International Journal of Life Sciences and Biotechnology
Sahin, R. V., Studies of Molecular Marker in Sunflower (*Helianthus annus* L.). International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 133-141.
https://doi.org/10.38001/ijlsb.1436687
Review article

Studies of Molecular Marker in Sunflower (*Helianthus annus* L.)

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ABSTRACT
Helianthus annus L., known as sunflower, is belong to *Asteraceae* family. This family is one of the biggest angiosperm plant families in between dicotyledons and cultivated sunflower is an important oil plant all around the world. Chloroplast DNA analysis reveals that the origin of the genus dates back to 4.75 to 22.7 million years ago. Sunflower seeds contain 18% protein, 15% cellulose, 9% water, 14% minerals and carbohydrates. In general, sunflower seeds contain 35% to 50% oil by weight. The oil content of sunflower had been specified from 36.9% to 50.2%. These features have made sunflower an important plant worldwide. In principle, the breeding of sunflowers aims to improve the oil content and to get a plant resistant to disease. All breeding methods both classical and biotechnological methods try to contribute these aims. With the help of technology DNA markers have provided useful information about polymorphism, genetic relatedness, and diversity. Technology advances in breeding, especially the use of molecular markers, offer new strategies to obtain high-yielding and resistant plants through DNA sequences located at a known location on the chromosome. Sunflower has been a model plant in *Asteraceae* family in molecular marker studies because of its economic importance. Many molecular marker studies have been conducted against biotic and abiotic stress conditions, increase oil content and nutritional value, and water consumption etc. Considering its economic value, current studies on the plant will shed light on future studies and improvements can be observed in many yield criteria such as water consumption, harvest efficiency, resistance to pests.

ARTICLE HISTORY
Received
13 February 2024
Accepted
16 April 2024

KEY WORDS
Helianthus annus L., sunflower, sunflower breeding, molecular markers

Introduction

Helianthus annuus L. is known as sunflower and belongs to the *Asteraceae family*. The family *Asteraceae*, also known as *Compositae*, is one of the largest families of angiosperm plants among the dicotyledons. Approximately 10% of all flowering plants worldwide belong to the *Asteraceae* family, and this large family includes 1620 genera and 23600 species [1, 2]. This family, which has 12 subfamilies, grows mostly in subtropical and temperate climates, especially in meadows, valleys, grassy plains, rolling plateaus, and mountain slopes [1,3,4,5].

This family includes species of economic importance in various fields such as human nutrition, bioenergy production, oil production and floriculture.

Examination of chloroplast DNA [6] reveals that the origin of this genus dates back to 4.75 to 22.7 million years ago. Species within this genus diverged relatively late, approximately 1.7 to 8.2 million years ago [6]. Mostly people prefers the sunflower *H.annus* as an annual plant. Generally speaking, an annual plant, heliotrope is a large spiked plant derived from the shape and figure of the flower often used to represent the sun. The stem of the plant is hard and hairy, and the leaves are broad and coarsely toothed; it also has disc-shaped flower heads [7]. The heads consist of many single flowers which mature into seeds on a receptacle base [8].

While the trunk of the sunflower plant can reach a height of up to 3 meters, the flower head can have dimensions approaching 30 cm in diameter. The sunflower inflorescence is a constricted cluster consisted of various independent sessile florets that all share the identical receptacle which is also known as the capitulum. Sunflowers have shining yellow ray florets on the external surface and yellowish circle florets inner side. Throughout the maturation, sunflowers alter their way towards the sun and then stop once they begin flowering. This movement of head named as heliotropism [9]. On the outermost circle of the head, there are five petals on the leaves. The bouquet is generally golden yellow and sterile. The flowers that cover

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the large disc on the head are called disc florets. Each disc flower on the disc is called a flower. Disc fibres contain the pistil and stamens. Discoid fibres in the outer ring are more common than those in the inner ring [5]. The plate-like shape of the sunflower is concave and convex, sloping towards the ground. While the cup diameter of sunflower plants typically varies between 18 and 25 cm, this range can vary between 5 and 50 cm across all genotypes. In addition, sunflower yields are remarkable. Head diameter is one of the factors that is hugely affected by ecological circumstances, alike plant height [10]. The sunflower tray is not a single flower but consists of 1,000 to 2,000 individual flowers that are mutually attached to the base of the tray. Self-fertile sunflower, pollen activity, and honey bee for fertilization. It needs insects, including [11].

