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Research Article

Identification of adaptive traits and mechanisms in tropical soybean lines under drought stress from flowering to pod formation[1]

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ABSTRACT

Drought stress reduces soybean [*Glycine max* (L.)] yield stability in tropical environments, particularly from flowering to pod formation. This study aimed to identify adaptive traits and underlying mechanisms of tropical soybean lines under water-limited conditions, as well as to assess the selection potential under non-stress environments. Twenty-five high-yield soybean genotypes carrying the *long juvenile* (*lj*) and *pod dehiscence1* (*pdh1*) genes were selected and evaluated under normal (± 80 % of the field capacity - FC) and water-deficit (± 50 % FC) conditions. Two potentially tolerant (which demonstrated a higher capacity to maintain physiological performance and metabolic stability under water deficit) and two susceptible genotypes were identified for further in-depth evaluation. Proline and malondialdehyde (MDA) were negatively and significantly correlated with grain yield under normal and water-deficit conditions. Both characters showed high broad-sense heritability across the environments, indicating strong genetic control and stable phenotypic expression. The simultaneous increase of proline and MDA is associated with reduced grain yield, indicating a stress-induced metabolic trade-off in which carbon and energy are redirected from reproductive development toward cellular protection mechanisms in the soybean genotypes.

KEYWORDS: *Glycine max* L., drought tolerance, osmotic adjustment, oxidative stress.

RESUMO

Identificação de características adaptativas e mecanismos em linhagens tropicais de soja sob estresse por seca do florescimento à formação de vagens

O estresse hídrico reduz a estabilidade da produtividade da soja [*Glycine max* (L.)] em ambientes tropicais, particularmente do florescimento à formação de vagens. Objetivou-se identificar as características adaptativas e os mecanismos subjacentes de linhagens tropicais de soja sob condições de limitação hídrica, bem como avaliar o potencial de seleção em ambientes sem estresse. Vinte e cinco genótipos de soja de alta produtividade portadores dos genes *long juvenile* (*lj*) e *pod dehiscence1* (*pdh1*) foram selecionados e avaliados sob condições normais (± 80 % da capacidade de campo - CC) e de déficit hídrico (± 50 % da CC). Foram identificados dois genótipos potencialmente tolerantes (que demonstraram maior capacidade de manter o desempenho fisiológico e a estabilidade metabólica sob déficit hídrico) e dois suscetíveis para avaliações mais aprofundadas. Prolina e malondialdeído (MDA) apresentaram correlação negativa e significativa com a produtividade de grãos, tanto em condições normais quanto sob déficit hídrico. Ambos os caracteres apresentaram alta herdabilidade em sentido amplo nos ambientes, indicando forte controle genético e expressão fenotípica estável. O aumento simultâneo de prolina e MDA está associado à redução da produtividade de grãos, indicando uma compensação metabólica induzida pelo estresse, no qual carbono e energia são redirecionados do desenvolvimento reprodutivo para mecanismos celulares de proteção nos genótipos de soja.

PALAVRAS-CHAVE: *Glycine max* L., tolerância à seca, ajuste osmótico, estresse oxidativo.

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] and other cultivated crops frequently experience drought stress as a major abiotic constraint limiting yield stability, especially in tropical regions with irregular rainfall (Alshaharni et al. 2025). Climate change, which increases the frequency and intensity of drought events, further intensifies the risk of yield failure, especially in rainfed agricultural systems (Anda et

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al. 2019). Therefore, the development of soybean varieties adapted to water-limited conditions represents a strategic priority for supporting sustainable production.

A genotype can be considered tolerant to drought stress when it can maintain growth and yield under stressed conditions through the presence of genetically controlled biological and physiological mechanisms (Spormann et al. 2026). Adaptive mechanisms to drought stress include escape, avoidance, tolerance, and the ability to recover after stress (recovery) (Alshaharni et al. 2025). Plant responses to drought stress are complex, involving morphological, physiological, and biochemical changes (Spormann et al. 2026). These genotypic variations in responses reflect differences in adaptive capacity to water limitation and provide an important basis for breeding programs aimed at developing varieties with improved yield stability and performance under drought-stress environments (Anda et al. 2019).

The flowering to pod formation stage in soybean is a highly sensitive developmental phase to drought stress (Desclaux et al. 2000). During this period, water deficit can disrupt flower initiation and maintenance, reduce fertilization success, increase flower and pod abortion, as well as suppress seed filling. Consequently, yield reduction is often more strongly determined by disturbances during the reproductive phase than the vegetative phase (Anda et al. 2019). The adaptive traits expressed during the flowering to pod formation stage play a crucial role in determining the plant's ability to maintain yield under drought stress conditions (Wang et al. 2024).

