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REVIEW ARTICLE

Trends in Biotechnology and Plant Sciences

Integrating Phenotypic and Genotypic Approaches in Sunflower to Enhance Drought Tolerance: A Review

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Abstract

Sunflower (*Helianthus annuus L.*), the world's fourth most important oilseed crop, faces productivity challenges under drought conditions that are intensified by climate change. Drought causes a negative impact on sunflower physiology, such as photosynthesis, stomatal conductance, and osmotic control, leading to low biomass and oil production. In this review, the combination of phenotypic and genotypic methods of improving drought tolerance in sunflowers is emphasized. Under both experimental and field environments, researchers can pinpoint adaptive responses including enhanced root depth, increased proline content, improved leaf water retention and stable yield performance under varying stress conditions. These characteristics form the basis of the choice of drought-resilient genotypes using such indices as stress tolerance index (STI) and stress susceptibility index (SSI). On the genomic level, SSRs, SNPs and AFLPs have made it possible to conduct QTL mapping and genome-wide association studies (GWAS) so as to establish loci-specific to drought-responsive phenotypes. Regulatory gene families such as *GASA, SAP, TIFY*, transcription factors *(bHLH, WRKY, NAC*) have also been identified by transcriptomic and proteomic studies, which mediate abscisic acid signals, reactive oxygen species (ROS) regulation and metabolic adaptation. The combination of the phenotypic measurements with the multi-omics and bioinformatics tools will improve the accuracy of genomic selection (GS), marker-assisted backcrossing (MABC) and CRISPR/Cas9-based gene editing. Together with the introgression of alleles of wild Helianthus species, the approaches provide a way forward in expanding the genetic foundation and reinforcing the ability to withstand drought. The future opportunities concerned the use of AI-based phenomics and predictive modeling to shorten breeding periods and provide sustainable production. Phenotypic precision, genomic innovation and computational tools, therefore, converge to give a comprehensive approach of coming up with climate-smart, drought-resistant sunflower cultivars that can remain stable in yield conditions that are characterized by water scarcity.

KEYWORDS
Drought tolerance, Phenotypic screening, Genomic selection (GS), Genome-wide association studies (GWAS), Transcriptomics, Crop wild relatives (CWR), Climate-smart agriculture

1 | INTRODUCTION

Sunflower ranks among the key oilseed crops cultivated globally, providing premium oil and dietary fiber that are essential for promoting human health. After soybeans, rapeseed, and safflower, sunflower ranks as the fourth leading oilseed crop globally because of its high profitability and economic value (Adeleke & Babalola, 2020). Pakistan's edible oil sector faces a significant supply-demand gap, with local production unable to meet national needs. In FY 2024–25, total oil availability is estimated at 3.07 million tonnes, while local sources produced only 0.486 million tonnes, or around 16 percent. The deficit was offset by imports to the tune of 2.58 million tonnes or 84 percent, at an estimated US 2.75 billion (Government of Pakistan, Ministry of Finance, 2025). There has been a discernible warming trend in the global climate in the last century. Climate change coupled with human aspects has resulted in extreme weather and excessive rains with higher intensity and intensity all over the world. The drastic rise in the human activities in the 20 th century led to the major changes in the natural environment that became a cause of the rising temperature. Frequently, the Asian world has experienced an average drought in every three years in countries like India, Pakistan, Afghanistan, and Sri Lanka. Pakistan is mainly arid; although it has few humid conditions in the North, which leads to drought. In much of Baluchistan, Sindh, and in the southern and central parts of Punjab, the amount of precipitation is less than 250 mm per year (Rafiq et al., 2023). The drought stress is one of the main abiotic factors, which have impacted plant growth and yield by more than 25 percent in the global agricultural production. Plants undergo significant physiological and developmental alterations under harsh drought stress. Water scarcity severely affects the growth and development of the sunflower by decreasing root length, shoot length, and leaf area. The accumulation of hydrogen peroxide, lipid peroxidation, and membrane leakage intensifies oxidative damage under drought stress. Due to drought stress, antioxidant compounds, enzymes, and osmolytes also become imbalanced. Sunflower, as a key oilseed crop, demonstrates moderate tolerance to drought. The cultivation of sunflowers is highly susceptible to water stress due to fluctuating climatic conditions that increase the occurrence and intensity of drought (Nosherwan et al., 2024).

Conventional phenotypic breeding in sunflower is pegged upon the quantifiable phenotypes (e.g., yield factors, water-use efficiency, physiological adaptations e.g., osmotic adjustment stomatal regulation, and root characteristics) which has been successful in making moderate changes in drought tolerance using germplasm screening and selection. (Hussain et al., 2018). Polygenic characteristics like drought tolerance decline their ability to enhance in response to changing climatic conditions because of the interaction between genotype and the environment, and lengthy selection processes. (Debaeke et al., 2021).

Genomic methods including whole-genome sequencing and quantitative trait loci (QTL) mapping help to improve the accuracy of the detection of molecular markers and gene networks of drought tolerance characteristics.(Badouin et al., 2017). The evolutionary duplication of water-use efficiency of the sunflower genome: introgression of wild germplasm and marker-assisted breeding to enhance water-use efficiency in key phenotypes, such as flowering time and oil metabolism. (Debaeke et al., 2021).

Genomic tools like genetic markers may not be adequate to reflect field performance, which has to be confirmed under the variety of conditions. There is exploitation of these limitations and an improvement in breeding of resilient genotypes by a combined method, which is a combination of phenotypic assessment like multi-environment trial to evaluate stability of traits and genetic assessment based on the genome of QTLs involved in osmotic adjustment and root traits.(Debaeke et al., 2021; Hussain et al., 2018). Precision breeding for drought-resilient sunflower cultivation while incorporating ecosystem services such as pollinator support and phytoremediation advances, due to integration of quantitative genetics with expression analyses (Badouin et al., 2017).

