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Performance and screening of bird pepper genotypes for drought tolerance using PEG

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Abstract The results showed that genotype differences and PEG concentration significantly affected all observed traits of bird pepper. The interaction between genotype and PEG concentration was significant only for number of leaves, leaf greenness, shoot fresh weight, shoot dry weight, root volume, root fresh weight, and root dry weight. Genotype A15 showed superior performance for most traits, except for number of leaves. Genotype A07 also performed well in both vegetative and generative traits, but showed lower performance in shoot fresh weight and root dry weight. Drought stress markedly reduced bird pepper growth, with severe stress (12% PEG) decreasing shoot fresh and dry weights by 96% and 94%, respectively. Based on the mean stress tolerance index (STI) values across all evaluated traits, genotypes A07, A15, A20, A28, and A41 were classified as drought tolerant. The integrated analysis of morphological, physiological, and STI parameters suggests that genotypes A15, A28, and A41 have strong potential as donor parents for breeding programs aimed at improving drought tolerance.

Keywords: Chili, Hydroponic, STI, Vegetative

Introduction

Chili peppers (*Capsicum* sp.), belonging to the Solanaceae family, are annual plants native to South America. The popularity of chili peppers has increased in recent years since the development of culinary dishes with different levels of spiciness. Their pungency is due to their capsaicin content. Chili peppers are used as a food spice and medicine due to their pungent flavor (Idrees *et al*., 2020). This plant has a wide adaptability, resulting in high genetic variation. Genetic diversity in chili peppers includes various shapes, colors, and capsaicin content (Martínez-Ávalos *et al*., 2018). Many cultivated species exist, but the most popular and widely used are *Capsicum annuum* and *Capsicum frutescens*. Fresh chilies are very rich in ascorbic acid, various carotenoid pigments, and a large number of phenolic compounds. Consumption of these bioactive compounds is associated with the prevention of cancer, cardiovascular disease, cataracts, diabetes, Alzheimer's disease, and Parkinson's disease (Shipra *et al*., 2024). The chemical compound that

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produces the spicy taste in chilies is capsaicin (Puvača, 2022). In general, fresh chilies contain 0.1-1.0% capsaicin, which can be found in the seeds, skin, placenta, and flesh (Sahid *et al*., 2020). Bird pepper, popular in Indonesia as cayenne pepper, contains higher levels of capsaicin than red chilies (Alghamdi *et al*., 2025).

According to BPS (2024), chili pepper productivity in Indonesia reached only 8.2 tons/ha in 2023. This production is considerably lower than that of Ghana, where, under a similar tropical climate, productivity reached 12.47 tons/ha (Inusah *et al*., 2015). This low productivity is attributed to the fact that most bird pepper cultivation in Indonesia takes place in dryland areas (Hikmat *et al*., 2022). In dryland agriculture, irrigation often relies on rainfall, which can expose plants to drought conditions.

Drought stress affects stomatal conductance, enzyme secretion, and the accumulation of osmotic adjusting substances in leaves (He *et al*., 2020). Stomatal conductance has been shown to have a positive correlation with crop yield under water deficit conditions (Malika *et al*., 2019). Consequently, drought stress causes a reduction in photosynthetic activity and inhibits plant growth (Widuri *et al*., 2020). Chili yield losses under drought stress of 50% field capacity can reach 46% (Suwignyo *et al*., 2017). According to Mardani *et al*. (2017), the critical threshold for water deficit in chili plants is around 20% field capacity.

Bird peppers' response to drought varies depending on genetic factors (Kopta *et al*., 2020). Several mechanisms exist for drought tolerance. Drought avoidance prevents plants from being exposed to drought stress through early growth induction. This mechanism provides resistance by increasing water uptake or reducing water loss. Tolerant genotypes maintain higher levels of cell membrane integrity, chlorophyll stability, osmolyte accumulation, and lipid peroxidation under drought conditions (George and Sujatha, 2019), thus maintaining physiological processes and producing higher economic yields (Aslam *et al*., 2015). Therefore, drought tolerance is an important characteristic of chili peppers to increase production in areas with abiotic stress (Sahitya *et al*., 2019). Various traits used as indicators to evaluate plant drought resistance include root characteristics, leaf characteristics, osmotic adjustment capacity, water potential, ABA content, and cell membrane stability (Fang and Xiong, 2015).

