14	4.01	0.66	0.31	0.10
IMR-133	10.06	15.39	6.39	7.15	5.44	2.58	30.71	21.30	10.18	0.77	0.30	0.18
IMR-137	17.24	15.89	7.3	3.55	3.02	2.74	18.32	11.20	5.92	0.47	0.13	0.08
IMR-138	9.92	13.69	7.62	5.01	4.19	2.51	20.16	10.99	7.20	1.06	0.43	0.37
IMR-139	5.76	9.59	2.17	3.57	3.74	2.29	24.60	14.82	7.36	0.88	0.41	0.22
IMR-14	16.49	17.84	8.53	4.74	4.56	3.85	34.62	12.63	5.72	0.82	0.28	0.08
IMR-149	11.54	8.7	6.56	3.09	4.25	2.84	51.05	35.15	19.26	0.52	0.27	0.14
IMR-150	19.26	17.57	6.18	5.62	4.43	1.49	23.87	14.44	6.38	0.46	0.13	0.10
IMR-178	14.14	14.94	8.36	5.72	2.95	3.62	56.86	38.37	21.52	1.32	0.53	0.44
IMR-185	8.14	6.94	4.73	4.70	3.37	3.12	32.15	23.86	13.01	0.47	0.20	0.14
IMR-19	9.29	9.54	5.49	9.02	7.13	3.28	39.25	23.82	10.89	0.94	0.37	0.23
IMR-20	14.28	20.33	11.13	5.41	2.53	2.01	26.41	10.97	4.08	0.52	0.17	0.12
IMR-203	16.66	17.74	6.71	5.70	4.53	3.70	35.64	26.63	13.23	0.82	0.34	0.14
IMR-204	14	16.25	6.08	6.75	5.69	3.89	39.85	32.70	16.76	0.57	0.20	0.12
IMR-205	13.73	14.11	8.85	5.25	3.06	4.17	21.49	12.20	5.48	1.02	0.46	0.23
IMR-212	12.16	15.78	5.79	3.81	2.46	1.85	32.93	20.92	12.69	0.39	0.14	0.02
IMR-222	17.4	17.93	11.25	3.54	3.47	1.93	40.73	24.83	13.74	1.14	0.47	0.23
IMR-230	15.56	12.1	6.97	7.49	5.76	3.53	34.75	21.31	10.76	0.90	0.42	0.26
IMR-231	11.05	13.92	8.79	6.47	5.21	2.91	47.61	33.21	13.13	0.77	0.29	0.20
IMR-235	15.43	20.58	12.82	4.31	2.95	1.85	26.51	16.74	13.72	0.85	0.44	0.24

