

REVIEW ARTICLE

# Advancements in sunflower genomics: Navigating the Biotech revolution for crop improvement

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## Abstract

Sunflowers are a staple crop in global agriculture, with significant production in temperate and semi-arid climates. The cultivated sunflower is notable for its diverse chromosomal configurations and its historical cultivation by Native American tribes. Recent advancements in sunflower genomics have revealed a complex and extensive genome, offering insights into traits critical for breeding, such as oil content, disease resistance, and environmental resilience. Genomic studies have also illuminated the pathways governing drought tolerance and fatty acid composition, improving the breeding of sunflowers tailored to specific agronomic and nutritional needs. Identifying genes associated with disease resistance, particularly against the parasitic weed broomrape, highlights the potential of genomics in safeguarding crop productivity. Overall, the article emphasizes the importance of sunflower genomics in shaping the future of crop improvement and agriculture.

## Keywords

genetic diversity; genomics; omics; QTL; sunflower

## Introduction

Sunflower (*Helianthus annuus* L.) is primarily cultivated in temperate, semi-arid climates. Temperate, semi-arid climates in regions such as the Great Plains (U.S.), Central Asia, and southern Australia are ideal for sunflower cultivation. These regions, with moderate temperatures and low rainfall (250-500 mm annually), support sunflower growth due to the crop's drought tolerance, making it a key player in oilseed production in countries such as Kazakhstan, and Ukraine. The United States is the world's fourth-largest producer of oil crops, following soybean, oil palm, and canola (1). In addition to its primary use in human diets, sunflower oil has a variety of applications in the chemical and pharmaceutical sectors. It is used in the production of bio-lubricants and cosmetics, and as a carrier oil in pharmaceuticals due to its emollient properties. In the cosmetics industry, sunflower oil is a key ingredient in moisturizers and creams, valued for its high vitamin E content. In the pharmaceutical industry, it serves as an excipient in drug formulations. Globally, about 10% of sunflower oil production is directed toward industrial uses, highlighting its versatility beyond food consumption (2). The global trend in sunflower cultivation is steadily increasing. According to the data shown in 2024, the major

sunflower-producing countries in 2023 include the Russian Federation (17.1 MMT), Ukraine (14.5 MMT), European Union (10.2 MMT), Argentina (4.1 MMT), China (2.2 MMT), Turkey (1.5 MMT), and the United States (0.995 MMT) (FAO 2024). Over the next 20 years, it is predicted that global oil output and consumption both for food and non-food purposes will double. In particular, it is projected that canola will expand by 1.8 times, oil palm by three times, sunflower by 2.2 times, and soybean by tripling, according to projections from the Food and Agriculture Organization (FAO) and the Organization for Economic Cooperation and Development (OECD). These estimates reflect the growing global demand for vegetable oils across food, biofuel, and industries. Sunflower seeds are an excellent source of nutrients since they are high in fiber, protein, minerals, and phenolic compounds. Sunflower crops can be planted late in the wet season as they are drought tolerant. They are also used in agricultural systems where they are rotated with corn, beans, or rice. Products made from sunflowers are in high demand, especially the seeds and oil. Notably, sales of sunflower oil in 2020 totalled USD 18.50 billion (3).

Seeds and oil are the two primary components that make up the sunflower market. Due to rising health consciousness and knowledge of oilseed's many health advantages, oilseed has taken center stage in the market (4). High production of seeds and oil, genetic resistance, and a high degree of tolerance to economically significant illnesses, pests, parasitic plants such as broomrape, and resilience to abiotic stressors (mostly drought) are the key breeding objectives for sunflowers.

As one of World's most important oilseed crops, increased efforts are needed to employ cutting-edge breeding techniques to meet the rising global demand for sunflower products. These techniques include genomic selection, CRISPR-Cas9 gene editing, marker-assisted selection (MAS), and hybrid breeding. Genomic selection can accelerate breeding by predicting offspring performance early in the breeding cycle, while CRISPR-Cas9 enables precise gene edits to improve traits such as disease resistance and drought tolerance. Marker-assisted selection improves breeding efficiency by using genetic markers linked to desirable traits. Hybrid breeding remains essential for enhancing yield potential and adaptability to changing climates. Together, these tools can significantly enhance sunflower productivity and quality (5). This will focus on issues restricting the phenotypic manifestation of genetic potential, which will increase both the quantity and the quality of sunflower production. The intricate inheritance patterns of the aforementioned traits require special consideration, particularly about resilience and tolerance to various pests and droughts (6).

Numerous biotic factors affect sunflower productivity globally, and a particularly destructive one is the parasitic plant broomrape (*Orobanche cumana* Wallr.). Broomrape (*Orobanche* spp.) is highly destructive to sunflower crops because it parasitizes the roots, depleting the plant of water and nutrients. This leads to significant

yield losses, often ranging from 20% to 80%, with severe infestations potentially causing complete crop failure. Broomrape also affects sunflower oil quality, making it a major challenge in regions such as Europe, the Middle East, and North Africa. Its control is difficult due to its long-lasting seeds, which can persist in the soil for years, complicating management strategies in affected areas. Many European and Asian nations, particularly those in Central and Eastern Europe, as well as Spain, Turkey, Israel, Iran, Kazakhstan, and China, have high rates of broom rape (7). Over the past few years, there has been close monitoring of the growth of broomrape, its entry into new countries, and the creation of new, stronger races (8). Sunflower broomrape has a high rate of mutation and dispersal (9).