Weather conditions have a significant impact on the development and growth of sunflowers. Sunflowers are native to North America, but they are grown in many different regions around the world, making them adaptable to a variety of climates. Sunflower plants dislike low temperatures and prefer a temperature range of 20-25°C during the day and 15-18°C at night for optimum growth. Too little sunlight will result in stunted growth and florets. Adequate water supply is critical to sunflower growth, especially during the germination and flowering stages. For optimal growth, sunflower prefers well-drained and nutrient-rich soils with a pH value ranging from 6.0 to 7.5. Poor soil quality can cause negative consequences such as slowed growth and shrinkage of buds. [12, 13]. Sunflower has a characteristic root system that spreads both deeply and widely, which provides convenience in terms of water and nutrient intake. Compared with leaf growth, sunflower root growth is faster, and if there are no limiting factors, the root system can grow to a depth of more than 3 meters to reach the water surface. Water and nutrient absorption are closely related to the structure of the root system [14]. Cultivated sunflowers generally bloom in 60-70 days and become physiologically mature in 80-100 days. The total growth period depends on its genetic structure and environmental conditions, ranging from 125-130 days [15].

Sunflower achenes are composed of seeds and shells, and their oil content is 44% higher than of canola and soybeans. Sunflower seeds contain 18% protein, 15% cellulose, 9% water, 14% minerals and carbohydrates [16]. The oil content of sunflowers depends on the variety, growing conditions and harvest time. In general, sunflower seeds contain 35% to 50% oil by weight. Studies have determined that the oil content of sunflower plants varies between 36.9% and 50.2%. [17].

Research has revealed the role of fatty acid composition and levels of compounds such as tocopherols, sterols, and carotenoids, which are important in determining sunflower oil quality. Generally, sunflower oil contains 55-65% linoleic acid and 20-30% oleic acid. The remaining 5-10% consists of palmitic and stearic acids. The sunflower plant is rich in linoleic acid, a polyunsaturated fatty acid, and has also been identified as a source of important nutrients such as calcium, phosphorus, vitamin E and niacin [18, 19]. It is known to decline low-intensity lipids, progress immunity, and assist to avoid against cardiovascular disorder [20].

Considering the widespread use of the sunflower plant in areas such as food source, feed additive, industrial raw material, breeding the plant with biotechnological methods is important in terms of saving time and financial resources.

The aim of this study is to make a general evaluation about the biotechnological methods used in sunflower breeding.

The Breeding of Sunflower

Archaeological studies have determined that sunflowers were first cultivated by American Indians in 4625 BC. [21]. Immediately after the discovery of America, sunflowers were brought to the botanical garden of Madrid by Spanish explorers. [22]. In 1716, after a patent was received in England to extract oil from sunflower seeds, the plant began to be used as an oil plant. The sunflower plant was introduced to Russia by Russian Tsar Peter I, who appreciated its aesthetic value. In 1829, Bokarev from the Belgorod region discovered the method of obtaining oil from sunflower seeds, which paved the way for the plant to be grown in agricultural fields and used in oil production [23]. The scientific studies about sunflower breeding began in 1912. At that time, the Kruglik Plant Breeding and Experimental Research Station was established [24] 1988. In 1932, several sunflower breeding stations were established by the former Soviet Union; These stations were opened first in Krasnodar (VNIIMK), then in Rostov, Kharkov and Odessa. High-yielding and high-oil content sunflower genotypes (Peredovik, VNIIMK 8931, Smena, etc.) developed in these centers have contributed significantly to the spread of sunflower as an oil crop worldwide and inspired the advancement of sunflower production worldwide. Sunflower cultivation was carried out in 1937 in Saskatchewan, Canada, and in 1950 at the Texas experiment station in the United States. [25]

After the historical progress of sunflower breeding has reached 3 phases. Within the scope of these methods, mass selection for variety development, individual selection method for variety development and hybrid development process are applied. Towards the end of the 19th century, sunflower became more widespread

