

Evaluation of Drought Tolerance in Kosovo Maize (*Zea mays* L.) Genotypes during Germination Using PEG-Induced Stress*

Kosova Mısır (*Zea mays* L.) Genotiplerinde Çimlenme Sırasında PEG Kaynaklı Stres Kullanılarak Kuraklık Toleransının Değerlendirilmesi

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Abstract

Drought represents one of the most critical abiotic stresses that severely restrict maize (*Zea mays* L.) productivity, especially during the germination phase and the early development of seedlings. In this study, drought tolerance was assessed in a total of 60 maize genotypes originating from different regions of Kosovo, along with one commercial maize genotype (Kompozit Şeker) from Türkiye, which was included intentionally as a reference genotype to enable a clearer comparison of stress responses. Polyethylene glycol (PEG 6000) was applied at concentrations of 10%, 20%, and 30% to simulate osmotic stress conditions and to uniformly induce water-deficit environments under controlled laboratory conditions. The traits evaluated in this experiment included germination percentage (G%), mean germination time (G/day), and several important root and shoot characteristics such as length, fresh weight (FW), and dry weight (DW). As PEG concentration increased, significant reductions in germination and substantial inhibition of seedling growth were observed, with the most pronounced negative effects occurring under the 30% PEG treatment. Principal component analysis (PCA) together with hierarchical clustering clearly distinguished the accessions into separate groups based on their measured stress responses, demonstrating a high degree of genotypic diversity among the evaluated materials. Several genotypes-most notably ACC16, ACC18, and ACC46-exhibited consistently superior performance across all traits and stress intensities, indicating strong drought tolerance at the early growth stage. These findings highlight that the local maize genetic resources of Kosovo represent a valuable genetic reservoir for drought-tolerant alleles and provide important contributions to the development of new maize varieties resilient to climate change. Additionally, the results serve as a guide for sustainable maize production, improving agricultural productivity, and planning future agricultural strategies. According to the available literature, this is one of the first studies evaluating drought tolerance in local maize genotypes from Kosovo under PEG-induced osmotic stress.

Keywords: PEG 6000, Germination, Genotypes, Osmotic stress, Genetic diversity

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Öz

Kuraklık, özellikle çimlenme dönemi ve fide gelişiminin erken evrelerinde, mısırın (*Zea mays* L.) verimliliğini ciddi şekilde kısıtlayan en önemli abiyotik streslerden biridir. Bu çalışmada, farklı bölgelerden toplanan 60 Kosova yerel mısır genotipi ile Türkiye’den (Kompozit Şeker) alınan ve karşılaştırma amacıyla referans genotip olarak kullanılan bir ticari mısır genotipinin çimlenme aşamasında kuraklık toleransı sistematik olarak değerlendirilmiştir. Kontrollü laboratuvar koşullarında, su eksikliği stresini simüle etmek amacıyla polietilen glikol (PEG 6000) farklı derişimlerde (%10, %20 ve %30) uygulanmıştır. Denemede çimlenme yüzdesi (G%), ortalama çimlenme süresi (G/gün), kök ve sürgün uzunluğu ile yaş ağırlık (YA) ve kuru ağırlık (KA) gibi temel morfolojik ve fizyolojik özellikler detaylı şekilde ölçülmüştür. PEG derişimi arttıkça, çimlenmede belirgin azalmalar ve fide gelişiminde güçlü bir baskı gözlenmiş olup, en olumsuz etkiler özellikle %30 PEG uygulamasında ortaya çıkmıştır. Temel bileşen analizi (PCA) ve hiyerarşik kümeleme analizi, genotiplerin stres yanıtlarına göre açık bir şekilde gruplandığını göstermiş ve materyaller arasında yüksek düzeyde genetik çeşitlilik olduğunu ortaya koymuştur. Özellikle ACC16, ACC18 ve ACC46 genotipleri, tüm stres düzeylerinde (%10, 20, 30 PEG) ve incelenen özelliklerde üstün performans sergileyerek erken gelişim aşamasında güçlü kuraklık toleransı göstermiştir. Bu bulgular, Kosova’ya özgü yerel mısır genetik kaynaklarının, kuraklığa dayanıklı aleller için değerli bir genetik rezerv olduğunu vurgulamakta ve iklim değişikliğine dayanıklı yeni mısır çeşitlerinin geliştirilmesine önemli katkılar sunmaktadır. Ayrıca, elde edilen sonuçlar sürdürülebilir mısır üretimi, tarımsal verimliliğin artırılması ve gelecekteki tarımsal stratejilerin planlanması açısından da yol gösterici niteliktedir. Mevcut literatüre göre, bu çalışma PEG kaynaklı ozmotik stres altında Kosova kökenli yerel mısır genotiplerinde kuraklık toleransını değerlendiren önemli ilk kapsamlı çalışmalardan biridir.

Anahtar Kelimeler: PEG 6000, Çimlenme, Genotip, Ozmotik stres, Genetik çeşitlilik

1. Introduction

Plants are constantly exposed to various stress factors that can negatively impact their yield potential and, in severe cases, threaten their survival in the soil (Suzuki et al., 2014; Partheeban et al., 2017; Özdemir and Sade, 2024). Among environmental stressors, drought is one of the most damaging, significantly reducing plant growth, development, and productivity (Xiong et al., 2006; Bilgin et al., 2008; Soltanbeigi, 2019). Global climate change is projected to reduce crop yields and diminish plants' ecological adaptability (Osborn and Ferguson, 2019; Badr et al., 2020). Climate projections indicate that major crops like winter wheat and maize will experience considerable yield losses, with maize being particularly vulnerable to drought stress (Webber et al., 2018). Developing crop varieties capable of withstanding various environmental stresses is crucial for securing future food supplies. Local genotypes are thought to be resilient to both abiotic and biotic challenges, making them an important resource for plant breeders who seek to create elite lines with enhanced drought tolerance (Abdel-Ghani et al., 2015; Sukma et al., 2018; Osborn and Ferguson, 2019; Mustamu et al., 2023). However, according to the Food and Agriculture Organization (FAO), genetic resource banks often lack sufficient data regarding early-stage plant traits such as germination capacity and seedling morphology under stress conditions (FAO, 2010). Several components of growth and development during germination can serve as useful selection criteria for identifying drought-tolerant genotypes (Nivethitha et al., 2020).