Impact of Drought Stress on Sunflower Physiological Effects

Drought stress strongly affected the Physiological functions of sunflower (*Helianthus annuus L.*), leading to reduced growth and yield. The key physiological response to drought, particularly in cold and arid regions with limited irrigation, includes a decline in net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs). Mild deficits (65–75% FC) caused a reduction in Pn, Tr, and Gs compared to full irrigation (75–85% FC) at the seedling and maturity stages, with more pronounced adverse effects under moderate (55–65% FC) and severe (45–55% FC) stress Stomatal closure is a major drought-avoidance component, which negatively affects this decrease that inhibits carbon dioxide uptake and can result in photo inhibition (Chen et al., 2023).

A reduction in the quantum yield of PSII (phP0), the electron transport yield (phE0), the performance index (PIABS) and an increase of absorption flux per reaction center (ABS/RC), the dissipation energy flux (DI0/RC) and K- and L-bands under drought conditions is shown by chlorophyll a fluorescence induction kinetics. These changes are associated with damage to the photosynthetic apparatus, which is characterized by a low activity of electron transport, and increased energy dissipation. In addition, the light-harvesting efficiency decreases because of the loss in the chlorophyll content, which worsens photo inhibition (Arslan et al., 2020).

Adaptive adjustments to moderate drought positively affected the leaf-level water use efficiency (WUE) Chen et al. (2023) have found that the impact of drought stress on WUE rises initially and switches to non-stomatal processes as the drought stress escalates. Cultivated sunflowers exhibit a 24% decrease in biomass and reduced drought resilience compared to their wild relatives, consistent with differences in specific leaf area, maximum stomatal conductance, and stomatal pore length. There were no significant differences in osmotic adjustment and succulence, which means that there was a small capacity to tolerate drought (Tran et al., 2024).

Abscisic acid increase occurs after stomatal closure has started and hydraulic cues like change in turgor and acoustic emission will pre-empt the process as early signals (Huber et al., 2019). gsmax plasticity in cultivated sunflower types has a low affinity with drought resistance, which means that it is less resilient than wild germplasm (Tran et al., 2024).

Agronomic effects

Sunflower agronomic performance is significantly affected by drought stress. Under drought stress, grain yield decreases by 30% compared to normal irrigation, with mean yields of 2.7 t/ha under optimal conditions and 1.8 t/ha under drought stress (Ghaffari et al., 2023). Grain yield and weight of 1000 grains decrease with increasing drought stress. Head diameter and grain yield of sunflower reduce with an increase in irrigation intervals during water stress. The grain yield reduced to 83% due to a decrease in the weight of 1000 grains and the number of grains per head in drought stress (Gholinezhad et al., 2009). Among oilseed crops, sunflower shows the maximum reduction in seed yield (16.3%) under limited water availability (Ebrahimian et al., 2019). Drought stress during anthesis can reduce yield by as much as 80%.

Various yield-contributing traits are also affected by drought stress. Grain weight decreases by 11%, and the number of grains per head declines by 22% under drought stress compared with normal irrigation (Ghaffari et al., 2023). The number of grains per head, plant height, and weight of 1000 grains are reduced in drought. A lower number of grains per head is observed under reduced irrigation. Higher plant densities result in shorter growth periods, reduced 1000-grain weight, fewer grains per head, and smaller stem and head diameters (Gholinezhad et al., 2009).50 % reduction in head diameter observed under controlled conditions of drought stress. The leaf growth and leaf area index also decline under drought stress. (Smaeili et al., 2022). The harvest index is also affected; it decreases significantly with increasing plant density and severe drought stress (Gholinezhad et al., 2009).

Drought stress reduces oil production and quality, with sunflower experiencing the highest average reduction of 22.5% across different irrigation water availability levels. At 40% PET, severe drought stress causes a sharp decrease in oil percentage in sunflower (Ebrahimian et al., 2019). Palmitic and linoleic acid levels increase by 11% and 3%, respectively, while stearic and oleic acids decrease by 6% and 11%, respectively, under drought stress conditions (Ghaffari et al., 2023).

The agronomic systems encounter water-deficient conditions that enhance crop water productivity (WPc). At irrigation of less than 90 mm, the maximum economic water productivity (WPe) will be reached, and biophysical water productivity (WPb) will reach the maximum of 90 mm and then the levels of 120 mm will follow (Smaeili et al., 2022). Drought stress also influences other agronomic characteristics, such as increasing maturity in sunflower (Ghaffari et al., 2023).

Growth Stage Sensitivity

Drought stress during the flowering, fertilization, and grain-filling stages results in the greatest yield loss (Gholinezhad et al., 2009). Water shortage during the seedling stage leads to a delay in germination, retards the growth of the seedlings and reduces the photosynthetic performance of the seedlings. The results of a two-year experiment in the arid Hexi Oasis indicated that drought stress lowered the leaf photosynthetic rate, stomatal conductance, and transpiration with greater reductions observed with higher severity of stress. Sunflower are medium sensitive to drought during the seedling stage. The process of photosynthesis was inhibited during moderate and heavy water stress (55-65% FC and 45-55% FC), respectively. It was observed that water use efficiency of the leaves improved under mild drought (65-75% FC). Mild water stress during seedling and maturity stages led to water use efficiency without yielding much. These results show that resources that have the possibility to optimize production are conserved (Chen et al., 2023).

Flowering, seed maturity and seed development stages are of greater susceptibility to the drought stress. Use of 80 percent of the normal water (ETc) showed a slight improvement but the use of 60 percent ETc resulted in significant losses in yield and quality during seed formation (Mostafa, 2022).

Phenotypic Traits for Drought Tolerance Screening in Sunflower

Phenotypic screening serves as an essential tool for identifying drought-tolerant genotypes in sunflower breeding and in water stress, it offers valuable insights into plant traits (Table 1) (SHREE et al., 2016). Sunflower is highly susceptible during critical stages, including germination, seedling establishment, and the reproductive phase, although it sh.ws some drought tolerance (Clapco et al., 2018). Using both controlled setups and field experiments, researchers evaluate these responses through induced drought stress (Shehzad et al., 2021).

Germination Percentage

The critical early indicator of drought tolerance is germination percentage, as during imbibition and radicle emergence, water scarcity hinders seedling establishment. Drought-tolerant genotypes have a higher percentage of germination under drought stress because of osmotic adjustment. The low germination of lines with PEG-6000 induced drought stress of less than 10% and 20% would be an indication of valuable genetic resources in early-stage drought tolerance. (Clapco et al., 2018).

