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Photosynthetic and related traits in variety-specific drought acclimation of soybean

Jiří Krucký[1], Václav Hejnák[1], Marek Popov[1], Jan Kubeš[1], Jana Česká[1], Ľubomír Harenčár[2], Milan Skalický[1], Pavla Vachová[1], František Hnilička[1]*

[1]*Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Institute of Plant Genetics and Biotechnology, Plant Science and Biodiversity Centre, Slovak Academy of Sciences, Nitra, Slovak Republic*
**Corresponding author: krucky@af.czu.cz*

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Abstract: This study evaluated the acclimation of eight soybean varieties to water deficit through changes in photosynthetic performance and physiological, biochemical, and epigenetic traits. Plants were grown under controlled conditions in two treatments: control (80% water-holding capacity; WHC) and drought (40% WHC). Water status was assessed using relative water content and osmotic potential, while photosynthetic performance was evaluated using gas exchange, water use efficiency, maximum quantum efficiency of photosystem II, chlorophylls, and carotenoids. Total flavonoid content, reducing power, proline accumulation, and 5-methylcytosine content were determined. Water deficit induced marked variety-specific changes across traits. The varieties Hana and Atacama maintained higher relative water content (RWC), less negative leaf osmotic potential (Ψ_s), and more favourable photosynthetic performance under drought, while the variety Atlas showed a favourable profile in several other traits. In contrast, the varieties Dornburg, York, and Impala showed a less favourable response, whereas the varieties Labrador and Zaria occupied intermediate positions depending on the trait evaluated. Multivariate and correlation analyses showed that photosynthetic performance was closely associated with plant water status, while stronger biochemical and epigenetic responses were more evident in less favourable or intermediate profiles. These results indicate that soybean acclimation to water deficit is coordinated and variety-specific and cannot be assessed using a single parameter.

Keywords: water relations; chlorophyll fluorescence; phenotypic plasticity; water stress; *Glycine max* L.

Plant acclimation to water deficit is particularly evident in water status parameters and photosynthetic performance (Hossain et al. 2024a, Jiang et al. 2025). Changes in plant water status are reflected in relative water content and leaf osmotic potential, and subsequently affect stomatal conductance, transpiration rate, photosynthetic rate, and water use efficiency (Parasuraman et al. 2024, Rodrigues et al. 2024). This level of response is also associated with the condition of the photosynthetic apparatus, which can be evaluated by measuring the maximum quantum efficiency of photosystem II and the content of photosynthetic pigments (Buezo et al. 2019, Basal et al. 2024). Together, indicators of plant water status, gas exchange, water-use efficiency, chlorophyll fluorescence, and photosynthetic pigment content therefore represent important components in evaluating plant responses to drought (Hossain et al. 2024a, Abdullah et al. 2025). At the same time, however, acclimation to water deficit cannot be reli-

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ably assessed by a single physiological trait; rather, it should be understood as the result of interrelated changes across multiple parameters (Aphalo and Sadras 2022, Alghabari 2026).

Beyond water status and photosynthetic performance, the plant response to drought also includes biochemical and epigenetic changes (Basal et al. 2024, Krucky et al. 2025). An important role may be played particularly by antioxidant and osmotically related metabolic responses, such as changes in flavonoid content, reducing power, or proline accumulation (Hossain et al. 2024b, Lin et al. 2024). In addition, epigenetic regulation may also contribute to the plant response, including changes in 5-methylcytosine content, which may reflect regulatory reorganisation of metabolism under stress conditions (Zi et al. 2024, Lodhi and Srivastava 2025). Although individual parameters of the soybean response to drought have been described in several studies (Rasheed et al. 2022, Alghabari 2026), a more comprehensive evaluation is still lacking, one that simultaneously integrates photosynthetic performance with water status parameters, biochemical responses, and epigenetic regulation across a larger set of varieties. It also remains less clear to what extent these traits are interconnected within the variety-dependent response (Alzahrani 2024).

In this study, acclimation is understood as a set of plastic changes by which plants respond to water deficit over their lifetimes (Aphalo and Sadras 2022, Jiang et al. 2025). Because the extent and nature of these changes depend on the interaction between variety and environment, the acclimation response can also be evaluated at the level of individual varieties under controlled conditions (Alseekh et al. 2025). This study aimed to evaluate the acclimation of soybean varieties to water deficit and to identify photosynthetic, physiological, biochemical, and epigenetic traits associated with their variety-specific responses and more favourable acclimation profiles. In line with this aim, the following hypotheses were formulated: (*i*) water deficit induces variety-specific changes in photosynthetic performance as well as in other monitored physiological, biochemical, and epigenetic traits; and (*ii*) photosynthetic performance parameters show significant mutual correlations and are also correlated with other monitored characteristics, especially with parameters of water status, biochemical response, and epigenetic regulation, and these relationships may contribute to distinguishing varieties with a more favourable acclimation profile.

MATERIAL AND METHODS

Plant material and growth conditions. Eight soybean varieties (*Glycine max* L.) of different geographic origins were used in the experiment: Atacama (Austria), Atlas (Romania), Dornburg (Germany), Hana (Czech Republic), Impala (South Africa), Labrador (France), York (USA), and Zaria (Bulgaria). These varieties were selected to compare their responses to water deficit. Seeds of all varieties were provided by the Gene Bank of the Slovak Republic, National Agricultural and Food Centre, Research Institute of Plant Production, Piestany, Slovak Republic.

Before sowing, the seeds were surface-disinfected in a 1% sodium hypochlorite (NaClO) solution for 5 min and then sown in plastic pots (11 × 11 × 23 cm) filled with a peat-based substrate (Klasmann TS2, Geeste, Germany). The substrate consisted of natural light peat with adjusted pH, had an electrical conductivity of 55 mS/m, a pH_{H_2O} of 5.5–6.5, and contained 2.0 kg/m^3 added NPK fertiliser (14:16:18). The plants were grown in a controlled-environment growth chamber (Conviron E8, Winnipeg, Canada) equipped with a CMP6050 control system under a photosynthetic photon flux density of 750 µmol/m^2/s, a 14/10 h day/night photoperiod, a 23/18 °C day/night temperature regime, and relative humidity of 50% during the day and 60% at night.

Experimental design and treatments. The cultivation phase lasted 45 days, during which all plants were regularly watered to ensure uniform growth until the third trifoliate leaf stage (BBCH 13). For each variety, 10 pots were established, with four plants per pot. Subsequently, for each variety, five pots were randomly assigned to the control treatment (well-watered) and five to the drought treatment (water-deficient). For subsequent measurements and sampling, one plant per pot was used for each analysed trait; thus, five biological replicates were evaluated for each variety × treatment combination (n = 5).

Substrate water-holding capacity (WHC) was determined gravimetrically using a modified saturation-based procedure according to Junker et al. (2015). Briefly, pots filled with the substrate were fully saturated with water and, after the weight had stabilised, the wet reference weight (W_wet) was recorded. The substrate was then dried to constant weight to obtain the dry reference weight (W_dry). The weight of the empty pot was also included in

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the calculation. Target pot weights (W_target) corresponding to the required substrate moisture level were calculated according to the following equation:

$$W\_target = W\_dry + f \times (W\_wet - W\_dry)$$

where f = 0.80 for the control and f = 0.40 for the drought treatment.

After treatment assignment, pots designated for drought were allowed to dry naturally to 40% WHC. Once this level had been reached, the 10-day experimental phase was initiated. The weight of all pots was checked daily and, as required, distilled water was added to maintain 80% WHC in the control treatment and 40% WHC in the drought treatment throughout the experimental phase. At the end of the experimental phase, measurements and sampling were performed at BBCH 15, after which the experiment was terminated.