Conducting field experiments on water stress is often challenging because drought frequently interacts with other abiotic stresses, making results difficult to interpret (George and Sujatha, 2019). An alternative approach is to induce water stress through polyethylene glycol (PEG) solutions for germplasm screening. Polyethylene glycols with a molecular mass of 6000 or above is a non-ionic, water-soluble polymers that are not expected to penetrate intact plant tissues. Instead, it reduces osmotic potential, thereby limiting water absorption by the roots. A controlled and measured artificial water stress environment using PEG is suitable for identifying tolerant genotypes.

PEG6000 is widely used by researchers to screen for drought-tolerant chili peppers. Soaking seeds with 20% PEG6000 inhibited germination in all chili pepper species. Significant differences were observed between landraces, with more domesticated types from intensive cultivation systems germinating faster than wild-types (Bernau *et al*., 2020). Wang *et al.* (2024) reported that simulated drought stress with 10% PEG6000 resulted in inhibition of seedling growth, cell membrane damage, and increased total phenol and flavonoid content of cucumber seedlings. PEG6000-induced water stress in vitro in chili peppers also resulted in a decrease in dry matter in shoots and roots, a decrease in stomatal density, and stomatal midpoint width (Rodrigues *et al*., 2025).

The objective of this study was to evaluate the performance and to screen bird pepper germplasms for drought tolerance as sources of gene(s) controlling drought-tolerance.

Materials and methods

This study used a split-plot design, with the main plot determined by PEG_{6000} concentration and the subplots determined by bird pepper genotype. Twenty-three bird pepper accessions (Table 1) with high variability (Rustikawati *et al*., 2025) from several regions in Indonesia and introduced varieties from various countries were evaluated for their performance under water stress conditions. Three levels of water stress (0% PEG as control, 6% PEG, and 12% PEG) were used to evaluate the bird pepper plants up to four weeks after treatment.

Three-week-old seedlings were transferred to a hydroponic system filled with AB mix nutrient solution. Each experimental unit consisted of three seedlings. One week later, they were transferred to a nutrient solution supplemented with PEG according to the treatment. Plants were supported with styrofoam to grow above the solution. Drought stress was applied periodically at one-week intervals for four weeks of treatment. Control plants were maintained in AB mix nutrient solution for the same period. Measurement of observation variables was recorded at the end of the stress treatment or in the fourth week. Growth parameters observed included plant height, stem diameter, number of leaves, leaf greenness, leaf area, shoot fresh weight, shoot dry weight, root length, root fresh weight, and root dry weight. The dry weight of shoots and roots was obtained by oven-drying the samples at a temperature of 70°C for 48 hours, then weighing them with a digital scale until a constant weight was obtained. Leaf greenness was measured using a SPAD chlorophyll meter and expressed as SPAD values.

To determine the effect of treatment on the observed variables, an analysis of variance (ANOVA) was performed. Meanwhile, to compare the average values between treatments, a further LSD test was carried out at α=5%. Accession tolerance to drought stress is calculated using the stress tolerance index (STI) based on the decrease in performance relative to normal conditions. The stress tolerance index has been used successfully to identify chili genotypes with the best response under drought stress conditions. The value of STI was calculated by the formula of (Ali and El-Sadek, 2016).

$$\text{STI} = \frac{(Yp.Ys)}{(\bar{Y}p)^2}$$

where STI, Ys, Yp and $\overline{\text{Yp}}$, were Stress Tolerance Index, the observed value in stress condition, the observed value in non-stress condition, the mean value over all genotypes evaluated in non-stress condition.