with the mass selection method and became a local variety grown largely in gardens. [26]. An important achievement of mass selection is the creation of varieties resistant to the sunflower moth (*Homoeosoma nebulella* Denis and Schiffermüller) and the leaf fluke (*Orobanche cumana* Wallr.). In the late 19th and early 20th centuries, sunflower production was seriously damaged by these insects and parasite species. These harmful insect and parasite species have greatly threatened sunflower production [27]. Being simple and economical are among the most important advantages of this method. The adequacy of this technique may vary depending on the heritability of the trait, genotype and environment interaction, and gene effects on the selected trait. This method has been reported to be more effective for traits that have high heritability and are controlled by spliced genes. Mass selection did not lead to an increase in sunflower yield; however, improvements were observed in sunflower oil content, earliness, and resistance to pests and diseases [28, 29]. Individual selection has been the most widespread and victorious technique for sunflower diversity formation. Individual selection to preserve seed reserves in sunflower breeding was initiated by V. S. Pustovoit in about the 1920s; hence this selection method is also known as Pustovoit's reserve method [30]. This method is based on the individual selection of the most suitable plants from the pioneer population. The seeds of the plants collected one by one are divided into two and some of them are used for planting purposes and the remaining part is used as spare seeds. Elite individuals of superior varieties, intervaried hybrids and the best results from previous selection cycles are used as the starting population. [26]. The application of heterosis or F1 viability in order to obtain high yield is stated as the main purpose of sunflower cultivation. From a genetic perspective, heterosis usually occurs as a result of inter-allele interaction (dominance and super-dominance) and to a lesser degree depends on the result of inter-allele interaction (epistasis). [26]. The first studies of sunflower heterosis applications were made in the 1940s, and a 60% increase in yield was observed compared to the varieties. [28, 31].

Breeding practices have been going on for more than fifty years to develop sunflower hybrids. In the 1960s, the first sunflower breeding efforts were initiated in Russia with the aim of developing varieties with high oil content [32]. To achieve this goal, cytoplasmic male sterility (CMS) was developed through hybridization studies between *Helianthus petiolaris* and cultivated sunflower [33]. Sunflower is an open-pollinated species with the help of insects and therefore heterogeneity related to genetic and phenotypic diversity may arise from random mating. [34]. The traditional breeding method requires significant space and resources for plant selection. [35]. Breeding work carried out to develop a new sunflower variety can take ten years. [36]. By creating appropriate breeding lines, restrictions arising from heterogeneity in the lines can be eliminated. Double haploid (DH) lines can be obtained by repeated crosses with parent lines containing preferred traits and progeny selection, or by development of haploids followed by chromosome doubling. [37]. Haploid plants cannot complete meiosis and are therefore sterile [38]. Fertility can vary with factors such as chemical or spontaneous chromosome doubling, and as a result of these factors, 100% homozygosity can be observed in a single generation. [38, 39]. The resulting double haploid (DH) line eliminates the need for backcrossing with a fascinating parent line during numerous crossbreeding processes and thus significantly speeds up the creation of true breeding lines. [40, 41]. Additionally, double haploids can be used to accelerate the incorporation of many mutations, advance additional mutagenesis screens, reduce ploidy levels (e.g., tetraploid vs. diploid), create homozygotes for gametophyte-lethal mutations, and reduce reproductive depression associated with self-pollination. [38, 41]. Double haploids can also be used to rapidly generate mapping populations such as chromosome substitution lines [40].

In sunflower breeding uses some haploid induction methods. Parthenogenesis is one of the methods. Some experiments have been conducted about resistance to broomrape, fungus, imidazoline, and downy mildew [42,43, 44]. The other haploid induction method is anther culture. This method has applicated for the fertility restoration [45, 46, 47].

In 1987, Ishino made a discovery while studying genes that are associated with the conversion of alkaline phosphatase's isozyme in E. coli, which led to the development of CRISPR [48]. For the past four decades, the transfer of sunflower plants via Agrobacterium has been on the rise [49, 50, 51, 52, 53, 54]. Studying molecular biology to improve transgenic sunflowers with properties like pest resistance, herbicide resistance, and increased oil yield is crucial. It is also pivotal to explore the ecological effect of these alterations [55]. Furthermore, an investigation study governed a survey that resulted in the development of CAS-3 and CAS-5 mutants with high levels of stearic acid and palmitic acid contents, respectively [56]. CAS-14 mutants resulted in an increase of stearic acid content up to 37% [57].

What is the Molecular Marker?

Molecular markers are used as an important tool in genetic research. They are often used to identify specific regions on chromosomes and are often associated with phenotypic traits. They are of great importance in

plant breeding programs, especially in plant breeding, in identifying and selecting plants with desired genetic characteristics. There are various types of molecular markers and these types are classified according to the type of genetic activity, method of detection, and mode of transmission [58]. For example, depending on the detection method, hybridization-based techniques or polymerase chain reaction (PCR)-based techniques can be used. There are also different modes depending on the transmission mode. These modes may vary depending on how pointers are transferred. This diversity of molecular markers enables more efficient management of genetic resources in plant breeding. [59, 60]. Markers point out polymorphism, which can increase through a chance of nucleotide or mutation in the genome loci [61] and make it possible to define genetic diversities between individual organisms or species [62]. Molecular marker techniques are used in many areas such as genetic mapping, patrilineal tests, detection of mutant genes associated with hereditary diseases, variety identification, marker-assisted breeding in plant breeding, population history, epidemiology, food safety and population studies. Genetic mapping is used to understand genetic relationships and genomic positions between species, while patrilineal tests are useful to determine ancestral links. It is important to monitor mutant genes to detect causes and carriers of inherited diseases. While variety identification helps determine the accuracy of plant varieties, pointer-assisted breeding is used to quickly transfer desired traits. Population history and epidemiology studies use molecular marker techniques to understand disease spread and genetic diversity. In food safety research, it is important for monitoring nutrients and tracking food-borne diseases. [63].