Water status. Relative water content (RWC) was determined gravimetrically on fully expanded trifoliate leaves. Five equally sized leaf segments were collected from each sampled leaf and weighed immediately to determine fresh weight (FW). The segments were then saturated in distilled water for 4 h, gently blotted dry with filter paper, and weighed again to determine turgid weight (TW). They were subsequently dried at 90 °C for 3 h to determine dry weight (DW). RWC was calculated according to the following equation:

$$RWC\ (\%) = (FW - DW)/(TW - DW) \times 100.$$

Leaf osmotic potential (Ψ_s) was determined using a PSYPRO instrument (Wescor Inc., Logan, USA) according to Krucky et al. (2025), and values were expressed in MPa.

Photosynthetic performance. The maximum quantum efficiency of photosystem II (F_v/F_m) was determined using a portable FluorPen FP110 fluorometer (PSI, Drásov, Czech Republic). Fully expanded trifoliate soybean leaves were dark-adapted for 20 min using removable leaf clips, and measurements were performed in the morning (9:00–11:00).

Leaf gas exchange parameters, including net photosynthetic rate A (μmol $CO_2/m^2/s$), transpiration rate E (mmol $H_2O/m^2/s$), and stomatal conductance g_s (mol $H_2O/m^2/s$), were measured on fully expanded trifoliate leaves using the portable LCpro+ gas exchange system (ADC BioScientific Ltd., Hoddesdon, UK). Measurements were performed in the morning (9:00–11:00) under steady-state conditions in a measurement chamber at 23 °C and a photon flux density of 750 μmol/m^2/s. Instantaneous water use efficiency (WUE) was calculated as the A/E ratio, and intrinsic water use efficiency (WUE_i) as the A/g_s ratio.

Photosynthetic pigment content (Chl *a*, Chl *b*, total chlorophyll, and carotenoids) was determined spectrophotometrically from equally sized leaf discs collected from soybean leaves. The discs were extracted in 1 mL N,N-dimethylformamide for 4 h in the dark. Subsequently, the extracts were shaken for 45 min on a shaker (GFL 3005, Verkon Ltd., Prague, Czech Republic), and absorbance was measured using a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, Waltham, USA) at wavelengths of 663.8, 646.8, and 480 nm. Chlorophyll concentrations were calculated according to Porra et al. (1989), and carotenoids according to Wellburn (1994), and expressed in mg/m^2.

Biochemical traits. Extracts for determining total flavonoid content (TFC) and reducing power (RP) in leaves and roots were prepared as described by Peiretti et al. (2019). TFC was determined according to Tsanova-Savova et al. (2018) and expressed as quercetin equivalents (mg QE/g FW). RP was determined according to Oyaizu (1986) and expressed as ascorbic acid equivalents (mg AAE/g FW).

Leaf proline content was determined according to Bates et al. (1973) and expressed as μmol/g FW.

DNA isolation and 5-methylcytosine quantification. Soybean leaf samples were homogenised in liquid nitrogen using a mortar and pestle. DNA was isolated from 100 mg of fresh leaf tissue using the NucleoSpin Plant II Kit (Macherey-Nagel GmbH & Co. KG, Dueren, Germany) according to the recommended miniprep protocol with PL1 lysis buffer. Global DNA methylation was determined from 100 ng DNA using a fluorometric MethylFlash Methylated DNA Quantification Kit (Epigentek Group Inc., Farmingdale, USA) according to the manufacturer's protocol. Fluorescence (530EX/590EM) was measured on a fluorescence microplate reader (Tecan Infinity M200, Tecan Deutschland GmbH, Crailsheim, Germany) and evaluated using Magellan software. Global DNA methylation was expressed as 5-methylcytosine content (5-mC, %).

Statistical analysis. Statistical analyses were performed in R using the RStudio integrated development environment (version 2026.01.1+403, Posit Software, PBC, Boston, USA). For all quantitative traits, data from five biological replicates were used for each variety × treatment combination (n = 5). Results are presented as mean ± standard deviation (SD). For individual traits, a two-way analysis of vari-

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ance (ANOVA) was applied with variety and treatment as fixed factors, including their interaction (variety × treatment). When the ANOVA indicated a statistically significant effect ($P < 0.05$), differences among variety × treatment combinations were assessed using Tukey's *HSD* (honestly significant difference) test, and statistically homogeneous groups were indicated by different letters.

For the multivariate analyses, variety-specific Δ values were calculated for each trait as the difference between the mean value under drought treatment and the corresponding mean value under control conditions (Δ = drought – control). These Δ values were used to visualise drought-induced responses by heatmap, principal component analysis (PCA), and Pearson correlation analysis. For the heatmap, Δ values were standardised by variable using z-scores. PCA was performed on centred and scaled Δ values. Relationships among drought-induced changes in the measured traits were assessed by calculating Pearson correlation coefficients (r) for all pairwise combinations of traits from Δ values across varieties, and their statistical significance was evaluated using corresponding P-values. In the correlation matrix, asterisks indicate significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

RESULTS

Water status. Drought significantly affected the water status of all evaluated soybean varieties, as reflected by a significant decrease in relative water content (RWC; Figure 1) and a simultaneous shift in leaf osmotic potential towards more negative values (Figure 2). Under control conditions, RWC values ranged within a narrow interval of 82.6–86.4%, whereas under drought, they decreased to 41.9–62.3%. Under drought conditions, the varieties Hana (62.3%) and Atacama (60.2%) maintained significantly higher RWC values, whereas the varieties Atlas (54.8%) and Labrador (53.1%) formed an intermediate group. In contrast, the lowest RWC values under drought were recorded in the varieties York (41.9%), Zaria (42.7%), Impala (43.3%), and Dornburg (44.0%), which formed the statistically lowest group.

A similar response was also observed for Ψ_s. Under control conditions, Ψ_s values were balanced among varieties (–0.9 to –0.7 MPa), whereas under drought, they significantly decreased to –2.8 to –1.4 MPa. The significantly highest Ψ_s value under drought was recorded in the variety Hana (–1.4 MPa). The varieties Atacama, Atlas, and Labrador showed significantly higher Ψ_s values than the varieties Dornburg (–2.8 MPa), Impala (–2.7 MPa), York (–2.6 MPa), and Zaria (–2.5 MPa), which showed the most negative Ψ_s values under drought conditions.

Photosynthetic performance. Drought significantly affected the maximum quantum efficiency of photosystem II (F_v/F_m), gas exchange parameters, and water use efficiency in the evaluated soybean varieties (Table 1). Under control conditions, F_v/F_m values ranged from 0.81 to 0.83, whereas under drought, they decreased to values ranging from 0.76 to 0.81.

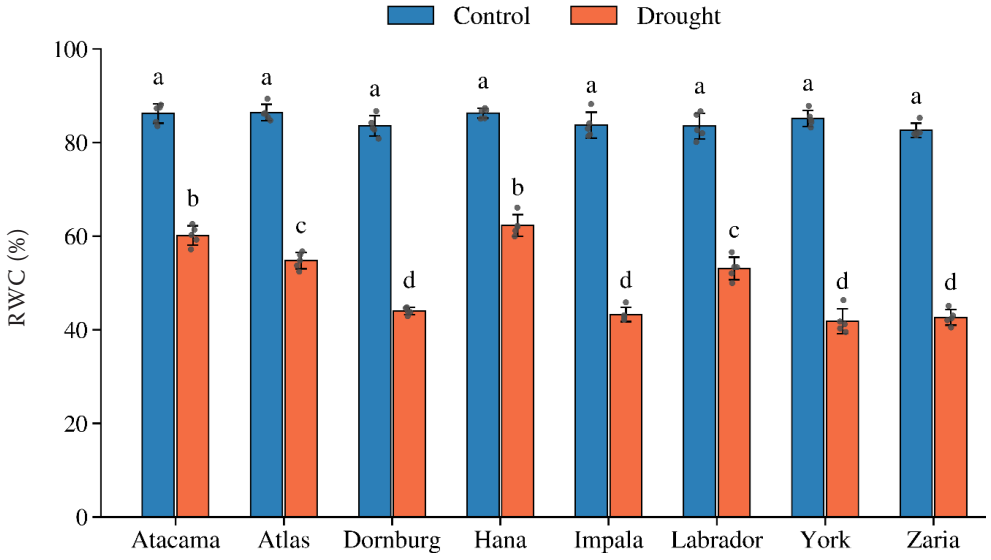

Figure 1. Relative water content (RWC) of soybean varieties under control and drought conditions. Data are presented as means ± SD ($n = 5$); ANOVA: $P < 0.001$. Different letters indicate statistically significant differences among variety × treatment combinations (Tukey's *HSD* test, $P < 0.05$)

