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EFFECTS OF CONSECUTIVE DROUGHT STRESS DURING ADJACENT GROWTH STAGES ON SOYBEAN MORPHO-PHYSIOLOGICAL TRAITS AND YIELD

FEIFEI HUANG[1], XIAOLIANG LI[1*], HONGWEI YUAN[2,3] AND JIWEI YANG[2,3]

[1]College of Resource and Environment, Anhui Science and Technology University, Fengyang 233100, China
[2]Anhui & Huaihe River Institute of Hydraulic Research, Hefei 230088, China
[3]Anhui Provincial Key Laboratory of Water Science and Intelligent Water Conservancy, Bengbu 233000, China

*Corresponding author's email: lixl@ahstu.edu.cn

Abstract

Consecutive drought events spanning adjacent soybean growth stages compound physiological stress and intensify yield penalties. The experimental design involved applying varying drought intensities to two adjacent growth stages, achieved by employing pairwise combinations of the stages seedling (S1), branching (S2), flowering-podding (S3), and seed filling (S4), to assess their impact on soybean morphology and physiology. The results demonstrated that drought stress significantly reduced all parameters in treated groups compared to the normal irrigation group, with losses increasing with severity. Under drought stress, soybean exhibited maximal reductions in plant height (PH, 25.70%) and tap root length (TRL, 24.45%) at S1-S2, while the S2-S3 stage saw greatest decreases in primary branch number (PBN, 75.00%), root dry weight (RDW, 44.16%), and stem dry weight (SDW, 33.89%). The most severe impacts occurred at S3-S4, with grain yield (GY, 67.19%), total pod number (TPN, 49.82%), total seed number (TSN, 58.94%), and pod wall weight (PWW, 64.82%) showing maximal reductions, accompanied by the highest empty pod rate (EPR, 18.68%). Notably, GY showed strong positive correlations with RDW and pod traits (TPN, TSN, PWW), but negative correlation with EPR ($p \leq 0.001$). 63.2% of the total variance in soybean drought response traits was collectively captured by these parameters within the first principal component (PC1). Thus, targeted irrigation during the S3-S4 stage is essential for mitigating drought-induced yield losses in soybean.

Key words: Drought stress; Growth stage sensitivity; Plant water relations; Biomass partitioning; Source-sink relationship

Introduction

Climate change is amplifying the scale and recurrence of drought across the globe (Li *et al.*, 2020; Khan & Gilani, 2021; Zhou *et al.*, 2023; Farooq *et al.*, 2025). Projections suggest that 4.5×10^6 hectares of agricultural land will be affected by drought annually (Cammalleri *et al.*, 2020), accompanied by a fivefold water demand-supply deficit (Neupane *et al.*, 2022). However, water scarcity alters crop phenotypes, physiological and biochemical profiles, ultimately reducing yield (Hossain *et al.*, 2024; Sarwar *et al.*, 2025). This drought stress consequently exacerbates global food shortages, with progressively severe impacts observed across major agricultural regions (Hendrawan *et al.*, 2023). Studies show drought severely reduced maize and soybean yields in China, the US, and Brazil (Liu *et al.*, 2024), while China's cereal losses threaten global food supply chains (Javed *et al.*, 2021). Among them, soybean [*Glycine max* (L.) Merr.], an ancient crop domesticated in China, is extensively cultivated in the Northeast Plain, Huang-Huai-Hai Plain, and Jianghan Plain (Dong *et al.*, 2023; Zhao *et al.*, 2024). As a vital source of plant-based protein and edible oil, it holds a pivotal position in both legume and industrial oilseed crops globally (Mannan *et al.*, 2023). However, owing to its high water demand and pronounced susceptibility to moisture deficit, drought stress significantly disrupts soybean organ allocation and grain yield (Zhou *et al.*, 2022). This triggers adaptive growth strategies to mitigate damage, consequently inducing drought-resistant 'stress memory' mechanisms (Dietz *et al.*, 2021; Sintaha *et al.*, 2022). The aforementioned 'memory' mechanism represents a pivotal breakthrough for mitigating drought-induced yield losses in soybean production. Deciphering drought response patterns in soybean is therefore essential for advancing current research.

Drought stress significantly constrains soybean agronomic traits and yield (Li *et al.*, 2024). Furthermore, variations in drought-affected growth stages, intensity levels, and exposure durations result in heterogeneous responses in plant developmental patterns (Wijewardana *et al.*, 2019; Cui *et al.*, 2021; Galić Subašić *et al.*, 2024). Drought exposure at any developmental stage impairs the photosynthetic capacity of soybean, (Elsalahy & Reckling, 2022), involving the loss of chlorophyll and impaired function of the chloroplast electron transport chain (El Amine *et al.*, 2025). The decline in photosynthetic performance directly impairs assimilate synthesis and translocation in reproductive organs, consequently reducing pollen viability, increasing floral abortion rates (Soba *et al.*, 2022; Du *et al.*, 2024), which in turn compromises seed set and weight per pod while raising the incidence of pod malformation or emptiness (Desclaux *et al.*, 2000; Brevedan & Egli, 2003; Wei *et al.*, 2018). Furthermore, drought stress accordingly reduces dry matter accumulation in vegetative organs (roots, stems, leaves) (Song *et al.*, 2022), and ultimately results in soybean yield

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reduction. The impact of light drought on soybean vegetative growth and yield is less pronounced at the seedling stage than that of severe deficit during the flowering and pod-filling stages (Cui *et al.*, 2019; Elsalahy & Reckling, 2022). This finding elucidates the predominant focus of previous research on the latter two critical growth stages in soybean drought stress studies, while comparatively neglecting early developmental phases such as the seedling stage (Wei *et al.*, 2018; Gao *et al.*, 2020; Elsalahy & Reckling, 2022; Saleem *et al.*, 2022; Poudel *et al.*, 2023). However, the conventional discrete drought stress studies focusing on isolated growth stages inadequately capture the high-frequency, continuous spatiotemporal dynamics of drought events under contemporary climate change scenarios.

