

Modern Methods for The Comprehensive Assessment of Physiological-Biochemical Traits, Seed Quality and Oil Productivity in The Drought Adaptation of Soybean Genotypes

Abduganiyeva M. Mumtoza

Researcher, Scientific Experimental Station of Oilseed and Fiber Crops, Uzbekistan

 Khakimov E. Abdumurod

Senior Researcher, Scientific Experimental Station of Oilseed and Fiber Crops, PhD in Agricultural Sciences, Uzbekistan

Received: 28 Mar 2026 | Received Revised Version: 16 Apr 2026 | Accepted: 05 May 2026 | Published: 31 May 2026

Volume 08 Issue 05 2026 | Crossref DOI: 10.37547/tajabe/Volume08Issue05-12

Abstract

Soybean (Glycine max (L.) Merr.) is one of the world's major sources of protein and vegetable oil, while its yield and seed quality are highly sensitive to water deficit. The effects of drought are not limited to yield reduction; drought also alters leaf water status, gas exchange, the photosynthetic apparatus, oxidative stress, osmotic adjustment, antioxidant defense, root architecture, seed protein and oil contents, and the fatty acid profile. The aim of this review is to systematize physiological, biochemical, seed-quality, molecular-genetic, and high-throughput phenotyping methods used for the comprehensive assessment of drought adaptation in soybean genotypes. In modern approaches, selection based on a single trait is insufficient; greater precision can be achieved by integrating relative water content, leaf water potential, photosynthesis and stomatal conductance, chlorophyll fluorescence, proline, malondialdehyde and antioxidant enzymes with NIR-based protein and oil analysis, gas-chromatographic fatty acid profiling, qRT-PCR/RNA-seq, GWAS, metabolomics, UAV multispectral imaging, and machine-learning methods. Analysis of the literature indicates that the most appropriate strategy for identifying drought-tolerant genotypes is the joint evaluation of yield retention under stress, physiological stability, and seed-quality traits.

Keywords: Glycine max, drought, physiological traits, proline, MDA, antioxidant enzymes, NIR, protein, oil, fatty acids, qRT-PCR, GWAS, UAV, phenotyping.

© 2026 Abduganiyeva M. Mumtoza, & Khakimov E. Abdumurod. This work is licensed under a Creative Commons Attribution 4.0 International License (CC BY 4.0). The authors retain copyright and allow others to share, adapt, or redistribute the work with proper attribution.

Cite This Article: Abduganiyeva M. Mumtoza, & Khakimov E. Abdumurod. (2026). Modern Methods for The Comprehensive Assessment of Physiological-Biochemical Traits, Seed Quality and Oil Productivity in The Drought Adaptation of Soybean Genotypes. The American Journal of Agriculture and Biomedical Engineering, 8(05), 71–77. <https://doi.org/10.37547/tajabe/Volume08Issue05-12>

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a strategic crop for food, feed, and oil industries, and its high seed protein and oil contents determine its major economic importance. In recent years, the increasing frequency of

drought, often occurring together with high temperature, has posed a serious threat to yield stability in soybean-growing regions. A comprehensive review published in 2026 identified drought as one of the major abiotic factors limiting soybean productivity and emphasized

that tolerance cannot be reliably evaluated using a single trait because of the complex interactions among genetic, physiological, and environmental factors [1].

Water deficit first reduces cell turgor, slows leaf growth, and induces stomatal closure. As a result, CO₂ diffusion and the rate of photosynthesis decrease, whereas under prolonged stress non-stomatal limitations associated with damage to photosystems and enzymatic processes become more pronounced. In soybean, vegetative growth may respond to water deficit even earlier than photosynthesis; during reproductive stages, particularly flowering, pod formation, and seed filling, drought causes flower abscission, seed abortion, and reduced seed mass [1,2].

In response to drought, soybean activates several protective mechanisms simultaneously, including reducing leaf area and transpiration, modifying water-use efficiency, accumulating proline and other osmolytes, detoxifying reactive oxygen species through antioxidant enzymes, maintaining membrane stability, and regulating the expression of stress-responsive genes. In modern experiments, parallel measurements of photosynthesis, stomatal conductance, chlorophyll, cell membrane stability, proline, glycine betaine, SOD, CAT, POD, water potential, and drought-related gene expression have enabled reliable discrimination among genotypes [3].