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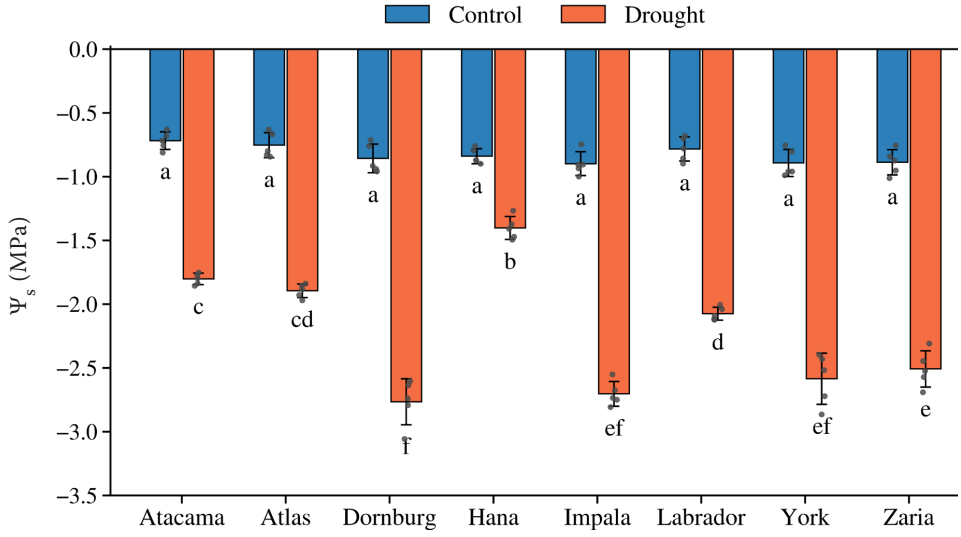

Figure 2. Leaf osmotic potential (Ψ_s) of soybean varieties under control and drought conditions. Data are presented as means ± SD (n = 5); ANOVA: $P < 0.001$. Different letters indicate statistically significant differences among variety × treatment combinations (Tukey's *HSD* test, $P < 0.05$)

Table 1. Chlorophyll fluorescence, gas exchange, and water use efficiency parameters of soybean varieties under control and drought conditions

Variety	Treatment	F_v/F_m	A (μmol $CO_2/m^2/s$)	E (mmol $H_2O/m^2/s$)	g_s (mol $H_2O/m^2/s$)	WUE	WUE_i
Atacama	control	0.82 ± 0.006^{abc}	17.5 ± 2.2^{abc}	2.4 ± 0.34^{a}	0.17 ± 0.026^{a}	7.5 ± 1.6^{bc}	105 ± 6^{bcd}
	drought	0.80 ± 0.010^{bcd}	8.0 ± 0.8^{d}	0.7 ± 0.03^{b}	0.04 ± 0.007^{b}	10.7 ± 1.2^{a}	208 ± 38^{a}
Atlas	control	0.83 ± 0.005^{a}	20.5 ± 3.4^{a}	2.5 ± 0.30^{a}	0.19 ± 0.029^{a}	8.2 ± 1.7^{ab}	107 ± 15^{bc}
	drought	0.81 ± 0.014^{abcd}	6.3 ± 0.9^{d}	0.7 ± 0.01^{bc}	0.03 ± 0.004^{b}	8.6 ± 1.2^{ab}	195 ± 43^{a}
Dornburg	control	0.81 ± 0.003^{abcd}	16.7 ± 1.2^{c}	2.4 ± 0.23^{a}	0.17 ± 0.025^{a}	6.9 ± 0.7^{bcd}	103 ± 16^{bcd}
	drought	0.76 ± 0.018^{e}	0.5 ± 0.1^{e}	0.3 ± 0.02^{d}	0.01 ± 0.005^{b}	1.6 ± 0.3^{f}	44 ± 17^{e}
Hana	control	0.82 ± 0.008^{abc}	19.8 ± 0.7^{ab}	2.5 ± 0.15^{a}	0.18 ± 0.019^{a}	8.1 ± 0.7^{b}	113 ± 12^{b}
	drought	0.81 ± 0.010^{abc}	8.0 ± 0.7^{d}	0.7 ± 0.02^{b}	0.04 ± 0.006^{b}	10.7 ± 0.9^{a}	231 ± 30^{a}
Impala	control	0.82 ± 0.004^{ab}	17.2 ± 1.9^{bc}	2.3 ± 0.22^{a}	0.17 ± 0.020^{a}	7.7 ± 1.5^{bc}	104 ± 15^{bcd}
	drought	0.79 ± 0.014^{de}	0.7 ± 0.2^{e}	0.3 ± 0.01^{d}	0.01 ± 0.003^{b}	2.3 ± 0.7^{ef}	59 ± 16de
Labrador	control	0.82 ± 0.007^{ab}	18.3 ± 1.6^{abc}	2.3 ± 0.20^{a}	0.17 ± 0.021^{a}	7.9 ± 1.0^{bc}	111 ± 16^{b}
	drought	0.80 ± 0.012^{bcd}	2.2 ± 0.5^{e}	0.3 ± 0.01^{d}	0.02 ± 0.007^{b}	7.6 ± 1.8^{bc}	126 ± 27^{b}
York	control	0.82 ± 0.009^{abc}	18.9 ± 1.2^{abc}	2.4 ± 0.27^{a}	0.17 ± 0.020^{a}	8.0 ± 1.1^{bc}	112 ± 9^{b}
	drought	0.79 ± 0.017^{cd}	0.8 ± 0.1^{e}	0.2 ± 0.01^{d}	0.01 ± 0.003^{b}	4.6 ± 0.6^{de}	61 ± 11^{cde}
Zaria	control	0.82 ± 0.009^{abc}	18.8 ± 1.8^{abc}	2.4 ± 0.20^{a}	0.17 ± 0.025^{a}	7.7 ± 0.9^{bc}	113 ± 12^{b}
	drought	0.78 ± 0.024^{de}	1.9 ± 0.3^{e}	0.4 ± 0.01^{cd}	0.02 ± 0.004^{b}	5.5 ± 0.9^{cd}	96 ± 16^{bcd}
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

F_v/F_m – maximum quantum efficiency of photosystem II; A – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; WUE – instantaneous water use efficiency; WUE_i – intrinsic water use efficiency; data are presented as means ± SD (n = 5); ANOVA: $P < 0.001$; different letters indicate statistically significant differences among variety × treatment combinations (Tukey's *HSD* test, $P < 0.05$)

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The highest F_v/F_m value under drought was recorded in the variety Hana (0.81), which was significantly higher than those of the varieties Dornburg (0.76), Zaria (0.78), and Impala (0.79).

A more pronounced effect of drought was evident in gas exchange parameters. Net photosynthetic rate (A) ranged from 16.7 to 20.5 μmol $CO_2/m^2/s$ under control conditions, whereas under drought, it decreased to 0.5–8.0 μmol $CO_2/m^2/s$. Under drought conditions, the varieties Atacama (8.0 μmol $CO_2/m^2/s$), Hana (8.0 μmol $CO_2/m^2/s$), and Atlas (6.3 μmol $CO_2/m^2/s$) formed a statistically higher group, whereas the varieties Dornburg, Impala, York, Zaria, and Labrador formed a statistically lower group. A similar response was recorded for transpiration rate (E), which under drought reached 0.2–0.7 mmol $H_2O/m^2/s$. The highest E values were recorded in the varieties Atacama, Hana, and Atlas (all 0.7 mmol $H_2O/m^2/s$), whereas the statistically lowest values were found in the varieties Dornburg, Impala, Labrador, and York; the variety Zaria occupied an intermediate position. Stomatal conductance (g_s) also significantly decreased under drought in all varieties, from 0.17–0.19 to 0.01–0.04 mol $H_2O/m^2/s$, but no statistically significant differences among varieties under drought were detected.