Climate projections reveal pronounced variations in drought frequency and intensity across soybean developmental stages in China. Notably, the drought frequency from sowing to seedling stage shows a general decline (excluding western regions), whereas drought risks during the branching-to-flowering and flowering-to-filling stages will significantly increase, particularly in northern and western regions (Han *et al.*, 2025). However, yield reduction induced by drought stress may occur at any phenological stage of soybean development (Gebre & Earl, 2021). While the Huaibei Plain is a vital soybean-producing region in China, its water management remains largely based on traditional empirical approaches and lacks targeted guidelines for addressing sequential drought across growth stages. Thus, employing a summer soybean pot experiment in the region, this study established a consecutive drought model to decipher the cross-stage impact processes, thereby seeking to inform future irrigation design for complex drought scenarios.

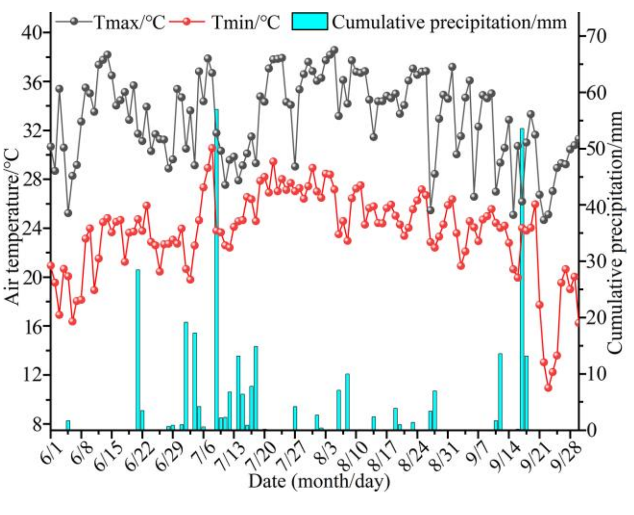


Fig. 1. Daily meteorological dynamics at the experimental station (June-September 2024).

Materials and Methods

Experimental site and environmental conditions: The pot experiment was conducted from June to September 2024 at Xinmaqiao Agricultural and Water Comprehensive Experimental Station (33°09′N, 117°22′E) in Bengbu City, Anhui Province, China. This experimental station occupies the southern periphery of the Huang-Huai-Hai Plain, belonging to the warm temperate semi-humid monsoon climatic zone. According to station records, the study area exhibits an annual average rainfall of 917 mm and evaporation of 916 mm, with lime concretion black soil (LCS) as the predominant soil type characterized by heavy texture and low permeability. The topsoil layer (0-40 cm) exhibited the following physicochemical properties: bulk density, 1.45 g/cm³; field capacity, 28%; pH, 6.75; total N, 0.73 g/kg; total P, 0.44 g/kg; total K, 6.06 g/kg. The daily meteorological data presented in Fig. 1 are the site-specific records for the entire experimental period, as captured by our experimental station's automated weather monitoring system.

Tested materials and cultivation management: Topsoil-derived LCS served as the growth medium in this pot study. The obtained 'standard soil' was sun-dried, sieved, and then transferred into plastic cultivation bucket (CB; top- and bottom-diameters of 33 and 22 cm and a height of 28 cm) at a uniform weight of 16 kg per bucket, followed by systematic compaction. On June 19th, soybean seeds of the 'Zhonghuang 13' cultivar were uniformly sown on the soil surface at a seeding density of 8-10 seeds per bucket, followed by the application of 4.0 g NPK compound fertilizer to each CB. After 7 days, three soybean seedlings exhibiting uniform growth and healthy development were retained per CB, a practice referred to as final thinning. Except for water management, all agronomic practices (including pest control and fertilization) were uniformly applied to pot-grown soybean plants throughout the experimental periods encompassing the seedling (S1), branching (S2), flowering-podding(S3), and seed filling (S4) stages. As illustrated in Fig. 2, the pot experiment was conducted in an open-air field setting, with a mobile rainout shelter installed to exclude precipitation effects. Additionally, all pot-grown soybean plants were positioned on elevated storage racks to prevent bottom-up water infiltration.

Experimental design

Table 1. Experimental design of drought treatments applied to different growth stage combinations (GSCs) in soybean.