Tolerance was categorized in relative over all genotype under study and classified into three classes, i.e. tolerance, moderately tolerance, and sensitive, respectively. The class interval was calculated by the following formula (Rustikawati *et al*., 2024):

$$\text{Interval} = \frac{(STIh - STIl)}{3}$$

Table 1. List of genotypes and their origin

Genotype	Origin	Genotype	Origin
A03	Lampung, Indonesia	A28	Bengkulu7, Indonesia
A04	Taiwan1	A29	Bengkulu8, Indonesia
A07	Bangka, Indonesia	A31	Rawit Bangkok, Indonesia
A10	Comercial Variety CR01	A33	Manna 1, Indonesia
A11	Temangung1, Indonesia	A34	Manna 2, Indonesia
A13	Bengkulu3, Indonesia	A35	Manna 3, Indonesia
A15	Temangung3, Indonesia	A37	Manna 5, Indonesia
A18	Taiwan3	A38	Manna 6, Indonesia
A20	Thailand1	A39	Manna 7, Indonesia
A25	Bogor2, Indonesia	A41	Manna 9, Indonesia
A26	Magelang, Indonesia	A43	Manna 11, Indonesia
A27	Bengkulu 6, Indonesia		

Results

Performance of bird pepper genotypes under drought stress

Result showed significant effects of PEG concentration and genotype on all observed traits, while the interaction between the two was only significant for plant height, stem diameter, leaf area and root length. The growth response of 23 bird pepper genotypes showed significant variation across all measured traits (Table 2).

The evaluated bird pepper genotypes displayed distinct variations in vegetative growth performance. In terms of plant height, genotypes A07, A15, A18, A28, and A41 exhibited superior growth. For stem diameter, genotypes A07, A10. A15, A18, A25, A28, A29, A39, and A41 showed better performance. Although genotype A20 produced the highest number of leaves, it performed poorly in other growth parameters compared to other genotypes. Leaf greenness did not vary significantly among genotypes, even though some exhibited relatively higher SPAD values, which ranged from 28.17 (A38) to 38.80 (A15). Genotypes with relatively larger leaf area included A07, A13, A15, A28, and A39. Overall, considering the five vegetative growth traits, genotypes A07 and A15, demonstrated consistently superior performance.

Three weeks after treatment, PEG application significantly reduced all growth parameters compared to the control. Severe stress at 12% PEG reduced plant height by 65%, stem diameter by 49%, number of leaves by 83%, and leaf area by 51%. Leaf greenness also decreased under stress, from 40.03 SPAD units in the control to 27.42 SPAD units at 12% PEG.

Table 2. Growth of 23 Genotypes of bird pepper at 3 weeks after transplanting

	Plant height[1] (cm)		Stem diameter[1] (mm)		Number of leaves[1]		Leaf greenness[1]		Leaf area[1] (cm^2)	
Genotypes										
A03	17.40	e-g	2.51	c-g	5.91	b-d	36.56	a-c	17.22	e-k
A04	11.25	kl	2.60	b-f	5.14	b-h	30.27	h-j	13.63	j-l
A07	20.21	a-d	2.85	a-c	5.60	b-f	37.14	ab	28.41	a
A10	17.72	c-g	2.76	a-e	5.89	b-d	32.62	d-h	14.72	i-l
A11	12.70	i-l	2.06	hi	4.09	g-i	33.39	c-h	10.89	l
A13	17.51	d-g	2.63	b-f	4.24	f-i	32.95	c-h	27.87	ab
A15	20.45	a-c	2.78	a-e	5.47	b-g	38.80	a	23.13	a-d
A18	20.84	a	2.93	ab	6.20	bc	33.89	b-h	15.71	h-l
A20	11.06	l	1.81	i	14.98	a	35.83	a-e	3.05	m
A25	15.17	g-i	2.79	a-d	4.40	e-i	34.23	b-g	22.37	b-f
A26	11.30	kl	2.43	d-g	4.33	e-i	30.33	h-j	17.06	f-k
A27	12.24	j-l	2.17	g-i	4.67	d-i	34.77	b-g	11.94	kl
A28	20.92	a	2.89	ab	4.44	e-i	34.64	b-g	26.37	a-c
A29	18.01	b-e	2.66	a-f	3.94	hi	35.56	a-f	22.74	b-e
A31	15.23	f-i	2.37	f-h	4.27	e-i	28.58	ij	16.99	f-k
A33	15.26	f-i	2.32	f-h	4.26	f-i	31.75	g-j	18.05	d-j
A34	15.85	e-h	2.61	b-f	5.49	b-g	32.33	e-h	20.95	c-h
A35	13.80	h-k	2.18	g-i	4.82	c-i	31.80	g-j	16.05	g-l
A37	15.87	e-h	2.42	e-h	6.48	b	35.91	a-e	10.89	l