A variety-specific response was also evident in water use efficiency. Instantaneous water use efficiency (WUE) ranged within a narrow interval of 6.9–8.2 under control conditions, whereas under drought, higher variability among varieties was evident, with values ranging from 1.6 to 10.7. The highest WUE values under drought were recorded in the varieties Atacama, Hana, and Atlas, which showed significantly higher values than the varieties Dornburg, Impala, York, and Zaria; the variety Labrador occupied an intermediate position. Similarly, intrinsic water use efficiency (WUE_i) showed balanced values under control conditions, ranging from 103 to 113, whereas under drought, it ranged more widely from 44 to 231. Under drought conditions, the varieties Hana (231), Atacama (208), and Atlas (195) formed the statistically highest group, whereas the lowest values were recorded in the varieties Dornburg (44), Impala (59), and York (61). The varieties Labrador and Zaria occupied intermediate positions according to the statistical grouping.

Drought significantly affected the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll, and carotenoids in the evaluated soybean varieties (Table 2). Differences among varieties were already apparent under control conditions, when

Table 2. Photosynthetic pigment content of soybean varieties under control and drought conditions

Variety	Treatment	Chl *a* (mg/m^2)	Chl *b* (mg/m^2)	Total chlorophyll (mg/m^2)	Carotenoids (mg/m^2)
Atacama	control	623 ± 13^{cde}	206 ± 5^{cd}	829 ± 13^{cde}	132 ± 3^{bcd}
	drought	502 ± 7^{gh}	149 ± 5^{ef}	651 ± 12^{gh}	102 ± 3^{fgh}
Atlas	control	736 ± 69^{a}	267 ± 37^{a}	1003 ± 105^{a}	151 ± 18^{a}
	drought	647 ± 14^{bcd}	237 ± 8^{abc}	884 ± 21^{bc}	131 ± 4^{bcd}
Dornburg	control	584 ± 27^{def}	181 ± 11^{de}	766 ± 37^{ef}	117 ± 9^{def}
	drought	457 ± 34^{h}	142 ± 5^{f}	599 ± 36^{h}	91 ± 8^{h}
Hana	control	699 ± 24^{ab}	259 ± 11^{ab}	958 ± 34^{ab}	140 ± 6^{ab}
	drought	683 ± 38^{abc}	247 ± 27^{ab}	929 ± 64^{ab}	121 ± 8^{cde}
Impala	control	587 ± 15^{def}	192 ± 7^{d}	779 ± 21^{def}	97 ± 5^{gh}
	drought	486 ± 28^{gh}	150 ± 9^{ef}	636 ± 36^{gh}	93 ± 8^{h}
Labrador	control	641 ± 21^{bcde}	230 ± 13^{bc}	870 ± 32^{bcd}	137 ± 7^{abc}
	drought	534 ± 21^{fg}	177 ± 8^{def}	710 ± 29^{fg}	106 ± 6^{efgh}
York	control	699 ± 13^{ab}	266 ± 10^{a}	965 ± 23^{ab}	140 ± 4^{ab}
	drought	483 ± 29^{gh}	152 ± 11^{ef}	635 ± 40^{gh}	98 ± 6^{gh}
Zaria	control	687 ± 42^{abc}	264 ± 28^{ab}	950 ± 69^{ab}	137 ± 8^{abc}
	drought	580 ± 14^{ef}	194 ± 8^{d}	774 ± 21^{def}	112 ± 3^{efg}
P		< 0.001	< 0.001	< 0.001	< 0.001

Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; data are presented as means ± SD ($n = 5$); ANOVA: $P < 0.001$; different letters indicate statistically significant differences among variety × treatment combinations (Tukey's *HSD* test, $P < 0.05$)

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higher chlorophyll pigment values were recorded mainly in the varieties Atlas, Hana, York, and Zaria. In contrast, lower values were found in the varieties Dornburg, Impala, Atacama, and Labrador. Under drought conditions, the varieties Hana and Atlas maintained the significantly highest chlorophyll pigment contents among all evaluated varieties and did not differ significantly from each other according to the statistical grouping. The variety Hana showed the highest contents of Chl *a* (683 mg/m^2), Chl *b* (247 mg/m^2), and total chlorophyll (929 mg/m^2), followed by the variety Atlas, with values of 647, 237, and 884 mg/m^2, respectively. The lowest values were recorded in the variety Dornburg (457, 142, and 599 mg/m^2).

Differences among varieties under control conditions were also apparent for carotenoids, with higher values recorded in the varieties Atlas, Hana, York, Zaria, Labrador, and Atacama, whereas lower values were found in the varieties Impala and Dornburg. Under drought conditions, the highest carotenoid content was recorded in the variety Atlas (131 mg/m^2), followed by the variety Hana (121 mg/m^2), whereas the lowest values were found in the varieties Dornburg (91 mg/m^2) and Impala (93 mg/m^2). The statistical grouping confirmed that the variety Atlas showed a significantly higher carotenoid content under drought than the varieties Dornburg, Impala, York, Atacama, Labrador, and Zaria, whereas no statistically significant difference was detected in comparison with the variety Hana.

Biochemical and epigenetic traits. Drought significantly affected total flavonoid content, reducing power, proline content, and 5-methylcytosine content in the evaluated soybean varieties (Table 3). Under control conditions, total flavonoid content in leaves (TFC leaves) ranged from 2.1 to 4.4 mg QE/g FW, whereas under drought, it increased to 6.1–13.9 mg QE/g FW. The highest TFC leaves value under drought was recorded in the variety Labrador (13.9 mg QE/g FW), which formed the statistically highest group. Higher TFC leaves values were also recorded in the varieties Dornburg, York, and Zaria, whereas the statistically lowest value under drought was found in the variety Atlas (6.1 mg QE/g FW). Total flavonoid content in roots

Table 3. Biochemical and epigenetic traits of soybean varieties under control and drought conditions