### The botany of sunflower

Within the genus *Helianthus*, there are 67 species, including the cultivated sunflower (*H. annuus* L.). It belongs to the Asteraceae (Compositae) family and is a dicotyledonous plant with a distinctive composite bloom (10). Depending on the cultivar, the inflorescence, also known as the sunflower head, contains 700–8,000 blooms. Two diploid, tetraploid, and hexaploid species have been identified (11). There are 34 chromosomes in cultivated sunflower ( $2n = 34$ ). Sunflowers are named after the Greek words *helios*, which means "sun," and *anthos*, which means "flower." The names for sunflowers—*girasol* in Spanish and *tournesol* in French—literally translate to "turn with the sun," which is a characteristic that sunflowers display. Native American tribes across North America farmed sunflowers as a common crop, 3,000 BC. It is believed to have existed in Arizona and New Mexico (12). Native American tribes across North America cultivated sunflowers not only as a common crop but also for their cultural, medicinal, and spiritual significance. The seeds were a vital food source, rich in fats and proteins, and were used in a variety of preparations. Tribes such as the Hopi, Lakota, and Mandan used sunflower seeds to make oil, which served as both a food product and a base for skin treatments. The sunflower's vibrant yellow blooms were symbolically linked to the sun and played a role in ceremonial practices. Additionally, various parts of the plant were used medicinally to treat ailments such as snake bites and chest pains. Sunflowers held deep respect as a symbol of life, sustenance, and spiritual energy in many native cultures.

Sunflowers are native primarily to both North and South America, and some species are developed as an ornament for their impressive size and bloom heads as well as for their consumable seeds (13). Ornamental sunflower species commonly used for their large size and vibrant blooms include *H. annuus* (common sunflower) and *H. maximiliani* (Maximilian sunflower). These species are popular in gardens and landscapes for their striking flower heads and towering height.

Compared to the cultivated sunflower (*H. annuus*), which is primarily grown for oil and edible seeds, ornamental varieties are often selected for aesthetic traits such as unique flower colors (yellow, orange, red, or bi-

colored), petal shapes, and varied sizes. Dwarf ornamental sunflowers, such as the "Teddy Bear" or "Sunspot" varieties, are significantly smaller than the commercial oilseed types, making them ideal for decorative purposes. Additionally, ornamental sunflowers may have multi-branched stems with numerous blooms, while cultivated sunflowers typically focus on a single large flower head optimized for seed production. Concurring to think about the advancement of haploid chromosome numbers within the sunflower family, sunflower is thought to have been tamed 3,000–5,000 a long time ago by Native Americans who essentially utilized it as an edible source of seed (14). Most domestication sites for sunflowers are located within the eastern forests of North America, particularly in a few dry caves and rock shelters in the central and eastern United States (15). Sunflowers developed over 5,000 long ago and have experienced a generally quick developmental move from wild to tame. Sunflowers are a financially vital seed trim within the family Asteraceae, and the oilseed sunflower contributes roughly 10 percent of the world's plant-derived consumable oil (15).

Sunflowers are known to have a tall recurrence of polyploidy, which is a duplication across the whole genome. A ponder on the evolution of haploid chromosome numbers within the sunflower family found that the evolution of haploid chromosome numbers in Asteraceae was a dynamic process, with genome duplications and plummeting dysploidy being the foremost visit genomic occasions within the advancement of this family (16). The study identified over one hundred whole-genome duplication events within the sunflower family (16). Polyploidy is regarded as one of the greatest instruments mindful for the developmental victory of numerous species, in specific by empowering the adjustment of recently emerged polyploids to distinctive living spaces (16). For illustration, the repetitive nearness of polyploids in environments distinctive from those of their diploid forebears constitutes solid proof of the capacity for the colonization of polyploids' unused natural specialties (17). Polyploidy in sunflowers enhances their adaptability by increasing genetic diversity, allowing them to thrive in diverse environments. Polyploid sunflower species are often more resilient to environmental stresses such as drought and poor soil conditions compared to their diploid relatives. For instance, hexaploid wheat (*Triticum aestivum*) demonstrates greater resilience to drought and cold, enabling cultivation in a wide range of conditions

### Genetic diversity and variation in sunflowers

Various studies have used geomorphological traits and three distinct molecular marker approaches (RAPD (random amplified polymorphic DNA), ISSR (inter simple sequence repeat), and SRAP (sequence related amplified polymorphism)) to evaluate the genetic variation among thirteen genotypes of sunflowers. The study revealed that the genetic dissimilarity based on molecular markers (0.089 with a range of 0.014–0.164) was much lower than that based on agro-morphological dissimilarity (4.05 with a range of 0.979–7.128) and was considerable.