A38	14.61	h-j	2.31	f-h	5.56	b-f	28.17	j	15.87	h-l
A39	17.93	b-f	3.02	a	4.62	d-i	37.29	ab	23.46	a-d
A41	20.45	ab	2.83	a-c	5.69	b-e	36.22	a-d	21.57	c-g
A43	15.47	e-h	2.51	c-g	3.57	i	31.88	f-i	19.38	d-h
PEG (%)										
0	26.97	a	3.68	a	10.48	a	40.03	a	26.96	a
6	11.95	b	2.07	b	3.88	b	33.62	b	14.17	b
12	9.50	c	1.87	b	1.81	c	27.42	c	13.44	b
Genotype X PEG										
	ns		ns		**		*		ns	

1/ Means in the same column within Genotypes or PEG followed by the same lowercase letters are not significantly different according to LSD ($P \leq 0.05$). ns= not significantly different, ** was significantly different at α=1%, * was significantly different at α=5% according to ANOVA.

The shoot and root characteristics of the evaluated bird pepper genotypes exhibited considerable variation, suggesting the influence of genetic factors. Root length showed the narrowest range, from 11.08 cm in A04 to 17.29 cm in A15. Genotype A41 demonstrated better shoot growth, whereas A07 displayed better root development. Overall, genotype A15 consistently showed the best performance among all tested bird peppers (Table 3).

Drought stress significantly reduced shoot and root components of the bird pepper genotype three weeks after treatment (Table 3). PEG application drastically reduced shoot biomass and all root parameters. Severe stress at 12% PEG reduced shoot fresh and dry weight by 96% and 94%, respectively. Meanwhile, root fresh and dry weight decreased by 88% and 87%, respectively. Root volume decreased by 50%. However, root length was less affected by increasing drought stress. The average root length was 10.50 cm and 10.86 cm at 6% PEG and 12% PEG, respectively.

Table 3. Shoot and root components of bird pepper at three weeks after PEG treatment

	Shoot fresh weight[1] (g)		**Shoot dry weight[1] (g)**		**Root length[1] (cm)**		**Root volume[1] (mm^3)**		**Root fresh weight[1] (g)**		**Root dry weight[1] (g)**	
Genotypes												
A3	3.58	d-h	0.58	b-g	13.32	b-f	1.67	e-h	0.84	d-g	0.07	c
A4	5.94	ab	0.64	b-d	11.08	f	1.10	h	1.29	b-d	0.09	bc
A7	4.37	c-f	0.69	bc	16.57	ab	2.72	a-c	1.35	bc	0.12	a-c
A10	4.26	c-g	0.63	b-d	12.93	c-f	1.79	e-g	1.06	b-g	0.09	c
A11	1.71	j	0.27	hi	15.31	a-e	1.67	e-h	0.60	g	0.05	c
A13	4.66	b-e	0.60	b-e	16.14	a-c	2.06	de	1.32	bc	0.11	a-c
A15	6.15	a	0.93	a	17.29	a	2.89	ab	1.88	a	0.16	ab