Maize (*Zea mays* L.) is a vital agricultural crop, ranking third in global cereal production after wheat and rice (Fatima et al., 2023). Its demand is steadily increasing due to its multiple uses in food, livestock feed, industry, and biofuel production (Tahir et al., 2023; Rather et al., 2024). Nevertheless, drought as an abiotic stressor leads to approximately 15% yield loss in maize annually on a global scale (Adewale et al., 2018). In major maize-producing areas, global warming is expected to intensify drought events and introduce new pests and diseases. Developing drought-resilient maize germplasm is therefore a priority (Edmeades, 2013; Tesfaye et al., 2018). Drought affects nearly every developmental stage in plants, including germination, growth, and flowering (Rauf and Ahmad Sadaqat, 2008; Khayatnezhad and Gholamin, 2012). In maize, it not only reduces germination rates but also increases the mean germination time (Babar et al., 2014). Similar negative effects of drought on seedling growth have been reported in various crops (Khodarahmpour, 2011; Mostafavi et al., 2011). Osmotic stress induced by polyethylene glycol (PEG) has been widely used to simulate drought conditions and assess seedling responses (Bibi et al., 2012). In wheat, positive correlations among root length, fresh and dry weight, photosynthetic pigment content, and membrane stability have been demonstrated under both normal and stress conditions (Ahmed et al., 2019). PEG 6000 is highly effective in simulating drought by controlling water potential and is commonly used to identify tolerant or sensitive genotypes during germination stages. It is an essential tool in breeding programs aimed at improving drought resilience (Dani and Siswoyo, 2019; Magar et al., 2019). Previous studies have reported significant variation in maize root and shoot responses to different PEG concentrations (Petcu et al., 2018; Islam et al., 2019; Majid et al., 2020).

The scientific background of the high genetic diversity of traditional maize genotypes in Kosovo has not yet been investigated. To our knowledge, this is the first study to systematically evaluate the drought tolerance of 60 maize genotypes from Kosovo during the germination stage. This original research provides novel insights into their phenotypic variability under simulated drought stress and highlights their potential for use in drought-resilient breeding programs. The main objectives of this study were: (a) to assess the response of local maize genotypes to drought stress induced by PEG 6000, and (b) to identify drought-tolerant and drought-sensitive genotypes. The hypothesis is that some genotypes will display superior drought tolerance at early developmental stages, opening the way for further genetic and physiological characterization.

2. Materials and Methods

2.1. Selection and collection of maize genotypes

Maize genotypes were selected based on standard morphological descriptors, including kernel color, shape, size, and texture. In addition to morphological traits, priority was given to the historical importance of maize cultivation in the respective regions. These genotypes were previously reported by farmers to exhibit high germination rates and resilience to diverse agroecological conditions, including both biotic and abiotic stress. Sixty maize genotypes were collected from 20 regions across Kosovo, each with three sub-locations (*Table 1*,

Figure 1). For comparison, one reference genotype (Kompozit Şeker) was obtained from the Maize Research Institute, Ministry of Agriculture and Forestry, Republic of Türkiye.

From each landrace, five mature kernels with brown husks and full physiological maturity were selected. Seeds were dried to a moisture content of 14% before further experimentation.