Geomorphological trait-based cluster analysis identified two main, different groupings, with one line (line 63) constituting an independent cluster. With an average of 12.2 bands per primer, 15 primers from all genotypes amplified 183 DNA (deoxyribonucleic acid) fragments in total 85 (46.45%) of the segments exhibited polymorphism. Three main unique groups were found using cluster analysis using a combined RAPD, ISSR, and SRAP data set. An independent cluster was formed by a single line (Line 63). Three molecular indicators showed a positive connection ( $r = 0.104$ ;  $p < 0.01$ ). Between the matrices of genetic dissimilarity based on SRAP, ISSR, and RAP (receptor associated protein) markers and the agro-morphological dissimilarity matrix, a significantly significant positive correlation ( $r = 0.606$ ,  $p < 0.01$ ) was discovered.

Twenty one microsatellite markers were used in the study to assess the genetic diversity across 68 genotypes of sunflowers (18). These markers produced 49 polymorphic bands with DNA fragment sizes ranging from 92 to 850 bp. The markers showed substantial variation in the polymorphic information content (PIC), with the greatest and lowest PIC values found in Ha806-ar and Ha494-ar, respectively. Every locus included 2.86 alleles on average. Notably, Iranian hybrids showed the least genetic variety, whereas cytoplasmic male sterility (CMS) lines displayed the greatest. Iranian hybrids were distinguished from other groups using principal coordinates analysis, and an analysis of molecular variance (AMOVA) revealed that most genetic variation (90%) was found within groups rather than among them (10%). The research highlighted the importance of SSR (simple sequence repeat) markers, in particular Ha806-ar, for genotype differentiation in sunflowers and highlighted the considerable genetic differences across individuals. Because of their low similarity coefficient, it was indicated that some paternal lines, such as R26 and CMS502, would be useful for breeding in the future. The sunflower groupings' high degree of genetic resemblance points to a narrow genetic basis and the necessity of adding new genes to increase the genetic diversity of the breeding stock.

To estimate the foremost heterozygotic cross-breed combinations and innate breeding, as well as to affirm genealogical joins, data concerning hereditary disparity on breeding assets is significant for trim breeding programs (19). Using straightforward arrangement rehashes (SSRs), this study evaluated the hereditary differing qualities in Kenyan sunflower breeding lines. A total of 83 alleles were identified across 32 SSR loci. The 24 sunflower accessions yielded a average of 2.7 alleles per locus, with an allele number per locus extending from 2 to 7. The normal polymorphic data substance (PIC) was 0.384. After performing a cluster examination utilizing the hereditary closeness coefficients, three bunches were recognized from the 24 sunflower breeding assets. The vital arrange investigation (PCoA) showed 47.38% of the overall fluctuation, or 34% and 13.38%, individually. The hereditary differences of the Kenyan sunflower breeding assets were found to be less than those of other sunflower

germplasm assets. This finding raises questions about the reasonability and centrality of presenting first-class genotypes from various roots to the Kenyan sunflower breeding program in arrange to choose breeding lines with a more extensive hereditary base.

101 genotypes consisting of 98 inbred, two hybrids, and one open-pollinated variety (20). All genotypes were grouped into ten clusters. Among the 11 investigated traits, the number of filled seeds, test weight, and plant height contributed maximum towards genetic divergence.

Genetic divergence among 102 genotypes and grouped into twelve clusters were assessed (21). Based on the inter-cluster distances and per se performance, the genotypes namely; GMU-4, GMU-11, GMU-14, GMU-16, GMU-25, GMU-40, and GMU-70 were selected for further intercrossing to obtain high heterosis and also to recover desirable transgressive segregants. The analysis suggested the maximum contribution of seed yield per plant (40.2%) followed by number of leaves per plant (25.8%) and 100-seed weight (17.0%) towards divergence.

In another study examining 24 breeding lines for eight traits, the genotypes were grouped into ten clusters, with the largest cluster containing 13 genotypes. The inter-cluster distance was the maximum between clusters VI and VIII, followed by clusters IV and VI, and clusters VI and IX. The study revealed that plant height contributed maximum towards divergence (45.29%), followed by seed yield per plant (25.72%) and oil content (15.94%). Based on the inter-cluster distance and per se performance, the genotypes such as 17A, 47A, CSFI 5325, CSFI 5415, CSFI 5436, and CSFI 5013 were identified as suitable parents which could be intercrossed to obtain high heterosis.

