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Integrated Physiological Evaluation of Drought Tolerance in Soybean (*Glycine max* L. Merrill) Across Segregating Populations

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Abstract
Drought is one of the major constraints affecting soybean yield stability. This study evaluated drought-response variation by combining field phenotyping under restricted irrigation with analysis of segregating populations. A total of 248 segregants (F_3: 106; F_4: 142) from seven biparental crosses, along with 13 F_5 advanced lines, were assessed for canopy temperature (CT), chlorophyll content (SPAD), relative leaf water content (RWC), normalized difference vegetation index (NDVI), hundred-seed weight (HSW), and days to maturity. Results revealed substantial variation: several progenies showed transgressive segregation for NDVI, SPAD, and RWC.

Keywords: Soybean, drought tolerance, canopy temperature, RWC, SPAD, NDVI

Introduction
Soybean (*Glycine max* L. Merrill), a member of the Fabaceae family, is the world's foremost oilseed crop with high agricultural, nutritional, and economic value. It contains 36-40% protein with all essential amino acids, health-promoting unsaturated fatty acids, essential micronutrients, and bioactive compounds such as isoflavones and flavonoids that contribute to human health. Agronomically, soybean improves soil fertility through biological nitrogen fixation, reducing dependence on synthetic fertilizers, while industrially it serves as raw material for oils, paints, fibers, and other products. Globally, the United States, Brazil, Argentina, China, and India account for more than 90% of production (Rodríguez Navarro *et al*., 2010) [16]. In India, soybean covers over 118 lakh hectares with major cultivation in Madhya Pradesh, Maharashtra, and Rajasthan. In Maharashtra, it is primarily grown as a rainfed monsoon crop (Anonymous, 2023) [1]. Soybean is highly sensitive to water stress, and drought is the most critical abiotic factor limiting productivity, with yield losses exceeding 40% under severe conditions (Specht *et al*., 1999; Purcell and Specht, 2016) [19]. The severity of drought impact depends on genotype and stress timing-vegetative-stage drought reduces plant height, stress during flowering reduces seed number, and late stress decreases seed weight. Drought also impairs nitrogen fixation, reducing biomass allocation and yield. Improving drought tolerance in soybean is essential for yield stability. This requires identifying drought-responsive genes, understanding physiological mechanisms such as canopy cooling, water-use efficiency, and symbiotic nitrogen fixation, and applying molecular markers for efficient selection (Turner *et al*., 2001; Blum, 2009; Sinclair, 2018; Ye *et al*., 2018) [21, 4, 17, 22]. Given climate change and increasing drought frequency, developing drought-resilient soybean cultivars is vital for food security and sustainable.

Materials and Methods
Study site
The experiment was conducted at the Botany Research Farm, Post Graduate Institute, MPKV, Rahuri, during the *summer*2024-25 season under restricted irrigation. The experiment was laid out under Randomized Block Design (RBD) with two replications under stress conditions. Sowing was done on 24 January 2025; total rainfall during the crop growth period was 4.4 mm.All necessary cultural practices were followed as recommended.

The experimental materials consisted of seven parental genotypes along with their segregants and advanced lines (Table No. 2.1). A total of 248 segregants from 7 crosses and 13 advanced F_5 lines were evaluated (Table No. 2.2).
All the observations were recorded on five randomly selected plants in each replica. Plants were evaluated for six different physiological parameters viz. Days to maturity, Hundred-seed weight (HSW), Chlorophyll content (SPAD) using SPAD-502 meter (Markwell *et al*., 1995) [12], Relative leaf water content (RWC) estimated using fresh, turgid, and dry weights, Canopy temperature (CT) recorded with an infrared thermometer (Guendouz *et al*., 2012) [6], and NDVI calculated from reflectance values. For this study, an infrared thermometer (CT Gun) was employed to measure canopy temperature. A SPAD Meter was used to quantify the chlorophyll content. The canopy temperature depression (CTD) was subsequently calculated as the difference between the canopy temperature and the air temperature. All data was collected between 11:00 and 15:00 under bright sunlight. The Relative Water Content (RWC) of leaves was estimated as a percentage by measuring the fresh weight (FW), saturated weight (SW), and dry weight (DW) of leaf samples in grams and applying a given formula.