Another important issue in soybean is the preservation of technological and nutritional seed quality together with yield under stress conditions. Depending on genotype and stress stage, water deficit may alter protein and oil contents as well as the proportions of palmitic, stearic, oleic, linoleic, and linolenic acids. Some high-protein lines-maintained seed protein levels of 46-55% under heat and drought, whereas oil content decreased in many genotypes [4]. Therefore, breeding should evaluate not only 'tolerance' itself, but also the simultaneous stability of yield, protein, oil content, and oil quality under stress.

The objective of this review is to analyze classical and modern methods for the comprehensive assessment of drought adaptation in soybean genotypes based on physiological-biochemical traits, seed quality, and oil productivity, and to highlight opportunities for integrating these approaches with molecular-genetic and digital phenotyping technologies.

2. Methods

2.1. Review design

This study was prepared as a narrative-analytical literature review. Particular attention was given to publications from 2019-2026 addressing soybean responses to drought, physiological and biochemical markers, seed composition, oil and fatty acids, drought-related genes, GWAS/QTL, transcriptomics, metabolomics, and remote phenotyping. Scientific information was synthesized from peer-reviewed publications available through Frontiers, PubMed/PMC, MDPI, and Elsevier platforms. The review was organized according to the IMRAD structure; in review articles of this type, separate 'Review design' and 'Evaluation criteria' subsections are commonly used.

2.2. Evaluation criteria

The methods were divided into five functional groups: (1) physiological traits describing plant water status and the photosynthetic apparatus; (2) biochemical markers reflecting osmotic adjustment, oxidative damage, and antioxidant defense; (3) yield components, seed protein, oil content, and fatty acid composition; (4) molecular-genetic and omics approaches; and (5) high-throughput phenotyping and integrated statistical analysis. Each method was evaluated in terms of its informativeness for breeding, destructive or non-destructive nature, throughput, suitability for field use, and ability to discriminate among genotypes.

3. Results and Discussion

3.1. Experimental modeling of drought and standardization of stress intensity

The first requirement for correct assessment of drought tolerance is precise and reproducible control of stress intensity. In greenhouse or growth-chamber experiments, drought regimes may be maintained using a defined percentage of field capacity, gravimetric irrigation, soil-moisture sensors, or water-potential measurements. Under field conditions, it is advisable to run irrigated and water-limited treatments in parallel, monitor soil moisture by depth, and account for precipitation and evapotranspiration. The phenological stage at which stress is imposed must also be clearly reported because water deficit during vegetative growth does not have the same final effects on yield and quality as stress during flowering or seed filling.

Reproductive stages are especially sensitive to drought. Water deficit during pod formation and seed filling increases seed abortion and reduces the number of pods per plant and 1000-seed weight. The effect becomes even

stronger when drought is accompanied by high temperature. Poudel et al. [5], comparing drought, heat, and their combination in ten soybean cultivars, found that combined stress reduced stomatal conductance more

strongly than either factor alone, increased canopy temperature, and had a marked negative effect on seed weight. Thus, heat background should also be recorded in drought-breeding experiments.



Figure 1. Soybean selection plots under field conditions.

3.2. Physiological traits: water status, photosynthesis, and chlorophyll fluorescence

Relative water content (RWC) is one of the most practical indicators for evaluating plant responses to drought. RWC expresses the current water content of leaf tissue relative to its fully turgid state and reflects the genotype's capacity to retain water. When water potential and osmotic potential are determined using a pressure chamber or appropriate osmometric methods, the degree of plant water deficit can be assessed more precisely. Drought-tolerant genotypes often maintain higher RWC and water potential under stress than sensitive genotypes [3].

In gas-exchange analysis, net photosynthetic rate (Pn), stomatal conductance (gs), transpiration rate (E), and internal CO₂ concentration (Ci) are measured using portable infrared gas analyzers. At the early stages of drought, the decline in Pn is mainly associated with stomatal limitation, whereas under severe and prolonged stress, non-stomatal limitations caused by damage to chloroplasts and metabolic systems become predominant. Water-use efficiency (WUE) should be calculated together with photosynthesis because some genotypes can markedly reduce water loss while maintaining biomass or yield relatively well [1,6].