### The sunflower genomics

The absence of a reference genome sequence in the Compositae family, which includes a wide range of commercially relevant flowering plants such as weeds, medicinal, and food and horticulture crops, hinders research and development activities within the family (22). This work presents efforts to sequence the genome of *H. annuus*, a major crop in the Compositae family and cultivated sunflower, with an estimated size of 3.5 Gb. The sequencing approach utilized involves the creation of comprehensive genetic and physical maps. This aids in the systematic assembly of shotgun sequences. Additionally, whole-genome shotgun sequencing is performed using the Solexa and 454 platforms. Together, these methods enhance the accuracy and efficiency of the genome sequencing process (23). The creation of a sequence-based physical map, which provides distinct sequence markers at roughly every 5–6 kb interval across the genome, considerably enhances the effectiveness of this method. About 85% of the sunflower genome is represented by the physical map as of right now (24). The high percentage of repetitive sequences, comprising about 78% of the sunflower genome, indicates a significant level of genome complexity. Repetitive sequences, often including transposable elements and duplications, can complicate genome assembly and analysis, as they make it harder to

distinguish between unique regions of the genome. These repeats also contribute to the genome's size, but they often do not directly code for proteins. Instead, they may play roles in gene regulation, genome stability, or evolution. The high coverage obtained by 454 (15.5x) and Solexa (80x) sequencing helps to ensure that even with these complexities, the genome can be accurately mapped and assembled. Moreover, over 76% of contigs longer than 5 kb have been successfully matched with either the genomic or physical maps, or both. The alignment implies that the used methodology is well-positioned to provide a precise and all-inclusive reference genome for the sunflower, therefore making a substantial contribution to the genetic resources accessible for the Compositae family (25).

The sunflower (*H. annuus*) has a base chromosomal count of 17, and its genome size is estimated to range between 2,871 and 3,189 million base pairs (Mbp). However, some sources indicate that the genome's actual size is closer to 3500 Mbp (26). The genome of sunflowers has 55,803 long non-coding spliced RNAs (ribonucleic acids) and 52,232 inferred protein-coding genes (27). The genome of a sunflower is the first to be fully sequenced, as is the genome of any member of the biggest plant family, Asteraceae, which has over 23,000 species identified (28). The 300,945-bp master circle consists of the 26S, 5S, and 18S rRNAs, 18 tRNAs, and 27 protein-coding sequences.

The mosaics found within the genome of sunflowers are renowned for preserving proof of previous and current hybridization with congeners, even in species where numerous intricate isolating mechanisms prohibit interbreeding (29).

Several genes that are probably related to drought tolerance have been found in sunflower, such as HAS1 (sunflower, asparagine synthetase) or HAS1.1, HaDhn1 (sunflower dehydrin gene), SunTIP (sunflower tonoplast intrinsic protein), HaDhn2, Sdi (sunflower drought-induced), and Hahb-4 (sunflower homeobox-leucine zipper gene). It has been suggested that these genes help sunflowers withstand drought stress because they have been shown to express at high levels under drought stress. These genes contribute to drought tolerance in sunflowers by facilitating nitrogen storage (HAS1, HAS1.1), protecting cells from dehydration (HaDhn1, HaDhn2), regulating water movement (SunTIP), activating drought-response mechanisms (Sdi), and controlling stress-related gene expression (Hahb-4), enabling the plant to survive water stress.

The study combined high-throughput lipidome phenotyping with genome-wide association studies (GWAS) to find genetic variations connected to sunflower seed fatty acid concentration. On chromosomes 3 and 14, the study found strong genetic correlations for eleven fatty acids, including uncommon fatty acids. Uncommon fatty acids identified in sunflower include palmitoleic acid, arachidic acid, behenic acid, and lignoceric acid. These fatty acids are typically found in smaller quantities compared to the more common linoleic and oleic acids, but they contribute to the overall diversity and nutritional

profile of sunflower oil. Fatty acids account for up to 34.5% of a variation in docosanoic acid (22:0) in sunflower oil. The study also identified 429 genes related to sunflower oil metabolism, with 124 of these genes are located near significant SNPs (single nucleotide polymorphisms). The findings of this study support genome-based selection, which can hasten the identification of genotypes that supply the required level of fatty acids in sunflower breeding programs (30).

The study found that the biggest number of nucleotide binding site-leucine-rich repeat (NBS-LRR) genes, which encode disease resistance proteins important in plant defense, are found on sunflower chromosome 13, followed by chromosomes 9, 4 and 2 (31, 32). The investigation also identified several potential genes associated with sunflower resistance to *Sclerotinia* head rot, a devastating disease that significantly impacts yield and quality in susceptible regions. These findings are particularly valuable for sunflower cultivation in areas prone to high humidity and frequent rainfall, conditions that favor the spread of *Sclerotinia*. By incorporating these resistance-linked genes into breeding programs, researchers can develop more resilient sunflower varieties, helping to reduce reliance on chemical treatments, minimize crop losses, and improve the economic viability of sunflower farming in affected regions (33).

### Marker-assisted selection

Using RAPD markers, the first map was created on a wild sunflower. The journey of genetic mapping in sunflowers represents a remarkable chapter in the field of plant genetics, reflecting broader trends and difficulties in agricultural biotechnology. The initial mapping efforts using RAPD markers marked the inception of a detailed genetic understanding of sunflowers, paving the way for more sophisticated mapping techniques. The subsequent adoption of RFLP (restriction fragment length polymorphism) markers by researchers signified a significant leap forward, albeit delayed compared to other major crops such as wheat and maize. This delay, as

noted, underscores the unique challenges faced by sunflower genetic mapping, including the involvement of private companies which may have contributed to the slower pace of development.