IMR-243	17.98	19.95	8.29	3.96	1.98	3.22	43.93	28.05	13.96	1.22	0.56	0.30
IMR-249	12.25	13.56	9.46	4.50	3.32	4.07	42.01	24.91	10.77	1.24	0.55	0.42
IMR-260	14.05	18.53	9.53	4.52	2.94	1.90	30.10	16.19	9.77	0.40	0.18	0.08
IMR-268	8.85	7.38	4.36	7.72	6.12	2.71	45.01	32.19	20.83	0.86	0.34	0.17
IMR-272	9.85	11.66	8.56	6.17	2.62	3.02	24.15	10.03	7.04	0.48	0.20	0.12
IMR-278	13.29	9.89	4.43	6.13	6.97	3.26	23.67	12.06	5.42	1.04	0.47	0.23
IMR-293	11.32	13.93	9.47	7.17	7.04	4.80	33.82	21.49	13.45	0.91	0.40	0.22
IMR-294	15.17	15.23	7.11	6.60	3.47	2.48	22.20	10.36	7.64	1.14	0.47	0.29
IMR-313	8.24	10.41	8.41	7.04	5.03	2.19	31.60	17.20	12.57	0.89	0.43	0.30
IMR-327	9.27	11.25	6.38	6.01	5.53	4.49	38.75	22.65	14.21	0.72	0.29	0.20
IMR-332	15.13	12.09	7.85	5.65	4.90	4.35	24.82	11.26	4.75	0.46	0.15	0.02
IMR-342	16.84	15.86	8.04	7.48	5.09	3.21	63.68	29.63	13.95	0.63	0.22	0.10
IMR-367	11.9	10.76	4.61	5.22	5.92	3.38	36.30	20.69	6.43	0.81	0.23	0.12
IMR-368	11.78	11.86	8.01	3.57	2.60	2.54	41.26	21.06	9.46	0.90	0.33	0.18
IMR-373	8.97	6.15	5.25	4.59	4.53	3.58	27.43	11.04	3.73	0.44	0.16	0.07
IMR-375	7.55	9.18	4.71	4.31	2.47	3.16	33.82	15.75	5.93	0.50	0.19	0.04
IMR-379	9.99	8.45	5.77	3.92	2.97	4.18	21.63	7.30	2.59	0.99	0.36	0.17
IMR-380	12.58	14.51	11.77	3.80	4.66	2.87	9.18	1.50	1.50	0.61	0.26	0.08
IMR-381	11.99	14.2	12.65	4.08	2.20	1.73	12.67	6.01	2.80	0.64	0.23	0.12
IMR-382	12.78	14.95	6.85	4.48	4.69	1.97	12.58	6.80	4.71	0.57	0.15	0.11
IMR-384	11.57	14.05	9.14	6.13	4.20	2.78	8.99	4.64	3.36	0.38	0.15	0.13
IMR-387	7.38	8.95	6.05	3.01	3.25	3.31	23.51	9.74	3.15	0.31	0.14	0.07
IMR-388	12.06	13.66	5.6	6.49	4.80	5.35	11.14	5.74	1.59	0.71	0.20	0.08
IMR-397	18.82	17.17	7.83	5.60	4.06	2.28	34.57	16.43	11.38	0.80	0.20	0.07
IMR-401	10.59	11.67	4.53	1.44	3.14	2.50	14.26	4.56	1.93	0.46	0.15	0.05
IMR-405	8.97	8.92	4.81	3.07	1.49	2.32	17.32	7.96	3.16	0.98	0.32	0.09
IMR-406	15.52	14.48	6.69	3.05	2.12	3.22	24.27	11.99	4.08	0.93	0.33	0.16
IMR-419	14.37	13.2	9.85	5.94	4.48	5.23	48.29	19.77	5.60	0.71	0.22	0.10
IMR-42	15.65	10.83	3.5	9.34	6.68	4.12	34.58	23.51	12.45	0.45	0.17	0.11
IMR-420	15.5	16.48	7.83	5.75	4.27	2.60	26.67	10.51	5.08	0.39	0.11	0.03
IMR-424-1	11.01	9.59	5.01	4.50	4.47	3.30	58.13	27.48	14.00	1.22	0.40	0.19
IMR-428-1	16.74	16.55	10.58	5.98	3.46	3.78	12.46	4.85	1.88	0.48	0.20	0.06
IMR-429	17.09	13.73	4.02	6.37	3.00	4.18	16.99	6.95	3.91	0.81	0.23	0.11
IMR-433	10.69	14.05	9.69	4.95	2.96	3.72	19.83	9.64	3.48	0.53	0.11	0.12
IMR-436	15.37	21.13	11.16	5.54	4.13	3.77	11.02	5.22	4.00	1.16	0.36	0.19
IMR-439	18.09	18.29	7.12	3.48	3.42	2.40	12.49	7.04	3.66	0.54	0.15	0.06
IMR-44	16.69	18.43	9.48	7.86	5.59	2.32	22.02	13.41	8.53	1.01	0.40	0.27
IMR-441	19.39	21.52	11.72	3.94	3.81	3.11	38.80	19.55	10.08	1.11	0.26	0.12
IMR-444	18.28	19.13	9.85	5.37	4.30	3.16	33.81	13.94	10.50	0.95	0.29	0.04
IMR-450	10.42	19.28	6.72	3.08	3.01	2.95	47.49	18.73	10.35	0.72	0.23	0.05
IMR-451	18.32	21.02	10.29	6.53	4.62	3.71	14.25	4.17	3.42	0.96	0.25	0.08
IMR-454	14.81	15.15	12.07	6.15	2.75	3.47	42.12	18.69	13.35	1.22	0.37	0.12
IMR-462	12.31	10.57	6.93	4.05	3.32	3.55	44.02	27.59	14.09	0.93	0.41	0.24
IMR-470	10.06	10.25	6.37	8.20	5.49	6.04	31.15	21.08	12.41	0.62	0.20	0.12
IMR-471	8.7	10.31	3.97	4.66	3.87	2.82	45.24	30.16	16.04	0.51	0.20	0.11
IMR-482	7.88	8.21	4.07	4.73	2.71	2.20	34.10	23.90	11.03	1.02	0.49	0.32
IMR-487	7.08	7.87	7.91	7.87	4.86	3.44	24.72	13.23	6.47	0.72	0.30	0.19
IMR-49	10.18	10.1	4.35	5.62	4.27	2.64	17.00	7.23	3.78	0.70	0.25	0.20
IMR-493	11.28	13.03	5.38	4.19	5.12	4.23	14.69	10.16	4.50	0.70	0.29	0.16
IMR-498	12.1	13.04	7.45	6.46	5.64	2.72	12.37	6.55	1.95	0.55	0.23	0.08
IMR-499	10.44	17.19	7.28	5.44	4.51	2.61	15.51	9.79	5.31	0.41	0.17	0.12
IMR-506	12.93	17.98	9.38	5.10	4.02	2.37	19.85	9.54	5.07	0.81	0.35	0.17
IMR-538	9.08	9.33	5.16	6.57	5.35	3.35	26.97	13.86	7.48	0.67	0.30	0.18
IMR-576	12.04	14.88	8.22	7.39	8.03	5.29	63.34	43.77	24.46	0.77	0.30	0.15
IMR-577	17.57	22.78	11.23	5.02	3.28	2.47	38.15	23.19	13.67	0.53	0.20	0.11
IMR-579	9.33	11.37	6.94	7.53	6.40	4.03	40.46	28.49	14.78	0.98	0.48	0.32
IMR-58	11.44	18.43	9.52	6.93	5.75	3.25	14.29	9.50	7.03	0.54	0.21	0.15