$$\text{Relative Water Content (RWC) \%} = \frac{FW - DW}{TW - DW} \times 100$$

Table 1: Parental genotypes and drought-response characterization

Sr. No.	Genotype	Character
1	TGX 709-50E	Drought tolerant (Delayed wilting)
2	EC 333901	Drought tolerant (Delayed wilting)
3	EC 602288	Drought tolerant
4	NRC 37	Drought sensitive
5	JS 20-98	Moderate Drought Tolerant Widely adopted variety
6	KDS 1173	High yielding advanced line moderately sensitive
7	KDS 1201	High yielding advanced line moderately sensitive

Table 2: Segregants analyzed in the study

Sr. No.	Cross	Total number of segregants (with generation)	
1	NRC 37 × EC 602288	9 (F_3)	18 (F_4)
2	KDS 1201 × EC 333901	17 (F_3)	18 (F_4)
3	KDS 1173 × EC 602288	18 (F_3)	11 (F_4)
4	JS20-98 × EC 333901	34 (F_3)	25 (F_4)
5	JS 20-98 × EC 602288	28 (F_3)	38 (F_4)
6	JS 20-98 × TGX 709-50E	14 (F_4)	13 (F_5)
7	KDS 1201 × TGX 709-50E	-	18 F_4)

Results and Discussion

A statistical analysis of the recorded observations of the parents/segregants was conducted, with the ensuing results presented. Physiological analysis under restricted irrigation revealed substantial variability across crosses and segregants. In the cross I (NRC 37 × EC 602288), canopy temperature (CT) of the F_3 segregants ranged from 30.54 to 32.96°C (mean 31.66°C), markedly lower than both parents (35.82°C in NRC 37 and 34.73°C in EC 602288). In Bazzer *et al*. (2020) got similar result in cross KS4895 × Jackson the drought resistance parent Jackson had lower canopy temperature than drought susceptible parent KS4895. By contrast, the F_4 generation recorded higher CT variation (33.90-36.52°C; mean 35.18 °C), with 2 segregants were cooler than EC 602288, 2 were warmer than susceptible NRC 37. Normalized Difference Vegetation Index values in the F_3 segregants it ranged from 0.68 to 0.81 (mean 0.76), with 8 individuals above 0.74 and only one at 0.68, aligning with EC 602288. In F_4, NDVI varied from 0.69 to 0.78 (mean 0.74), where 2 segregants matched the tolerant parent (0.69) and the16 exceeded parental values. Chlorophyll content (SPAD) of all F_3 segregants (38.44-46.02; mean 41.79) surpassed EC 602288 (38.35). However, in F_4, values ranged widely (25.40-44.38; mean 34.04), with 3 skewed toward EC 602288 and 15 others transgressive below the susceptible parent NRC 37 (37.36). Relative leaf water content was 75.68% in NRC 37 and 76.27% in EC 602288 (Table No. 3.1). Among F_4 segregants, RWC ranged from 74.07% to 80.47% (mean 77.42%). Two individuals were closer to NRC 37, 3 to EC 602288, while 13 exceeded both, indicating improved water retention. Hundred-seed weight averaged 11.68 g in F_3 segregants (8.84-14.13 g), with 8 segregants above NRC 37 (9.53 g). In contrast, F_4 segregants showed lower seed weight overall (6.19-10.22 g; mean 7.74 g), with only 2 above NRC 37 and the majority below EC 602288 (9.10 g). Previously Kumar *et al*. (2017) [11] found that HSW of soybean decreases in drought stress. Days to maturity differed significantly. NRC 37 matured in 98 days, whereas EC 602288 required 108 days. F_3 segregants matured between 103-105 days (mean 111 days), with most surpassing P_2. In F_4, maturity ranged from 98 to 110 days (mean 106 days); one segregant equalled P_1, 4equalled P_2, 7 were intermediate, and 6 matured after P_2. This cross demonstrated strong recombination effects for drought-responsive traits: F_3 segregants often displayed transgressive behaviour in NDVI, SPAD, and HSW, while F_4 lines revealed a mix of skewed and superior segregants for RWC and CTD, reflecting genetic variability in drought-response traits.
In the F_3 generation of cross II (KDS 1201 × EC 333901), canopy temperature of segregants ranged from 31.98 to 34.66 °C (mean 33.61 °C), all lower than both parents (37.28°C in KDS 1201 and 35.19°C in EC 333901), indicating clear transgressive segregation. In F_4, CT ranged from 32.58 to 37.46 °C (mean 34.87 °C); 11 segregants were cooler than the parental range. According to Khan *et al*. (2011), average maximum temperatures ranging from 31 to 37°C negatively impacted seedling dry weight and field emergence of soybean during the growth stage from full bloom to seed initiation. This finding is consistent with the results of the present study. Normalized Difference Vegetation Index of parents was 0.57 (KDS 1201) and 0.61 (EC 333901). In F_3, 17 segregants ranged from 0.54 to 0.77 (mean 0.68), with 15 segregants exceeding parental range. In F_4, NDVI values spanned 0.69-0.80 (mean 0.73), with all 18 segregants outperforming both parents. Previously Jones *et al*. (2025) [8] stated that vegetation indices were able to mimic breeder selections for wilting, with red green blue vegetation index achieving up to 87.5% similarity in two years. Chlorophyll content (SPAD) was 40.57 in KDS 1201 and 43.24 in EC 333901. F_3 segregants ranged from 32.68 to 47.34 (mean 39.05), where 9 were transgressive below both parents. In F_4, SPAD ranged from 29.38 to 46.96 (mean 38.87); while 11 of them showed values lower than both parents indicates transgressive segregation. Relative leaf water content was 79.81% in KDS 1201 and 72.88% in EC 333901. In F_4 segregants, RWC ranged from 61.40% to 76.48% (mean 68.75%). The 13 segregants were