Various morphological, physiological, and biochemical traits contribute to the maintenance of cellular function and plant metabolism under water-deficit conditions (Wang et al. 2024). However, the expression of these adaptive traits is often environment-specific and becomes more pronounced when plants are subjected to stress (Saleem et al. 2022). This condition poses a challenge in breeding programs, as direct selection under stress environments is not always feasible, requires high costs, and may result in inconsistent data due to extreme environmental variability (Alshaharni et al. 2025). The situation indicates the need for approaches capable of identifying adaptive traits and tolerance mechanisms that are physiologically relevant under drought stress but stable and detectable in normal conditions. Therefore, this study aimed to identify adaptive traits and underlying mechanisms of tropical soybean lines in response to drought stress during the flowering to pod formation stage.

MATERIAL AND METHODS

The experiment was conducted in the screen house of the National Research and Innovation Agency (NRIA), in Bogor, Indonesia, from November 2023 to April 2024, using a randomized complete block design with three replications. Treatments were arranged in a factorial design with two factors, namely soybean genotype and water availability.

The genotype factor consisted of 25 genotypes (Table 1) selected for high yield and medium to large seed size carrying the *long juvenile* (*lj*) gene, renowned for extending the vegetative phase, and the *pod dehiscence1* (*pdh1*) gene, which facilitates resistance to pod shattering. The water availability factor consisted of well-watered conditions (D0;

Table 1. List of the 25 genotypes used in the study.

Genotypes	Background	Note	*lj* gene	*pdh1* gene
Gro	Gro	CV	−	−
Anj	Anj	CV	−	+
Der	Der	CV	−	−
Mel	Mel	CV	+	+
Ver	Ver	CV	+	+
AP17	Gro × Mel	SL	+	−
AP21	Gro × Mel	SL	+	+
AP16	Gro × Mel	SL	+	−
AP19	Gro × Mel	SL	+	−
AP33	Gro × Mel	SL	+	−
AP38	Gro × Mel	SL	+	−
AP28	Gro × Mel	SL	+	+
AP32	Gro × Mel	SL	+	−
AP35	Gro × Mel	SL	+	−
AP18	Gro × Mel	SL	+	+
AP26	Gro × Mel	SL	+	+
AP3	Gro × Mel	SL	+	−
AP29	Gro × Mel	SL	+	+
AP30	Gro × Mel	SL	+	−
AP46	Gro × Ver	SL	+	+
AP49	Gro × Gly	SL	+	−
AP24	Gro × Mel	SL	+	−
AP9	Gro × Mel	SL	+	−
AP39	Gro × Mel	SL	+	−
AP58	Gro × Par	SL	+	−

Gro: Grobogan; Anj: Anjasmoro; Der: Dering; Mel: Melrose; Ver: Vernal; Gly: Glycine H; Par: Paranagoiana; CV: check variety; SL: soybean line; *lj* gene: long juvenile gene; *pdh1* gene: pod shattering resistance gene; +: presence of the gene; - : absence of the gene.

± 80 % of the field capacity - FC), applied from planting to harvest, and drought stress (D1; ± 50 % FC), imposed from the flowering to pod formation stage, with irrigation withheld at 10 days before flowering. A total of 150 experimental units were established, with each unit consisting of three polybags.

Before the main experiment was initiated, a preliminary drought stress evaluation was conducted to determine the planting substrate weight corresponding to 80 and 50 % FC. This evaluation used nine polybags (20 cm in diameter and 45 cm in height) without plants, each containing 10 kg of planting substrate (topsoil, goat manure, and sand; 1:1:1, w/w). A gravimetric method was applied to these polybags to determine the soil weight at 80 and 50 % FC. The soil water potential was measured at a depth of 20 cm, using a WP4 dewpoint potential meter. The results were used as the basis for establishing the experimental treatments, with irrigation adjusted based on daily monitoring of the planting substrate weight.

During the main experiment, the same planting substrate used in the preliminary drought stress evaluation was placed into polybags containing 10 kg of substrate. Four seeds were sown per polybag and thinned at 10 days after sowing (DAS), to retain two healthy plants per polybag. NPK 14-14-14 Osmocote was used at a rate of 2.5 g per polybag for basal fertilization, followed by foliar fertilizer applications containing 20 % of N, 15 % of P_2O_5, 15 % of K_2O, and 1 % of $MgSO_4$ during the vegetative stage, as well as 6 % of N, 20 % of P_2O_5, 30 % of K_2O, and 1 % of $MgSO_4$ after flowering. Pest control was conducted regularly, and approximately 90 % of the pods were harvested on reaching maturity. Microclimatic conditions were recorded at 15-min intervals using a data logger (BTH 81, BSide, China), with mean daily air temperature and relative humidity of 28.5 ºC and 83 %, respectively.

Experimental observations were conducted on morphological, physiological, and biochemical traits, which were measured at the end of the drought stress treatment period, namely at the pod formation stage for each experimental unit. Morphological traits included seed yield per plant, number of pods, number of seeds, seed size, and dry shoot weight. The growth duration was measured from the flowering stage to harvest. The root architectural traits included total root area, root length, root dry weight, and root density. To obtain quantitative parameters of the root system, architecture analysis was performed using an imaging system with the ImageJ software (Wibisono & Nurcholis 2023). In addition, pollen viability was assessed at 10 days after the appearance of the first flower using the potassium iodide staining method (Djanaguiraman et al. 2012).