A18	4.52	c-f	0.48	c-h	11.77	f	2.50	b-d	0.97	b-g	0.18	a
A20	1.60	j	0.21	i	14.20	a-f	1.56	e-h	0.74	e-g	0.06	c
A25	2.79	h-j	0.37	f-i	11.80	f	1.94	d-f	0.92	c-g	0.07	c
A26	3.66	d-h	0.39	e-i	12.68	d-f	1.67	e-h	1.13	b-e	0.09	bc
A27	1.66	j	0.27	hi	12.98	c-f	1.39	f-h	0.59	g	0.05	c
A28	4.25	c-g	0.66	bc	16.96	a	3.28	a	1.40	b	0.12	a-c
A29	3.94	d-h	0.69	bc	16.54	ab	1.94	d-f	1.17	b-e	0.10	bc
A31	2.69	h-j	0.28	hi	16.07	a-d	1.78	e-g	0.70	e-g	0.04	c
A33	2.32	ij	0.27	hi	16.16	a-c	1.71	e-h	0.65	fg	0.05	c
A34	3.06	g-i	0.36	f-i	15.68	a-e	1.78	e-g	0.90	c-g	0.07	c
A35	3.46	e-i	0.46	c-h	14.16	a-f	1.71	e-h	1.00	b-g	0.07	c
A37	3.33	f-i	0.42	d-h	16.16	a-c	2.08	c-e	1.07	b-f	0.09	c
A38	3.43	e-i	0.36	f-i	12.22	ef	1.17	gh	0.64	fg	0.06	c
A39	4.86	b-d	0.58	b-f	16.67	ab	3.02	ab	1.34	bc	0.09	bc
A41	5.44	a-c	0.72	ab	15.94	a-d	2.06	de	1.31	b-d	0.09	bc
A43	2.86	h-j	0.35	g-i	14.12	a-f	1.33	f-h	0.90	c-g	0.07	c
PEG (%)												
0	9.84	a	1.216	a	22.57	a	2.66	a	2.34	a	0.19	a
6	0.81	b	0.17	b	10.86	b	1.83	b	0.47	b	0.04	b
12	0.38	b	0.074	b	10.50	b	1.35	c	0.29	b	0.02	b
Genotype X PEG												
	**		**		ns		**		**		*	

1/ Means in the same column within Genotypes or PEG followed by the same lowercase letters are not significantly different according to LSD (P≤0.05). ns= not significantly different, ** was significantly different at α=1%, * was significantly different at α=5% according to ANOVA.

Genotype variation was evident, with significant genotype × PEG interactions for shoot fresh and dry weight, root volume, root fresh weight, and root dry weight. In particular, genotypes A15, A28, A39, and A41 exhibited higher shoot and root biomass even under stress. A15 was superior in all shoot and root component parameters. In contrast, genotypes A11, A20, A27, and A33 showed low biomass.

Tolerance of bird pepper genotypes to drought stress

The stress tolerance index calculated from morphological and biomass traits revealed substantial variation among the 23 bird pepper genotypes in response to PEG-induced osmotic stress (Table 4). Significant differences in STI values were observed across various traits. Plant height, leaf number, leaf greenness, and root dry weight had STI values above 0.5, while leaf area and root volume even exceeded 1.00. These traits determine the average STI value. For example, genotypes with high root volume STI such as A07 (1.13), A15 (1.21), A28 (2.10), and A41 (1.06), were grouped as tolerant because they had high average STI values. Genotype A20 was also classified as tolerant (average STI of 0.59), due to its very high leaf number STI (3.87). A20 typically exhibits a bushy growth habit with numerous small leaves; therefore, despite reductions under osmotic stress, its leaf number remained higher than the population average. Eight other genotypes with an average STI of 0.41–0.58 were categorized as moderately tolerant.

The tolerant genotypes (mean STI ≈ 0.58–0.75) comprised A07, A15, A20, A28, and A41, all of which sustained better vegetative growth and biomass accumulation under PEG stress. Conversely, nine genotypes A04, A10, A11, A26, A27, A31, A33, A35, and A43 were categorized as sensitive (mean STI < 0.41), as reflected by pronounced declines in shoot dry weight, root dry weight, and leaf area.