Table 1. Characteristics of maize genotypes from Kosovo

Landrace's label	Locality	Sublocality	Latitude (°, ' ")	Longitude (°, ' ")	Altitude (m)	Vernacular name	Landscape	Kernel colour	Kernel shape
ACC1	Kacanik	Ivaj	42 11 50.21	21 14 50.60	790	Kolomboq	Mountain	White	Circle
ACC2	Kacanik	Kacanik I vjeter	42 17 19.84	21 13 10.33	540	Kolomboq	Flat	White	Oval
ACC3	Kacanik	Kovacec	42 14 47.00	21 13 41.62	515	Kolomboq	Flat	White	Circle
ACC4	Ferizaj	Jezerc	42 21 30.00	21 01 08.84	759	Kolomboq	Mountain	White	Oval
ACC5	Ferizaj	Sazli	42 24 28.46	21 11 10.06	575	Kolomboq	Flat	Orange	Oval
ACC6	Ferizaj	Zaskok	42 20 54.00	21 08 05.08	763	Kolomboq	Mountain	White	Circle
ACC7	Podujeve	Ballovc	42 53 13.00	21 14 12.33	617	Kolomboq	Flat	White	Circle
ACC8	Podujeve	Podujeve	42 54 51.00	21 12 02.21	637	Kolomboq	Flat	White	Circle
ACC9	Podujeve	Gerdovc	42 49 21.70	21 12 58.20	586	Kolomboq	Flat	White	Oval
ACC10	Gjakove	Plancor	42 26 49.00	20 21 43.19	444	Miser	Flat	White	Circle
ACC11	Gjakove	Piskote	42 25 06.06	20 23 54.06	399	Miser	Flat	White	Circle
ACC12	Gjakove	Skivjan	42 26 12.35	20 22 29.13	425	Miser	Flat	White	Circle
ACC13	Drenas	Arllat	42 32 30.5	20 49 33.60	659	Kolomboq	Mountain	White	Circle
ACC14	Drenas	Necoc	42 32 02.1	20 54 38.50	606	Kolomboq	Flat	White	Circle
ACC15	Drenas	Kishnareke	42 33 15.5	20 53 48.50	605	Kolomboq	Flat	Yellow	Triangle
ACC16	Lipjan	Smallushe	42 30 20.42	21 06 04.10	566	Miser	Flat	White	Circle
ACC17	Lipjan	Slovine	42 31 21.38	21 13 21.78	603	Miser	Hilly	White	Oval
ACC18	Lipjan	Gracke	42 30 35.93	21 12 20.90	591	Miser	Flat	White	Circle
ACC19	Shterpc	Brod	42 15 52.74	21 06 53.13	721	Kolomboq	Mountain	White	Circle
ACC20	Shterpc	Firaje	42 15 42.59	21 06 04.42	756	Kolomboq	Mountain	White	Oval
ACC21	Shterpc	Brezovic	42 12 33.58	20 57 05.12	1025	Kolomboq	Mountain	White	Circle
ACC22	Malishev	Banje	42 27 57.90	20 47 37.67	557	Kolomboq	Flat	White	Circle
ACC23	Malishev	Senik	42 28 47.38	20 49 34.04	658	Kolomboq	Hilly	Red	Oval
ACC24	Malishev	Bubli	42 31 22.55	20 40 09.03	504	Kolomboq	Hilly	White	Circle
ACC25	Decan	Baballoq	42 28 42.87	20 18 18.56	502	Miser	Flat	White	Oval
ACC26	Decan	Lloqan	42 31 33.86	20 17 24.66	599	Miser	Hilly	White	Circle
ACC27	Decan	Carabreg	42 31 42.31	20 17 54.12	582	Miser	Hilly	Yellow	Circle
ACC28	Kline	Gremnik	42 34 52.36	20 35 06.45	408	Miser	Mountain	White	Circle
ACC29	Kline	Dersnik	42 36 36.49	20 35 49.45	429	Miser	Mountain	White	Circle
ACC30	Kline	Sferke	42 32 48.89	20 36 44.02	546	Miser	Hilly	White	Circle
ACC31	Prishtine	Barileve	42 45 07.84	21 07 23.11	543	Kolomboq	Hilly	White	Oval
ACC32	Prishtine	Kastriot	42 43 29.10	21 05 13.77	539	Kolomboq	Flat	Yellow	Triangle
ACC33	Prishtine	Prugovc	42 44 53.64	21 07 54.50	544	Kolomboq	Hilly	White	Circle
ACC34	Mitrovic	Zhabar eperm	42 51 59.75	20 49 44.28	529	Miser	Hilly	White	Circle
ACC35	Mitrovic	Suhodoll	42 52 40.53	20 49 58.03	520	Miser	Hilly	White	Circle
ACC36	Mitrovic	Gushavc	42 52 28.48	20 49 21.98	521	Miser	Hilly	White	Oval
ACC37	Shtime	Belince	42 26 13.43	21 01 06.96	593	Miser	Hilly	White	Oval
ACC38	Shtime	Caraleva	42 27 04.32	20 58 08.82	654	Kolomboq	Hilly	White	Circle
ACC39	Shtime	Pjetershtice	42 28 45.95	20 57 30.66	690	Kolomboq	Hilly	White	Circle
ACC40	Peje	Loxhe	42 38 06.09	20 19 07.68	507	Miser	Hilly	White	Circle
ACC41	Peje	Vitimirice	42 41 35.89	20 18 56.36	533	Miser	Hilly	White	Circle
ACC42	Peje	Raushiq	42 37 10.60	20 18 10.25	546	Miser	Hilly	White	Circle
ACC43	Gjilan	Zheger	42 22 44.81	21 28 44.26	493	Kolomboq	Flat	Yellow	Triangle
ACC44	Gjilan	Prilepnice	42 30 21.26	21 30 53.88	554	Kolomboq	Hilly	White	Oval
ACC45	Gjilan	Bresalc	42 30 14.81	21 23 38.38	688	Kolomboq	Hilly	White	Oval
ACC46	Istog	Vrelle	42 45 58.26	20 24 27.70	466	Kolomboq	Flat	White	Oval
ACC47	Istog	Gjurakoc	42 43 29.78	20 28 57.69	440	Kolomboq	Flat	White	Circle
ACC48	Istog	Banje	42 42 47.99	20 23 29.91	470	Kolomboq	Flat	White	Circle
ACC49	Vushtrri	Balinca	42 33 07.29	20 46 53.88	624	Kolomboq	Hilly	White	Circle
ACC50	Vushtrri	Smrekonic	43 51 51.17	20 56 33.00	561	Kolomboq	Hilly	White	Triangle
ACC51	Vushtrri	Bukosh	42 48 49.27	20 56 44.80	517	Kolomboq	Flat	White	Oval
ACC52	Dragash	Shajne	42 03 59.16	20 39 30.41	1067	Kolomboq	Mountain	White	Circle
ACC53	Dragash	Brezna	42 07 18.54	20 38 34.82	961	Kolomboq	Mountain	White	Oval
ACC54	Dragash	Brodosane	42 06 33.83	20 43 05.39	1109	Kolomboq	Mountain	Yellow	Triangle
ACC55	Prizren	Caparc	42 16 54.05	20 46 09.94	383	Kolomboq	Flat	Yellow	Rectangle
ACC56	Prizren	Lutogllave	42 17 15.92	20 47 36.69	390	Kolomboq	Flat	White	Oval
ACC57	Prizren	Zhur	42 10 00.17	20 36 31.20	394	Kolomboq	Flat	White	Triangle
ACC58	Kamenic	Kolloleq	42 35 38.96	21 38 25.30	512	Miser	Hilly	White	Circle
ACC59	Kamenic	Dajkoc	42 33 27.20	21 39 09.56	465	Miser	Hilly	White	Triangle
ACC60	Kamenic	Shipashnic e poshtme	42 34 37.41	21 39 11.37	479	Miser	Hilly	White	Triangle