Type		Lower limits of SMC during different growth stages (%)			
Combination	Treatment	S1	S2	S3	S4
S1-S2	T1	55	55	75	75
	T2	55	45	75	75
	T3	45	55	75	75
	T4	45	45	75	75
S2-S3	T5	75	55	55	75
	T6	75	55	45	75
	T7	75	45	55	75
	T8	75	45	45	75
S3-S4	T9	75	75	55	55
	T10	75	75	55	45
	T11	75	75	45	55
	T12	75	75	45	45
CK	CK	75	75	75	75

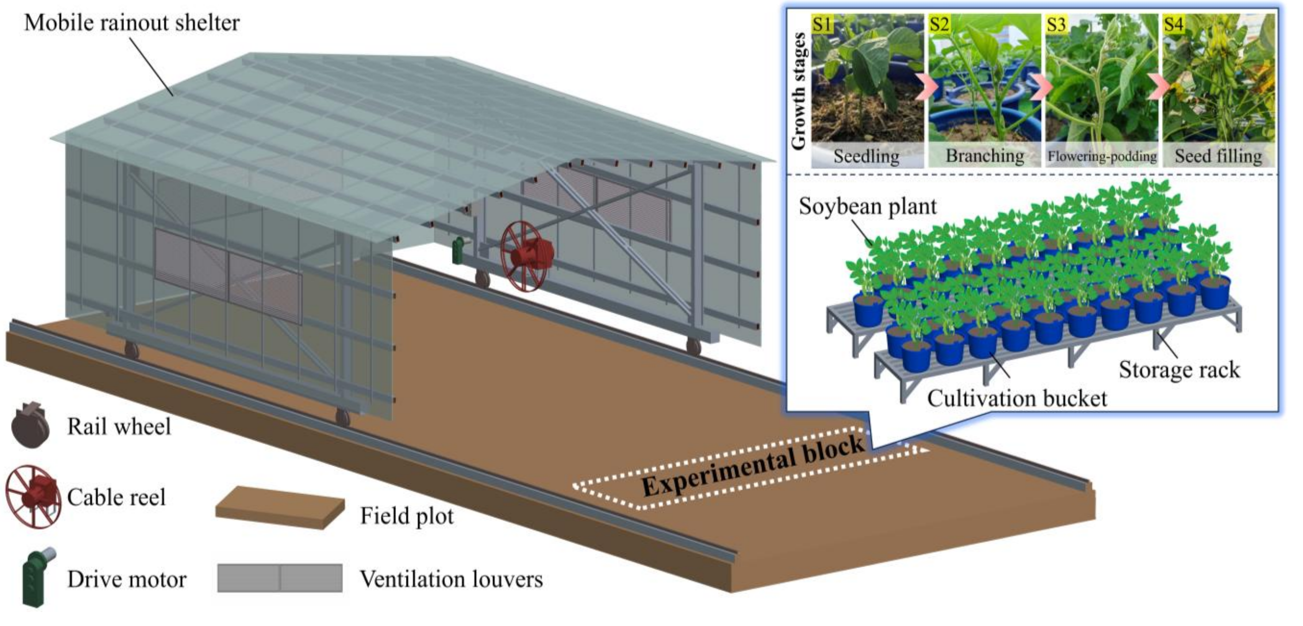
This study utilized soybean plants exposed to consecutive drought stress across consecutive growth stages, with controlled variations applied to drought intensity and developmental phase. Drought intensity was regulated by manipulating the lower limit of soil moisture content (SMC), integrating long-term field drought monitoring data from the experimental station and established methodologies from prior research (Patanè & Cosentino, 2010; Wei *et al.*, 2018; Cui *et al.*, 2021). In this pot experiment, three lower limits of SMC were established: 75% (no drought), 55% (light drought), and 45% (moderate drought). To evaluate how sustained water deficit during specific growth phases affects soybean development, this study treated two adjacent growth stages as a cohesive experimental unit. Consequently, three different GSCs were established in this experimental design, which comprised one control group (CK) and 12 drought treatment groups, each with three biological replicates. Table 2 summarizes the detailed experimental protocol.

Measurement items and methods

Morphological indicators: At maturity, soybean plants were harvested and measured for plant height (PH) and tap root length (TRL) using a standard ruler (1 m in range). The primary branch number (PBN), total pod number (TPN), empty pod number (EPN), and total seed number (TSN) were manually recorded through visual inspection. Concurrently, grain yield (GY) and pod wall weight (PWW) were measured using a PR1602ZH/E electronic balance (Ohaus Instruments, Changzhou, China). Additionally, the empty pod rate (EPR) of soybean pods was determined using the following formula:

$$EPR = \frac{EPN}{TPN} \times 100\%$$

(A) Schematic of the experimental design



(B) Photograph of the experimental design

Fig. 2. Schematic of soybean drought stress experimental arrangement.

Table 2. Effect of different drought stress patterns on GY and its component traits.

Type		GY (g)	TPN	EPR (%)	TSN	PWW (g)
Combination	Treatment					
S1-S2	T1	32.37 ± 3.26b	38.00 ± 6.08ab	6.18 ± 1.30bc	169.50 ± 6.36b	16.12 ± 0.13ab
	T2	31.93 ± 4.58b	36.67 ± 2.52abc	7.13 ± 5.18bc	165.00 ± 29.70bc	15.29 ± 1.62cd
	T3	29.60 ± 4.62bc	34.67 ± 4.51bcd	7.61 ± 5.48bc	133.50 ± 23.33bcd	13.64 ± 2.92bcd
	T4	24.41 ± 4.57cd	29.00 ± 3.46cde	8.03 ± 4.37bc	121.00 ± 12.73de	13.21 ± 2.36bcd
S2-S3	T5	24.63 ± 3.88cd	30.67 ± 4.16bcde	9.01 ± 0.32bc	125.00 ± 26.87cde	11.81 ± 1.90cd
	T6	19.68 ± 5.23de	27.00 ± 7.00de	9.76 ± 0.34abc	102.00 ± 24.04de	10.44 ± 2.39de
	T7	23.22 ± 3.73cd	27.67 ± 3.06de	10.84 ± 1.64abc	129.50 ± 19.09bcde	10.68 ± 2.33de
	T8	19.01 ± 1.20de	25.33 ± 3.79e	12.76 ± 9.69ab	100.50 ± 6.36de	10.63 ± 2.93de
S3-S4	T9	20.60 ± 1.13de	25.00 ± 1.73e	11.48 ± 5.77abc	111.33 ± 2.08de	9.87 ± 0.31de
	T10	13.18 ± 0.95e	22.00 ± 1.41e	18.68 ± 3.23a	96.50 ± 4.95de	6.87 ± 1.09e
	T11	20.16 ± 1.32de	24.33 ± 9.07e	12.00 ± 3.31ab	97.50 ± 9.19de	10.83 ± 1.59de
	T12	17.00 ± 1.08de	22.33 ± 0.58e	15.49 ± 4.51ab	87.67 ± 2.52e	9.73 ± 1.12de
CK	CK	40.17 ± 5.65a	44.50 ± 0.71a	2.25 ± 0.04c	213.50 ± 41.72a	19.53 ± 3.32a