Discussion

Chili pepper is a vegetable crop plant that can grow well in various regions in Indonesia (Herison *et al*., 2017). However, its growth and yield are greatly decreased when it is grown in drought land. Sensitiveness of the plant to environmental stress greatly depends on the phase of plant growth and stress duration. The fast-growing vegetative phase is more sensitive than the seedling and generative phases (Rustikawati *et al*., 2023).

PEG 6000 is widely used by researchers to simulate drought stress due to its osmotically active properties. When dissolved in water, PEG 6000 binds water molecules, thereby decreasing the amount of free water available to plant roots. As a result, plants experience conditions resembling drought, where water uptake becomes restricted. Increasing the concentration of PEG and the duration of exposure intensifies the level of stress and leads to greater plant damage (Qi *et al*., 2023). In this study, 12% PEG stress resulted in decreased growth and biomass of all tested chili genotypes. The same PEG concentration was also successfully used for chili seedling selection (Gangotri *et al*., 2022). Disruption of plant physiological processes, including reduced chlorophyll content, was one factor responsible for this decline (Daningsih, 2024). Growth impairment occurs because high PEG concentrations in the media trigger a decrease in leaf water potential, thereby reducing the relative leaf water content and, consequently, plant turgor. Under these conditions, plants close their stomata and limit CO_2 assimilation to reduce the rate of photosynthesis (Chen *et al*., 2022); (Ashraf and Harris, 2013). Sensitive plants exhibit wilting symptoms due to decreased plant turgor. Potassium ions (K+) play a role in maintaining intracellular turgor. These ions are transported into plant cells against the concentration gradient via the K+ transporter (Fang and Xiong, 2015). Therefore, young leaves are more sensitive and easily wilt.

Table 4. Results of stress tolerance index calculations on the tested genotypes

Genotypes	Plant height	Stem diameter	Number of leaves	Leaf greenness	Leaf area	Shoot fresh weight	Shoot dry weight	Root length	Root Volume	Root fresh weight	Root dry weight	Average	Tolerance[1]
A03	0.51	0.56	0.33	1.00	0.51	0.13	0.29	0.40	0.46	0.22	0.22	0.42	M
A04	0.21	0.63	0.34	0.62	0.30	0.06	0.08	0.27	0.14	0.06	0.10	0.26	S
A07	0.66	0.67	0.32	0.89	1.29	0.12	0.23	0.57	1.13	0.28	0.28	0.59	T
A10	0.56	0.66	0.48	0.82	0.34	0.11	0.18	0.33	0.60	0.12	0.14	0.39	S
A11	0.26	0.38	0.23	0.79	0.17	0.03	0.08	0.46	0.56	0.08	0.11	0.29	S
A13	0.50	0.64	0.26	0.80	0.94	0.10	0.18	0.47	0.73	0.21	0.22	0.46	M
A15	0.73	0.66	0.34	1.11	0.93	0.14	0.28	0.66	1.21	0.37	0.45	0.63	T
A18	0.77	0.71	0.38	0.94	0.35	0.08	0.11	0.30	1.07	0.15	0.52	0.49	M
A20	0.21	0.31	3.87	0.90	0.01	0.02	0.04	0.47	0.39	0.16	0.17	0.59	T
A25	0.42	0.73	0.26	0.91	0.86	0.07	0.10	0.32	0.77	0.21	0.19	0.44	M
A26	0.18	0.43	0.22	0.58	0.50	0.04	0.04	0.34	0.52	0.14	0.22	0.29	S
A27	0.24	0.47	0.30	0.87	0.23	0.03	0.07	0.35	0.25	0.09	0.08	0.27	S
A28	0.76	0.76	0.29	0.87	1.23	0.20	0.37	0.67	2.01	0.54	0.58	0.75	T
A29	0.55	0.62	0.21	0.87	0.92	0.09	0.20	0.66	0.75	0.30	0.26	0.49	M
A31	0.37	0.50	0.21	0.63	0.38	0.03	0.04	0.58	0.44	0.08	0.04	0.30	S
A33	0.42	0.43	0.29	0.81	0.57	0.06	0.08	0.57	0.43	0.11	0.11	0.35	S
A34	0.44	0.56	0.39	0.82	0.69	0.06	0.09	0.57	0.56	0.19	0.16	0.41	M
A35	0.32	0.44	0.21	0.71	0.39	0.05	0.09	0.51	0.52	0.14	0.10	0.31	S
A37	0.48	0.54	0.52	1.01	0.19	0.09	0.16	0.63	0.90	0.28	0.32	0.47	M
A38	0.35	0.43	0.26	0.60	0.33	0.02	0.04	0.35	0.21	0.06	0.06	0.25	S
A39	0.52	0.73	0.24	0.96	0.85	0.13	0.20	0.65	1.18	0.24	0.20	0.54	M
A41	0.69	0.66	0.41	1.13	0.77	0.22	0.37	0.61	1.06	0.35	0.41	0.61	T
A43	0.37	0.50	0.11	0.76	0.57	0.03	0.05	0.43	0.31	0.13	0.15	0.31	S