Shoot Length

Shoot length under drought stress is the morphological measure of the distribution of aboveground biomass (Hasan et al., 2020) (Shehzad et al., 2021). Shoot development is a critical characteristic related to an increased drought tolerance since in tolerant genotypes, the shoot development is maintained under -0.6 MPa osmotic stress.(Shehzad et al., 2021). It can be used in specific breeding since it resulted in a significant variation in the length of shoots when the plants were under drought stress, with an additive genetic effects. (Hasan et al., 2020).

Root Length

Root length is an adaptive characteristic in drought-tolerant sunflower genotypes to absorb water in deeper layers of soil. (Hasan et al., 2020; Shehzad et al., 2021). The accessions with drought tolerance showed higher root length during -0.6 MPa osmotic stress, which was related to low cell membrane damage. (Shehzad et al., 2021). Genetic diversity evaluation of 70 genotypes also supports the existence of high variability, which proves that it is essential in breeding programs. (Hasan et al., 2020).

Fresh Leaf weight

The weight of fresh shoot can be used in water-deficient environments as a predictive factor of accumulating biomass. Out of the 70 lines of sunflower, there were more tolerant genotypes with better fresh shoot weight which implies that they have more water to retain. (Hasan et al., 2020). Its potential can be highlighted by the variability observed in its study of genotypes, making it useful in identifying candidates that are resistant to drought.

Dry Root Weight

During drought stress conditions, dry root weight is a relevant parameter in the evaluation of the root structural integrity. Among 70 genotypes, tolerant lines that are more emphatic than the other on root development as a significant drought-adaptative trait were identified (Hasan et al., 2020). Their performance was magnified by the fact that they remained stable in the screening program.

Total Leaf Area (TLA)

One of the primary indicators of photosynthetic capacity (decreased in drought conditions) is total leaf area (TLA), which leads to adaptive strategies, such as leaf rolling. The case of 71 lines has shown that the drought tolerant genotypes had retained more total leaf area (TLA), which was drug to be associated with increased water use efficiency. These lines were positively correlated to yield and had significant variability and TLA was retained (SHREE et al., 2016).

Specific Leaf Area

Specific leaf area (SLA) is the ratio of the leaf area to dry weight; it is a parameter used to measure the adaptation of drought using leaf structural efficiency. Drought-tolerant lines demonstrated higher SLA under stress, meaning they had better photosynthetic functioning among 71 lines (SHREE et al., 2016).

Achene Yield

The yield of achene is a significant economic characteristic, which shows physiological and morphological reactions of plants to drought (Tyagi et al., 2018). Drought tolerant genotypes held their yield stability and yield declined by lines 4.15 t/ha under non-stress situations to 3.58 t/ha under drought stress conditions (Ucak et al., 2017). In stress situations, hybrids obtained on the basis of tolerant CMS lines, in turn associated with their high combining ability, became more productive in field tests (Tyagi et al., 2018). Intolerant genotypes had a higher yield in deficit irrigation of 80 and 60 percent field capacity (Mostafa, 2022; Saremi-Rad and Mostafavi, 2020).

Chlorophyll Content

The photosynthetic capacity of plants under the influence of drought is manifested in chlorophyll concentration. The chlorophyll concentration of the tolerant genotypes was 1.5 to 2.0mg/g during drought, which underscores their adaptive ability (Ucak et al., 2017). Genetic diversity among genotypes aids in the selection of drought-tolerant lines.

Proline Content

Proline contributes to the maintenance of cell integrity and acts as a biochemical marker under drought conditions. In water-stress-tolerant genotypes, the proline level ranged from 0.5 to 1.2 μmol/g, which was associated with increased drought tolerance (Ucak et al., 2017). This variability brings out its purpose in screening.

The combination of these traits underpins multi-trait selection indices, while the observed genetic variability facilitates targeted crosses for developing drought-tolerant sunflower hybrids (Tyagi et al., 2018).

Table 1: Methods for Measuring Key Phenotypic Traits in Sunflower Drought Tolerance Screening

Trait	Measurement Method	Key Steps/Protocol	Environment/Stage	Reference
Germination Percentage	Count of germinated seeds as a percentage.	Place 50 seeds on filter paper with 10% and 20% PEG-6000. Incubate at 25°C for 7 days. Count seeds with radicle >2 mm.	Laboratory; Germination stage.	(Clapco et al., 2018)
Shoot Length	Linear measurement with a ruler.	Grow seedlings under -0.6 MPa osmotic stress. Measure from soil line to tip at 14–21 days.	Hydroponics; Seedling stage.	(Shehzad et al., 2021)
Root Length	Linear measurement with ruler.	Grow seedlings under -0.6 MPa osmotic stress. Measure main root at 14–21 days.	Hydroponics; Seedling stage.	(Shehzad et al., 2021)
Fresh Shoot Weight	Weight with balance.	Grow seedlings under drought. Weigh fresh shoots at 14–21 days.	Greenhouse; Seedling stage.	(Hasan et al., 2020)
Dry Root Weight	Weight with balance.	Grow seedlings under drought. Dry roots at 70°C for 48 h; weigh.	Greenhouse; Seedling stage.	(Hasan et al., 2020)
Total Leaf Area (TLA)	Leaf area meter.	Measure the area of leaves from 71 genotypes under drought.	Field; Vegetative stage.	(SHREE et al., 2016)
Specific Leaf Area	Ratio of area to dry weight.	Measure leaf area and dry weight from 71 genotypes under drought.	Field; Vegetative stage.	(SHREE et al., 2016)
Achene Yield	Weight of seeds per plot.	Harvest at maturity under 80% and 60% field capacity. Weigh after drying to 10% moisture.	Field; Maturity stage.	(Mostafa & Afify, 2022; Ucak et al., 2017)
Chlorophyll Content	Spectrophotometric method.	Extract with 80% acetone from leaves under water stress. Measure at 645 nm and 663 nm.	Field; Vegetative stage.	(Ucak et al., 2017)
Proline Content	Colorimetric method.	Extract with sulfosalicylic acid from leaves under water stress. Measure at 520 nm.	Field; Vegetative stage.	(Ucak et al., 2017)

Summary of Major Phenotypic Traits Used for Drought Tolerance Evaluation in Sunflower *(Helianthus annuus* L.)