Physiological traits included leaf water potential and leaf relative water content, which were measured using a WP4 dewpoint potential meter (Lestari et al. 2023). Leaf temperature was analyzed using a forward-looking infrared camera, Model CX-series (FLIR, USA) (Pradawet et al. 2023). Photosynthetic system traits comprised stomatal conductance, transpiration, assimilation rate, and water-use efficiency, which were measured using an LI-6800 instrument. All physiological measurements were performed on the fully expanded third leaf.

Biochemical traits included chlorophyll a and chlorophyll b, anthocyanin, carotenoid, extracted from two frozen (-80 ºC) leaf discs (0.56 cm² each) using a methanol-based extraction method (Warren 2008), as well as proline, and malondialdehyde (MDA). The samples were ground and homogenized in 1 mL of methanol, and centrifuged at 16,000 g for 2 min. The supernatant was collected and re-extracted once under the same conditions, and the pooled extracts were used for pigment determination. Microplate measurements were made by pipetting 200 µL of sample into a 96-well flat-bottom polystyrene microplate. Pigment contents were quantified spectrophotometrically using a microplate reader-based method with a Varioskan Lux (Thermo Scientific, Russia) at 470, 537, 647, and 663 nm wavelengths. Pigment contents were estimated using the equations described by Lestari et al. (2023).

Proline content was determined using the ninhydrin-based microplate assay (Carillo & Gibon 2011). Fresh leaf samples (50 mg) were extracted using a cold extraction procedure in ethanol:water (40:60, v/v; 2 mL). The resulting mixture was left overnight at 4 ºC and then centrifuged at 14,000 g (5 min). The sample extract was diluted 20 times on a fresh weight basis (w/v) in a 70:30 (v/v) ethanol:water mixture. The reaction mixture consisted of 1 % (w/v) ninhydrin dissolved in 60 % (v/v) acetic acid and 20 % (v/v) ethanol. About 100 µL of reaction mixture was combined with 50 µL of diluted extract or 50 µL of 1, 0.4, 0.2, 0.1, and 0.04 mM proline standard in a microtube, mixed thoroughly, and incubated at 80 ºC

for 20 min in a water bath. After cooling to room temperature, the tubes were centrifuged at 2,500 rpm for 1 min, and 200 µL of the reaction solution were transferred to a 96-well flat-bottom polystyrene microplate. Absorbance was measured at 520 nm using a Varioskan LUX (Thermo Scientific, USA). Proline concentration was quantified using a standard curve prepared with 0.04-1 mM L-proline standards ($y = 0.2696x + 0.0436$; $R^2 = 0.9987$) and expressed as µmol g^{-1} of fresh weight (fw) (Carillo & Gibon 2011).

The MDA content was determined using the thiobarbituric acid (TBA) assay with minor modifications from Wang et al. (2013). Fresh leaf tissue (0.2 g) was homogenized in 2 mL of 5 % (w/v) trichloroacetic acid (TCA) and centrifuged at 5,000 g for 15 min. An aliquot of the supernatant (200 µL) was mixed with 300 µL of 0.5 % (w/v) TBA prepared in 5 % TCA, vortexed thoroughly, and incubated in a water bath at 80 ºC for 30 min. The reaction mixture was immediately cooled in ice bath and centrifuged at 5,000 g for 15 min. Subsequently, 200 µL of the supernatant were transferred into a 96-well flat-bottom polystyrene microplate, and absorbance was measured at 450, 532, and 600 nm using a Varioskan LUX (Thermo Scientific, USA). The MDA concentration was calculated according to Wang et al. (2013).

All observation characteristics were compiled and tabulated using the Microsoft Excel 2019 (Microsoft, USA). The biplot analysis of the principal component analysis (PCA), based on seed yield and drought tolerance indices, resulted in the identification of two drought-tolerant and two drought-susceptible genotypes, which showed contrasting responses to drought stress. These responses were evaluated using 15 drought tolerance indices (El-Azeem et al. 2023), including yield stability index, stress susceptibility index, tolerance index, stress tolerance index, mean production, geometric mean production, harmonic mean, sensitivity drought index, drought resistance index, relative drought index, stress susceptibility percentage index, yield index, drought tolerance efficiency, modified 1 stress tolerance index, and modified 2 stress tolerance index.