The introduction of AFLP (amplified fragment length polymorphisms) markers further enriched the genetic maps, according to the work of demonstrating the field's evolving nature and the continuous search for more efficient and informative markers. The establishment of linkage maps with 17 linkage groups, mirroring the haploid chromosome number in sunflowers, marked a critical point in creating a structured genetic framework for this crop (34). The transition to SSR markers, represented a significant advancement in the precision and utility of sunflower genetic maps (35). The first composite SSR map was a monumental achievement, integrating hundreds of SSR markers and covering a substantial portion of the sunflower genome (36). This map not only served as a cornerstone for subsequent genetic research in sunflowers but also facilitated the mapping and tagging of key agronomic traits (37). The expansion of SSR markers derived from genomic sequences and expressed sequence tags, significantly enhanced the density and utility of sunflower genetic maps (38, 39). The integration of these markers into existing maps, exemplifies the dynamic and iterative process of genetic mapping, where new tools and markers continuously refine and expand our understanding of the genome (40).

The culmination of these efforts in the assembly of the sunflower genome sequence, particularly the HanXRQ7 reference genome represents a watershed moment in sunflower genetics (27). This comprehensive assembly, covering a significant portion of the sunflower genome, provides an invaluable resource for geneticists and breeders alike, facilitating a deeper understanding of the genetic basis of key traits and enabling more targeted and efficient breeding strategies (Table 1).

**Table 1.** The three studies on quantitative trait locus (QTL) analysis and genomic regions controlling various traits in sunflower

Study	Basal stalk rot resistance (41)	Seed protein content and other traits in water-stress conditions	Drought tolerance in sunflower (43)
Objective	Identify QTL (quantitative trait locus) for BSR (basal stalk rot) resistance	Identify genomic regions controlling various traits	Conduct QTL analysis for drought tolerance
Population studied	RIL population derived from HA 441 × RHA 439 cross	Recombinant inbred lines (RILs) in split-plot design	Evaluation of phenotypic traits during germination and seedling stages
Genotyping approach	Genotyping-by-sequencing (GBS)	SSR markers	-
Marker development	Adapted GBS approach to discover SNP markers	Two SSR markers were identified for protein content	-
Genetic linkage map	1053 SNP markers on 17 linkage groups (LGs)	-	33 QTLs identified on eight chromosomes
Environmental testing	Tested in five environments for BSR resistance	Evaluated under well-watered and water-stress conditions	Evaluated under well-watered and drought-stress conditions
Identified QTLs	Six QTLs identified across environments	Specific and nonspecific QTLs detected	33 QTLs identified with LOD (logarithm of odds) ranging from 2.017 to 7.439
Significant QTLs	Qbsr-10.1 and Qbsr-17.1 on LGs 10 and 17	Specific QTL for protein content	-
Additional QTLs	Qbsr-4.1, Qbsr-9.1, Qbsr-11.1, Qbsr-16.1 detected in single environment	Overlapping QTLs for protein content and seed density	Putative drought-related genes identified within QTL confidence intervals
Potential application	Discussion of potential use in marker-assisted selection breeding	Insight for sunflower drought tolerance breeding and improvement	-

## Mutation studies

Mutagenesis has become an important tool in plant hereditary qualities inquired about, advertising a capable strategy for actuating hereditary variety. In sunflowers, actuated transformations have been instrumental in creating mutant cultivars, driving noteworthy changes in trim abdicate and quality (44).

## Application in sunflower breeding

Actuated transformations have been broadly utilized in sunflower breeding programs due to their cost-effectiveness and flexibility to different breeding targets. Whereas where a small rate of initiated changes may be valuable for breeders, they contribute to producing a broader hereditary inconstancy inside sunflower populaces (45, 46).

## Classification of mutations

**Point mutations:** These include modifications at a single nucleotide level. The foremost common sort is base combine substitutions, where a purine base may be supplanted by another purine base (e.g., G-A move) or a pyrimidine base may be supplanted by another pyrimidine base (e.g., C-T move). Such substitutions can lead to changes in codons or amino acids.

**Structural mutations:** These changes result from chromosome breaks and rearrangements, leading to modifications within the structure of chromosomes. Cases incorporate reversals, translocations, duplications, and cancellations.

**Chromosome number changes:** Changes can moreover influence the number of chromosomes, leading to aneuploidy or polyploidy (Table 2).