GY, TSN, and PWW were statistically evaluated at the CB level, while TPN and EPR were analyzed per individual plant

SPAD: SPAD readings (SPAD-502 Plus, Konica Minolta Inc., Japan) were taken between 09:00 and 12:00 on sunny days to assess relative chlorophyll content. Following the one-leaf-per-CB protocol, SPAD measurements were taken on the third mature trifoliate leaf (counting from the apex) of each plant. The described procedure was adhered to in accordance with a standardized protocol to ensure measurement consistency.

Dry matter accumulation: Following thorough rinsing of soybean roots and stems with clean water and sub-sequent air-drying at ambient conditions, individual organs were excised using scissors and then transferred to corresponding pre-labeled kraft paper bags for storage. To inactivate enzymes, samples were oven-dried (Jinghong Experimental Equipment Co., Ltd., China) at 105°C for 30 min, and subsequently at 80°C until reaching constant weight. The final dry weight was determined (electronic balance, see Section 2.4.1) and recorded.

Data analysis: In this study, Microsoft Excel 2010 (Redmond, WA, USA) was utilized for initial data preprocessing, followed by IBM SPSS Statistics 27 (Chicago, IL, USA) for ANOVA analysis. Graphical representations including column charts, correlation heatmaps, and principal component analysis (PCA) plots were generated using Origin 2021 (Northampton, MA, USA). In particular, PCA was conducted on the correlation matrix to ensure equal weighting of all traits.

Results

Response patterns of PH, TRL, and PBN: As demonstrated in Fig. 3, drought stress imposed at different GSCs significantly inhibited soybean PH, TRL, and PBN, with all drought treatments showing varying degrees of reduction compared to the CK. The progression from S1 to S4 saw the inhibitory effect of drought on PH and TRL become progressively less pronounced, with the reduction magnitude following the sequence: S1-S2 > S2-S3 > S3-S4. Notably, the inhibitory effect on PBN peaked during S2-S3, displaying the reduction order: S2-S3 > S1-S2 > S3-S4. During the S1-S2 stage, soybean PH, TRL, and PBN decreased by 20.22%-25.70%, 18.37%-24.45%, and 31.25%-50.00%, respectively. During the S2-S3 stage, the corresponding reductions were 16.85%-20.44%, 15.32%-19.74%, and 43.75%-75.00%, while further reductions of 3.23%-6.04%, 4.95%-14.19%, and 18.75%-37.50% were observed in the S3-S4 stage. Statistically significant differences ($p<0.05$) were observed in all treatment groups relative to the CK. Across all treatments except T7, soybean PH, TRL, and PBN under different GSCs consistently followed the order L-L > L-M > M-L > M-M, indicating a negative correlation with increasing drought intensity.

Response patterns of SPAD: Collectively, SPAD values for drought-stressed soybean under different GSCs showed non-significant reductions relative to the CK ($p>0.05$). As illustrated in Fig. 4, the reduction ranges observed during different GSC (S1-S2, S2-S3, and S3-S4) were 0.28%-10.51%, 0.32%-9.86%, and 5.21%-13.23%, respectively. Within each GSC, the SPAD values consistently followed the order M-L > M-M, with both significantly lower than those of L-L and L-M, demonstrating that elevated drought stress during the pre-ceding growth stage induced more pronounced degradation of soybean chlorophyll content.

Response patterns of RDW and SDW: Figure 5 demonstrates that as soybean developmental stages progressed, both RDW and SDW in all GSCs (S1-S2, S2-S3, S3-S4) exhibited significant reductions compared to the CK. Comprehensive comparison revealed that the reduction rates of RDW and SDW followed the order S2-S3 > S1-S2 > S3-S4, demonstrating that the S2-S3 stage was most vulnerable to drought-induced dry matter accumulation suppression in soybean. In terms of RDW, the reduction rate in S1-S2 stage ranged from 10.56% to 13.86% without significant difference compared to CK ($p<0.05$). For the S2-S3 stage, reductions ranged from 31.62% to 39.70%, with T8 showing a greater reduction of 44.16%. Similarly, reductions of 27.64% to 29.44% were observed in the S3-S4 stage, while T10 exhibited an even higher reduction of 44.42%. All these values differed significantly from the CK ($p<0.05$). Regarding SDW, the reduction rate in S1-S2 stage ranged from 0.50% to 24.59%, with only T3 and T4 showing significant differences from CK ($p<0.05$). Most treatments during the S2-S3 and S3-S4 stages exhibited reductions within the ranges of 30.65%-33.89% and 21.43%-30.19%, respectively. Exceptions included T7 (22.24%) and T12 (34.40%). Consistent with this pattern, all treatments except T7, T9, and T10 differed significantly from the CK ($p<0.05$). Within each GSC, soybean RDW and SDW broadly exhibited a consistent pattern across the treatments: L-L > L-M and M-L > M-M. This gradient demonstrates that progressive drought intensity in later growth stages significantly suppressed plant dry matter accumulation.