transgressive with lower values than either parent. Hundred-seed weight (HSW) was 10.15 g in KDS 1201 and 9.33 g in EC 333901. In F_3, HSW ranged from 7.49-10.60 g (mean 9.12 g), 7 segregants had HSW below both parents. In F_4, HSW spanned 6.97-11.06 g (mean 8.62 g); 2 exceeded KDS 1201, and 13 were lower than EC 333 901showing transgressive segregation. Days to maturity differed significantly between parents (90 days in KDS 1201 and 102 days in EC 333901). F_3 segregants matured between 89 and 115 days (mean 103 days). One matured earlier than P_1 and 8 exceeded P_2. In F_4, DTM ranged from 86 to 110 days (mean 101 days); 11 segregants required longer days than EC 333 901. Overall, in this cross consistent transgressive segregation was observed for drought-related traits, with segregants consistently having lower canopy temperatures and higher NDVI.

In the F_3 generation of cross III (KDS 1173 × EC 602288), the advanced line KDS 1173 (P_1) recorded a canopy temperature of 32.41°C, while the tolerant parent EC 602288 (P_2) showed 33.43 °C. In the F_3 generation, CT ranged from 29.42 to 35.14 °C (mean 33.34°C). The 6 segregants were beyond the parental range. In the F_4 generation, CT varied between 32.86 and 34.86°C (mean 33.79°C) 5 showing transgressive segregation with lower temperature. These findings highlight significant genetic variability for canopy cooling. NDVI values of P_1 and P_2 were 0.69 and 0.68, respectively. The F_3 segregants showed NDVI between 0.62 and 0.76 (mean 0.68), with 7 segregants having lower NDVI than both parents which were transgressive segregants. In the F_4 generation, NDVI values ranged from 0.72 to 0.81 (mean 0.76), with all segregants surpassing both parents, indicating strong selection for drought-resilient individuals. SPAD readings were 41.99 for P_1 and 34.78 for P_2. In the F_3 generation, values ranged from 31.30 to 44.20 (mean 39.44 SPAD), 1 segregant had value lower than P_2 and 5 exceeding P_1. The F_4 segregants showed SPAD values of 34.18 to 44.44 (mean 37.83), with 10 in the parental range, 1 higher than P_1, and 1 lower than P_2, suggesting segregation for chlorophyll content under drought. RWC of P_1 and P_2 was 73.73% and 76.64%, respectively. F_3 segregants ranged from 67.92% to 81.77% (mean 75.65%), with 3 segregants had lower RWC than P_1 and 7 higher than both parents indicating genetic variability. F_4 segregants ranged from 73.14% to 83.66% (mean 78.26%), 7 exceeding both, confirming improvement in water retention. HSW was nearly equal in both parents (7.81 g in P_1 and 7.79 g in P_2). The Kumar *et al*. (2019) [10] investigated RLWC through generation mean analysis in soybean. They found that RLWC exhibited significant additive effects, with only minor contributions from dominance, indicating that selection for RLWC can be effectively implemented in early segregating generations. In the F_3 generation, HSW ranged from 5.79 to 11.10 g (mean 7.47 g), with 5 exceeding both parents, and 12 below parental range. The F_4 segregants showed 5.05 to 7.76 g (mean 6.63 g), with 10 below parental range, suggesting drought stress reduced seed weight in later generations. P_1 matured in 98 days, while P_2 required 108 days. In the F_3 generation, DTM ranged from 91 to 110 days (mean 99 days), with 12 matching P_1, 1 matching P_2, 2 later than P_2 and 1 earlier than P_1. F_4 segregants matured in 98-109 days (mean 104 days), with 2 later than P_2 and 5 earlier than P_1. This variation indicates both drought escape and tolerance strategies among segregants.