IMR-592	9.37	12.94	4.53	4.61	3.32	1.65	26.27	18.98	11.94	1.02	0.43	0.32
IMR-597	15.68	21.47	8.67	4.46	2.67	2.56	34.66	21.70	8.49	0.64	0.28	0.13
IMR-599	16.04	15.1	7.91	4.47	3.44	3.15	19.82	11.44	5.21	0.46	0.15	0.09
IMR-602	16.21	18.37	10.48	6.27	6.17	4.29	22.23	13.60	7.37	1.26	0.57	0.41
IMR-604	10.32	11.35	5.28	6.16	6.31	4.10	14.15	11.13	5.61	0.44	0.22	0.07
IMR-609	16.72	18.59	10.64	5.45	4.61	2.83	23.97	12.29	6.11	0.85	0.35	0.26
IMR-610	18.69	24.05	12.69	3.37	2.00	2.52	18.82	10.48	4.23	0.59	0.19	0.11
IMR-611	11.24	17.52	7.28	5.99	2.86	3.85	24.18	15.12	8.48	1.04	0.46	0.35
IMR-612	15.6	14.56	12.16	4.44	4.34	3.60	13.31	12.16	7.31	0.62	0.30	0.19
IMR-614	16.47	18.84	6.18	5.60	4.76	2.54	34.43	25.22	14.86	1.09	0.54	0.36
IMR-616	16.22	19.68	7.47	7.83	6.48	4.46	25.99	13.81	8.90	0.98	0.43	0.34
IMR-617	13.67	17.93	5.99	8.60	8.10	5.14	27.06	10.47	7.03	0.90	0.34	0.26
IMR-618	10.71	13.77	8.24	4.59	2.56	2.00	31.24	22.56	13.89	0.91	0.41	0.31
IMR-619	16.66	23.36	12.98	6.30	4.87	3.48	47.65	28.47	16.72	1.20	0.58	0.48
IMR-620	16.14	26.73	13.26	7.97	7.34	5.14	27.61	18.56	9.66	0.57	0.18	0.08
IMR-63	6.91	9.97	3.52	6.58	5.48	3.42	20.90	14.12	9.89	0.51	0.22	0.11
IMR-8	13.51	22.58	12.09	8.84	6.32	6.76	62.84	35.08	19.82	0.57	0.28	0.11
IMR-87	15.57	13.63	4.63	4.71	2.60	2.30	31.20	21.59	12.09	0.52	0.13	0.11
MEAN	13.07	1475.21	774.48	5.48	4.29	3.23	29.43	17.01	9.06	76.05	0.30	0.17
CD (p<0.05)	Genotypes (G) =1.17 Regimes (R) =0.204 Genotypes*Regimes (G*R) =2.04			Genotypes (G) = 1.18 Regimes (R) =0.205 Genotypes*Regimes (G*R) =2.05			Genotypes (G) = 1.24 Regimes (R) =0.215 Genotypes*Regimes (G*R) =2.15			Genotypes (G) =0.038 Regimes (R) =0.006 Genotypes*Regimes (G*R) =0.066		