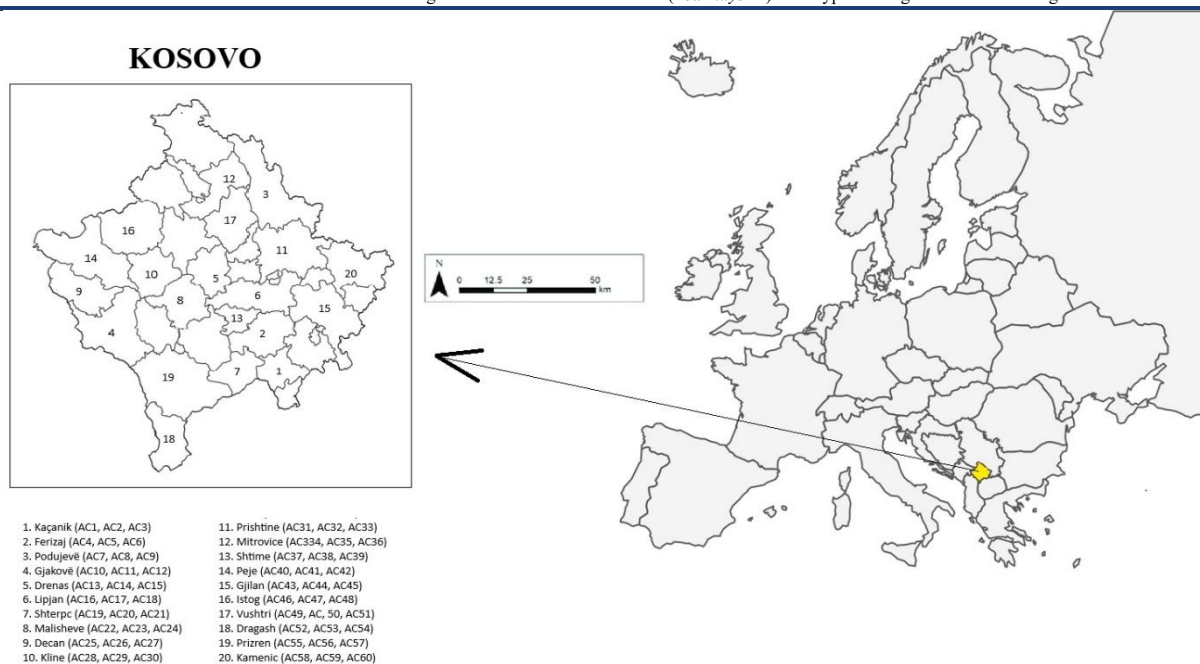


Figure. 1. Map of Kosovo indicating locations where maize landrace seeds were collected. Numbers indicate locations where maize genotypes were found

2.2. Experimental design

The experiment was conducted in two stages. First, the Department of Crop Science, Faculty of Agriculture, University of Prishtina identified cultivation sites of traditional maize genotypes across Kosovo. Seeds were then transported to the Department of Agricultural Biotechnology, Tekirdağ Namık Kemal University, Türkiye.

A randomized factorial design with three replications was implemented. The two experimental factors were: (1) genotype, and (2) osmotic stress levels induced by polyethylene glycol (PEG 6000) at 0%, 10%, 20%, and 30%. In total, 732 experimental units (Petri dishes), comprising 7320 seeds, were evaluated under controlled conditions

2.3. PEG 6000 treatment

Seeds were tested under PEG-induced osmotic stress, commonly used to simulate water deficit conditions under controlled environments. Although PEG does not mimic drought stress directly, it induces osmotic stress by lowering the water potential of the medium without being absorbed by plant tissues (Verslues et al., 1998).

Ten seeds per genotype were randomly selected, surface sterilized with 1% (v/v) sodium hypochlorite for 5 minutes and rinsed with distilled water. Seeds were placed in Petri dishes lined with filter paper and treated with PEG 6000 solutions (0%, 10%, 20%, and 30%). Dishes were incubated in a growth chamber at $22 \pm 2^\circ\text{C}$ with 50% relative humidity.

Germination was assessed daily for seven days, recording radicle emergence of ≥ 3 mm. Final germination percentage (G%) was calculated according to the International Seed Testing Association (ISTA, 1996). Root and shoot traits were measured as described in Table 2.

2.4. Data analyses

All measured parameters were recorded from three independent replications, each consisting of ten seeds per genotype and treatment. For each replication, germination and seedling traits were measured and averaged. The mean values $\pm$ standard error (SE) were calculated and used for statistical analysis.

Analysis of variance (ANOVA) at $P < 0.05$ was performed for all traits to assess the significance of treatment, and genotypes using IBM SPSS Statistics 2019 v26 analytical software. In addition to ANOVA, detailed mean

values of all measured traits for each genotype and treatment were used to enable direct comparison of osmotic stress responses among genotypes. Correlation heatmap and PCA analysis were performed using Python v3.10.12. For data processing and analysis, the Pandas library (v2.2.2) was used; for numerical operations and data manipulation, NumPy (v1.26.4) was utilized. The Seaborn library (v0.13.2) was applied for correlation matrix and graphical analysis, while Matplotlib (v3.8.0) for plotting and data visualization. The PCA and data standardization were performed using the Scikit-learn library (v1.5.2). To identify tolerant maize genotypes under osmotic stress, mean trait values were used to group genotypes with similar phenotypes using the "pheatmap" library in RStudio.