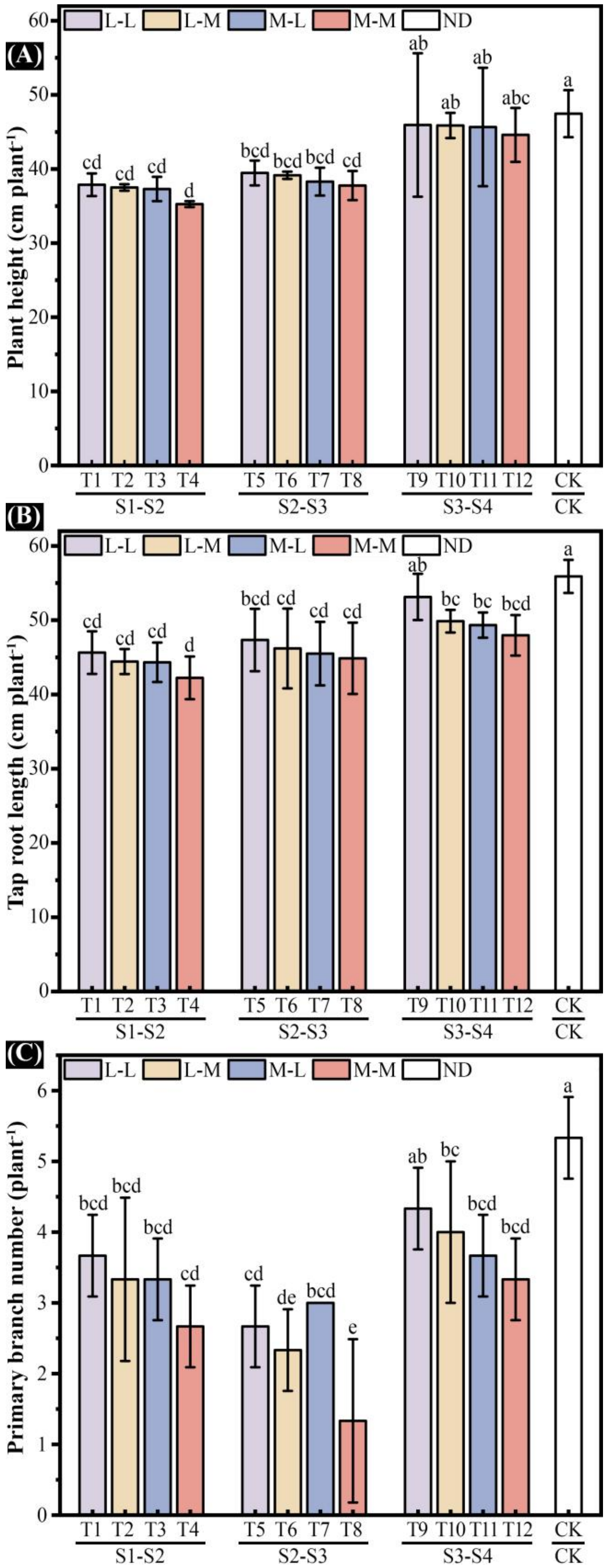


Fig. 3. Effects of different drought stress patterns on PH, TRL, and PBN. In the figure, L represents light drought stress, while M represents moderate drought stress. L-L: Light drought in both the preceding and subsequent growth stages; L-M: Light drought in the preceding stage followed by moderate drought in the subsequent stage. M-L and M-M follow analogous representation conventions. ND: The non-drought control condition. Additionally, S1-S4 represent the four growth stages, and T1-T12 the twelve treatment groups, as detailed in Table 1, with error bars indicating ± standard deviation (SD). The same notation applies hereafter.

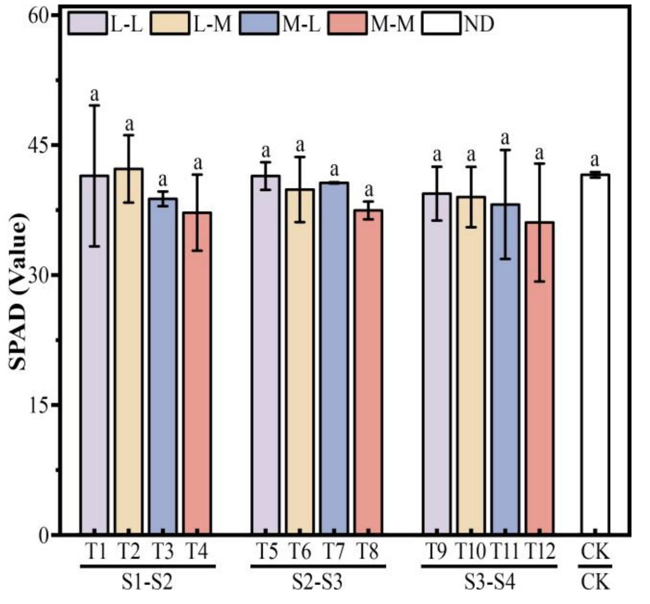


Fig. 4. Effect of different drought stress patterns on SPAD.