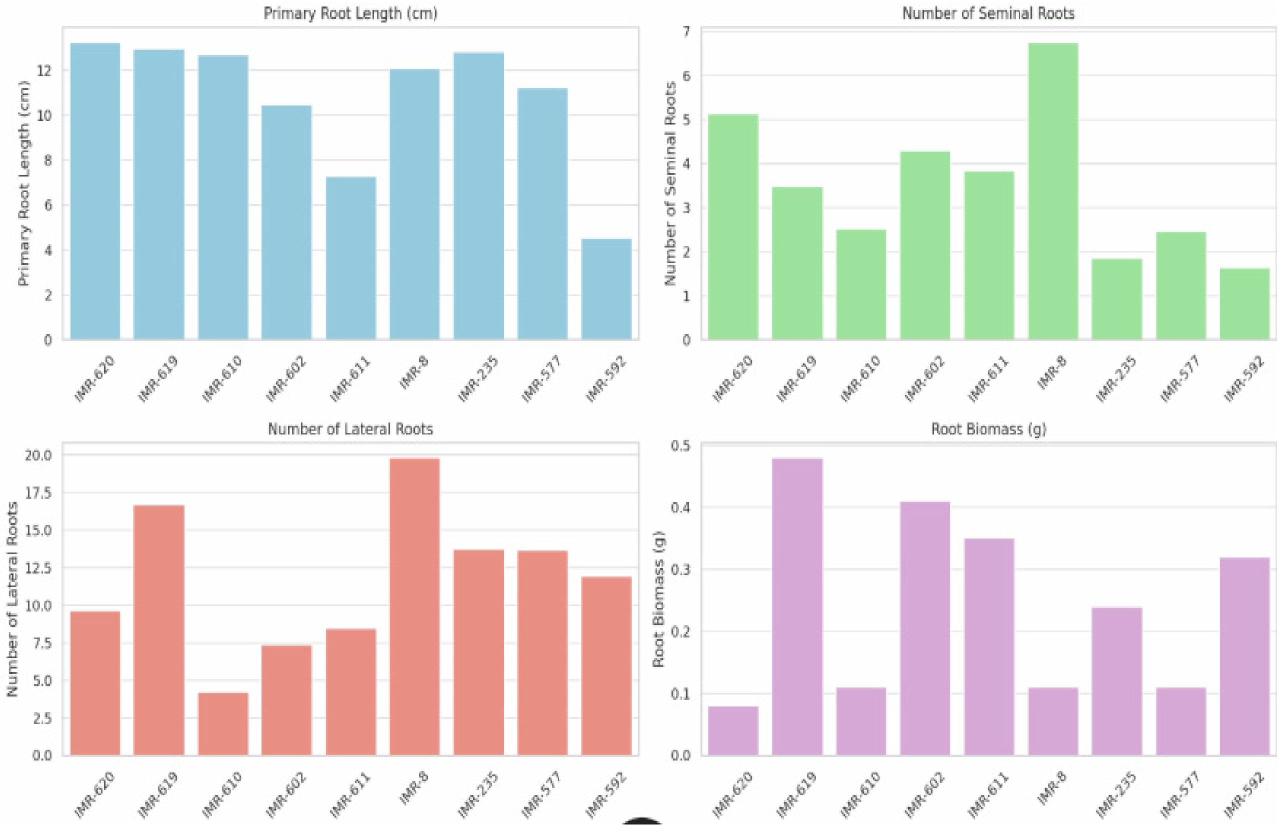

Fig. 1: The top-performing maize genotypes under 20% PEG-induced drought stress are shown graphically here, with four important root features highlighted