Table 2. Acronyms and descriptions of germination, root, and shoot traits evaluated under osmotic stress conditions

Traits	Description/Method
Germination (G%)	Calculated as $G\% = (n / N) \times 100$, where n represents the number of germinated seeds (radicle ≥ 3 mm) and N represents the total number of seeds sown (Tanaka-Oda et al., 2009).
Germination time (G/Day)	The average germination time was estimated using the formula given by Scott et al. (1984) as; $MGT\ days = \Sigma TiNi / S$, where Ti is the number of days since the start of the experiment, Ni is the number of seeds germinated per day, and S is the total number of germinated seeds (Scott et al., 1984).
Shoot length (ShL)	Maximum shoot length, measured from the root-shoot junction to the tip of the grain (cm).
Shoot fresh weight (ShFW)	The weight of the fresh shoot detached from the grain and immediately measured in grams.
Shoot dry weight (ShDW)	The weight of dried fresh shoots placed in a kraft bag and dried in an oven at $70 \pm 5^\circ\text{C}$ for 48 hours.
Root length (RL)	The maximum length of fresh roots, measured from the root-shoot junction to the tip of the longest root (cm)
Root fresh weight (RFW)	The fresh weight of roots from each plant separated from the seed and immediately measured in grams.
Root dry weight (RDW)	The weight of dried fresh roots placed in kraft bag and dried in an oven at $70 \pm 5^\circ\text{C}$ for 48 h to achieve complete drying, measured in grams.

3. Results and Discussion

3.1. Variation in germination, root, and shoot traits

The performance of the evaluated maize genotypes for germination and seedling traits is presented in Table 3.

Osmotic stress induced by PEG caused a clear and consistent negative effect on all measured traits. At 10% PEG, significant differences ($P < 0.05$) were observed for all traits except germination percentage (G%). With increasing PEG concentrations (20% and 30%), the inhibitory effect became more pronounced and statistically significant across all parameters. These results support prior observations that maize is more susceptible to drought stress compared to crops such as wheat and barley (Tarawneh et al., 2019; Thabet et al., 2018). A reduction in germination rate is often one of the earliest indicators of stress intolerance, leading to weak and unstable seedling development (Kaydan and Yagmur, 2008; Abdel-Ghani et al., 2015). In this study, G% declined significantly with increasing PEG concentrations, showing a mean reduction of 23.85% at 20% PEG and 63.35% at 30% PEG relative to the control. As in other crops, root development in maize is crucial for water and nutrient uptake. Stress-induced reductions were observed in root length (RL), root fresh weight (RFW), and root dry weight (RDW), reflecting genotypic sensitivity to water deficit (Fenta et al., 2014; Mwamlima et al., 2019). The magnitude of decline in RL was proportional to stress level, with mean differences of 2.23 cm, 6.87 cm, and 9.83 cm at 10%, 20%, and 30% PEG, respectively. Similarly, shoot traits (ShL, ShFW, ShDW) were reduced under drought, with ShL declining by 1.9 cm at 10% PEG and over 4 cm at higher stress levels. This

decline is mainly due to reduced water uptake by roots, which leads to a decrease in cell turgor under osmotic stress (Anjum et al., 2017). These findings are consistent with reports that root system strength is closely linked to drought response in cereals (Chloupek et al., 2010; Avramova et al., 2016; Ahmed et al., 2019).

Table 3. ANOVA results for germination, root, and shoot traits of maize genotypes under control and PEG-induced osmotic stress conditions

Trait	Control vs. 10% PEG		Control vs. 20% PEG		Control vs. 30% PEG	
	Mean difference	Significance	Mean difference	Significance	Mean difference	Significance
G%	5.24	ns	23.85	*	63.35	*
G (day)	-0.46	*	-0.48	*	1.33	*
RL	2.23	*	6.87	*	9.83	*
RFW	0.04	*	0.16	*	0.21	*
RDW	0.003	*	0.02	*	0.03	*
ShL	1.9	*	3.86	*	4.01	*
ShFW	0.09	*	0.19	*	0.22	*
ShDW	0.007	*	0.02	*	0.23	*

*=significant (P<0.05), ns = non-significant.

Several studies have used higher PEG concentrations (up to 40–50%) to simulate severe osmotic stress in maize and other cereals. Mustamu et al. (2023) reported significant declines in germination and seedling vigor beyond 30% PEG, marking the stress tolerance threshold. Similarly, Fatima et al. (2023) observed germination dropping below 50% at 30% PEG, aligning with our results. Thus, the 30% PEG used here represents a realistic yet severe stress level, suitable for differentiating genotypes without completely inhibiting growth. The chosen PEG levels (10%, 20%, 30%) are consistent with previous studies and effective for assessing stress responses in maize genotypes.

3.2. Trait relationships under PEG induced osmotic stress

Correlations among morphological and germination traits of maize genotypes-including mean germination time (G/day), germination percentage (G%), RL, ShL, RFW, RDW, ShFW, and ShDW under PEG-induced drought stress are shown in *Figure 2*. In general, most traits exhibited positive correlations within the same treatment (control or osmotic stress) but showed weaker or even negative relationships across treatments. Positive correlations (shown in red) reflect values approaching +1, indicating that as one trait increases, so does the other. Negative correlations (in blue) approach -1, indicating inverse relationship between traits. Under control conditions, strong positive correlations were observed between shoot and root traits, such as between ShDW and ShFW ($r = 0.84$), and RDW and RFW ($r = 0.73$), suggesting coordinated biomass development. Under stress conditions, although positive correlations remained, their strength decreased- e.g., RDW and RFW dropped to $r = 0.67$ -highlighting the impact of water stress on trait integration. Interestingly, certain trait correlations across environmental conditions became negative. For example, the correlation between ShL under drought and ShFW under control was $r = -0.10$, indicating divergent trait responses across environments. This suggests that plants may reduce shoot elongation as an adaptive mechanism under stress, while maximizing shoot biomass under non-stress conditions (Keerthi et al., 2025).

The very weak negative correlation ($r = -0.082$) between RFW under stress conditions and RDW under control conditions suggests limited association between these traits across environments. This likely reflects differential physiological responses: under osmotic stress, root tissues often have reduced water content due to stress, resulting in lower fresh biomass. In contrast, under well-watered conditions, plants accumulate more dry matter because of optimal nutrient and water availability, promoting continuous root development and biomass partitioning. These findings align with previous research in maize and other cereals where changes in correlation structure reflect genotype-by-environment interaction and stress-induced plasticity (Thabet et al., 2018; Ahmed et al., 2019; Badr et al., 2020).