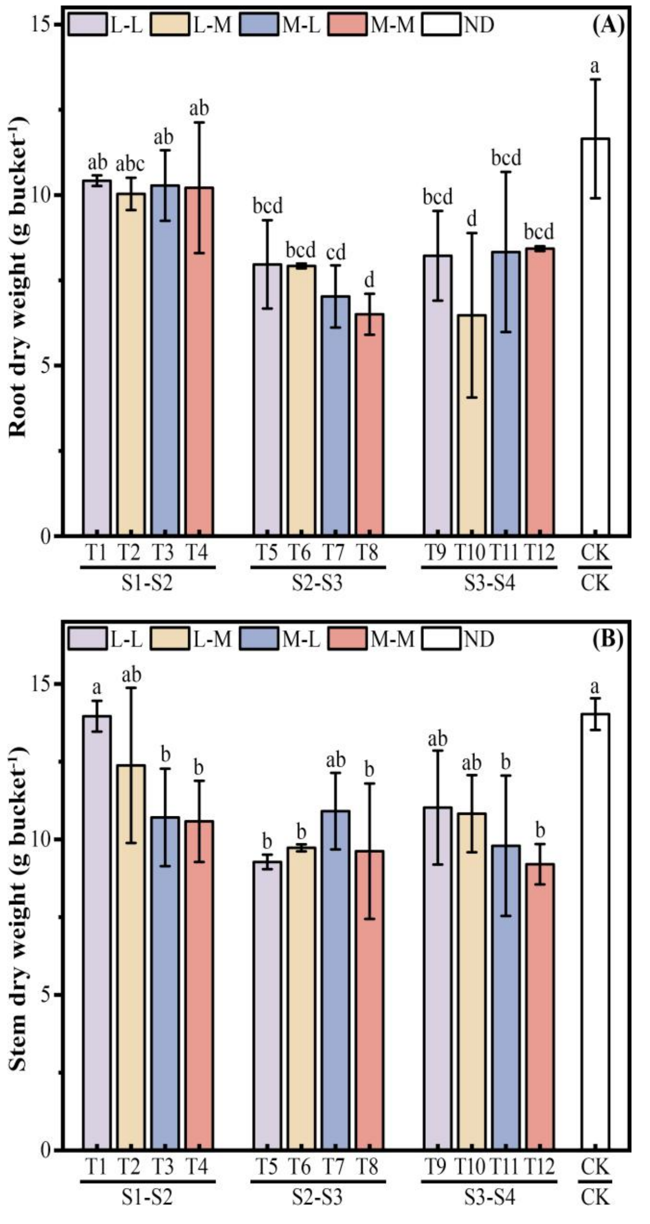


Fig. 5. Effect of different drought stress patterns on RDW and SDW.

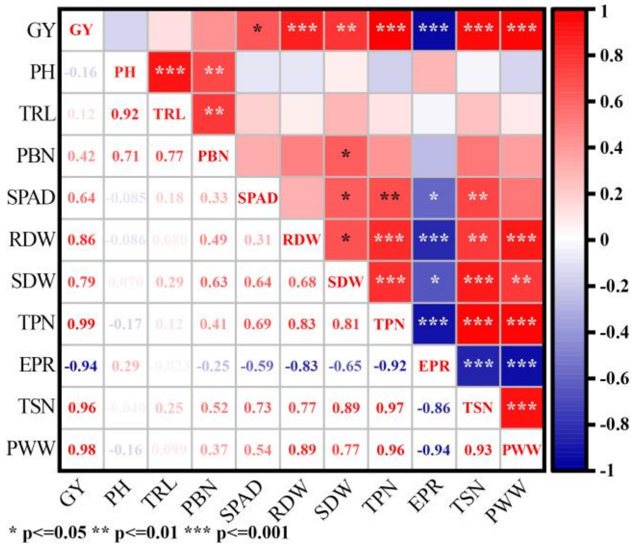


Fig. 6. Correlation analysis of various soybean indicators under different drought stress patterns.

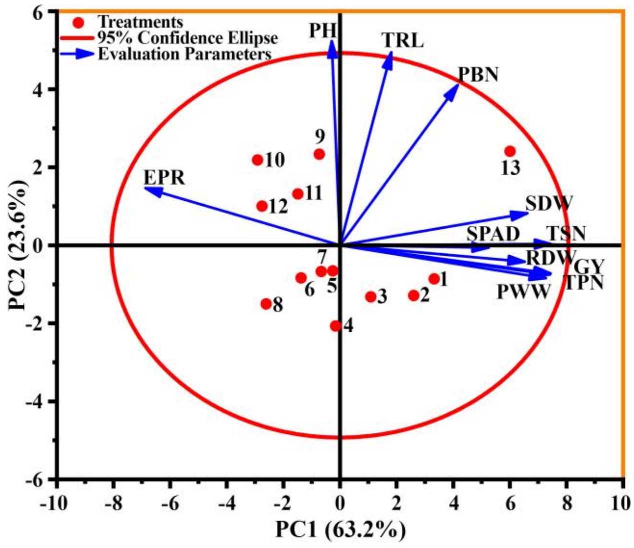


Fig. 7. PCA of various soybean indicators under different drought stress patterns.