Data normality was assessed using the Shapiro-Wilk test ($p > 0.05$). All observational data of the selected genotypes were analyzed using multivariate analysis of variance (Manova) at a significance level of $\alpha = 0.05$, with SPSS 22. Traits showing significant genotype × environment (G × E) interactions were further analyzed to evaluate adaptive responses under normal and drought stress conditions, as well as the percentage changes between treatments in the tolerant and susceptible genotype groups, using an F-test at $\alpha = 0.05$. The Pearson's correlation analysis was performed to determine the relationships between these traits and seed yield at $\alpha = 0.05$. Heritability analysis was also conducted to assess the potential use as a selection criterion in drought-tolerant soybean breeding programs (Lestari et al. 2023).

RESULTS AND DISCUSSION

The analyzed traits were examined to identify adaptive mechanisms associated with drought tolerance and to determine the relationships with seed yield, thereby providing potential selection criteria for soybean breeding under water-limited environments. To ensure that the imposed treatments represented distinct soil water regimes, the status was characterized based on substrate weight and soil water potential.

Soil substrate weight and soil water potential at 20 cm depth were measured to confirm differences between normal and drought stress conditions. Normal conditions (± 80 % FC) showed a soil substrate weight of ± 12.7 kg (Figure 1A) and water potential of approximately -0.47 MPa (Figure 1C). Under drought stress conditions (± 50 % FC), the soil weight decreased to ± 10.2 kg (Figure 1A), with a soil water potential of approximately -1.42 MPa (Figure 1C). The shift from -0.47 to -1.42 MPa occurred over a period of ± 10 days without irrigation in the screen house (Figure 1B), with a mean daily water loss of ± 200 mL. These results are consistent with Lestari et al. (2023), who reported that a soil water potential of -1.46 MPa reflects drought stress conditions equivalent to 50 % FC. Similarly, Desclaux et al. (2000) applied drought stress in soybean using a range of soil water potentials from -0.05 MPa (normal) to below -0.75 MPa (drought), further supporting the relevance of soil water potential as a criterion for simulating drought stress. The mean air temperature during the experiment was 28.5 ºC. This temperature was higher than that commonly observed in subtropical soybean production regions, such as the United States, where the mean temperature during the optimal soybean growing season (June-October 2024) was 22.5 ºC (NOAA 2025).

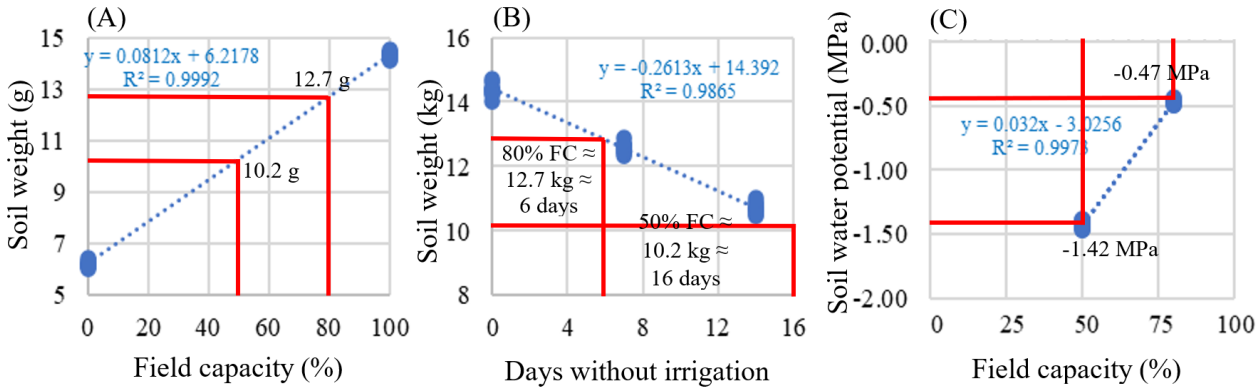

Figure 1. Relationship between field capacity and soil substrate weight (A); changes in soil substrate weight during the drought stress imposition period (B); and relationship between field capacity and soil water potential at a depth of 20 cm from the soil surface (C).

The seed yield of the 25 soybean genotypes showed considerable variation among the samples. An overall reduction in seed yield was observed under drought stress conditions (Figure 2A). The GRO and AP58 cultivars showed sharp reductions in seed yield (6.85 and 4.98 g, respectively), indicating a high level of susceptibility to drought stress. AP21 and AP46 produced the highest seed yield under drought stress (12.35 and 11.80 g, respectively), when compared with all other genotypes, indicating their potential as drought-tolerant candidates (Figure 2A). The biplot analysis of the principal component analysis (PCA) based on seed yield and drought tolerance indices (Figure 2B; Table 2) resulted in the identification of two drought-tolerant (AP21 and AP46) and two drought-susceptible (GRO and AP58) genotypes, which showed contrasting responses to drought stress.

Manova (Table 3) showed a significant G × E interaction, indicating that genotypic responses to stress varied across environmental conditions. This interaction enabled the identification of differences in drought adaptation among genotypes based on the evaluated traits (Mahadevaiah et al. 2021). Significant G × E interactions ($p < 0.05$) were observed for eight traits, namely, pollen viability, leaf relative water content, shoot dry weight, assimilation rate, proline, MDA, anthocyanin, and carotenoid (Table 3).