Variety	Treatment	TFC leaves (mg QE/g FW)	RP leaves (mg AAE/g FW)	TFC roots (mg QE/g FW)	RP roots (mg AAE/g FW)	Proline (μmol/g FW)	5-mC (%)
Atacama	control	3.1 ± 0.17^{j}	28.2 ± 0.3^{k}	0.81 ± 0.06^{fg}	5.4 ± 0.2^{k}	3.4 ± 0.3^{f}	32.7 ± 0.6^{e}
	drought	8.9 ± 0.03^{e}	65.8 ± 0.4^{e}	0.91 ± 0.03^{de}	12.3 ± 0.3^{efg}	18.0 ± 5.3^{bc}	46.9 ± 0.4^{b}
Atlas	control	2.2 ± 0.12^{l}	16.7 ± 0.1^{m}	0.79 ± 0.02^{fg}	8.0 ± 0.6^{j}	4.7 ± 0.3^{ef}	26.9 ± 0.4^{f}
	drought	6.1 ± 0.07^{g}	61.7 ± 1.1^{f}	1.00 ± 0.03^{c}	12.8 ± 0.2^{ef}	55.8 ± 3.4^{a}	44.6 ± 0.8^{c}
Dornburg	control	2.7 ± 0.02^{k}	28.4 ± 4.0^{k}	0.81 ± 0.03^{fg}	11.6 ± 0.3^{fgh}	3.3 ± 0.1^{f}	27.0 ± 0.7^{f}
	drought	12.4 ± 0.17^{b}	83.9 ± 0.8^{b}	0.86 ± 0.01^{efg}	10.7 ± 0.6^{hi}	25.8 ± 10.5^{b}	48.1 ± 1.1^{ab}
Hana	control	2.1 ± 0.04^{l}	20.8 ± 1.3^{l}	0.78 ± 0.03^{g}	9.4 ± 0.2^{ij}	5.2 ± 1.4^{def}	21.8 ± 0.8^{g}
	drought	7.1 ± 0.27^{f}	51.8 ± 1.2^{g}	0.85 ± 0.05^{efg}	14.9 ± 0.3^{d}	12.8 ± 2.6^{cde}	35.4 ± 1.1^{d}
Impala	control	3.9 ± 0.01^{i}	34.3 ± 0.4^{i}	0.99 ± 0.07^{cd}	13.9 ± 0.5^{de}	5.4 ± 0.9^{def}	32.3 ± 0.5^{e}
	drought	8.5 ± 0.16^{e}	96.1 ± 0.2^{a}	1.50 ± 0.01^{a}	28.7 ± 0.9^{a}	13.2 ± 2.9^{cd}	49.6 ± 0.7^{a}
Labrador	control	4.4 ± 0.05^{h}	39.0 ± 0.9^{h}	0.86 ± 0.01^{efg}	11.2 ± 1.3^{gh}	4.9 ± 0.6^{def}	20.8 ± 0.5^{g}
	drought	13.9 ± 0.29^{a}	75.1 ± 1.0^{c}	1.38 ± 0.02^{b}	22.4 ± 0.6^{b}	13.2 ± 1.5^{cd}	35.6 ± 0.5^{d}
York	control	3.8 ± 0.10^{i}	31.4 ± 1.1^{j}	0.93 ± 0.02^{cde}	12.1 ± 0.2^{fgh}	5.5 ± 0.3^{def}	31.9 ± 0.7^{e}
	drought	11.2 ± 0.33^{c}	69.3 ± 0.1^{d}	0.99 ± 0.08^{cd}	29.6 ± 1.5^{a}	20.9 ± 4.8^{bc}	49.3 ± 0.3^{a}
Zaria	control	2.5 ± 0.04^{kl}	22.0 ± 0.6^{l}	0.66 ± 0.02^{h}	5.7 ± 0.3^{k}	6.0 ± 0.9^{def}	21.0 ± 0.5^{g}
	drought	9.4 ± 0.27^{d}	75.6 ± 0.3^{c}	0.88 ± 0.04^{ef}	18.7 ± 1.4^{c}	18.6 ± 5.2^{bc}	43.4 ± 0.5^{c}
P		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

TFC leaves – total flavonoid content in leaves; RP leaves – reducing power in leaves; TFC roots – total flavonoid content in roots; RP roots – reducing power in roots; 5-mC – 5-methylcytosine content; QE – quercetin equivalents; AAE – ascorbic acid equivalents; FW – fresh weight; data are presented as means ± SD (n = 5); ANOVA: P < 0.001; different letters indicate statistically significant differences among variety × treatment combinations (Tukey's *HSD* test, P < 0.05)

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(TFC roots) showed a weaker variety-specific response. Under control conditions, TFC roots values ranged from 0.66 to 0.99 mg QE/g FW, whereas under drought, they increased to 0.85–1.50 mg QE/g FW. Under drought, the varieties Impala (1.50 mg QE/g FW) and Labrador (1.38 mg QE/g FW) showed significantly higher TFC roots values than all other varieties, whereas the lowest value was recorded in the variety Hana (0.85 mg QE/g FW).

Reducing power also showed a significant response to drought. In leaves (RP leaves), their values ranged from 16.7 to 39.0 mg AAE/g FW under control conditions, whereas under drought, they increased to 51.8–96.1 mg AAE/g FW. The statistically highest RP leaves value under drought was recorded in the variety Impala (96.1 mg AAE/g FW), whereas the statistically lowest value was found in the variety Hana (51.8 mg AAE/g FW). Higher RP leaves values were also recorded in the varieties Dornburg, Labrador, and Zaria. In roots (RP roots), values ranged from 5.4 to 13.9 mg AAE/g FW under control conditions and reached 10.7–29.6 mg AAE/g FW under drought. Under drought, the varieties York (29.6 mg AAE/g FW) and Impala (28.7 mg AAE/g FW) formed the statistically highest group, whereas the statistically lowest value was recorded in the variety Dornburg (10.7 mg AAE/g FW).

Under control conditions, leaf proline content ranged from 3.3 to 6.0 µmol/g FW, whereas under drought, it reached 12.8–55.8 µmol/g FW. The highest proline value under drought was recorded in the variety Atlas (55.8 µmol/g FW), which differed significantly from all other varieties. Higher values were also recorded in the varieties Dornburg, York, Zaria, and Atacama, whereas the lowest values were found in the varieties Hana (12.8 µmol/g FW), Impala (13.2 µmol/g FW), and Labrador (13.2 µmol/g FW).

The content of 5-methylcytosine (5-mC) was significantly increased by drought in all varieties. Under control conditions, 5-mC values ranged from 20.8% to 32.7%, whereas under drought conditions, they ranged from 35.4% to 49.6%. The highest 5-mC values under drought were recorded in the varieties Impala (49.6%) and York (49.3%), which formed the statistically highest group. The variety Dornburg (48.1%) did not differ significantly from this pair. The statistically lowest 5-mC values were recorded in the varieties Hana (35.4%) and Labrador (35.6%), whereas the varieties Atlas and

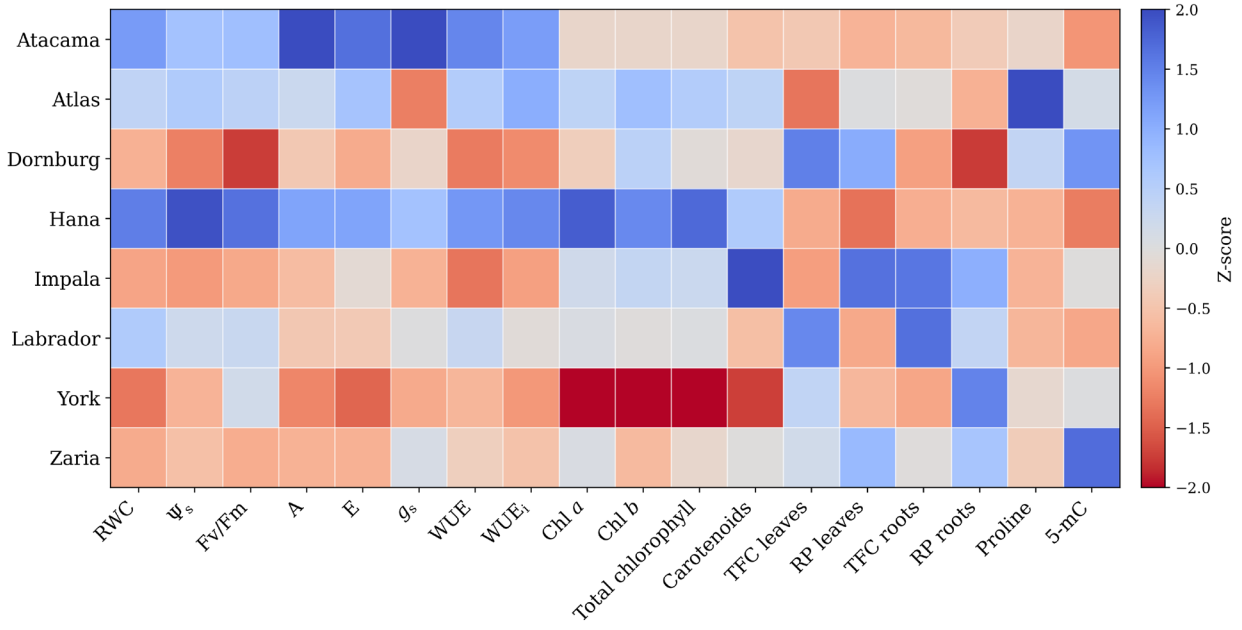

Figure 3. Heatmap of standardised variety-specific Δ values of measured traits in soybean varieties in response to drought. Δ values were calculated as the difference between drought and control treatment (Δ = drought – control) and standardised by variable using z-scores. RWC – relative water content; Ψ_s – leaf osmotic potential; F_v/F_m – maximum quantum efficiency of photosystem II; A – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; WUE – instantaneous water use efficiency; WUE_i – intrinsic water use efficiency; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; TFC leaves – total flavonoid content in leaves; RP leaves – reducing power in leaves; TFC roots – total flavonoid content in roots; RP roots – reducing power in roots; 5-mC – 5-methylcytosine content

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Zaria showed lower intermediate values and the variety Atacama showed a higher intermediate value.