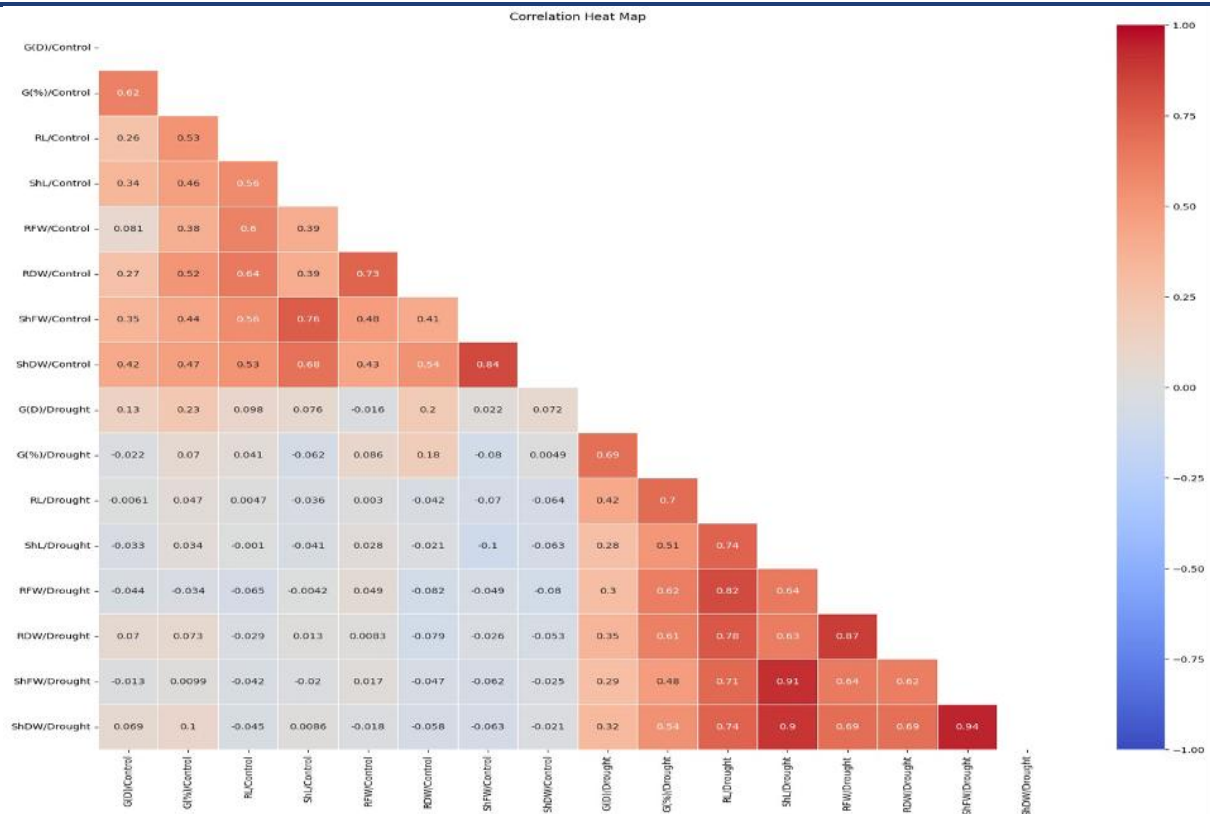


Figure 2. Heatmap illustrating Pearson correlation coefficients among germination and seedling traits of maize genotypes under control and PEG-induced osmotic stress conditions. Positive correlations are shown in red, while negative correlations are in blue. Strong trait associations (e.g., between ShFW and ShDW under control conditions, $r = 0.84$) are evident in the absence of stress, whereas reduced correlations under stress indicate disruption of trait coordination caused by osmotic stress

3.3. Principal component analysis

Principal component analysis (PCA) was conducted to explore the relationships among morphological and germination traits of maize genotypes under both control and osmotic stress conditions. The first two principal components, PC1 and PC2, accounted for 31.27% and 25.01% of the total variation, respectively, explaining a combined 56.28% of the total trait variability (Figure 3).

The PCA biplot revealed a clear separation between traits measured under control versus treatment conditions, indicating the substantial influence of water deficit on phenotypic expression. Under control conditions, biomass-related traits—including RFW, RDW, ShFW, and ShDW—clustered together, demonstrating their coordinated contribution to overall plant growth. These strong associations suggest that, in the absence of stress, plants allocate resources efficiently across root and shoot systems (Mohi-Ud-Din et al., 2021). Although PC1 and PC2 explain 56.28% of the total variation, the remaining ~44% may result from other biological processes not measured here, such as osmolyte accumulation, antioxidant enzyme activity, or other cellular stress responses, which can influence germination and seedling growth under osmotic stress.

However, under osmotic stress, these traits diverged in their contribution, reflecting a breakdown in coordinated biomass accumulation due to water limitation. For instance, the very weak negative correlation ($r = -0.082$) observed between RFW under osmotic stress and RDW under control suggests that these parameters are poorly associated across conditions. This likely reflects a shift in physiological priorities under drought, reduced water availability leads to decreased root water content and thus lower fresh biomass, whereas under control conditions, optimal water and nutrient availability supports greater dry matter accumulation and root development.