Drought stress significantly affected all the traits (Table 4). In both tolerant and susceptible

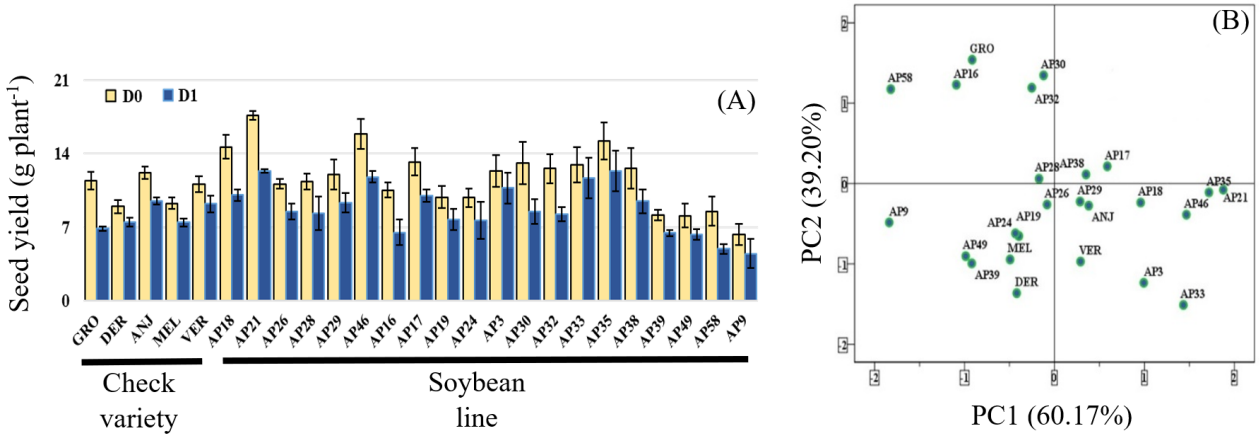

Figure 2. Seed yield of 25 soybean genotypes under normal (D0) and drought stress (D1) conditions (mean ± standard deviation; A); biplot analysis of the principal component analysis (PCA) based on seed yield and drought tolerance indices (Table 2) used to classify soybean genotypes according to their drought response (B). PC1 and PC2 represent the first and second principal components, respectively.

Table 2. Principal component analysis based on seed yield of 25 soybean genotypes and drought tolerance indices.

Component	PC1	PC2
Ys	0.99	0.04
Yn	0.88	0.47
YSI	0.45	-0.89
SSI	-0.46	0.89
TOL	0.17	0.98
STI	0.96	0.27
MP	0.96	0.29
GMP	0.97	0.25
HM	0.98	0.22
SDI	-0.45	0.89
DI	0.96	-0.28
RDI	0.46	-0.89
SSPI	0.17	0.98
DTE	0.45	-0.89
YI	0.99	0.04
K1STI	0.87	0.48
K2STI	0.99	0.06
Eigenvalues	10.23	6.66
% of var	60.17	39.20
% Cum	60.17	99.37

PC: principal component; Ys: yield under stress condition; Yn: yield under normal condition; YSI: yield stability index; SSI: stress susceptibility index; TOL: tolerance index, STI: stress tolerance index; MP: mean production; GMP: geometric mean production; HM: harmonic mean; SDI: sensitivity drought index; DI: drought resistance index; RDI: relative drought index; SSPI: stress susceptibility percentage index; DTE: drought tolerance efficiency; YI: yield index; K1STI: modified 1 stress tolerance index; K2STI: modified 2 stress tolerance index; Var: variance; Cum: cumulative.

Table 3. Multivariate analysis of variance (Manova; mean squares) for morphological, physiological, and biochemical traits of drought-tolerant and drought-susceptible soybean genotypes under normal and drought stress conditions.

Traits	G	E	G × E
SY	257.8**	142.016**	1.161^{ns}
NP	4,347.04**	672.04**	5.042^{ns}
NS	13,490.04**	3,775.04**	12.042^{ns}
SS	7.363^{ns}	32.63**	6.815^{ns}
GD	216**	121.5*	32.67^{ns}
PV	2,184.16**	4,149.53**	805.67**
LWP	35.11**	47.29**	9.95^{ns}
LRWC	238.59**	3,237.73**	165.35**
LT	0.304^{ns}	33.844**	0.004^{ns}
RDW	0.007^{ns}	1.04^{ns}	0.139^{ns}
RL	2,044.67**	651.63*	0.000^{ns}
TRA	3,218.77**	2,638.45*	27.61^{ns}
RD	0.019^{ns}	1.709^{ns}	0.066^{ns}
SDW	66.124**	62.504**	13.301*
SC	0.044^{ns}	0.063*	0.025^{ns}
TR	6.68^{ns}	9.44*	2.23^{ns}
A	3.42**	46.61**	1.983**
WUE	870.07^{ns}	17,435.05^{ns}	1,295.69^{ns}
Proline	1.732**	1.001**	0.545*
MDA	0.98**	2.696**	0.469**
Cha	0.332**	11.287**	0.115^{ns}
Chb	0.007^{ns}	3.123**	0.012^{ns}
ANT	1.398**	4.772**	1.095**
CAR	0.091**	1.565**	0.125**