## CRISPR-Cas9

High oleic acid sunflowers are always preferred for their industrial applications and health advantages. In sunflowers, oleic acid is converted to linoleic acid by the enzyme FAD2-1. The FAD2-1 gene may be edited to alter its fatty acid composition using the CRISPR/Cas9 approach, which has been used lately as a novel breeding method to increase agricultural output and sustainability (47). Therefore, the FAD2-1 gene was knocked out using CRISPR/Cas9 genome editing techniques to boost the synthesis of oleic acid content. Two genotypes of low-oleic sunflowers were modified with two sgRNAs that target the FAD2-1 gene for this reason. In a single reaction that is followed by A, sgRNA expression cassettes were integrated into the binary vector using golden gate assembly—transformation mediated by *Agrobacterium tumefaciens* and *in vitro* germination (48). *A. tumefaciens*-mediated

selection of putatively transformed shoots was conducted using an optimum kanamycin concentration of 100 mg/L in the medium. A summary of the difficulties in transforming sunflowers was provided, along with potential fixes. According to this work, sunflowers can still be altered using the CRISPR/Cas9 genome editing method to produce high-oleic sunflowers (49).

## Genome-wide association studies (GWAS)

To move quickly with sunflower gene discovery and breeding, genomic technologies including transcriptomics and genome-wide association studies (GWAS) are being employed. GWAS is a useful method for locating genetic variations in sunflowers linked to complex characteristics. Through genome analysis of several sunflower plants, scientists can find genetic markers linked to desirable characteristics such as oil content or disease resistance. Utilizing these markers, new sunflower varieties with enhanced characteristics can be created. A GWAS using 8723 SNPs in 333 sunflower lines identified 24 SNPs on a specific chromosome significantly associated with fertility restoration, an essential component in hybrid breeding. This helped researchers fine-tune breeding programs for improved hybrids (50).

## Genome sequencing

**Next-generation sequencing (NGS) and its applications:** Numerous plant genomic applications, including transcriptome sequencing for SNP (single nucleotide polymorphism) identification in rye, whole-genome sequence variation studies on *A. thaliana* accessions de novo sequencing of BACs (bacterial artificial chromosomes) in barley and genome sequencing in cucumber have all made use of NGS technology (51-53). Using high-throughput sequencing, the xylitol dehydrogenase gene, which is in charge of xylose consumption, in yeast, a species with a relatively small genome (54, 55). The cloned grain protein content gene GPC-B1 in tetraploid wheat was successfully fine-mapped using a combination of BSA and Illumina RNAseq technologies (56). By matching individual readings to a 40,349 unigene sequence reference wheat transcriptome from the NCBI (National Centre for Biotechnology Information), the SNP finding was made possible (56).

## Transcriptomics

Transcriptomics is the study of all the RNA transcripts produced by the genome of an organism under specific conditions or at a particular time. It involves analyzing the expression levels of genes by measuring the abundance of mRNA molecules (57). Transcriptomic analysis provides insights into which genes are active, how they are

**Table 2.** Highly resistant plants for *Alternaria* leaf blight

Treatment	Mutation type	Dosage/Concentration	Mortality rate	Variability in M2 generation	Frequency of highly resistant plants
Gamma rays	Irradiation	200 Gy	Low	Less	High
Gamma rays	Irradiation	250 Gy	High	Not applicable	Not applicable
EMS (ethyl methane sulfonate)	Chemical	0.015 mols/dm <sup>3</sup>	Low	Moderate	Moderate
EMS	Chemical	0.020 mols/dm <sup>3</sup>	Low	High	Low

regulated, and their roles in various biological processes (58). By studying transcriptomes, researchers can understand gene expression patterns, identify differentially expressed genes, and unravel the molecular mechanisms underlying physiological responses, development, and diseases in organisms.

In plant biology, transcriptomics is a crucial instrument for examining gene expression patterns at the transcript level (59). By analyzing the transcriptome, researchers can gain insights into biological processes, regulatory networks and the dynamics of gene expression. Transcriptome analysis was utilized in a study on natural leaf senescence in sunflowers to look at changes in gene expression that occur throughout leaf development and senescence. To measure the amounts of gene expression and find genes that are differently expressed, methods such as RNA sequencing and microarray analysis were used. A thorough understanding of molecular processes during senescence is possible by combining transcriptome data with metabolomics, which connects changes in gene expression to physiological changes and metabolic pathways. Transcriptomics provides light on molecular mechanisms underlying plant growth and responses to environmental stimuli, as well as regulatory networks and important genes (60).

A recent thorough spatiotemporal dissection of metabolic changes during *A. thaliana* senescence was carried out. This study included over 260 metabolites, such as various lipids, carbohydrates, amino acids, organic acids, secondary metabolites, and ionic nutrients. It also included diverse colors.