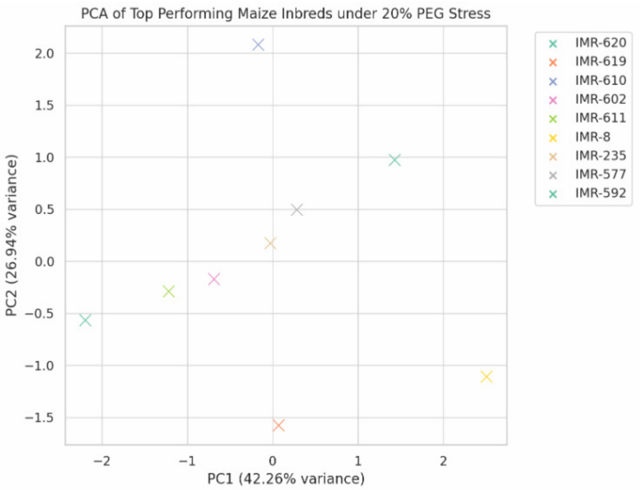

Fig. 2: Principal component analysis of 100 maize genotypes

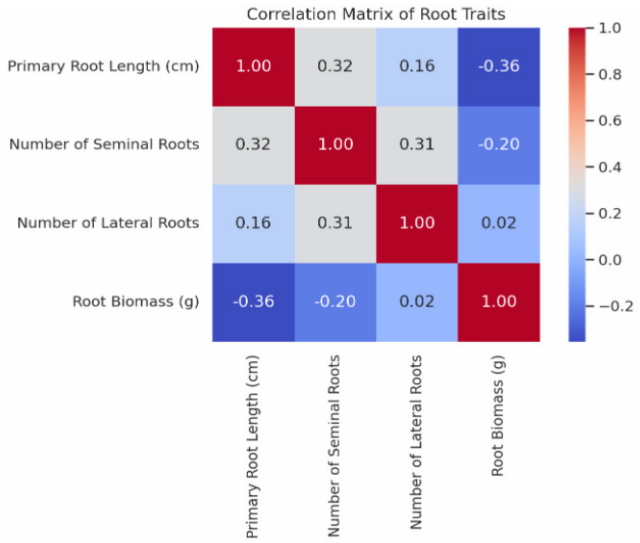

Fig. 3: Correlation between different root parameters

Table 2: Correlation Matrix Interpretation

Trait 1	Trait 2	Correlation (r)
Primary Root Length	Root Biomass	–0.35
Seminal Roots	Lateral Roots	0.31
Seminal Roots	Root Biomass	–0.20
Lateral Roots	Root Biomass	0.02

Table 3: PCA loadings of different principal components

Trait	PC1	PC2
Primary Root Length	+0.579	+0.242
Seminal Roots	+0.566	–0.263
Lateral Roots	+0.373	–0.712
Root Biomass	–0.454	–0.604

Table 4: Eigen values and variance of different PCs

Principal Component	Eigenvalue	Variance Explained (%)
PC1	1.902	42.26%
PC2	1.212	26.94%

Discussion

Drought stress poses a significant challenge to maize productivity, particularly during early developmental stages when seedling vigor and root development are critical for survival (Farooq *et al.,* 2009; Anjum *et al.,* 2011; Lobell *et al.,* 2014). Polyethylene glycol (PEG)-induced osmotic stress offers a reproducible and non-invasive method to simulate drought conditions in controlled environments and screen for tolerant genotypes (Michel & Kaufmann, 1973; Verslues *et al.,* 2006; Islam *et al.,* 2024).