Chlorophyll content can be monitored non-destructively using SPAD meters or spectral indices, while chlorophyll a, chlorophyll b, and carotenoids can be quantified

spectrophotometrically in the laboratory. Chlorophyll fluorescence enables assessment of photosystem II status before stress effects become evident in yield. Parameters such as Fv/Fm, PhiPSII, qP, and NPQ indicate reductions in photochemical efficiency caused by drought and the activation of protective energy dissipation. A 2026 review summarized reductions in Fv/Fm and changes in stomatal conductance and photosynthesis as major physiological components of the drought phenotype [1].

Measurement of leaf or canopy temperature by infrared thermometry is also highly informative. When stomata close and transpirational cooling declines, leaf temperature increases. Therefore, canopy temperature depression or the difference in canopy temperature relative to a control can provide a rapid indicator of drought response. This trait is particularly suitable for evaluating large collections using UAV-based thermal imaging.

3.3. Biochemical markers: osmoprotectants, lipid peroxidation, and antioxidant defense

Drought enhances the formation of reactive oxygen species (ROS) in cells. Excess ROS oxidize membrane lipids and damage proteins and nucleic acids. Malondialdehyde (MDA) is a widely used marker of lipid peroxidation, and its accumulation indicates increased membrane injury. Relatively low MDA accumulation, reduced electrolyte leakage, and high membrane stability are important diagnostic traits of

tolerant genotypes [3,7].

Proline is one of the most widely used osmolytes for assessing drought adaptation. It contributes to osmotic adjustment, stabilizes proteins and membranes, and helps limit ROS. However, a high proline concentration should not automatically be interpreted as 'tolerance', because strong stress itself can also induce pronounced proline accumulation. Therefore, proline should be interpreted together with RWC, MDA, photosynthesis, and yield retention. In a comprehensive 2024 study, tolerant genotypes were more clearly distinguished when proline, glycine betaine, SOD, CAT, POD, and stress-responsive genes were analyzed together [3].

Among antioxidant enzymes, superoxide dismutase (SOD) converts superoxide radicals to H₂O₂, while catalase (CAT) and ascorbate peroxidase (APX) participate in H₂O₂ detoxification; peroxidase (POD) also plays an important role in cellular redox balance. In drought-tolerant genotypes, increased activity of these enzymes under moderate stress can limit oxidative damage. In modern comprehensive assessments, it is also useful to measure H₂O₂, total antioxidant capacity, soluble sugars, and phenolic compounds in addition to enzymatic markers.

Metabolomics takes biochemical analysis to a new level. GC-MS, LC-MS, and NMR platforms can detect hundreds of metabolites simultaneously and provide a metabolic 'signature' of the drought response. Wang et al. [8] integrated physiology, transcriptomics, and metabolomics and demonstrated the roles of proline, spermidine, and amino-acid metabolism in drought adaptation. This approach does not replace classical proline or MDA analysis; rather, it links these markers to underlying molecular pathways.

3.4. Assessment of seed quality, protein and oil contents, and oil productivity

When selecting drought-tolerant genotypes, nutritional and technological seed quality should be evaluated together with yield retention. Although protein and oil contents in soybean seeds are strongly genotype-dependent, stress stage and intensity can substantially alter their proportions and final output. Wijewardana et al. [9] showed that soil-moisture deficit during the reproductive period modifies seed protein, sugars, and several fatty acids. Subsequent studies confirmed that some high-protein lines can maintain high seed protein levels even under stress [4].

Near-infrared spectroscopy (NIR) is highly convenient for rapid assessment of protein and oil in breeding programs. NIR can determine protein, oil, and moisture within a short time without grinding the seeds. Whole-seed NIR systems are especially efficient for screening large numbers of genebank and breeding samples; when calibration and bias control are properly performed, results show high agreement with conventional chemical methods [10]. Its main advantages are non-destructive analysis, preservation of seed for subsequent planting, and rapid screening of large collections.

Oil quality is not determined by total oil percentage alone. The proportions of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids determine nutritional and technological value. Fatty acids are usually converted to methyl esters and analyzed by gas chromatography (GC-FID or GC-MS). Heat and drought during seed filling can affect desaturation processes and alter the balance between oleic and polyunsaturated fatty acids [4,5].