### Other omics studies in sunflower

#### Pangenome

Pangenomics is a thorough genetic analysis method that looks at genetic differences among several people to analyze a species' whole genetic makeup. Pangenomics, as opposed to traditional genomics, which concentrates on a single reference genome, identifies core genes that are present in every individual and variable genes that are lacking in at least one individual, thus capturing the genetic variety within a community (61). Understanding genetic variances, structural variations, the existence or absence of genes, and changes in gene copy number can all be facilitated by this method. Pangenomics provides the ability to identify lost genes, restore genetic diversity from wild relatives, and explore genomic resources for the development of climate-resilient crop varieties. It is useful for investigating characteristics, evolutionary processes, domestication, and breeding advances in crops. All things considered, Pangenomics is essential to expanding our knowledge of genetic variety, adaptability, and evolution in plant species. It also offers opportunities for crop development, sustainable agriculture, and tackling issues brought on by shifting environmental conditions. Pangenomics is a thorough genetic analysis method that looks at genetic differences among several people to analyze a species' whole genetic makeup (62). Pangenomics, as opposed to traditional genomics, which

concentrates on a single reference genome, identifies core genes that are present in every individual and variable genes that are lacking in at least one individual, therefore capturing the genetic variety within a community. Understanding genetic variances, structural variations, the existence or absence of genes, and changes in gene copy number can all be facilitated by this method. Pangenomics provides the ability to identify lost genes, restore genetic diversity from wild relatives, and explore genomic resources for the development of climate-resilient crop varieties (63). "Lost genes" refer to genes that have been eliminated from cultivated plants due to selective breeding but still exist in wild relatives. Pangenomics helps identify these genes and reintroduce them into breeding programs to restore genetic diversity and improve traits such as disease resistance and stress tolerance. It is useful for investigating characteristics, evolutionary processes, domestication, and breeding advances in crops. All things considered, pangenomics is essential to expanding our knowledge of genetic variety, adaptability, and evolution in plant species. Pangenomics has revolutionized sunflower breeding by uncovering valuable genes from wild species, like those for disease resistance and drought tolerance. These genes are reintroduced into modern sunflowers, boosting resilience and yield, and enhancing adaptability to changing environments. It also offers opportunities for crop development, sustainable agriculture, and tackling issues brought on by shifting environmental conditions (27).

#### Metabolomics

The current study identified and quantified 38 primary metabolites within sunflower leaves, encompassing organic acids, fatty acids, amino acids, and sugars. Malate, citrate, and chlorogenic acid emerged as the most abundant organic acids, while linolenic acid, linoleic acid, and palmitic acid dominated the fatty acid profile (64). Serine, alanine, and glutamate were the primary free amino acids detected. Glucose and sucrose were the major soluble sugars; however, fructose concentrations were significantly lower compared to other Asteraceae family members. The lower fructose concentration in sunflowers compared to other Asteraceae family members is notable because fructose plays a key role in plant metabolism, particularly in energy storage and stress responses. In sunflower breeding, this reduced fructose level may influence the plant's carbohydrate metabolism, affecting traits such as seed development, oil content, and drought tolerance. Understanding this difference could help breeders select for improved metabolic efficiency or stress adaptation, potentially enhancing sunflower productivity and resilience under varying environmental conditions. This observation suggests a potential sunflower-specific metabolic pathway where fructose is preferentially transported to the stem for storage rather than being converted into inulin (65).

Further analysis using LC-ESI-QTOF-MS targeted specialized metabolites. Putative annotations were assigned to peaks exhibiting the highest intensities, revealing compounds belonging to three distinct families:

cafeoylquinates, methyl-flavonoids, and sesquiterpenoids. These findings align with previous sunflower biochemical studies (66). Caffeoylquinic acid, a ubiquitous sunflower component, plays a role in lignification and exhibits a correlation with leaf age. Additionally, it represents the dominant phenolic acid in sunflower florets and is present in seeds as well. When encountered in sunflower oil, caffeoylequinates, including oxidized chlorogenic acid, can interact with sunflower proteins, forming undesirable green-colored complexes. This oxidative reaction partly explains the underutilization of sunflower proteins in the food industry, despite their appealing qualities such as affordability and lack of allergens. Several putative methylated flavonoids were also identified, known to serve as chemotaxonomic markers for the Asteraceae family. Finally, sunflower-specific sesquiterpenoids were detected, with one tentatively identified as a diversion, a compound exhibiting potential insecticidal properties (67).

### Challenges in sunflower genome sequencing and assembly

#### Large number of multi-gene families

Due to the presence of numerous multi-gene families, decoding the sunflower genome is a challenging task. For example, sunflowers require the heat shock protein (*hsp*) gene family to develop and survive under abiotic stress environments including high temperatures, salt, and drought. *HSF*, *sHsp*, *Hsp40*, *Hsp60*, *Hsp70*, *Hsp90*, and *Hsp100* domains were discovered to be represented by the genes 88, 72, 192, 52, 85, 49, and 148, respectively, in a recent work that characterized and examined *HSF* and *Hsp* gene family members in the sunflower genome. Additionally, 81% of the sunflower genome is made up of many families of Gypsy and Copia retrotransposons, which contribute to the repetitive component of the genome (68). The genomic sequencing of sunflowers has created new prospects despite these obstacles.