In the F_3 generation of cross IV (JS20-98 × EC 333901), the moderately tolerant parent JS 20-98 (P_1) recorded a canopy temperature of 34.36°C, while the tolerant parent EC 333901 (P_2) showed 35.19 °C. In the F_3 generation, CT ranged from 30.58 °C to 34.78 °C (mean 32.75 °C). Of the 34 segregants, 31 displayed cooler canopy temperatures than both parents. In the F_4 generation, CT ranged from 31.88 °C to 36.58 °C (mean 34.17 °C). Among 25 segregants, 3 exceeded P_2 and 14 were cooler than both parents. These results indicate effective transgressive segregation for cooler canopy temperatures under drought. NDVI values of P_1 and P_2 were 0.65 and 0.61, respectively. The F_3 segregants showed NDVI values between 0.65 and 0.83 (mean 0.78), with 33 of them surpassing it. In the F_4 generation, NDVI ranged from 0.70 to 0.85 (mean 0.78), with all 25 segregants showing values higher than both parents, highlighting selection for vigorous and photosynthetically efficient genotypes. According to Farias *et al*. (2023) [5] increase in NDVI with higher shoot biomass is attributed to soybean's spectral response, where greater photosynthetically active biomass enhances NIR reflectance and reduces red reflectance. This shift, as noted by Smith *et al*. (2017) [18], reflects higher chlorophyll content and overall biomass. Red wavelengths are absorbed by chlorophyll, leading to lower reflectance with increasing chlorophyll levels. SPAD readings were 36.59 for P_1 and 43.24 for P_2. In the F_3 generation, SPAD ranged from 26.98 to 44.94 (mean 36.79). Of 34 segregants, 3 exceeded P_2 and 14 were below both parents. The F_4 segregants showed SPAD values between 26.10 and 40.32 (mean 34.17), with 17 below parental range showing transgressive segregation. The decline in mean SPAD in F_4 suggests stress-driven reduction in chlorophyll content. RWC of P_1 and P_2 was 77.07% and 72.88%, respectively. In the F_3 generation, segregants ranged from 60.68% to 85.31% (mean 73.24%). The 13 segregants were transgressive with lower values. In the F_4 generation, RWC ranged from 65.17% to 82.80% (mean 75.27%). Of 25 segregants, 4 were lower and 9 exceeded both parents. These findings suggest the presence of high-RWC segregants with improved water retention. HSW was 11.12 g in P_1 and 9.33 g in P_2. The F_3 generation ranged from 6.45 to 12.67 g (mean 9.54 g), 12 lower than P_2 and 6 higher than both parents. In F_4, HSW spanned 7.89 to 12.77 g (mean 9.72 g). The 6segregants had lower HSW than both parents and 6 exceeded P_1. This wide distribution reflects transgressive segregation with potential yield improvement. P_1 matured in 100 days and P_2 in 102 days. F_3 segregants matured between 89 and 115 days (mean 106 days), with 22 maturing later than P_2 and 12 earlier than P_1. In F_4, maturity ranged from 100 to 111 days (mean 107 days), 24 later than P_2. The overall delay in maturity suggests both drought avoidance and extended seed-filling strategies among segregants.