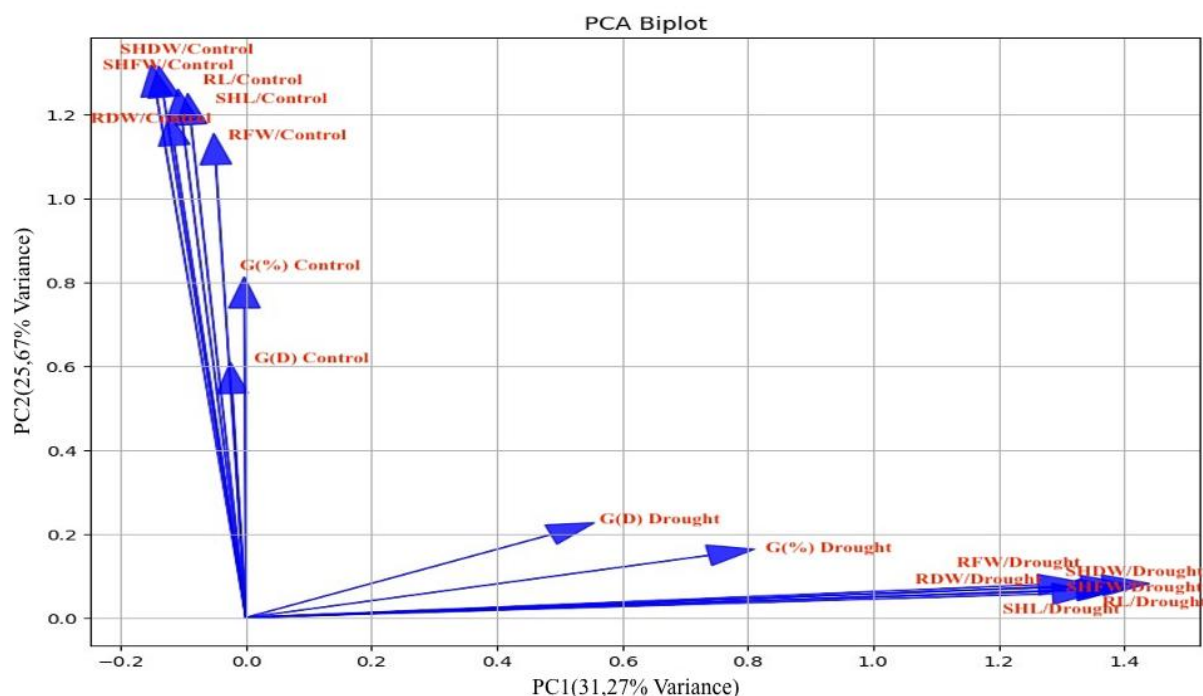


Figure 3. Principal component biplot showing the distribution of maize traits under control and PEG-induced osmotic stress conditions. The first two principal components (PC1 and PC2) explain 56.28% of the total variation. Traits measured under control conditions cluster together, while those under stress exhibit directional shifts, indicating altered physiological responses to water deficit

Likewise, G% and mean germination time (G/day) were positively associated under control conditions, indicating that faster germination supports successful seedling establishment. Under drought, however, these traits were redistributed in the PCA space, suggesting genotype-specific differences in germination response to water deficit. Shoot and root length (ShL and RL), which were also positively correlated under control conditions, displayed altered associations under drought stress, pointing to differential trait plasticity. These observations are consistent with previous work on maize hybrids, where PCA effectively differentiated stress-tolerant and sensitive genotypes based on water use efficiency (WUE) and related indices (Arisandy et al., 2017; Hefny et al., 2017).

3.4. Identification of tolerant maize genotypes under PEG-induced osmotic stress

To identify drought-tolerant and sensitive genotypes, 60 maize genotypes from Kosovo and one commercial genotype from Türkiye were evaluated for their responses to PEG-induced osmotic stress at concentrations of 10%, 20%, and 30%. The reference genotype Kompozit Şeker showed intermediate performance compared to the Kosovo genotypes and served as a comparative baseline, with its response under PEG treatments supporting the selection of contrasting genotypes, where tolerant genotypes exhibited superior germination and morphological stability, while sensitive ones showed significant reductions under stress conditions. Key root, shoot, and germination traits were used in a hierarchical clustering analysis to categorize accessions based on performance under stress conditions (Figure 4).

The clustering analysis grouped all accessions into three major clusters: G1 (22 accessions), G2 (24 accessions), and G3 (15 accessions). At lower PEG concentrations (10% and 20%), groups G1 and G2 showed comparable germination percentages (G%), whereas at 30% PEG, G1 exhibited significantly higher G% values than G2 and G3, suggesting superior tolerance. Mean germination time (G/day) followed a similar trend, with G1 maintaining faster germination rates at higher stress levels.

For root-related traits (RL, RFW, RDW), at 10% PEG, G2 outperformed G1 and G3, while at 20% PEG, G1 and G2 were similar but superior to G3. However, at 30% PEG, G1 showed consistently higher values across all root parameters, indicating strong root system resilience under severe drought stress. Similarly, shoot traits (ShL,

ShFW, ShDW) were better expressed in G1 at 10% and 20% PEG. At 30% PEG, shoot growth was reduced across all groups, although one accession in G1 (ACC4) maintained relatively higher shoot trait values. Correlations observed between shoot and root traits reinforce the importance of subsoil development in supporting above-ground growth under water-limited conditions. The root-to-shoot ratio proved informative for distinguishing tolerant genotypes.

The most drought-tolerant genotypes-ACC2, ACC7, ACC8, ACC9, ACC16, ACC18, and ACC46-were all placed in G1, with ACC16, ACC18, and ACC46 standing out as top performers based on combined germination, root, and shoot metrics. In contrast, genotypes such as ACC30, ACC31, and ACC51, grouped in G3, showed the poorest performance, particularly under 30% PEG stress, and are considered drought sensitive. Interestingly, ACC4 displayed a high root-to-shoot ratio at 20% and 30% PEG but not at 10%, suggesting condition-dependent adaptation. Accessions from G2 such as ACC20, ACC22, ACC48, ACC56, and ACC57 showed intermediate performance, especially under moderate stress levels.

These findings support the use of early-stage germination and seedling traits including germination percentage, mean germination time, root and shoot length, and biomass allocation-as reliable indicators for drought tolerance screening. The consistent performance of specific genotypes across multiple traits under stress conditions highlights their potential for use in future breeding programs targeting drought-prone environments.