1/ T=tolerance, M= moderately tolerance, S=sensitive

Drought stress significantly reduced plant fresh weight, chlorophyll content, and root vitality. Conversely, the contents of soluble protein, proline, and malondialdehyde increased gradually with increasing PEG concentration and stress duration. Furthermore, drought also increased the activity of antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT), reaching peak values on the sixth day (Qi *et al.,* 2023)

The results of shoot and root component analyses also showed different genotype responses. Although most genotypes experienced a drastic reduction in shoot biomass under 6% and 12% PEG, tolerant accessions such as A15, A28, and A41 maintained higher shoot dry weight and root volume. This suggests efficient osmotic adjustment in these genotypes. Root traits, particularly root length and volume, are critical for water acquisition under stress, and the superior root growth of A28 and A39 under PEG indicates an adaptive root system architecture, consistent with earlier findings that robust root traits enhance drought tolerance in pepper and related crops (McNamara, 2021). Even alfalfa varieties exhibit increased root length under drought stress (Wang *et al*., 2025).

Drought stress causes growth impairment in surviving plants. Plant responses vary depending on genetic factors. Each genotype has a distinct response to PEG-induced osmotic stress conditions, as reflected by all observed variables. Therefore, determining tolerance levels based on one or a few variables is inaccurate. Genotype evaluation of environmental stress has been developed by many researchers (Ali and El-Sadek, 2016). Various methods to determine plant tolerance and used STI confirmed by other methods. Drought tolerance is conditioned by polygenes whose expression depends on genotype, environment, and genotype × environment interactions. Therefore, integrating multiple variables as selection criteria helps identify drought-adapted and climate-smart crop varieties (Mutanda *et al*., 2024). In this study, 13 vegetative and generative variables were used to determine STI values.

The calculated stress tolerance index values further validated these observations. Based on the average STI, A07, A15, A20, A28 and A41 were classified as tolerant, while eight genotypes were moderately tolerant. Sensitive genotypes, including A04, A10, A11, A26, A27, A31, A33, A35, A38, and A43, exhibited poor performance across most traits. Interestingly, A20 exhibits extreme sensitivity to shoot-related traits, yet maintains a high leaf count under stress. Genetically, G20 has numerous, small, and slightly thick leaves. This suggests a possible escape mechanism, rather than true physiological tolerance.

PEG-induced stress effectively differentiated tolerant and sensitive genotypes, supporting its utility as a screening method for early-stage drought evaluation in Bird pepper. Combined analysis of morphological, physiological, and STI data indicated that genotypes A15, A28, and A41 have potential as donor parents in breeding programs for drought tolerance. Their ability to maintain biomass, leaf greenness, and root development under stress suggests a comprehensive tolerance mechanism. Future studies should integrate physiological and molecular analyses, such as osmolyte accumulation, antioxidant enzyme activity, and drought-responsive gene expression, to elucidate the mechanisms underlying tolerance, thereby facilitating marker-assisted selection and accelerating the development of drought-tolerant bird pepper cultivars.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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