Under water stress, the cross V (JS 20-98 × EC 602288) in which JS 20-98 (P_1) recorded canopy temperature 34.36 °C, while EC 602288 (P_2) showed 33.43 °C. In the F_3 generation, CT ranged from 25.84 °C to 33.36 °C (mean 31.98 °C). Of 28 segregants, 21 exhibited cooler CT than both parents. In the F_4 generation, CT values varied from 30.66 °C to 38.16 °C (mean 34.32 °C). Among 38 segregants, 7 were cooler than P_2 and 16 exceeded both parents. This variability indicates scope for selection of genotypes with efficient canopy temperature regulation under drought. NDVI values were 0.65 for P_1 and 0.68 for

P_2. The F_3 segregants ranged from 0.62 to 0.80 (mean 0.73), with 1 below P_1 and 23 transgressive segregants surpassing both parents. The F_4 segregants showed a similar trend, ranging from 0.62 to 0.82 (mean 0.74). One was below P_1, and 33 exceeded both parental values. These results reflect strong transgressive segregation and potential for identifying high-vigor drought-tolerant lines. Nataraj *et al*. (2020) [13] found that NDVI and root-to-shoot ratio (RSR) are primarily governed by additive × additive interactions, suggesting these key drought-related traits can be effectively selected in segregating populations. SPAD values were 36.59 for P_1 and 34.78 for P_2. In F_3, segregants ranged from 26.10 to 44.04 (mean 36.56). Three had less SPAD value than P_2 and 13 surpassed both parents. The F_4 generation showed SPAD values between 26.94 and 43.86 (mean 36.21). Ten were below P_2 and 20 had greater chlorophyll content than both parents. The higher frequency of superior segregants highlights potential for improved photosynthetic performance. RWC of P_1 and P_2 was 77.07% and 76.27%, respectively. In F_3, values spanned 59.45-93.24% (mean 78.54%). The 9 were below P_2, 15 exceeded P_1 and one showed the highest RWC (93.24%) in the study. In F_4, RWC ranged from 54.02% to 77.23% (mean 77.42%). All 37 were below both parents. Patil *et al*. (2013) reported predominant additive gene action for RLWC in F_2 populations from drought-tolerant × susceptible crosses, with minor dominance effects, suggesting its utility in enhancing drought tolerance through selection. HSW of P_1 was 11.12 g and P_2 9.18 g. In F_3, values ranged from 5.86-11.96 g (mean 7.97 g). The 21 had lower HSW than both parents. In F_4, HSW ranged from 5.56-11.30 g (mean 7.74 g). Of 38 segregants 12 had lower HSW than P_2. The distribution indicates yield sensitivity under stress, with occasional transgressive segregants surpassing parents. P_1 matured in 100 days, while P_2 required 108 days. In F_3, segregants ranged from 98-117 days (mean 107.63). Six matured earlier than P_1 and 15 matured later than P_2. In F_4, maturity ranged from 86-114 days (mean 104 days). Nine matured before P_1 and 16 exceeded P_2. The observed spread suggests both early- and late-maturing lines may contribute to drought escape or extended seed-filling. The results highlight the potential to select for improved canopy temperature, NDVI, and SPAD, but also the challenges in maintaining RWC and HSW under stress.