G: genotype; E: environment; SY: seed yield (g plant^{-1}); NP: number of pods (pod plant^{-1}); NS: number of seeds (seed plant^{-1}); SS: seed size (g 100 seeds^{-1}); GD: growth duration R1-R8 (days); PV: pollen viability (%); LWP: leaf water potential (MPa); LRWC: leaf relative water content (%); LT: leaf temperature (ºC); RDW: root dry weight (g); RL: root length (cm); TRA: total root area (cm^2); RD: root density (g cm^{-2}); SDW: shoot dry weight (g); SC: stomatal conductance (mol H_2O m^{-2} s^{-1}); TR: transpiration rate (mol H_2O m^{-2} s^{-1}); A: assimilation rate (µmol CO_2 m^{-2} s^{-1}); WUE: water-use efficiency (µmol CO_2 mol^{-1} H_2O); proline (µmol mg^{-1} of fresh weight - fw); MDA: malondialdehyde (µmol mL^{-1} fw); Cha: chlorophyll a (µg mg^{-1} fw); Chb: chlorophyll b (µg mg^{-1} fw); ANT: anthocyanin (µg mg^{-1} fw); CAR: carotenoid (µg mg^{-1} fw). * Significant at 0.05; ** significant at 0.01; ns: not significant.

genotypes, pollen viability, leaf relative water content, shoot dry weight, assimilation rate, and carotenoid decreased significantly ($p < 0.05$), whereas proline, MDA, and anthocyanin increased significantly ($p < 0.05$). However, the magnitude of these responses differed between the genotype groups. Under drought stress, tolerant genotypes maintained a significantly higher pollen viability, leaf relative water content, assimilation rate, shoot dry weight, and carotenoid than susceptible genotypes ($p < 0.05$). Conversely, susceptible genotypes showed higher reductions in the traits and accumulated significantly higher levels of proline, MDA, and anthocyanin ($p < 0.05$). These contrasting responses indicate clear differences in physiological and metabolic adaptation to water deficit between the drought-tolerant and drought-susceptible soybean genotypes.

Pollen viability, proline, and MDA also differed significantly ($p < 0.05$) between tolerant and susceptible genotypes under normal conditions (Table 4). This result suggests that the traits are influenced not only by drought-induced plasticity, but also by genetic factors. The factors may serve as adaptive selection traits that can be evaluated under non-stress conditions (Saleem et al. 2022). The results are consistent with those of Moussa & Abdel-Aziz (2008), who reported differences in basal proline and MDA levels between drought-tolerant and drought-susceptible maize genotypes under normal conditions. In this study, susceptible genotypes showed a 24.4 % higher proline content and 25 % higher MDA content than tolerant genotypes.

To further evaluate whether pollen viability, proline, and MDA could serve as effective

Table 4. Adaptive responses of tolerant (T) and susceptible (S) soybean genotypes under normal and drought stress conditions and the changes.

Characters	Drought			Normal			Change (%)	
	T	S	Sig	T	S	sig	T	S
PV	84.11	53.44	**	98.82	91.33	*	-14.89**	-41.49**
LRWC	61.07	49.51	**	79.05	77.99	ns	-22.75**	-36.52**
SDW	11.03	6.22	**	12.77	10.94	ns	-13.63*	-43.14**
A	3.49	2.16	**	5.69	5.52	ns	-38.66**	-60.87**
Proline	0.76	1.59	**	0.65	0.89	*	16.92**	78.65*
MDA	2.02	2.70	**	1.63	1.75	*	23.93**	54.29**
ANT	4.84	5.75	**	4.38	4.43	ns	10.50**	29.80**
CAR	1.75	1.49	**	2.12	2.14	ns	-17.45**	-30.37**

PV: pollen viability (%); LRWC: leaf relative water content (%); SDW: shoot dry weight (g); A: assimilation rate (μmol CO_2 m^{-2} s^{-1}); proline (μmol mg^{-1} of fresh weight - fw); MDA: malondialdehyde (μmol mL^{-1} fw); ANT: anthocyanin (μg mg^{-1} fw); CAR: carotenoid (μg mg^{-1} fw). * Significant at 0.05; ** significant at 0.01; ns: not significant.

selection traits, the relationships with seed yield were examined using the Pearson's correlation analysis (Table 5). Pollen viability showed a positive relationship with seed yield under normal conditions, but the correlation was not significant ($p > 0.05$). In contrast, pollen viability was significantly correlated with seed yield under drought stress conditions ($p < 0.05$). This result indicates that pollen viability is not an appropriate selection trait under normal conditions. However, pollen viability still functions as a supporting trait that enhances reproductive success under drought stress (Djanaguiraman et al. 2012). Proline and MDA showed significant negative correlations with seed yield ($p < 0.05$), indicating that the higher accumulation of these traits was associated with lower seed yield. Therefore, proline and MDA have the potential to be used as adaptive selection traits under normal conditions, enabling more efficient and consistent indirect selection in soybean breeding programs for drought tolerance (Hanif et al. 2021).