Multivariate and correlation analysis. The heatmap of standardised Δ values showed a distinct variety-specific drought-response profile across the monitored traits (Figure 3). The most balanced and overall, more favourable profile was observed in the varieties Hana and Atacama, which showed positive z-scores for water status parameters (RWC, Ψ_s) and for most photosynthetic performance traits, particularly F_v/F_m, A, E, g_s, WUE, and WUE_i. The variety Atlas also showed a favourable profile, mainly due to positive values for water status and most photosynthetic performance traits, whereas Labrador occupied a rather intermediate position. In contrast, the varieties Dornburg, Impala, York, and Zaria were characterised by predominantly negative z-scores for water status and most photosynthetic performance traits, while their response was more often associated with higher values of some biochemical and epigenetic traits. The heatmap thus indicated a distinction between varieties with a more favourable physiological profile and varieties in which drought-induced biochemical and epigenetic changes were more strongly expressed.

Principal component analysis enabled differentiation of varieties based on Δ values of the drought response (Figure 4). The first principal component (PC1), explaining 50.8% of the variability, separated varieties associated with more favourable water status and photosynthetic performance from varieties with a more pronounced biochemical and epigenetic response to drought. The positive part of PC1 was occupied mainly by the varieties Hana, Atacama, and Atlas, while Labrador was close to this group. In

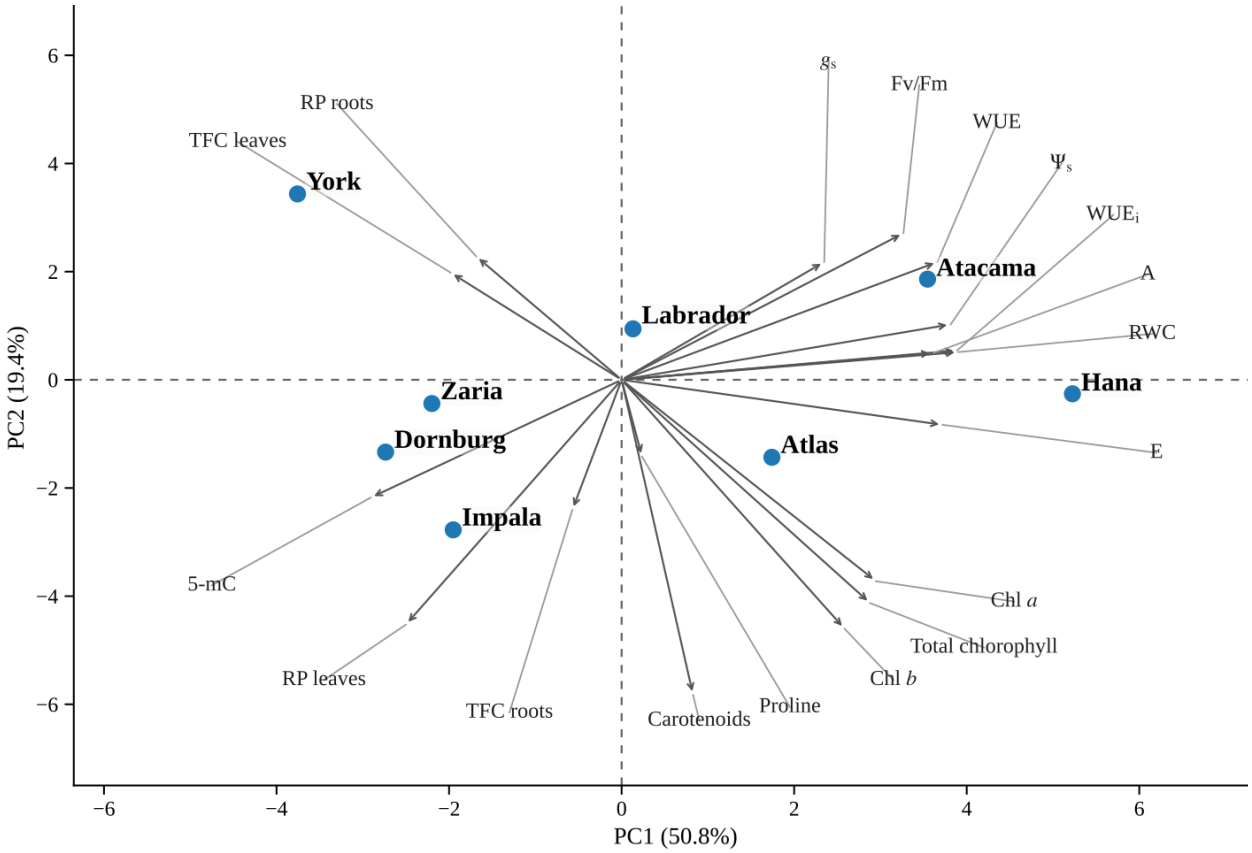

Figure 4. Principal component analysis (PCA) based on variety-specific Δ values of measured traits in soybean varieties in response to drought. Δ values were calculated as the difference between drought and control treatment (Δ = drought – control) and centred and scaled before analysis. RWC – relative water content; Ψ_s – leaf osmotic potential; F_v/F_m – maximum quantum efficiency of photosystem II; A – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; WUE – instantaneous water use efficiency; WUE_i – intrinsic water use efficiency; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; TFC leaves – total flavonoid content in leaves; RP leaves – reducing power in leaves; TFC roots – total flavonoid content in roots; RP roots – reducing power in roots; 5-mC – 5-methylcytosine content