In cross VI (JS 20-98 × TGX 709-50E), the canopy temperature of JS 20-98 (P_1) was 34.36 °C, while TGX 709-50E (P_2) recorded 33.30 °C. In the F_4 generation, 14 segregants showed CT between 30.74 °C and 35.22 °C (mean 33.19 °C). The2 had more CT than P_1 and 5 were cooler than both parents. In advanced lines of F_5 generation, CT ranged from 30.78 °C to 35.06 °C (mean 32.76 °C). Of 13 segregants, 1 exceeded P_1, while 7 were cooler than P_2. The lower mean CT in F_5 suggests improved transpiration cooling and potential drought adaptation. NDVI values were 0.65 for P_1 and 0.74 for P_2. In F_4, segregants recorded values between 0.57 and 0.78 (mean 0.69). One fell below P_1 and 2 exceeded the parental range. In advanced lines of F_5 generation, NDVI improved, ranging from 0.73 to 0.82 (mean 0.79). Ten segregants surpassed P_2. Previously, Bendig *et al*. (2019) [3] demonstrated that drought-tolerant soybean genotypes typically maintain higher NDVI values and exhibit superior canopy retention during mid-to-late reproductive stages. SPAD values were 36.59 for P_1 and 40.81 for P_2. The F_4 segregants ranged from 32.62-46.96 (mean 40.48). Two fell below P_1 and 8 exceeded both parents. In F_5, SPAD ranged from 30.90-48.28 (mean 39.84). Three segregants below P_1 and 7 surpassed both parents. The predominance of higher SPAD segregants indicates improved chlorophyll retention, a desirable trait for sustaining photosynthesis under drought. Parental values of RWC were 77.07% (P_1) and 75.99% (P_2). In F_5, segregants showed a range of 69.25-78.05% (mean 74.02%). One exceeded P_1, while 8 were lower than P_2. The improved mean RWC highlights the presence of individuals with superior water retention, beneficial for maintaining turgor under stress. HSW of P_1 recorded 11.12 g and P_2 9.80 g. In F_4, HSW ranged from 5.79-12.28 g (mean 8.81 g). Two segregants exceeded HSW than P_1 and 7 were below P_2. Similarly in Hauser *et al*. (2025) [7] individual seed weight decreased with increasing temperature. In advanced lines of F_5 generation, values extended from 7.68-14.90 g (mean 9.80 g). One exceeded P_1 and 4 fell below P_2. The wider distribution and higher extremes in F_5 indicate transgressive segregation and potential for yield improvement. JS 20-98 (P_1) matured in 100 days, while TGX 709-50E (P_2) matured in 88 days. In F_4, maturity ranged 89-113 days (mean 101 days). Six segregants matured after P_1. In advanced lines of F_5 generation, maturity extended to 91-114 days (mean 104 days). Twelve segregants took more time than both parents to get matured. The shift toward later maturity in advanced lines may indicate drought escape mechanisms through prolonged seed filling. The presence of segregants exceeding parental values for traits like SPAD, NDVI, HSW, and maturity in later generations (F_4 and F_5) demonstrates successful genetic recombination.

In cross VII (KDS 1201× TGX 709-50E), the canopy temperature of KDS 1201 (P_1) was 37.28°C, while TGX 709-50E (P_2) recorded 33.30°C. The F_4 segregants exhibited CT values ranging from 31.54°C to 35.32°C (mean 33.38°C). Among them 8 were cooler than P_2. The predominance of lower CT segregants suggests inheritance of thermal regulation traits under stress. NDVI values were 0.57 in KDS 1201 (P_1) and 0.74 in TGX 709-50E (P_2). The F_4 population ranged from 0.66 to 0.82, with a mean of 0.76. Nine segregants had exceeded NDVI than P_2. The majority of lines showed transgressive segregation with enhanced NDVI, indicating improved canopy vigour and photosynthetic performance under drought stress. Chlorophyll content was 40.57 SPAD in P_1 and 40.81 SPAD in P_2. The F_4 segregants varied between 31.06 and 43.57 SPAD (mean 36.60). The14 segregants displayed lower value of SPAD. Despite reduced chlorophyll in several segregants, the presence of individuals with superior retention reflects differential expression of photosynthetic traits under stress. RWC was 79.81% in P_1 and 75.99% in P_2. F_4 segregants ranged from 62.75% to 81.75%, with a mean of 72.86%. The9 segregants recorded lower values than both parents. Tiwari *et al*. (2021) [20] also conducted generation mean analysis to assess the genetic architecture of RLWC in soybean. Their results revealed a predominance of additive gene effects with relatively minor contributions from dominance effects. These findings indicate that genetic gain for RLWC can be effectively achieved through selection in early segregating generations. HSW of P_1 recorded 10.15 g and P_2 9.80 g. In the F_4 generation, HSW ranged between 5.63 g and 9.82 g (mean 7.46 g). The 16 were below both parents. The reduction in seed weight highlights drought-induced constraints on seed filling, though a subset-maintained parent-comparable performance. Maturity was 90 days for P_1 and 88 days for P_2. The F_4 segregants matured within 87-112 days (mean 93 days). Two matured earlier than both parents (87 days), while 8 exceeded P_1, maturing later than 90 days. The extended maturity duration in several lines may indicate drought escape strategies or genotypic variation for phenological plasticity. The results highlight both favorable transgressive segregation for certain traits and negative impacts of drought stress on others. Overall impact: The presence of transgressive segregants for RWC, SPAD, and NDVI highlights the potential of recombination to generate superior drought-tolerant phenotypes.