Molecular Markers for Enhancing Drought Tolerance in Sunflower

Simple Sequence Repeats (SSRs): Targeted Tools for Drought Tolerance

For drought tolerance screening of sunflower germplasm and QTL mapping of drought-responsive genes, researchers have used SSRs in conjunction with RFLPs and AFLPs (Chugh et al., 2022). Using 30 SSR primer pairs, 100 inbred sunflower lines were fingerprinted under normal and water-deficient conditions. In non-stress environments, 22 SSR markers were associated with plant height, head diameter, yield, and other morphological traits, while 21 markers were linked under water-deficit stress, with some consistently expressed across both conditions. Under water-limited conditions, broad-sense heritability estimates varied within the range of 0.10 to 0.34 (Gezeljeh Ali et al., 2018). Another study utilized a linkage map comprising 210 SSR markers distributed across 1,653.1 cm to assess 70 recombinant inbred lines (RILs) developed from the PAC2 × RHA266 cross. Two QTLs controlling relative water content (RWC) and seven for chlorophyll content were identified under water-limited conditions, explaining between 0.39% and 52.48% of the phenotypic variance. Under both stressed and non-stressed conditions, QTLs for both traits were mapped to linkage groups 10 and 16 (Abdi et al., 2013).

In 2016 and 2017, 64 sunflower genotypes were screened using inter-simple sequence repeat (ISSR) markers under both non-stress and water-stress conditions, with drought stress imposed during flowering.45 ISSR markers that showed associations with yield-related traits were identified using a general linear model (GLM), 23 of which were also validated through a mixed linear model (MLM). 32 related to morpho-physiological traits were identified 24 were supported by MLM, and 19 markers for phenological traits, with 10 confirmed through MLM (Darbani et al., 2020).

Single Nucleotide Polymorphisms (SNPs): Mapping Drought Traits

Extensive SNP data sets have been produced through next-generation sequencing in sunflower to map the drought-related QTLs (Dimitrijevic and Horn, 2018).The SNP markers to map the drought-related QTLs (Kole, 2019). In the PAC2 × RHA266 RIL population, a linkage map integrating 11 SNP markers with SSRs identified two QTLs for relative water content (RWC) and seven for chlorophyll concentration, with R^2 values reaching 52.48% under drought conditions. They identified common QTLs on linkage group 10 with the various irrigation treatments (Abdi et al., 2013).

Amplified Fragment Length Polymorphisms (AFLPs): Assessing Genetic Distance

For the mapping of drought-responsive QTLs, the integration of AFLPs, SSRs, and SNPs has been employed (Chugh et al., 2022). In a diallel population of five sunflower parental lines, 160 AFLP markers were used to calculate Jaccard's distance (Smaeili et al.) alongside Euclidean distance based on 12 morphological traits under well-watered and water-stressed conditions. High JD correlated with high Euclidean distance, with both metrics showing significant relations with F1 performance, specific combining ability (Clapco et al.), and mid-parent heterosis for traits like seed yield and RWC. LR25 was identified as a key parent for optimal yield under stress (Darvishzadeh, 2012). SSRs, SNPs, and AFLPs have been applied in sunflower to map drought-related traits and screen germplasm, with SSRs identifying associated markers, SNPs enabling high-density mapping, and AFLPs assessing genetic distances for heterosis.

Controlled cross-based biparental QTL mapping offers precise information on the genetic basis of drought-responsive traits. Studies on early developmental stages have uncovered major loci regulating germination and seedling responses. In the K55 × K58 recombinant inbred line (RIL) population, 33 QTLs were mapped across eight chromosomes for traits including germination rate, germination potential, germination index, and root-to-shoot ratio, explaining 0.016–10.712% of the phenotypic variance. Chromosomes 9, 13 and 17 showed significant clustering of QTL and suggesting pleiotropic or tightly linked loci and the presence of consistent QTLs in chromosomes 13 and 17 was seen with irrigated as well as drought-stressed environment. Some of the candidate genes found in these regions include aquaporins and GABA transporters, which represent water transport and stress responses mechanisms.(Shi et al., 2023).

Research has identified loci linked to physiological and morphological characteristics on crop wild hybrids (CMS HA89 x wild H. annuus var. annuus). A 1968.2 cm linkage map with 498 markers was used to map 44 QTLs of fecundity, stem diameter, and leaf water content in which the linkage group 17 exhibited significant clustering under various water treatments. (Owart et al., 2014). These loci have a low level of interaction in their QTL x environment making them the most suitable loci of target practice in a breeding program that would put forward a widely adaptable cultivar.

In sunflower, GWAS complements biparental mapping by leveraging natural genetic diversity within diverse germplasm panels. RIL populations, physiological traits such as WUE and CID have also been mapped. Nine QTLs associated with WUE and eight with CID were detected with consistent CID loci on LG06 and LG13, accounting for 7–21% of variance within the XRQ × PSC8 cross (Adiredjo et al., 2014). While biomass and water transpiration loci share genetic regulation, WUE QTLs are specific to certain conditions and reveal how drought type affects genetic structure (Segarra, 2020).

Genomic tools for drought tolerance in sunflower

The application of GWAS in sunflower supplements biparental mapping work by utilizing natural genetic diversity in varied germplasm panels. Analysis of 80 significant associations of eight drought-related traits in 226 inbred sunflower lines using 94,162 SNPs associated with 118 candidate genes with RNA-seq as a source of molecular-level information (Wu et al., 2022). GWAS was run on 226,779 SNPs in 342 accessions of sunflower, which revealed loci with effects on flowering time and height of plants under drought stress, which are potential targets of interventions to improve yields. GWAS in 288 accessions of sunflower found 13 genomic regions, linked to total root length and biomass allocation, involving both cytochrome p450 and auxin-responsive proteins in root adaptation to stress (Masalia et al., 2018).