The effectiveness of proline and MDA as selection traits was further evaluated through heritability analysis. Heritability estimates showed that proline and MDA showed a high heritability ($h^2bs > 50$ %) under both drought stress and normal conditions (Table 6). These results suggest a strong genetic control over osmolyte accumulation and lipid peroxidation responses, while also indicating that the genes associated with drought tolerance remain expressed under non-stress conditions (Wang et al. 2024). Ghaffari et al. (2019) also reported that the heritability values for proline and MDA were higher under drought conditions than under normal conditions and fell into the high heritability category. Consequently, the phenotypic variation observed for these traits reflects genuine genetic differences among genotypes, suggesting a significant potential as adaptive traits for indirect

Table 5. Pearson's correlations between the characteristics of drought-tolerant and drought-susceptible soybean genotypes under drought stress conditions (above the diagonal) and normal conditions (below the diagonal).

Characters	SY	PV	LRWC	SDW	A	Proline	MDA	ANT	CAR
SY	1	.88**	.93**	.71*	.96**	-.87**	-.84**	-.98**	.92**
PV	.55	1	.65*	.85**	.89**	-.61*	-.91**	-.82**	.73**
LRWC	.22	-.03	1	.43	.83**	-.96**	-.61*	-.95**	.92**
SDW	.37	.29	.19	1	.76**	-.35	-.97**	-.59*	.47
A	.48	.17	.03	-.62*	1	-.72**	-.87**	-.95**	.92**
Proline	-.79**	-.36	-.41	.08	-.74**	1	.53	.89**	-.84**
MDA	-.72**	-.35	-.56	-.24	-.35	.88**	1	.75**	-.63*
ANT	-.16	-.31	.53	-.07	-.11	-.28	-.55	1	-.98**
CAR	.09	-.16	.27	-.71*	.73**	-.66*	-.48	.55	1

SY: seed yield (g $plant^{-1}$); PV: pollen viability (%); LRWC: leaf relative water content (%); SDW: shoot dry weight (g); A: assimilation rate (μmol CO_2 m^{-2} s^{-1}); proline (μmol mg^{-1} of fresh weight - fw); MDA: malondialdehyde (μmol mL^{-1} fw); ANT: anthocyanin (μg mg^{-1} fw); CAR: carotenoid (μg mg^{-1} fw). * Significant at 0.05; ** significant at 0.01.

Table 6. Estimates of variance components and heritability of tolerant and susceptible soybean genotypes under normal and drought stress conditions.

Characters	Normal				Drought			
	V_e	V_g	V_p	h^2_{bs}	V_e	V_g	V_p	h^2_{bs}
PV	22.732	48.547	71.279	0.681	46.865	924.863	971.728	0.952
LRWC	5.123	0.592	5.715	0.104	18.763	127.275	146.038	0.872
SDW	2.301	2.585	4.886	0.529	2.696	22.225	24.921	0.892
A	0.108	0.004	0.112	0.033	0.132	1.757	1.889	0.930
Proline	0.020	0.049	0.069	0.710	0.167	0.647	0.814	0.795
MDA	0.005	0.014	0.019	0.737	0.017	0.462	0.479	0.965
ANT	0.025	0.005	0.030	0.176	0.042	0.814	0.856	0.951
CAR	0.013	0.004	0.017	0.235	0.028	0.069	0.097	0.711

PV: pollen viability (%); LRWC: leaf relative water content (%); SDW: shoot dry weight (g); A: assimilation rate (μmol CO_2 m^{-2} s^{-1}); proline (μmol mg^{-1} of fresh weight - fw); MDA: malondialdehyde (μmol mL^{-1} fw); ANT: anthocyanin (μg mg^{-1} fw); CAR: carotenoid (μg mg^{-1} fw); V_e: environmental variance; V_g: genetic variance; V_p: phenotypic variance; h^2_{bs}: broad-sense heritability.

selection in soybean breeding programs for drought tolerance.