#### Evolutionary adaptation of sunflowers

Sunflowers are known for their capacity to adjust to diverse situations, including a range of soil types, variable moisture levels, and resistance to environmental stresses such as drought, salinity, and temperature extremes. Their adaptability makes them suitable for cultivation across different climates and growing conditions. They have a wide hereditary base that permits them to endure and adapt to abiotic stresses such as drought, salinity, and high temperatures (69). Sunflowers have a heliotropic head that moves to take after the sun, which is an adjustment to shade bigotry. The bristly stem of the sunflower is created as a implies of securing against creature predators and making a difference it to hold water in an assortment of conditions. The leaves on the stem of the sunflower are arranged in a way that allows the plant to efficiently capture sunlight for photosynthesis. This unique leaf orientation optimizes light absorption, enabling the sunflower to maximize energy production and thrive across various environments. Wild sunflowers show broad variety, both between and inside species, and

researchers have detailed that variety is protected by pieces of “supergenes” that allow adjustment to diverse situations (70). Such developmental changes are ordinarily broken and separated over eras of mating. Sunflowers have been tamed over 5,000 a long time and have experienced a generally fast developmental move from wild to tamed (71). Sunflowers are a financially vital seed trim within the Asteraceae family, and Approximately 10% of the world's oilseeds are sunflowers plant-derived eatable oil.

#### Functional diversity of sunflowers

Sunflower has a wide extend of useful differences. A recent study analysed the microbial structure and functional profile of the sunflower rhizosphere grown in two areas in South Africa. The study found that microbial communities occupying the rhizosphere play significant parts in deciding plant well-being and abdication. The study also showed that microbial communities in sunflower rhizosphere and bulk soils were different, and the degree of variation was based on the type of carbon substrates and the soil microbial composition (72). Wild sunflowers are adjusted to an assortment of diverse living spaces and show an exceptional sum of phenotypic and hereditary differing qualities, which makes them a show framework for considering adjustment, speciation, and taming (73). Sunflowers have a wide hereditary base that permits them to endure and adjust to abiotic stresses such as dry spells, saltiness, and tall temperatures (73). Sunflowers have a heliotropic head that moves to take after the sun, which is an adjustment to shade narrow-mindedness. The bristly stem of the sunflower is created as a implies of ensure against creature predators and make a difference it to hold water in an assortment of conditions. The leaves on the sunflower stem are positioned to efficiently capture sunlight, maximizing photosynthesis and supporting the plant's growth. Sunflowers have been tamed over 5,000 a long time and have experienced a generally quick developmental move from wild to tamed (73).

#### High frequency of polyploidy in sunflowers

Sunflowers are known to have a tall incidence of polyploidy, which is a duplication across the whole genome. A study on the evolution of haploid chromosome numbers within the sunflower family found that the evolution of haploid chromosome numbers in Asteraceae was a dynamic process, with genome duplications and plummeting dysploidy being the most frequent genomic occasions within the advancement of this family (16). The study concluded above one hundred whole-genome duplication occasions within the sunflower family. Polyploidy is regarded as one of the greatest mechanisms responsible for the developmental victory of numerous species, in specific by empowering the adjustment of recently emerged polyploids to distinctive living spaces. For illustration, the repetitive nearness of polyploids in environments different from those of their diploid ancestors constitutes strong evidence of the capacity for polyploids to colonize new ecological niches (17).

## Conclusion

The exploration of sunflower genomics within the context of the biotechnological revolution presents a promising frontier for addressing both current and future agricultural challenges. The sunflower (*H. annuus*) holds a pivotal role in the global agricultural economy, not only as a significant oilseed crop but also for its potential in the nutraceutical market and its adaptability to a range of environmental conditions. As highlighted in this review, advancements in genomic research and breeding strategies have substantially contributed to our understanding and enhancement of sunflower traits, ranging from disease resistance and herbicide tolerance to the development of high-oleic and nutraceutical varieties. The genetic mapping and characterization of key traits, such as the high oleic acid content through the prevention of mutation and the development of herbicide-tolerant cultivars through the identification of mutant alleles at the *Ahas1* locus, exemplify the strides made in sunflower genomics. These advancements not only offer a deeper understanding of the sunflower genome but also equip breeders with the tools necessary for the precise and efficient development of superior sunflower cultivars. The integration of genomic technologies, such as GWAS and transcriptomics, offers promising avenues for accelerating gene discovery and breeding, enabling the identification of genetic markers associated with desirable traits. Combined with the development of molecular markers for traits such as disease resistance and environmental adaptation, this approach can significantly enhance the efficiency and precision of sunflower breeding programs. Future research in this area promises to unlock new genetic potentials, paving the way for sunflowers that are even more resilient and productive, ultimately contributing to a more sustainable agricultural industry.

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## Authors' contributions

SG conceived the review article, drafted the paper's outline, and wrote the first draft of the manuscript with a pictorial representation of key concepts. RK reviewed the manuscript critically, added to its intellectual content, and provided significant revisions. RS did an extensive literature review, arranged relevant data, and contributed to visual representation. MS<sup>1</sup> & MS<sup>2</sup> provided supervision, ensured that the manuscript met all the publication standards, and made a critical review and editing. All authors read and approved the final manuscript. (MS<sup>1</sup> stands for

M Senthivelu & MS<sup>2</sup> stands for M Sudha)

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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