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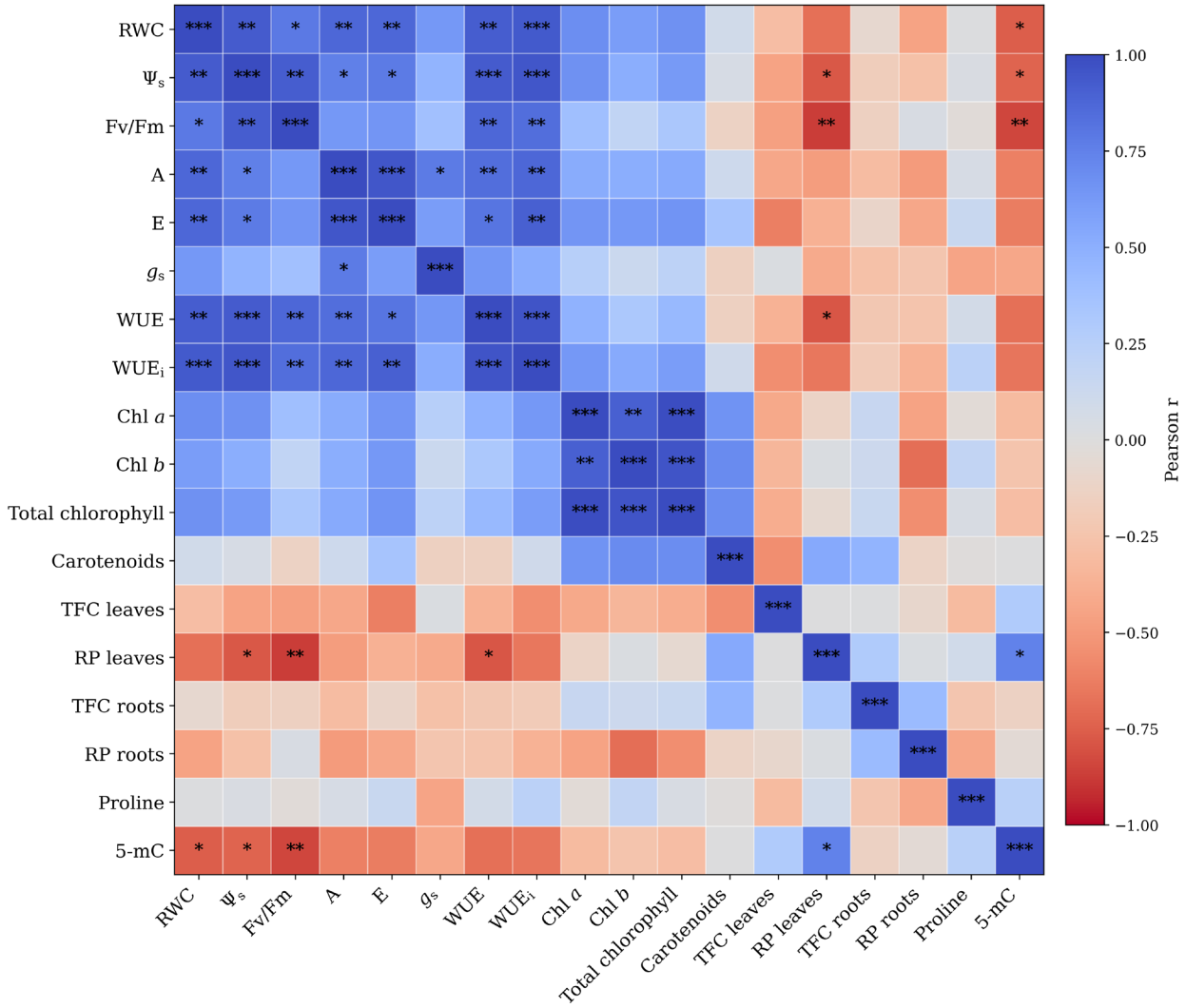

Figure 5. Pearson correlation matrix of drought-induced changes in measured traits in soybean varieties. Correlations were calculated from variety-specific Δ values across varieties (Δ = drought – control). Cell colours indicate Pearson correlation coefficients (*r*); asterisks indicate significance levels (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). RWC – relative water content; Ψ_s – leaf osmotic potential; F_v/F_m – maximum quantum efficiency of photosystem II; A – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; WUE – instantaneous water use efficiency; WUE_i – intrinsic water use efficiency; Chl *a* – chlorophyll *a*; Chl *b* – chlorophyll *b*; TFC leaves – total flavonoid content in leaves; RP leaves – reducing power in leaves; TFC roots – total flavonoid content in roots; RP roots – reducing power in roots; 5-mC – 5-methylcytosine content

contrast, negative PC1 values were associated mainly with the varieties York, Dornburg, Zaria, and Impala. The second principal component (PC2), explaining 19.4% of the variability, further distinguished varieties according to more specific combinations of traits, with York and Atacama positioned mainly in its positive part, whereas Impala and Atlas were positioned mainly in its negative part. PCA thus indicated that the varieties differed not only in the overall profile of drought response, but also in the combination of physiological, biochemical, and epigenetic traits involved in this response.

The Pearson correlation matrix based on variety-specific Δ values showed several significant relationships among the monitored traits in response to drought (Figure 5). Significant positive correlations were observed mainly between water status parameters (RWC, Ψ_s) and photosynthetic performance traits, including F_v/F_m, A, E, WUE, and WUE_i. Significant positive relationships were also found

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among chlorophyll pigments. In contrast, some biochemical and epigenetic traits, particularly RP leaves and 5-mC, showed significant negative correlations with selected water status and photosynthetic performance parameters. Proline and carotenoids occupied a more specific position in the correlation structure, because their relationships with the other traits were not fully consistent with most other drought-induced indicators. The correlation analysis thus indicated that the response of varieties to drought was not formed by isolated changes in individual parameters, but by a coordinated network of relationships among physiological, biochemical, and epigenetic traits.

DISCUSSION

The most pronounced differences among varieties were observed in water status and gas exchange parameters. Under drought conditions, the varieties Hana and Atacama maintained the statistically highest relative water content (RWC) values, whereas the varieties Atlas and Labrador formed an intermediate group, and the statistically lowest values were recorded in the varieties York, Zaria, Impala, and Dornburg. A similar distinction was also evident for leaf osmotic potential (Ψ_s), where the variety Hana showed the statistically highest and least negative Ψ_s value, whereas the varieties Atacama, Atlas, and Labrador had significantly higher Ψ_s values than the varieties Dornburg, Impala, York, and Zaria. These results indicate that, under drought conditions, particularly the varieties Hana and Atacama maintained a more favourable leaf water status than the more sensitively responding varieties, because higher RWC and less negative Ψ_s reflect the ability of plants to better preserve leaf hydration and cellular turgor (Alzahrani 2024, Rodrigues et al. 2024).

This distinction among varieties was also supported by gas exchange parameters. Under drought conditions, the varieties Atacama, Hana, and Atlas formed a statistically higher group for net photosynthetic rate, whereas the varieties Dornburg, Impala, York, Zaria, and Labrador formed a statistically lower group. Similarly, the highest transpiration rate values were recorded in the varieties Atacama, Hana, and Atlas, whereas the statistically lowest values were found in the varieties Dornburg, Impala, Labrador, and York; the variety Zaria occupied an intermediate position. Although stomatal conductance significantly decreased under drought in all varieties, no statistically significant differences among varieties under drought were detected. These results therefore indicate that the more favourable response of the varieties Hana, Atacama, and Atlas was not based solely on restricting water loss, but also on the ability to maintain relatively higher assimilatory activity under water deficit (Hossain et al. 2024a, Alghabari 2026).

In this context, it is important to interpret instantaneous water use efficiency and intrinsic water use efficiency not in isolation, but in relation to A, E, and g_s (Krucky et al. 2025). The highest WUE values under drought were recorded in the varieties Atacama, Hana, and Atlas, which showed significantly higher values than Dornburg, Impala, York, and Zaria, whereas Labrador occupied an intermediate position. Similarly, for WUE_i, the varieties Hana, Atacama, and Atlas formed the statistically highest group. The higher WUE and WUE_i in these varieties therefore cannot be interpreted only as a consequence of reduced stomatal conductance, but rather as a manifestation of a more balanced relationship between limiting transpiration and maintaining photosynthetic function (Rodrigues et al. 2024, Abdullah et al. 2025). In contrast, in the varieties Dornburg, York, and Impala, the less favourable water status (RWC, Ψ_s) was associated with statistically lower A, E, WUE, and WUE_i values, corresponding to a stronger drought-induced physiological limitation (Alzahrani 2024, Hossain et al. 2024a).

The differences in water status and gas exchange were further accompanied by changes in the maximum quantum efficiency of photosystem II and photosynthetic pigment content. Although drought-induced changes in F_v/F_m were less pronounced than changes in gas exchange parameters, a variety-specific response was also evident for this trait. The highest F_v/F_m value under drought was recorded in the variety Hana, which showed a significantly higher value than the varieties Dornburg, Zaria, and Impala. This result indicates that, in Hana, the functional stability of photosystem II under water deficit was significantly better preserved than in the more sensitively responding varieties (Buezo et al. 2019, Alghabari 2026).