Table 3: Physiological performance of genotypes and segregants of different generations for different parameters acrossseven biparental crosses

Cross I- NRC 37 (P_1) × EC 602288 (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_3 range**	**F_3 mean**	**F_4 range**	**F_4 mean**
CT (°C)	35.82	34.73	30.54 -32.96	31.66	33.90 - 36.52	35.18
CC (SPAD)	37.36	38.35	38.44 - 46.02	41.79	25.40 - 44.38	34.04
NDVI	0.70	0.69	0.68 - 0.81	0.76	0.69 - 0.78	0.74
RWC (%)	75.68	76.27	-	-	74.07 - 80.47	77.42
HSW (g)	9.53	9.10	8.85 - 14.13	11.68	6.19 - 10.22	7.74
DTM (days)	98	108	103 - 115	111	98-110	106
Cross II - KDS 1201 (P_1) × EC 333 901 (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_3 range**	**F_3 mean**	**F_4 range**	**F_4 mean**
CT (°C)	37.28	35.19	31.98 - 34.66	33.61	32.58 - 37.46	34.87
CC (SPAD)	40.57	43.24	32.68 - 47.34	39.05	29.38 - 46.96	38.87
NDVI	0.57	0.61	0.54 - 0.77	0.68	0.69 - 0.80	0.73
RWC (%)	79.81	72.88	-	-	61.40 - 76.48	68.75
HSW (g)	10.15	9.33	7.49 - 10.60	9.12	6.97 - 11.06	8.62
DTM (days)	90	102	89-115	103	86-110	101
Cross III - KDS 1173 (P_1) × EC 602288 (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_3 range**	**F_3 mean**	**F_4 range**	**F_4 mean**
CT (°C)	32.41	34.73	29.42 - 35.14	33.34	32.86 - 34.86	33.79
CC (SPAD)	41.99	38.35	31.30 - 44.20	39.44	34.18 - 44.44	37.83
NDVI	0.69	0.69	0.62 - 0.76	0.68	0.72 - 0.81	0.76
RWC (%)	73.73	76.27	67.92 - 81.77	75.65	73.14 - 83.66	78.26
HSW (g)	7.81	9.10	5.79 - 11.10	7.47	5.05 - 7.76	6.63
DTM (days)	98	108	91-110	99	98 -109	104
Cross IV - JS 20 98 (P_1) × EC 333 901 (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_3 range**	**F_3 mean**	**F_4 range**	**F_4 mean**
CT (°C)	34.36	35.19	30.58 - 34.78	32.75	31.88 - 36.58	34.17
CC (SPAD)	36.59	43.24	26.98 - 44.94	36.79	26.10 - 40.32	34.17
NDVI	0.65	0.61	0.65 - 0.83	0.78	0.70 - 0.85	0.78
RWC (%)	77.07	72.88	60.68 - 85.31	73.24	65.17 - 82.80	75.27
HSW (g)	11.12	9.33	6.45 - 12.67	9.54	7.89 - 12.77	9.72
DTM (days)	100	102	89-115	106	100 - 111	107
Cross V - JS 20 98 (P_1) × EC 602288 (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_3 range**	**F_3 mean**	**F_4 range**	**F_4 mean**
CT (°C)	34.36	34.73	25.84 - 33.36	31.98	30.66 - 38.16	34.32
CC (SPAD)	36.59	38.35	26.10 - 44.04	36.56	26.94 - 42.86	36.21
NDVI	0.65	0.69	0.62 - 0.80	0.73	0.62 - 0.82	0.74
RWC (%)	77.07	76.27	59.45 - 93.24	78.54	54.02 - 77.23	68.70
HSW (g)	11.12	9.10	5.86 - 11.96	7.97	5.56 - 11.30	8.09
DTM (days)	100	108	98-117	108	86 - 114	104
Cross VI - JS 20 98 (P_1) × TGX 709-50E (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_4 range**	**F_4 mean**	**F_5 range**	**F_5 mean**
CT (°C)	34.36	33.30	30.74 - 35.22	33.19	30.78 - 35.06	32.76
CC (SPAD)	36.59	40.81	32.62 - 46.96	40.48	30.90 - 48.28	39.84
NDVI	0.65	0.74	0.57 - 0.78	0.69	0.73 - 0.82	0.79
RWC (%)	77.07	75.99	-	-	69.25 - 78.05	74.02
HSW (g)	11.12	9.80	5.79-12.28	8.81	7.68-14.90	9.80
DTM (days)	100	88	89-113	101	91 - 114	104
Cross VII - KDS 1201 (P_1) × TGX 709-50E (P_2)						
Trait	**P_1 value**	**P_2 value**	**F_4 range**		**F_4 mean**	
CT (°C)	37.28	33.30	31.54 - 35.32		33.38	
CC (SPAD)	40.57	40.81	31.06 - 43.57		36.60	
NDVI	0.57	0.74	0.66 - 0.82		0.76	
RWC (%)	79.81	75.99	62.75 - 81.75		72.86	
HSW (g)	10.15	9.80	5.63 - 9.82		7.46	
DTM (days)	90	88	87-112		93.47	