For evaluating oil productivity, oil percentage alone should not be the primary breeding criterion; the net oil yield per hectare is more informative. It can be calculated as follows: Oil yield (kg/ha) = seed yield (kg/ha) x oil content (%) / 100. Protein yield can be calculated in the same way. This approach prevents genotypes with high oil percentage but very low seed yield from being incorrectly ranked as superior. Under stress, the optimal genotype is one that maintains a balanced combination of seed yield, oil percentage, oil quality, and total oil output.

3.5. Molecular-genetic and omics methods

While physiological and biochemical traits describe the outcome of stress, molecular methods help identify the genetic mechanisms regulating these responses. Targeted qRT-PCR can compare expression of stress-related genes such as GmDREB, GmNAC, GmWRKY, GmP5CS, GmSOD, GmCAT, and GmPOD under control and drought conditions. For example, GmP5CS is associated with proline biosynthesis, whereas the DREB and NAC families regulate broad transcriptional networks involved in drought response [3,11]. A practical design is to first screen a large set of genotypes phenotypically and physiologically, then perform detailed gene-expression analysis in 3-5 contrasting genotypes.

RNA-seq transcriptomics measures the expression of thousands of genes simultaneously and identifies

differentially expressed genes and signaling pathways. When integrated with metabolomics, changes in gene expression can be linked with the accumulation of proline, phenylpropanoids, amino acids, polyamines, and other metabolites [8]. Proteomics reveals responses at the level of enzymes and structural proteins. Therefore, transcriptome-metabolome-phenotype integration provides a substantially more complete view of the causal chain underlying drought tolerance than any single indicator.

In large genetic collections, GWAS and QTL mapping are used to identify SNP markers associated with yield, biomass, pod number, seed weight, slow wilting, and other traits under drought. Li et al. [12] evaluated 188 soybean genotypes under well-watered and drought-stressed conditions and identified a number of SNPs and QTL regions associated with yield components. Importantly, genetic associations under control and stress conditions may not fully coincide; therefore, breeding for drought tolerance should be linked directly to phenotypes measured in the target stress environment.

Molecular markers do not replace phenotyping in breeding. Drought tolerance is a polygenic trait with a strong genotype x environment interaction. The most effective strategy is to combine marker data with physiological traits, multi-location yield trials, and BLUP estimates or integrated indices derived from quality parameters.

3.6. UAV, multispectral and hyperspectral

phenotyping, and machine learning

Repeated gas-exchange, RWC, and biochemical measurements for every genotype in large genebank or breeding collections are expensive and labor-intensive. Therefore, high-throughput phenotyping has become a major direction in modern soybean research. UAV-mounted RGB, multispectral, thermal, or LiDAR sensors can simultaneously obtain canopy cover, plant height, NDVI, RECI, ARVI, temperature, and other spectral traits from hundreds of plots.

Liang et al. [13] used UAV multimodal data and machine-learning algorithms across hundreds of soybean genotypes and showed that drought tolerance can be predicted using a yield-based index. A support vector regression model successfully estimated a yield-related drought index, and the approach also produced useful results when transferred across locations. In a 2026 field study, UAV-derived vegetation indices discriminated yield retention better than visual canopy-wilting scores under some conditions [14].

Hyperspectral sensors record hundreds of narrow spectral bands and can detect changes in pigments, plant water status, and biochemical properties before visible wilting begins. Machine learning can use red-edge and SWIR regions to classify stress and even estimate certain biochemical traits [15]. However, remote-sensing methods must be calibrated against laboratory 'gold-standard' measurements; otherwise, model transferability across years and locations declines.