The specialized metabolites like antioxidants were also involved in drought responses. The link between secondary metabolism and drought resilience in 450 sunflower hybrids was demonstrated through GWAS-based detection of 30 secondary metabolites co-localizing with abiotic stress QTLs and associated with seven candidate genes mainly encoding cytochromes P450 and glycosyl transferases (Moroldo et al., 2024). Association mapping of 64 sunflower genotypes under moisture stress found loci related to yield elements and morpho-physiological characteristics with head and stem diameters becoming the key characteristics (Darbani et al., 2020).

Transcriptome profiling of the sunflower under drought stress shows activation of photosynthesis-associated genes by drought stress to delay senescence, which is regulated by core transcription factors, *bHLH, WRKY, and bZIP* (Moschen et al., 2017). In response to drought, researchers found 2,589 differentially expressed genes (DEGs) that involved the hormone signaling of plants, MAPK signaling, and the synthesis of secondary metabolites, where transcription factors *bHLH025, NAC53*, and *SINAT3* were important mediators of drought adaptation. The flexibility in the dynamic control of gene splicing by alternative splicing enhances during periods of drought and rehydration by modulating transcripts (Shen et al., 2023).

RNA-seq and qRT-PCR findings indicate that the expression of gene in question is marked by appropriate governance of the gene expression to mediate drought tolerance through the abscisic acid (ABA) genes NCED3, NCED5, ABI1, and PYL4 (Sarazin et al., 2017).In normal watering case, sensitive genotypes displayed high ABA levels, confirming that the regulation is essential in drought tolerance (Sarazin et al., 2017). A feedback interaction between MAPK and ABA pathways enhances the adaptation of the plant to stress (Shen et al., 2023). DEGs specific to genotype are material to develop drought-resistant lines and transcription factors and pathways are seen to be conserved across sunflower genotypes (Moschen et al., 2017; Sarazin et al., 2017; Shen et al., 2023). Proteomics can be used as a promising technique in order to reveal protein pathways that deal with plant physiology and stress adaptation. Based on the research of plant proteomics, it is possible to reach an improvement in learning about the pathways of stress endurance (Ahmad et al., 2016).

Carbohydrate metabolism and photosynthesis-related proteins become low in the susceptible genotypes to drought stress, whereas tolerance genotypes remain stable. Differential proteomics have been used to examine the adaptation of sunflowers to drought. Two different genotypes that respond differently to drought stress were employed. Database searches using peptide mass fingerprints identified two genotype-specific proteins, while 23 and 5 stress-responsive protein spots were detected in the susceptible and tolerant genotypes, respectively. The susceptible genotype showed a decline in protein spots corresponding to photosynthesis and carbohydrate metabolism in drought stress, suggesting suppressed energy metabolism. These alterations did not show in tolerant genotypes, indicating unaltered metabolic activity under stress (Castillejo et al., 2008). Flowering-stage drought stress was applied across two years to study proteomic responses in two sunflower inbred lines. According to proteomic analysis, drought-induced changes were observed in 18 proteins in sensitive lines, whereas 24 proteins were altered in tolerant lines. Simultaneous down regulation of oxygen-evolving enhancer and ferredoxin-NADP reductase was identified as an early drought stress signal. The primary mechanisms involved in drought tolerance in sunflower are reactive oxygen species scavenging, energy and water conservation and maintaining cell structural stability (Ghaffari et al., 2017). An experiment was conducted to evaluate how water loss alters protein levels in sunflower. The analysis revealed 467 consistent bands in the tolerant line, while 417 were observed in the sensitive line. Maintaining stable metabolism, retaining cell moisture, reinforcing cellular structure, and enhancing antioxidant activity were the primary traits attributed to the tolerant line for withstanding environmental stress (Damavandi et al., 2021).

Table 2: Key Gene Families and Candidate Genes Associated with Drought Tolerance in Sunflower

Gene Family/Type	Specific Genes	Role/Response in Drought	Source Reference
GASA	*HaGASA2, HaGASA10, HaGASA11*	Significantly upregulated; prominent role in drought tolerance mechanisms.	(Ullah et al., 2024)
GASA	*HaGASA6, HaGASA12, HaGASA16, HaGASA17, HaGASA20, HaGASA21, HaGASA24*	Significant variation in expression (up/down-regulated).	(Ullah et al., 2024)
ABA/Signaling (Candidates from GWAS/RNA-seq)	*LOC110941963* (microtubule-associated)	Candidate gene for drought response.	(Wu et al., 2022)
ABA/Signaling (Candidates from GWAS/RNA-seq)	*LOC110885273* (protein kinase)	Abscisic acid-related; may play an important role in drought response.	(Wu et al., 2022)
ABA/Signaling (Candidates from GWAS/RNA-seq)	*LOC110891369* (receptor-like kinase)	Abscisic acid-related; may play an important role in drought response.	(Wu et al., 2022)
ABA/Signaling (Candidates from GWAS/RNA-seq)	*LOC110894816* (nucleotide transporter)	Candidate gene for drought response.	(Wu et al., 2022)
SAP	*HaSAP1, HaSAP3, HaSAP8, HaSAP15, HaSAP16, HaSAP21, HaSAP23, HaSAP26*	Strongly induced/upregulated at transcriptional level under drought stress.	(Zhang et al., 2024)
SAP	*HaSAP10, HaSAP22*	Upregulated under drought (further investigated via qRT-PCR).	(Zhang et al., 2024)
TFs/Signaling	*bHLH025, NAC53, SINAT3*	Central roles in response to severe drought; positive response to rehydration; involved in hormone/MAPK/secondary metabolite pathways.	(Shen et al., 2023)
TIFY/JAZ/PPD	*HaPPD1, HaPPD4*	Significantly upregulated at early stages of drought; sensitive to drought stress.	(Zhang et al., 2025)
TIFY/JAZ/PPD	*HaJAZ2, HaJAZ4, HaJAZ5, HaJAZ9, HaJAZ12*	Highly expressed (from JAZ V subfamily); potential roles in drought tolerance.	(Zhang et al., 2025)
SPL	25 *HaSPL* genes	Broad involvement in response to drought stresses; differential expression under water stress.	(Jadhao et al., 2023)
TFs (General)	*AP2/ERF, MYB, bHLH, WRKY*	Enriched among DEGs; important in drought response (high-abundance families).	(Shi et al., 2024)