Response patterns of GY and its component traits: Table 2 demonstrates that under drought conditions (T1-T12), soybean yield components including GY, TPN, TSN, and PWW were significantly reduced compared to the CK, whereas EPR exhibited a concomitant increase. Collectively, the amplitude variations of the aforementioned five indicators exhibited a graded response pattern (S1-S2 < S2-S3 < S3-S4), demonstrating that drought stress during the S3-S4 growth stage exerted the most pronounced detrimental effects on soybean GY and its component traits, with quantitative responses across growth stages are shown below: (1) The GY decline percentages exhibited 19.42%-39.23%, 38.69%-52.68%, and 48.72%-67.19%, respectively. Each drought treatment group differed significantly from the CK ($p < 0.05$). (2) The TPN reduction percentages were 14.61%-34.83%, 31.08%-43.08%, and 43.82%-49.82%, respectively. Drought treatments T3 through T12 differed significantly from the CK ($p<0.05$). (3) The TSN reduction percentages were 20.61%-43.33%, 41.45%-52.93%, and 47.85%-58.94%, respectively. Results for all drought treatments were statistically significant ($p < 0.05$). (4) The PWW reduction percentages were 17.46%-32.36%, 39.53%-46.54%, and 44.55%-64.82%, respectively. A consistent trend across the drought treatments was the exhibition of statistically significant differences from the CK ($p < 0.05$). (5) The EPR increased by 174.67%-256.89%, 300.44%-467.11%, and 410.22%-730.22%, respectively. During the S1-S2 and S2-S3 stages, no significant differences from the CK were detected, with the exception of treatment T8. In contrast, the S3-S4 stage exhibited statistically significant divergence from the CK ($p < 0.05$) across all treatments apart from T9. Within each GSC, the EPR followed L-L < L-M and M-L < M-M patterns, while other traits showed opposite responses. These results confirmed that soybean GY and its component traits were progressively more adversely affected by escalating drought stress intensity.

Comprehensive analysis

Correlation analysis between GY and other indicators: Pearson correlation analysis revealed differential interrelationships among evaluated indicators in soybean under different drought stress patterns (Fig. 6). Significant correlations were observed between GY and other drought-responsive indicators: 1) moderate positive with SPAD ($r = 0.64$, $p \leq 0.05$); 2) strong positive with SDW ($r = 0.79$, $p \leq 0.01$); 3) highly significant positive with RDW, TPN, TSN and PWW ($r = 0.86$-0.99, $p \leq 0.001$); (4) extreme negative with EPR ($r = -0.94$, $p \leq 0.001$). For GY, correlations with PH ($r = -0.16$), TRL ($r = 0.12$), and PBN ($r = 0.42$) were not significant, despite the presence of directional trends.

Principal component analysis (PCA): PCA performed on 11 indicators under drought stress revealed that the first two components, PC1 and PC2, collectively explained 86.8% of the observed variance (Fig. 7). The eight traits (GY, TPN, SDW, TSN, RDW, PWW, SPAD, EPR) dominated PC1, explaining 63.2% of its variance. Meanwhile, PH, TRL, and PBN were the primary determinants of PC2, accounting for 23.6% of its explained variance. The spatial distribution of sample points in the PCA plot reflected their relative similarity in the feature space, with closer proximity indicating higher phenotypic resemblance (He *et al.*, 2024). As depicted in Fig. 6, treatments 1-13 clustered in distinct quad-rants according to their assigned groups (S1-S2, S2-S3, S3-S4, and CK), indicating that treatments within the same group exerted similar effects on soybean evaluation indicators.

Discussion

Drought stress systematically alters soybean morphological development throughout phenological phases (Wei *et al.*, 2018; Hossain *et al.*, 2024), exhibiting consistent depression in PH, TRL, and PBN (Bui *et al.*, 2022; Song *et al.*, 2022; Li *et al.*, 2024). The inhibitory effect exhibits progressive intensification corresponding to incremental stress gradients (Dong *et al.*, 2019). Wei *et al.*, (2018) focusing on drought at individual stages, reported that stress occurring during S2 led to a reduction of about 25.37% in PH relative to the control. Research has confirmed that increasing drought severity across a single growth stage inhibits soybean root architecture development, with effects being most pronounced at its most severe (García-Rodríguez *et al.*, 2024). Li *et al.*, (2024) reported a 6.11-fold greater reduction in PBN when prolonged (71 days) versus short-term (37 days) water deficit was imposed prior to the S3 stage. This study revealed that consecutive drought stress across adjacent soybean growth stages (S1-S2, S2-S3, S3-S4) significantly reduced PH, TRL, and PBN compared to the CK, with varying magnitudes of reduction observed among different GSCs. Notably, drought stress imposed specifically during the S1-S2 stage caused the most pronounced reductions in PH and TRL, with maximum decline rates of 25.70% and 24.45%, respectively. In contrast, the S2-S3 stage exhibited the greatest detrimental effect on PBN, showing a 75.00% maximal reduction under drought conditions. Within the current phase of various GSCs, the detrimental effects of different drought patterns consistently exhibited the trend L-L < L-M < M-L < M-M across multiple morphological indicators, suggesting a progressive deterioration in plant form and structure with increasing drought intensity.