Table 1. Recommended indicators for the comprehensive assessment of drought tolerance in soybean

Category	Main indicators	Recommended method	Breeding relevance
Water status	RWC, water potential, osmotic potential	Gravimetry, pressure chamber, osmometry	Water retention and turgor
Photosynthesis	Pn, gs, E, Ci, WUE	Portable IRGA	Stability of gas exchange
Photochemistry	Fv/Fm, PhiPSII, NPQ	Chlorophyll fluorometer	PSII injury and recovery
Pigments	SPAD, Chl a/b, carotenoids	SPAD/spectrophotometry	Status of the photosynthetic apparatus
Oxidative stress	MDA, H2O2, electrolyte leakage	Spectrophotometric analysis	Membrane injury
Osmotic/antioxidant	Proline, SOD, CAT, POD, APX	Biochemical assays	Protective response
Seed quality	Protein, oil, moisture	NIR + reference chemical method	Rapid, non-destructive screening
Oil quality	16:0, 18:0, 18:1, 18:2, 18:3	GC-FID/GC-MS	Fatty acid profile

Molecular	GmDREB, GmNAC, GmP5CS, etc.	qRT-PCR/RNA-seq	Stress-responsive genes
Genomics	SNP, QTL, haplotype	GWAS/sequencing	Marker- assisted/genomic selection
Digital phenotyping	NDVI, RECI, canopy temperature, height	UAV multispectral/thermal/LiDAR	Rapid assessment of large collections

3.7. Integrated assessment and selection of promising genotypes

The final goal of comprehensive assessment is to convert numerous measurements into practical breeding decisions. Based on genotype yield under control and drought conditions, stress tolerance index (STI), geometric mean productivity (GMP), yield stability index (YSI), tolerance index (TOL), and other indices can be calculated. STI and GMP are particularly useful for identifying genotypes with high yield in both control and stress environments, whereas TOL reflects the magnitude of yield loss. GMP has also been used to define drought-response classes in UAV-based studies [13].

As the number of traits increases, correlation matrices, principal component analysis (PCA), heatmaps, and cluster analysis reveal the joint structure of physiological-biochemical and quality traits. In multi-year and multi-location trials, mixed models, BLUP/BLUE estimates, and genotype x environment (GxE) analysis are essential because a genotype that appears tolerant in one year may not show the same advantage in another environment. In modern breeding, the most valuable genotype is one that retains yield under stress, maintains RWC and photosynthesis, limits MDA accumulation, exhibits appropriate antioxidant and osmotic responses, and avoids severe losses in seed protein-oil quality and oil productivity.

From a practical perspective, a two-stage screening strategy is efficient. In the first stage, a large collection is rapidly screened in the field or greenhouse for yield, SPAD, canopy temperature, NDVI, RWC, and NIR-based protein and oil traits. In the second stage, selected contrasting genotypes are subjected to detailed gas-exchange, chlorophyll-fluorescence, proline, MDA, SOD/CAT/POD/APX, fatty-acid-profile, and qRT-PCR or RNA-seq analyses. This approach reduces costs and directs molecular research toward the most informative material.

Thus, modern methodology views drought adaptation not through a 'one marker-one conclusion' approach, but as an integrated phenotype-physiology-biochemistry-quality-genomics continuum. Such integration has the greatest practical value for evaluating genebank materials, selecting parental forms, and developing drought-tolerant, high-quality soybean cultivars.

4. Conclusion

Drought adaptation in soybean genotypes is a complex and multicomponent trait that cannot be reliably assessed using yield, proline, or a single molecular marker alone. The most informative approach is to integrate plant water status, photosynthesis, chlorophyll fluorescence, canopy temperature, osmotic and antioxidant defense, lipid peroxidation, yield components, seed protein and oil, fatty acid profile, and stress-responsive genes within a unified analytical framework.

For large collections, UAV, multispectral/hyperspectral imaging, NIR, and machine learning substantially accelerate primary screening. In subsequent in-depth stages, qRT-PCR, RNA-seq, metabolomics, and GWAS reveal the biological and genetic basis of tolerance. For seed quality, oil productivity and fatty acid composition should be considered together with oil percentage.

For future evaluation of soybean genetic resources under Uzbekistan conditions, it is advisable to establish control and water-deficit treatments involving multiple genotypes across at least two environments or years; RWC, photosynthesis/fluorescence, proline, MDA, antioxidant enzymes, NIR-based protein-oil analysis, and yield traits can serve as a core evaluation panel. Molecular-genetic analysis of the most promising and sensitive genotypes would further increase scientific novelty and breeding value.

Acknowledgments. This article was prepared within the framework of the applied research project AL-11725099312, "Development of new high-yielding varieties of oilseed crops adapted to the natural and climatic conditions of the Republic, establishment of

primary seed production, and their introduction into production."