Table of Sunflower Gene Family, Individual Genes, and Its Reported Role in Drought Stress Responses

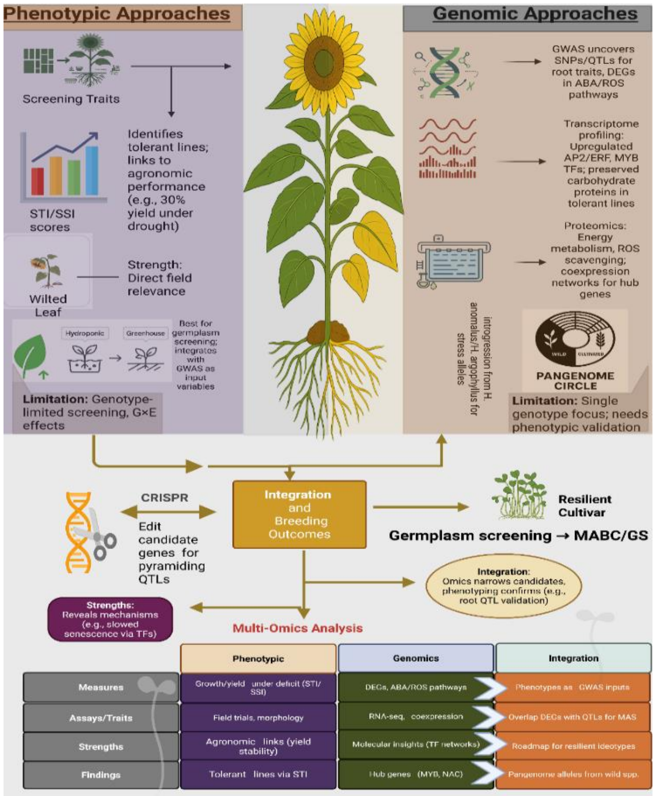
Integration of Phenotypic and Genomic Approaches

The integration of phenotypic traits with genomic information facilitates drought-resilient breeding, where STI and SSI indices help GWAS to uncover SNPs and candidate genes within regions of linkage disequilibrium. GWAS results are validated through transcriptome profiling in tolerant lines, highlighting candidate genes by overlapping DEGs with SNP-associated genes (Table 3) (Wu et al., 2022). Genomics has provided opportunities to use marker-assisted backcrossing (MABC) and genomic selection (GS) to use QTLs of wild species such as Helianthus anomalus to pyramid into cultivated sunflower lines. With CRISPR/Cas9 and combining the use of natural gene resources with technologies, the breeding of strong sunflower lines is improved. Multi-omics analyses can be described as a combination of genomic, transcriptomic, and proteomic data sets that present a combined view of the study of the mechanism of drought tolerance. The transcriptome analysis of regulatory networks performed on tolerant sunflower genotypes revealed that the stress-induced senescence is delayed due to higher expression of photosynthesis-related genes and transcription factors (e.g., *AP2/ERF, MYB*) supported by regulatory networks. (Moschen et al., 2017). Analysis at the proteome level provides insights into drought responses, highlighting proteins involved in energy metabolism and reactive oxygen species scavenging. In tolerant sunflower lines, carbohydrate metabolism proteins are largely preserved under drought, whereas notable down regulation is observed in sensitive genotypes (Damavandi et al., 2021). These findings help in MAS and GS for the selection of candidate genes.

Researchers can pinpoint genomic regions influencing drought-related morphological features through the integration of GWAS with comprehensive phenotypic assessments of root traits under normal and drought conditions (Masalia et al., 2018). Root structure and water uptake under stress are enhanced as integrative strategies provide a roadmap for breeding lines to identify loci. The introgression of genomic segments from wild relatives, including *Helianthus argophyllus*, into domesticated sunflower genotypes is shown from the analysis of the sunflower pangenome. The genetic pool broadens for breeding from these introgressed alleles and can contribute to adaptation to environmental stress (Badouin et al., 2017).

The identification of genes and markers for complex traits and insights into their regulatory networks using the integrative strategies that various multiple omics approaches with bioinformatic tools. The use of bioinformatics and statistical tools is gaining importance in sunflower breeding. Ridge regression mixed model predictions from the R package *rrBLUP* and linear mixed modeling with customized covariance matrices for G×E interaction modeling and genomic estimated breeding value prediction from lme4GS (Caamal-Pat et al., 2021; Dimitrijevic & Horn, 2018; Endelman, 2011).

The integration of phenotypic and genomic information, supported by bioinformatics and multi-omics analyses, provides a comprehensive framework for breeding drought-resilient sunflower cultivars *(Fig 1)*.



	Phenotypic	Genomics	Integration
Measures	Growth/yield under deficit (STI/SSI)	DEGs, ABA/ROS pathways	Phenotypes as GWAS inputs
Assays/Traits	Field trials, morphology	RNA-seq, coexpression	Overlap DEGs with QTLs for MAS
Strengths	Agronomic links (yield stability)	Molecular insights (TF networks)	Roadmap for resilient ideotypes
Findings	Tolerant lines via STI	Hub genes (MYB, NAC)	Pangenome alleles from wild spp.

Fig. 1: Integrating phenotypic and genomic approaches for drought-resilient sunflower breeding. The framework illustrates how phenotypic screening (e.g., STI/SSI indices, trait evaluation) aligns with genomic tools such as GWAS, RNA-seq, and proteomics. Multi-omics analysis, CRISPR-based gene editing, and marker-assisted breeding (MABC/GS) contribute to the development of resilient cultivars.

Challenges and Advances in Drought Tolerance Breeding

The limited genetic diversity of cultivated sunflower (Helianthus annuus L.) hinders the breeding of drought-tolerant varieties. The genetic bottleneck caused by sunflower's domestication in North America and worldwide cultivation has led to a narrow genetic base, increasing their sensitivity to abiotic stresses like drought due to the loss of traits from crop wild relatives (CWR). Despite the fact that wild sunflower relatives have a broad environmental adaptability and a deep variation to abiotic stresses, such as salt tolerance, breeding of drought wildlife has not been observed, although most economic advantages of wild crop have focused on disease and herb variations, male sterility, in addition to salt tolerance (Seiler et al., 2017).