3.5. Limitations and practical implications

Although the present study was conducted under controlled laboratory conditions using PEG to simulate osmotic stress, these findings need to be validated in field environments where multiple abiotic and biotic factors interact. Nonetheless, the consistent performance of several genotypes under PEG-induced stress strongly suggests their potential for real-world applications. The identified drought-tolerant genotypes-particularly ACC16, ACC18, and ACC46 represent promising candidates for integration into breeding programs aimed at developing drought-resilient maize varieties. Their use as parental material in hybrid development or marker-assisted selection may accelerate the deployment of climate-resilient cultivars, especially in drought-prone regions such as the Western Balkans.

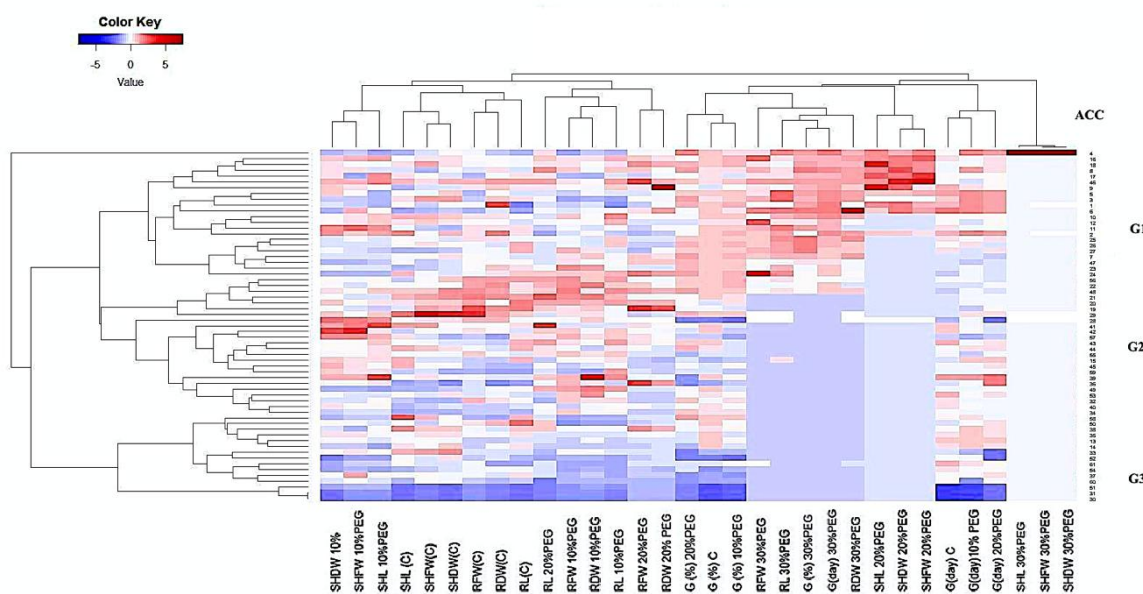


Figure 4. Grouping of maize genotypes was performed using hierarchical clustering based on the mean values of germination and seedling traits under PEG-induced osmotic stress. Each row represents an accession (as listed in Table 1), and each column shows the average value of traits measured at different PEG 6000 concentrations: G%, G/day, RL, ShL, RFW, ShFW, RDW, and SDW. Dark red indicates the highest-performing genotypes for each trait, while dark blue represents the lowest performers.

4. Conclusions

Osmotic stress during the germination stage has a pronounced impact on maize establishment, significantly affecting germination dynamics, root architecture, and shoot development. This study, representing the first systematic evaluation of maize genotypes from Kosovo under PEG-induced osmotic stress, revealed substantial variation in stress response across 60 genotypes and one reference genotype.

Among all genotypes tested, ACC16, ACC18, and ACC46 demonstrated exceptional tolerance to increasing levels of water deficit simulated by PEG. These genotypes consistently maintained high germination rates, stronger root and shoot systems, and resilience in biomass accumulation, particularly at 30% PEG-the most severe stress condition. Their performance not only underscores their adaptive potential but also highlights their suitability for direct integration into pre-breeding and hybrid development pipelines aimed at enhancing drought resilience in maize. In contrast, accessions ACC30, ACC31, and ACC51 exhibited high sensitivity to stress, as reflected by reduced germination and impaired seedling growth. Although less promising for breeding purposes, these genotypes offer valuable material for investigating the physiological and genetic basis of stress susceptibility.

The results of this study reinforce the utility of early-stage phenotyping using germination and seedling traits such as germination percentage, mean germination time, root/shoot length, and biomass traits as reliable indicators for screening osmotic stress tolerance. Furthermore, PCA and hierarchical clustering successfully distinguished tolerant and sensitive accessions, providing a strong framework for selection.

The high degree of variation observed among genotypes confirm the existence of valuable untapped genetic resources in Kosovo's maize germplasm. These findings contribute to global efforts in climate-resilient agriculture by identifying genotypes with intrinsic stress adaptation, which are especially relevant for semi-arid and drought-prone regions. Future research should focus on in-depth physiological, transcriptomic, and genome-wide analyses of the most tolerant and sensitive genotypes to uncover regulatory mechanisms of stress adaptation and accelerate their deployment in modern maize improvement programs. However, results obtained under controlled PEG-induced conditions should be validated through field trials, where multiple environmental factors interact, to confirm the stability and practical relevance of the identified drought-tolerant genotypes.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors

Authorship Contribution Statement

Concept: Jakupi, M., Demirbaş, S., Aliu, S.; Design: Jakupi, M., Demirbaş, S., Aliu, S.; Data Collection or Processing: Jakupi, M.; Statistical Analyses: Jakupi, M.; Literature Search: Jakupi, M., Demirbaş, S.; Writing, Review and Editing: Jakupi, M; Demirbaş, S; Yılmaz, S., Aliu, S.

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