References

1. Burner N., Harris D.K., Nyam C.K., Ahmad I., Lee N., Suh J.H., Fallen B., Li Z. Drought tolerance in soybean: genetics, metabolomics, remote sensing, and breeding for enhanced drought tolerance. *Frontiers in Plant Science*. 2026;17:1733525. doi:10.3389/fpls.2026.1733525.
2. Markulj Kulundžić A., Josipović A., Matoša Kočar M., et al. Physiological insights on soybean response to drought. *Agricultural Water Management*. 2022;268:107620.
3. Alzahrani Y. Evaluation of drought and salinity tolerance potentials of different soybean genotypes based upon physiological, biochemical, and genetic indicators. *Frontiers in Plant Science*. 2024;15:1466363. doi:10.3389/fpls.2024.1466363.
4. Kakati J.P., Fallen B.D., Armstrong P.R., Yan S., Bridges W., Narayanan S. High-protein soybean lines with stable seed protein content under heat and drought stresses. *Journal of Agriculture and Food Research*. 2024;18:101469. doi:10.1016/j.jafr.2024.101469.
5. Poudel S., Vennam R.R., Sankarapillai L.V., Liu J., Reddy K.R., Wijewardane N.K., Mukhtar M.S., Bheemanahalli R. Negative synergistic effects of drought and heat during flowering and seed setting in soybean. *Environmental and Experimental Botany*. 2024;222:105769. doi:10.1016/j.envexpbot.2024.105769.
6. Wang X., Wu Z., Zhou Q., Wang X., Song S., Dong S. Physiological response of soybean plants to water deficit. *Frontiers in Plant Science*. 2021;12:809692.
7. Arya H., Singh M.B., Bhalla P.L. Towards developing drought-smart soybeans. *Frontiers in Plant Science*. 2021;12:750664.
8. Wang L., He P., Hui M., Li H., Sun A., Yin H., Gao X. Metabolomics combined with transcriptomics and physiology reveals the regulatory responses of soybean plants to drought stress. *Frontiers in Genetics*. 2024;15:1458656. doi:10.3389/fgene.2024.1458656.
9. Wijewardana C., Reddy K.R., Bellaloui N. Soybean seed physiology, quality, and chemical composition under soil moisture stress. *Food Chemistry*. 2019;278:92-100. doi:10.1016/j.foodchem.2018.11.035.
10. Jiang G.-L. Comparison and application of non-destructive NIR evaluations of seed protein and oil content in soybean breeding. *Agronomy*. 2020;10(1):77. doi:10.3390/agronomy10010077.
11. Cui X., Tang M., Li L., et al. Expression patterns and molecular mechanisms regulating drought tolerance of soybean conferred by transcription factor gene GmNAC19. *International Journal of Molecular Sciences*. 2024;25(4):2396. doi:10.3390/ijms25042396.
12. Li S., Cao Y., Wang C., Yan C., Sun X., Zhang L., Wang W., Song S. Genome-wide association mapping for yield-related traits in soybean (*Glycine max*) under well-watered and drought-stressed conditions. *Frontiers in Plant Science*. 2023;14:1265574. doi:10.3389/fpls.2023.1265574.
13. Liang H., Zhou Y., Lu Y., Pei S., Xu D., Lu Z., Yao W., Liu Q., Yu L., Li H. Evaluation of soybean drought tolerance using multimodal data from an unmanned aerial vehicle and machine learning. *Remote Sensing*. 2024;16(11):2043. doi:10.3390/rs16112043.
14. Pawar P.S., Wu C., Harrison D., Rogers D., Fallen B., Canella Vieira C. UAV-based phenotyping outperforms visual canopy wilting for evaluating soybean drought tolerance and yield retention under rainfed conditions. *Frontiers in Plant Science*. 2026;17:1835549. doi:10.3389/fpls.2026.1835549.
15. Oliveira C.A. de, Vedana N.G., Mendonça W.A., Gonçalves J.V.F., Matos D.H.S. de, Furlanetto R.H., et al. High-throughput identification and prediction of early stress markers in soybean under progressive water regimes via hyperspectral spectroscopy and machine learning. *Remote Sensing*. 2025;17(20):3409. doi:10.3390/rs17203409.