In this study, substantial genotypic variation was observed among 100 maize inbred lines evaluated under 10% PEG stress. Although all root traits (primary root length, number of seminal and lateral roots, and root biomass) generally declined, some genotypes exhibited resilience by maintaining or enhancing specific traits. Root depth plays a vital role in accessing water stored in deeper soil layers. Genotypes such as IMR-620 and IMR-619 demonstrated significantly greater primary root elongation under PEG stress, indicating a drought-avoidance strategy (Sharp *et al.,* 2004; Comas *et al.,* 2013; Yue *et al.,* 2019). These findings are consistent with recent reports emphasizing root elongation as a key trait for drought resilience in cereals (Zheng *et al.,* 2022; Gao & Lynch, 2016). Genotypes like IMR-8 displayed a higher number of seminal and lateral roots, enhancing soil exploration and water absorption. Increased lateral root formation is linked to better nutrient and water uptake under stress conditions (Smith & De Smet, 2012; Janiak *et al.,* 2016). A moderate positive correlation (r = 0.31) between seminal and lateral roots suggests coordinated development strategies (Kadam *et al.,* 2015; Coudert *et al.,* 2010; Gao *et al.,* 2022). Additionally, seminal roots provide early anchorage and support seedling establishment in dry soils (O’Toole & Bland, 1987). Genotypes IMR-602, IMR-611, and IMR-619 maintained higher root biomass under stress. Biomass retention reflects sustained carbon allocation to roots and metabolic efficiency, both essential under water-limiting environments (Wasson *et al.,* 2012; Trachsel *et al.,* 2011; Uga *et al.,* 2013). Interestingly, a negative correlation (r = –0.35) was found between root length and biomass, suggesting a trade-off between elongation and root thickness, which has also been reported in wheat and rice (Zhan *et al.,* 2015; Blum, 2011). Principal Component Analysis (PCA) highlighted primary root length and lateral roots as major contributors to genotypic variation. Genotypes like IMR-235, IMR-620, and IMR-8 clustered in distinct quadrants, reflecting unique trait combinations and drought-adaptive strategies (Li *et al.,* 2015; Gao *et al.,* 2022). Such multivariate approaches facilitate ideotype selection for complex traits (Lynch, 2013; Islam *et al.,* 2024). PEG-induced osmotic stress proved to be an effective tool for rapid screening and phenotyping of early-stage drought responses in maize. Genotypes IMR-619, IMR-620, IMR-8, and IMR-235 showed promising root traits under stress, making them suitable candidates for breeding programs targeting drought tolerance. These findings align with previous efforts to integrate phenotypic and genotypic information to accelerate the development of climate-resilient cultivars (Burton *et al.,* 2013; Castaneda *et al.,* 2018; Lobell & Gourdji, 2012). Further studies incorporating physiological, anatomical, and molecular validation are recommended to confirm the functional basis of these root traits and enable their use in marker-assisted selection (MAS) and genomic selection pipelines.

References

Ali, Q., Ahsan, M., Ali, F., Aslam, M., & Maqsood, M. (2021). Genetic analysis and stability of maize hybrids under drought stress conditions. *SABRAO Journal of Breeding and Genetics,* **53**(2), 218–229.

Ali, Q. *et al*. (2021). Screening of maize inbred lines for drought tolerance using PEG. *Agronomy,* **11**(1), 98.

Anjum, S. A., Wang, L., Farooq, M., Hussain, M., Xue, L., & Zou, C. (2011). Brassinolide application improves drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. *Journal of Agronomy and Crop Science,* **197**(3), 177–185. https://doi.org/10.1111/j.1439-037X.2010.00459.x

Blum, A. (2011). *Plant breeding for water-limited environments*. Springer Science & Business Media.

Burton, M., Mitchelmore, D., & Lemke, C. (2013). Climate-resilient maize for drought-prone regions. *Field Crops Research,* **146**, 15–23. https://doi.org/10.1016/j.fcr. 2013. 03.005

Campos, H., Cooper, M., Habben, J. E., Edmeades, G. O., & Schussler, J. R. (2004). Improving drought tolerance in maize: A view from industry. *Field Crops Research,* **90**(1), 19–34. https://doi.org/10.1016/j.fcr.2004.07.003

Castaneda, M., Bolaños, E. A., & San Vicente, F. (2018). Drought-tolerant maize for African smallholders. *Outlook on Agriculture,* **47**(3), 163–169.

Comas, L. H., Becker, S. R., Von Mark, V. C., Byrne, P. F., & Dierig, D. A. (2013). Root traits contributing to plant productivity under drought. *Frontiers in Plant Science,* **4**, 442. https://doi.org/10.3389/fpls.2013.00442

Coudert, Y., Périn, C., Courtois, B., Khong, N. G., & Gantet, P. (2010). Genetic control of root development in rice, the model cereal. *Trends in Plant Science,* **15**(4), 219–226.

Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PLOS ONE,* **11**(5), e0156362. https://doi.org/ 10.1371/ journal.pone.0156362

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., & Basra, S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development,* **29**, 185–212. https://doi.org/10.1051/agro: 2008021

Gao, Y., & Lynch, J. P. (2016). Reduced crown root number improves water acquisition under water deficit stress in maize (*Zea mays* L.). *Journal of Experimental Botany,* **67**(15), 4545–4557.

Gao, Y., Wu, X., Wang, Y., & Zheng, Y. (2022). Comprehensive evaluation of maize drought tolerance using root phenomics and multivariate analysis. *Crop Science,* **62**(3), 1435–1448.

Gao, Y., *et al* . (2022). Uncovering elite alleles for maize root traits using genome-wide association studies. *Plant Physiology and Biochemistry,* **182**, 145–156.