Conclusion

The evaluation of seven soybean crosses under drought stress revealed substantial variability across physiological and yield-related traits, highlighting the potential of specific parental combinations for developing drought-resilient genotypes.

Canopy Temperature (CT): The cross JS 20-98 × EC 602288 showed the lowest mean canopy temperature in F_3 (31.98 °C) and presence of highly cooler segregants, making it the most promising cross for heat avoidance and transpirational cooling.

Normalized Difference Vegetation Index (NDVI): The cross JS 20-98 × EC 333901 consistently recorded the highest NDVI across F_3 and F_4 generations (mean ~0.78), surpassing both parents, thereby indicating superior photosynthetic efficiency and canopy vigour.

Chlorophyll Content (SPAD): The cross JS 20-98 × TGX 709-50E produced transgressive segregants with SPAD values up to 48.28, higher than both parents, suggesting greater chlorophyll retention and sustained photosynthetic capacity under stress.

Relative Water Content (RWC): The highest RWC was observed in JS 20-98 × EC 602288, where one F_3 segregant recorded 93.24%, reflecting superior water conservation ability. This cross therefore holds strong potential for water-use efficiency under drought.

Hundred Seed Weight (HSW): The cross JS 20-98 × TGX 709-50E showed the highest seed weight in advanced generation (up to 14.90 g), outperforming both parents, indicating strong potential for yield stability under stress.

Days to Maturity (DTM): The cross KDS 1201 × TGX 709-50E produced segregants maturing as early as 87 days, suggesting drought escape potential, while other crosses such as JS 20-98 × EC 602288 generated lines with delayed maturity (up to 117 days), useful for prolonged seed filling.
Overall, the crosses JS 20-98 × EC 602288 and JS 20-98 × TGX 709-50E emerged as the most promising, contributing superior segregants across multiple traits, particularly for cooler canopy temperature, higher RWC, greater seed weight, and extended photosynthetic efficiency. These cross offer valuable genetic resources for breeding drought-tolerant soybean cultivars and can be utilized in future breeding pipelines aimed at enhancing yield stability under water-limited environments.

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