Table 3: Comparative Overview of Phenotypic and Genomic Approaches for Drought Tolerance in Sunflower

Aspect	Phenotypic Approaches	Genomic Approaches
What it measures	Plant performance under water deficit: growth, yield, STI/SSI	DNA/RNA variation underlying drought response: DEGs, signaling pathways (ABA, ROS), candidate genes. (Wu et al., 2022; Shi et al., 2024)
Typical assays / traits	Indices like STI/SSI in the field and controlled drought; growth, morphology, and yield components. (Ghaffari et al., 2012)	RNA-seq under drought; coexpression networks; candidate gene families (e.g., ABA-related genes) (Wu et al., 2022; Shi et al., 2024)
Strengths	Links traits to agronomic performance (e.g., yield under drought). (Ghaffari et al., 2012)	Reveals molecular mechanisms (ABA signaling, ROS balance). (Shen et al., 2023; Shi et al., 2024)
Limitations	Small number of genotypes ((Ghaffari et al., 2012)	Single genotype or limited time points. (Wu et al., 2022; Shi et al., 2024)
Example findings in sunflower	STI/SSI identifies tolerant vs sensitive lines. (Ghaffari et al., 2012) Drought reduces yield in the reproductive stage. (Jocković et al., 2024)	GWAS + RNA-seq reveal ABA-related candidates. (Wu et al., 2022) Coexpression networks identify drought genes. (Shi et al., 2024)
Best-use cases	Screening germplasm; breeding with tolerant lines. (Jocković et al., 2024; Ghaffari et al., 2012)	Marker discovery (e.g., SNPs); gene prioritization for breeding. (Wu et al., 2022; Shi et al., 2024)
Integration point	Phenotypes (STI/SSI, yield) as variables for genomic analyses. (Barnhart et al., 2022; Shen et al., 2023)	Omics narrows candidates; phenotyping confirms relevance. (Wu et al., 2022; Shi et al., 2024)

Strengths, limitations, and integration points of phenotypic and genomic methods for improving drought resilience in sunflower breeding programs

Besides, it is difficult to be sure that yield stability under changing environmental conditions can be maintained when producing drought-tolerant types of sunflower especially as the pressure of climate change increases the drought stress. Growing sunflower as a spring crop under rainfed conditions exposes the latter to rising temperatures and water shortages, and it is important to note that according to the ideotype, basic changes in the new environment are necessary. Varietal assessment systems in the main production regions such as France focus on yield, oil level, early maturity, and resistance to diseases and do not provide much knowledge regarding drought resistance and yield stability, thus restricting the creation of genotypes adapted to climate (Debaeke et al., 2021).

The genetic nature of drought tolerance is very complicated with redundant and homeostatic molecular and physiological pathways in contrast to relatively simple genetic framework of transgenic characters to manage biotic stress. The relative failure of the bottom-up methodologies, in which the changes do not become apparent at the whole-plant and field levels, points at the necessity of the shift to the top-down ones, in which the key factor in the study is the crop performance under a variety of conditions. Phenotyping drought characteristics are associated with high levels of difficulty, because it needs sophisticated technologies to record expression at the crop level, whereas the conventional screens are imprecise and sophisticated strategies are limited only to a few genotype collections. Even though multi-level screening techniques are a good approach to parental line selection, there is a need to have genetic markers that can be highly relied upon in terms of trait monitoring between breeding cycles (Sinclair, 2011).

The intensive GxE interaction of seed yield with the drought-afflicted genotypes of sunflower highlights the importance of stability analysis with the parametric and non-parametric indices to select the stable genotype with high yield. The analysis of 100 oilseed sunflower genotypes in both normal and drought conditions showed a high level of genotype and GxE effects in ANOVA, which indicates the difficulty in the selection of stable cultivars. Selecting for drought yield traits is challenging due to their polygenic nature, making the process lengthy and costly, and requiring the integration of both types of stability indices for comprehensive evaluation (Akbari & Darvishzadeh, 2024)

Evaluating the yield advantages of drought-adaptive traits is critical but commonly underestimated, though commercial success depends on traits that enhance water efficiency and stress resilience rather than survival under extreme water scarcity (Sinclair, 2011). Crop modeling enables the prediction of yield performance across seasons and locations. However, applying it to sunflower breeding requires integrating novel production systems, such as low-input agriculture and agroforestry, to develop ideotypes that balance productivity with ecological functions (Debaeke et al., 2021; Sinclair, 2011).

The critical bottlenecks of remote sensing based high-throughput phenotyping of drought and heat stresses are the low UAV flight frequency at which precise flowering-time measurements can be obtained, the impossibility of scaling photosynthetic efficiency ideotypes of small plots to large diversity panels, the difficulty of monitoring carbohydrate translocation across leaves, stems, and grains under different stresses, and reduced accuracy of machine learning based yield estimates because stressed plants do not produce their seed uniformly. (Hein et al., 2021).

Advances in Drought Breeding

Knowledge of the genetic basis of drought tolerance has shown possible ways of crop improvement with the development of genetic engineering, marker-assisted selection and genomic selection and therefore, this can be incorporated into drought resilience mechanisms. Genomic selection involves the use of predictive methods of identifying desirable genotypes without phenotypic assessments. This approach will give a strong algorithm to enhance complex phenotypes like drought tolerance by evaluating alleles with both major and minor impacts. It has been used to enhance yield in maize and resistance to cyst nematodes in soybean as well as to amplify amylase activity in barley and agronomic behaviors like yield and stature in rice, GS in crops like maize and barley has decreased the number of selection cycles by almost half over traditional phenotypic selection.

These modern tools collectively enable the development of climate-smart, drought-tolerant sunflower cultivars by integrating genomics, AI, and genome editing *approaches* (Fig 2).