A similar distinction was also evident for chlorophyll pigments. Under drought conditions, the statistically highest contents of Chl *a*, Chl *b*, and total chlorophyll were maintained in the varieties Hana and Atlas, which did not differ significantly from each other according to the statistical grouping, whereas the lowest values were recorded in the variety Dornburg. These results support the interpretation

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that the more favourable response of some varieties was associated not only with better maintenance of water status and gas exchange, but also with greater stability of the photosynthetic apparatus (Guzzo et al. 2021, Basal et al. 2024, Hossain et al. 2024b). Conversely, the lower F_v/F_m and chlorophyll pigment values in the more sensitively responding varieties indicate that drought was reflected not only in stomatal limitation, but also in a more pronounced disruption of the photosynthetic apparatus (Hussain et al. 2019, Ahmad et al. 2024).

Carotenoids showed a more specific response to drought. The highest carotenoid content under drought was recorded in the variety Atlas, which showed a significantly higher value than most other varieties, whereas no statistically significant difference was detected in comparison with the variety Hana. The higher carotenoid content in these varieties may be related to their protective role in stabilising the photosynthetic apparatus under stress conditions, particularly through limiting oxidative damage and protecting pigment-protein complexes (Buezo et al. 2019, Alghabari 2026).

Marked varietal differences were also evident in the biochemical response to drought. Water deficit significantly affected total flavonoid content and reducing power in leaves and roots, while the extent of this response differed markedly among varieties (Guzzo et al. 2021, Hossain et al. 2024b). For total flavonoid content in leaves, the statistically highest value under drought was recorded in the variety Labrador, whereas high values were also observed in the varieties Dornburg, York, and Zaria. For total flavonoid content in roots, significantly higher values than in all other varieties were found in the varieties Impala and Labrador. Similarly, for reducing power in leaves, the variety Impala formed the statistically highest group, whereas for reducing power in roots, the statistically highest values were recorded in the varieties York and Impala.

Higher TFC and RP values were therefore often recorded in varieties with a less favourable or intermediate physiological profile, particularly in Impala, York, Zaria, and Labrador, and in some parameters also in Dornburg. The variety Labrador represented a specific case, as it occupied a rather intermediate position in terms of water status but at the same time showed the statistically highest TFC leaves value and a high TFC roots value. This suggests that a pronounced activation of the flavonoid component of the response does not have to be associated only with clearly the most sensitive varieties but may also reflect a specific strategy of response to water deficit. Higher flavonoid accumulation and higher reducing power therefore do not necessarily indicate a more favourable response to drought but may reflect a higher stress burden and a greater need for antioxidant defence (Desoky et al. 2021, Lin et al. 2024). Conversely, lower values of these biochemical parameters in physiologically more favourably responding varieties, particularly Hana, Atacama, and Atlas, do not necessarily indicate a weaker defence response, but rather a lower need to compensate for drought-induced oxidative load (Hossain et al. 2024a, Krucky et al. 2025). These results therefore support the interpretation that biochemical traits should be evaluated in relation to the overall physiological profile of a variety, rather than as independent indicators of a more favourable or less favourable response to water deficit (Guzzo et al. 2021, Alzahrani 2024).

A specific pattern of drought response was also evident in proline and 5-methylcytosine content. Leaf proline content was significantly affected by drought, while the extent of its accumulation was strongly variety-dependent (Abdullah et al. 2025, Alghabari 2026). The highest proline value was recorded in the variety Atlas, which differed significantly from all other varieties. This suggests that, in Atlas, proline may have played a more pronounced role in osmotic regulation and the protection of cellular structures. In contrast, the variety Hana maintained relatively low proline content despite its physiologically favourable profile. This difference supports the interpretation that proline cannot be regarded merely as a simple indicator of stress intensity, but rather as part of variety-specific response strategies to water deficit (Guzzo et al. 2021, Hossain et al. 2024a, Lin et al. 2024). Thus, in the variety Atlas, proline may have contributed more strongly to osmotic regulation, whereas in the variety Hana, the stabilisation of plant functions may have been ensured to a greater extent by the more favourable maintenance of water status and photosynthetic performance (Hussain et al. 2019, Basal et al. 2024, Zi et al. 2024).

The content of 5-methylcytosine (5-mC) was significantly increased by drought in all evaluated varieties, indicating that water deficit induced not only physiological and biochemical changes but also an epigenetic response (Krucky et al. 2025). The highest 5-mC values were recorded in the varieties Impala and York, while Dornburg did not differ significantly from

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this pair. These varieties also belonged to those with less favourable water status and a more pronounced limitation of photosynthetic performance. Higher 5-mC values therefore probably reflected a stronger stress impact and a more intensive regulatory response rather than more favourable acclimation to drought (Rao et al. 2024, Zi et al. 2024). These results may therefore be interpreted as indicating that changes in 5-mC content represented one manifestation of the variety-specific response of plants to water deficit (Krucky et al. 2025). Because global DNA methylation expressed as 5-mC content was assessed in this study, future research could focus on DNA methylation changes in specific genomic regions, particularly in regulatory regions of genes associated with photosynthesis, stomatal regulation, osmotic adjustment, and antioxidant defence, in relation to a more favourable acclimation of soybean to water deficit (Zi et al. 2024, Lodhi and Srivastava 2025).

The integrative significance of the monitored traits was particularly evident from the multivariate and correlation analyses. The heatmap showed that the varieties Hana and Atacama, and in some traits also Atlas, were associated with a more favourable profile of changes in water status and photosynthetic performance. In contrast, the varieties Dornburg, York, Impala, and Zaria showed a profile more strongly associated with a more pronounced biochemical and epigenetic response, whereas Labrador occupied a rather intermediate position. This distinction was further supported by principal component analysis (PCA), which indicated that the varieties differed not only in the intensity of the drought response, but also in the combination of traits involved in this response (Guzzo et al. 2021, Alzahrani 2024, Alghabari 2026).

The Pearson correlation matrix further confirmed significant positive correlations between water status parameters (RWC, Ψ_s) and photosynthetic performance traits, including F_v/F_m, A, E, WUE, and WUE_i. This supports the interpretation that the maintenance of photosynthetic performance in response to drought was closely associated with the maintenance of plant water status (Hussain et al. 2019, Hossain et al. 2024a). Significant positive relationships were also found among chlorophyll pigments, further indicating the interconnection among individual components of the photosynthetic apparatus. In contrast, some biochemical and epigenetic traits, particularly RP leaves and 5-mC, showed significant negative correlations with selected water status and photosynthetic performance parameters. This relationship supports the interpretation that a more pronounced disruption of water status and photosynthetic performance was associated with a more intensive defence and regulatory response (Desoky et al. 2021, Alzahrani 2024, Rao et al. 2024). The specific position of proline and carotenoids further indicates that some traits cannot be unequivocally assigned to a more favourable or less favourable response pattern, because their significance depended on the overall profile of the particular variety (Buezo et al. 2019, Lin et al. 2024, Alghabari 2026).

Taken together, the results of this study fulfilled the main aim of the work and generally supported both proposed hypotheses. It was shown that (*i*) water deficit induced variety-specific changes in photosynthetic performance as well as in related physiological, biochemical, and epigenetic traits in the evaluated soybean varieties, and that (*ii*) photosynthetic performance parameters were closely interconnected with plant water status and other monitored characteristics, as demonstrated particularly by the multivariate and correlation analyses. Under the conditions of this experiment, the varieties Hana and Atacama appeared to be the varieties with a relatively more favourable acclimation profile, and in some monitored traits also the variety Atlas. In contrast, the varieties Dornburg, York, and Impala exhibited an overall less favourable response to drought, whereas the varieties Labrador and Zaria occupied rather intermediate positions depending on the trait evaluated.

These variety-specific responses also indicate that soybean acclimation to water deficit cannot be assessed on the basis of a single parameter, but should be understood as the result of the coordinated action of multiple interconnected traits. Therefore, it will be important to verify these findings under different intensities and durations of water deficit and to assess the transferability of conclusions obtained under controlled growth-chamber conditions to small-plot and field conditions. This would enable more effective evaluation of soybean variety responses to drought and their potential for use in subsequent breeding programmes.

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