Islam, A., Khan, M. F., & Ahmed, M. (2024). Screening maize genotypes under PEG-induced stress: Implications for drought tolerance. *Frontiers in Agronomy,* **6**, 102–113. https://doi.org/10.3389/fagro.2024.102113

Janiak, A., Kwaśniewski, M., & Szarejko, I. (2016). Gene expression regulation in roots under drought. *Journal of Experimental Botany,* **67**(4), 1003–1014.

Kadam, N. N., Yin, X., Bindraban, P. S., Struik, P. C., & Jagadish, K. S. V. (2015). Does morphological and anatomical plasticity during the vegetative stage make wheat more tolerant of water deficit stress than rice? *Plant Physiology,* **169**(2), 1086–1102.

Klein, S. P., Schneider, H. M., Perkins, A. C., Brown, K. M., & Lynch, J. P. (2020). Multiple integrated root phenotypes are associated with improved drought tolerance. *Plant Physiology,* **183**(3), 1011–1025.

Li, X., Ingvardsen, C. R., Wulff, T., Schmidt, M., & Frei, U. (2015). Association mapping of drought tolerance and agronomic traits in maize. *Theoretical and Applied Genetics,* **128**, 133–145.

Lobell, D. B., & Gourdji, S. M. (2012). The influence of climate change on global crop productivity. *Plant Physiology,* **160**(4), 1686–1697.

Lobell, D. B., Schlenker, W., & Costa-Roberts, J. (2014). Climate trends and global crop production since 1980. *Science,* **333**(6042), 616–620.

Luo, H. (2010). Effects of PEG on seedling growth and physiological traits in maize. *African Journal of Agricultural Research,* **5**(7), 585–591.

Luo, Z., *et al* . (2022). PEG-induced drought stress in maize: Physio-biochemical and molecular responses. *Plant Stress,* **3**, 100059.

Lynch, J. P. (2013). Steep, cheap and deep: An ideotype to optimize water and N acquisition by maize root systems. *Annals of Botany,* **112**(2), 347–357.

Michel, B. E., & Kaufmann, M. R. (1973). The osmotic potential of polyethylene glycol 6000. *Plant Physiology,* **51**(5), 914–916.

O'Toole, J. C., & Bland, W. L. (1987). Genotypic variation in crop plant root systems. *Advances in Agronomy,* **41**, 91–145.

Raziuddin, *et al* . (2010). PEG-simulated drought stress and antioxidant enzymes in maize. *Plant Omics,* **3**(3), 97–104.

Ren, B., Liu, D., Wang, Y., & Yang, W. (2022). Dynamic response of maize root morphology to PEG-induced drought. *Plants, 11*(5), 654.

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: Physiology to functional genomics. *Journal of Experimental Botany,* **55**(407), 2343–2351.

Smith, S., & De Smet, I. (2012). Root system architecture: Insights from Arabidopsis and cereal crops. *Philosophical Transactions of the Royal Society B,* **367**(1595), 1441–1452.

Song, Y., Zhang, W., Xiong, D., Gu, X., & Zhang, C. (2020). Morphological and anatomical root traits in maize under drought. *Journal of Integrative Agriculture,* **19**(8), 1947–1959.

Trachsel, S., Kaeppler, S. M., Brown, K. M., & Lynch, J. P. (2011). Shovelomics: High throughput phenotyping of maize root architecture in the field. *Plant and Soil,* **341**(1–2), 75–87.

Uga, Y., Sugimoto, K., Ogawa, S., Rane, J., Ishitani, M., Hara, N., ... & Yano, M. (2013). Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics,* **45**(9), 1097–1102.

Verslues, P. E., Ober, E. S., & Sharp, R. E. (2006). Root growth and oxygen relations at low water potentials. *Plant Physiology,* **140**(2), 426–439.

Wasson, A. P., Richards, R. A., Chatrath, R., Misra, S. C., Prasad, S. V. S., Rebetzke, G. J., ... & Kirkegaard, J. A. (2012). Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. *Journal of Experimental Botany,* **63**(9), 3485–3498.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., Jin, D., Xing, Y., & Zhang, Q. (2019). Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. *Genetics,* **172**(2), 1213–1228.

Zhan, A., Schneider, H. M., & Lynch, J. P. (2015). Reduced lateral root branching density improves drought tolerance in maize. *Plant Physiology,* **168**(4), 1603–1615.

Zheng, J. *et al*. (2022). Genetic variation in maize root architecture traits and association with drought tolerance. *The Crop Journal,* **10**(1), 146–157.