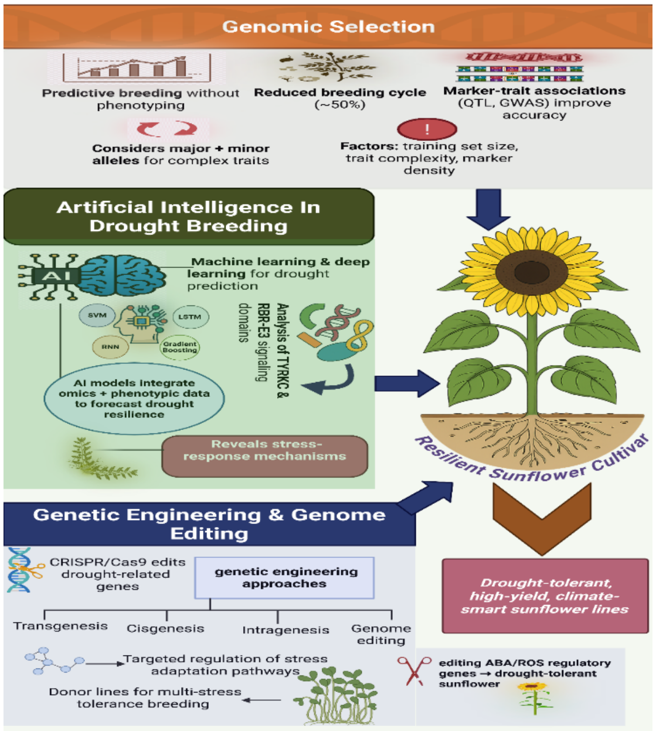


Fig. 2: Recent advances in drought breeding of sunflower. The illustration highlights genomic selection, AI-assisted drought prediction, and genetic engineering approaches such as CRISPR/Cas9, transgenesis, and cisgenesis for creating high-yield, climate-smart sunflower lines.

In comparison with traditional phenotypic selection, GS in crops (including maize and barley) has shortened selection cycles by almost 50, with uses including ameliorating grain yield in maize and soybean cyst nematode resistance and improving amylase activity in barley and agronomic measures of yield and plant height in rice. Genomic selection has been used in maize and chickpea with low-to-moderate predictive accuracy on yield and drought-related characteristics to increase breeding efficiency. The genomic selection performance depends on factors that include the breeding population type, training set size, complexity of traits and the genetic marker number used. The use of marker-trait associations of QTL and GWAS mapping into prediction models increases the prediction accuracy. The genetic architecture should be studied through GWAS and QTL mapping for a better understanding of complex traits (Rosero et al., 2020).

Advancement of research on plant stress mitigation is possible through artificial Intelligence techniques, including machine learning and deep learning. The combination of TYRKC and RBR-E3 domain analyses with integrating state-of-the-art algorithms (gradient boosting, support vector machines (SVM), recurrent neural networks (RNN), and long short-term memory (LSTM) demonstrated the essential aspects of drought stress response in crops, which is a significant step towards sustainable agriculture (Ali et al., 2024).

Genetically engineered organisms (GMOs) are multifaceted in genetic engineering methods involving transgenesis, cisgenesis, intragenesis, and genome editing to promote agricultural development. Through these strategies, the understanding of regulatory genes that control the response of plants to stress has become an exciting way of enhancing drought tolerance in crops. Through regulating key metabolic pathways, this approach relies on the plant's innate ability to adapt to stress (Simsek et al., 2024). Genome-editing tools, such as CRISPR/Cas9 and associated CRISPR endonucleases, are used to alter the genes of sunflower that are involved in stress-response.

This method can be used to develop sunflower cultivars that will have enhanced tolerance to various stresses and can be used as donors in cross-crop breeding programs (Mmbando , 2025).

Future Prospects

Reliable markers applicable to various populations can boost selection efficiency and significantly advance breeding efforts. Strong association between molecular markers and the desired trait facilitates the choice of desirable genotypes (Chugh et al., 2022). Utilizing existing plant genetic resources along with modern molecular approaches like GWAS and genomic selection offers great potential to improve sunflower performance for stress resilience and climate change adaptation (Miladinović et al., 2019).

Sunflower, cultivated as a rainfed summer crop on shallow to moderately deep soils, has yet to undergo drought tolerance assessment, with earliness to anthesis potentially serving as a drought escape trait. Current evaluation standards overlook yield stability, and homogeneous testing conditions prevent capturing the variability of water deficit challenges experienced by farmers in most regions (Debaeke et al., 2021). The use of multiple omics technologies combined with bioinformatics can reveal target genes and markers associated with complex traits and provide improved insight into trait regulation (Dimitrijevic & Horn, 2018).

Crucially, supportive policies and collaborative breeding networks will play a key role in implementing genetic and genomic advancements on the farm. Using wild Helianthus species adapted to extreme environments and novel breeding approaches, the HelEx project seeks to develop sunflowers resilient to heat and drought, concurrently evaluating their ecological and socio-economic implications (Inrae, 2023). Through the Crop Wild Relatives program, the Sunflower Pre-breeding Project has interbred wild sunflower relatives with elite cultivars, tested them in diverse locations including Argentina, Uganda, India, and Europe, and pinpointed important traits like early flowering and bigger heads to enhance drought resilience (Crop, 2021).

Conclusion

The review describes the effects of drought on sunflower, including physiological effects (decreased chlorophyll fluorescence and water-use efficiency), agronomic effects (poor oil quality and harvest index), and the necessity to use both phenotypic and genomic approaches to develop resistant cultivars. Phenotypic analysis of the important characteristics including root biomass and chlorophyll stability is very informative in improving germplasm. The genomic methods also shed light on the regulatory interactions, such as SAP/GASA gene activity and proteome-based cellular resilience that have been verified in biparental and association populations. Polygenic inheritance constraints and environmental effects can be overcome through integrating the application of marker-assisted backcrossing, prediction, and multi-omics systems. This synthesis increases the speed of pyramid breeding of drought tolerance genes of wild relatives into high yield sunflower varieties. With the increasing severity of drought and arid climatic conditions across the planet, machine learning in the phenotyping of stress and the design of regulatory genes are progressively leading to the production of climate-resilient sunflower varieties that guarantee stability in yield and sustainability of the ecosystem.

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Generative AI Statement: The authors declare that this manuscript has been written without the use of generative artificial intelligence tools.

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