Intracellular proline levels in plants are regulated by the balance among biosynthesis, catabolism, and transport between cellular compartments (Szabados & Savouré 2009). In plants, proline biosynthesis primarily occurs via the glutamate pathway, in which glutamate is converted into pyrroline-5-carboxylate (P5C) in the cytosol or chloroplast and subsequently reduced to proline by the enzymes pyrroline-5-carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) (Verslues & Sharma 2010). This pathway is active under both normal conditions and during nitrogen deficiency and osmotic stress, with P5CS generally encoded by two genes, whereas P5CR is encoded by a single gene in most plant species (Armengaud et al. 2004). Under stress conditions, plants can rapidly accumulate proline and subsequently degrade it once normal conditions are restored (Trovato et al. 2008). Proline is also considered an energy reservoir, as the oxidation of one molecule can generate approximately 30 ATP equivalents during the recovery phase (Trovato et al. 2008). However, excessive proline accumulation may lead to increased levels of P5C, which is toxic due to the induction of reactive oxygen species (ROS), apoptosis, and even programmed cell death (Székely et al. 2008), suggesting that proline homeostasis is critically important. Moreover, proline accumulation has an optimal threshold, beyond which it may become toxic.

Proline degradation occurs in the mitochondria through the action of proline dehydrogenase (PDH) or proline oxidase (POX), which converts proline into P5C. Subsequently, P5C dehydrogenase (P5CDH) converts P5C into glutamate (Szabados & Savouré 2009). This reversible process is known as the proline cycle (Verslues & Sharma 2010). In this study, the susceptible genotypes showed higher proline content than the tolerant genotypes under drought stress conditions (Table 4), indicating that proline accumulation does not necessarily reflect tolerance, but rather a response to higher stress intensity, including increased ROS due to impaired redox homeostasis. The condition is presumably associated with inefficiency in the proline cycle, particularly impaired PDH and P5CDH activities, leading to insufficient proline degradation. This is supported by *Arabidopsis* mutant studies, where loss of PDH function results in the inability to eliminate excess proline (Nanjo et al. 2003). P5CDH deficiency increases sensitivity due to toxic P5C accumulation, whereas the overexpression enhances tolerance by accelerating detoxification (Deuschle et al. 2001). Moreover, the simultaneous increase in proline and decrease in seed yield (Table 5) under drought stress reflects a stress-induced metabolic trade-off, in which carbon and energy are redirected from reproductive development toward osmotic adjustment and cellular protection rather than a direct causal relationship between proline accumulation and yield reduction.

MDA is an end product of lipid peroxidation formed through the degradation of polyunsaturated fatty acids in cell membranes under environmental stresses, including drought. In general, drought stress increases the production of ROS, which triggers oxidative damage to cellular membranes, and consequently leads to elevated MDA levels

(Hossain & Fujita 2010). Therefore, MDA is widely used as an indicator of lipid peroxidation, oxidative stress, and membrane damage in plants subjected to drought stress (Farissi et al. 2013). A high MDA content reflects increased membrane damage caused by ROS accumulation, whereas lower levels indicate a more effective antioxidant defense system capable of limiting oxidative damage. Excessive lipid peroxidation can impair membrane integrity, disrupt photosynthetic processes, and assimilate transport, ultimately reducing the supply of photoassimilates available for seed development and filling. Consequently, genotypes with higher MDA accumulation generally show lower seed yield under drought stress, resulting in a negative association between MDA content and yield.

The tolerance mechanisms identified in this study are not limited to the measured traits, but possibly represent the integrated outcome of multiple interacting adaptive pathways that were not directly quantified. At least three major components are involved in drought tolerance, namely osmotic adjustment, detoxification of ROS, and hormonal regulation. Osmotic adjustment is achieved through the accumulation of compatible solutes, including amino acid-based osmoprotectants, namely proline, polyol, and sugar-based compounds (mannitol, D-mannitol, trehalose, sucrose, and raffinose), as well as onium compounds in the form of glycine betaine and dimethylsulfoniopropionate, which collectively help to maintain the cellular water status under dehydration (Dar et al. 2016). In parallel, detoxification mechanisms contribute to tolerance through efficient ROS scavenging systems, as reflected by lower MDA levels in tolerant genotypes, indicating reduced lipid peroxidation and better membrane stability (Table 4). This process is primarily supported by enzymatic antioxidants, including superoxide dismutase, catalase, and ascorbate peroxidase, as well as non-enzymatic antioxidants such as ascorbic acid, glutathione, phenolics, flavonoids, and anthocyanins (Gupta et al. 2020). In addition, hormonal regulation, particularly abscisic acid (ABA), plays a central role in coordinating drought responses by activating bZIP transcription factors that bind to ABA-responsive elements (ABREs), thereby regulating genes involved in stomatal control, cellular protection, water status maintenance, and osmotic adjustment (Yu et al. 2003).

CONCLUSIONS

1. Drought-tolerant soybean genotypes maintain higher physiological and metabolic stability during flowering to pod formation under both normal and drought conditions;
2. Proline contributes to osmotic and redox regulation, whereas excessive proline together with malondialdehyde (MDA) indicates oxidative stress, disrupted reactive oxygen species homeostasis, and reduced seed yield;
3. Proline and MDA showed high heritability and negative correlations with yield across environments, supporting their use as stable genetic indicators for drought tolerance selection.

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