Under drought stress, the reduction in photosynthesis in soybean plants is primarily attributed to a decline in chlorophyll content (Wijewardana *et al.*, 2019), which occurs irrespective of whether the drought stress is imposed during the early or late growth stages (Elsalahy & Reckling, 2022). This study demonstrated that the SPAD values across the three GSCs (S1-S2, S2-S3, and S3-S4) were all reduced compared to the CK. The minimum and maximum decreases in SPAD values during S1-S2 and S2-S3 were (0.28%, 10.51%) and (0.32%, 9.86%), respectively, both of which were lower than those observed in S3-S4 (5.21%, 13.23%). This phenomenon may be attributed to the compensatory effect (Dong *et al.*, 2019) following rewatering in both the S1-S2 and S2-S3, as mild to moderate drought primarily impairs stomatal function without inducing irreversible damage to the photosynthetic apparatus, unlike severe drought (Zandalinas *et al.*, 2020; Saleem *et al.*, 2022). However, within the current phase of various GSCs, this study demonstrated that an increase in drought intensity during the later growth stage resulted in more pronounced reduction in soybean SPAD values when plants had been subjected to a similar level of drought stress earlier, echoing the conclusions of Basal *et al.*, (2020).

Impaired growth and development in drought-stressed soybean stems from the disruption of fundamental processes such as nutrient assimilation, translocation, and distribution (Cao *et al.*, 2021). Such alterations impair dry matter accumulation in roots and stems, with biomass reduction dictated largely by the severity of the imposed water deficit (Sincik *et al.*, 2008). In a study on drought at specific soybean growth stages, Wei *et al.*, (2018) found that stages S2 and S3 experienced the most substantial aboveground dry matter loss in stems, branches, and pods, with severe drought leading to reductions of 50.00% and 66.94%, respectively, compared to the CK after the stress period. Irreversible suppression of dry matter in key soybean organs (roots, stems, leaves) due to severe drought at a single pivotal stage has been shown (De Almeida *et al.*, 2024; García-Rodríguez *et al.*, 2024), with losses sustained post-rewatering. A consistent trend was observed: RDW and SDW declined under all GSCs compared to the CK, with the severity of reduction declining as follows: S2-S3 > S1-S2 > S3-S4. Accordingly, the S2-S3 stage was identified as the most severely affected period under drought stress, during which RDW and SDW decreased by 31.62-39.70% and 30.65-33.89%, respectively. Furthermore, the consistent pattern observed across each of GSC—where L-L > L-M and M-L > M-M (with minor exceptions)—clearly demonstrates a negative correlation between both RDW and SDW and the intensity of drought stress.

As the primary water-storing organs, pods and seeds directly determine soybean grain yield through their size and number (Desclaux *et al.*, 2000; Egli & Bruening, 2004). However, drought stress impaired the source-sink relationship of photoassimilates between pods and grains in soybean (Du *et al.*, 2024). This phenomenon occurs most frequently during the critical period from pod expansion to grain filling (Schoving *et al.*, 2022), during which both flowering intensity and pod development are adversely affected (Da Silva *et al.*, 2018), ultimately leading to reductions in GY. Wei *et al.*, (2018) demonstrated that the S3 stage exhibits the greatest sensitivity to water deficit with respect to yield reduction in soybean, followed by S4, with severe drought stress resulting in maximum yield losses ranging from 73% to 82% per plant. This study revealed that the magnitude of GY reduction in soybean under drought stress, when compared to the CK, followed the order: S1-S2 < S2-S3 < S3-S4. Specifically, the S3-S4 stage was the most severely affected, with yield losses ranging from 48.72% to 67.19%. Consistent with this finding, Foroud *et al.*, (1993) and Karam *et al.*, (2005) also reported that water deficit during the early flowering stage exerted less pronounced effects on soybean yield compared to that occurring in the late flowering stage. This study demonstrated that during the S3-S4 stage, TPN, TSN, PWW, and EPR were most severely impacted by drought stress. Among these, the first three indicators decreased by 43.82-49.82%, 47.85-58.94%, and 44.55-64.82%, respectively, while EPR per plant increased 11.48-18.68%. Research confirms that water deficit at the reproductive phase (flowering and pod-setting) substantially compromises soybean yield components (Fang *et al.*, 2010; He *et al.*, 2017), resulting in reductions of up to 42% in TPN, up to 3% in TSN per pod (Gebre & Earl, 2021), and a 13% increase in the EPR per plant (Wei *et al.*, 2018). Corroborating Song *et al.*, (2022), the current study within various GSCs found that higher drought intensity caused a greater decline in both overall soybean yield and its component traits.

This study has certain limitations. Our design specifically investigated consecutive drought, so direct quantitative comparison with single-stage drought effects was not possible. The discussed "compensatory effect" remains a hypothesis derived primarily from SPAD data, warranting targeted validation. Furthermore, findings from this controlled pot experiment require verification under field conditions to account for greater environmental complexity. Future work should integrate single and consecutive stress designs, probe recovery mechanisms, and conduct field trials to better inform drought adaptation strategies.

Conclusions

To assess the impact of continuous drought across two adjacent soybean growth stages, a pot experiment was performed in the Huaibei Plain. The results demonstrated that drought stress during S1-S2 severely inhibited PH and TRL, whereas during S2-S3 it notably decreased PBN, RDW, and SDW, and during S3–S4 exerted the most pronounced adverse effects on GY and its associated traits, including TPN, EPR, TSN, and PWW. In contrast, SPAD values did not differ significantly among GSCs, likely due to recovery after rewatering. Furthermore, under the same initial water deficit, increased drought intensity in a later stage caused more severe reductions in the aforementioned indicators. Comprehensive analysis revealed that soybean GY exhibited the most marked positive correlation with dry matter accumulation and yield components (except EPR), which also contributed the most substantially to the variance of the principal components. Therefore, optimizing the irrigation regime during the late growth stages, particularly S3-S4, is critical for safeguarding soybean yield. This study also provides a scientific basis for water resource management in soybean production under long-term consecutive drought conditions.

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