Genetic variety can be evaluated with either biochemical or DNA markers. DNA markers are nucleotide sequences that determine differences between the genomes of various individuals. Polymorphism can be caused by a variety of factors, such as insertions, deletions, point mutations, duplications, and translocations. However, it does not inhibit the activity of certain genes [64, 65]. DNA markers have ensured beneficial knowledge about polymorphism, genetic relatedness, and range [66].

The implementation of molecular markers to support the selection of resistance genes will make easy the refinement of improved germ-plasm. PCR-based genetic markers have been extensively operated in the mapping and analysis of agronomic properties in many crops [67, 68].

Based on different criteria, molecular markers are divided into various categories. These include the type of gene activity (dominant or codominant markers), the method of detection (hybridization-based techniques or polymerase chain reaction-based techniques), and the mode of transmission (inheritance from maternal organelles, inheritance from paternal organelles, biparental nuclear inheritance, or maternal nuclear inheritance) [64, 65, 69].

Molecular Marker Studies in *Helianthus annus* L.

In the *Asteraceae* family, sunflower is a model system for genomic studies because of its importance [33, 70]. Genetic analysis of sunflowers is essential because their germplasm has a wide range of characteristics such as yield, plant height, seed number, sensitivity, and earliness to abiotic and biotic stresses [70, 71]. According to various estimates, the *Helianthus* genus includes 49 to 67 species of annual and perennial herbaceous plants native to North America. [72, 73, 74]. This change in the number of species requires a more comprehensive examination of the speciation of this taxon. Sunflowers contain diploids, tetraploids and hexaploids, and the basic chromosome number is n = 17 [73]. Generally, morphological and hybridological analyses have been used to determine the relationship between sunflower species. [74, 75]. Common sunflower is a diploid crop with 2n=34 chromosomal number and it has 3000 Mb haploid genome [76].

Helianthus genus; contains 51 wild species with useful allelic variation and agronomic traits such as yield, resistance to abiotic and biotic stresses [77]. In their RFLP analysis, Rieseberg and Seiler found that cultivated sunflower genotypes were derived from a single origin during acculturation, that these lines had low allozyme variability and were all characterized by a single cpDNA [10]. There is also 40-50% nucleotide difference between the gene pool populations of cultivated sunflowers and wild sunflowers [78].

Numerous kinds of molecular markers are utilised in fingerprinting sunflowers like RAPD, AFLP, SNPs [79] and SSRs are one of the most frequently used molecular markers, such as phylogenetics, genome mapping fingerprinting, population studies and, genetic polymorphism prediction and marker-assisted selection, due to their many advantages and simplicity, co-dominant inheritance, low cost and high polymorphism reproducibility [65, 80].

The first mixed genetic SSR map spanned 1423 cM and identified 278 single-locus SSR markers, as well as 379 additional markers (public and privately owned). This initial map is currently used as the reference genetic map for sunflower and has subsequently been further enriched with additional SSR markers to investigate three new map populations [81] Heesacker (2008) [82] created more than 2000 SSR from

genomic sequences (gSSR) and EST (EST-SSR) and are now suitable for mapping and genotyping. *Helianthus* maps have been developed thanks to gSSRs, EST-SSRs, INDELs, and TRAPs markers.

The first molecular genetic linkage map for cultivated sunflower was created using randomly amplified polymorphic DNA (RAPD) markers and RFLP [83, 84, 85, 86, 87, 88]. Various genetic linkage maps have been constructed using increased fragment length polymorphisms. [89, 90].

The current progress of several hundred microsatellite markers for sunflowers has evolved the way to the analysis of molecular genetic variation in this crop [91, 92].

In terms of genetic data, Rieseberg and Seiler (1990) [93] indicated that, a large collection of wild and domesticated sunflower lines has been investigated and reported that domesticated ones exhibit reduced allozyme variability, all characterized by a single cpDNA (chloroplast DNA) restriction fragment length polymorphism (RFLP) haplotype. Although this information makes a single domestication origin seem likely, it is far from certain because the domesticated cpDNA haplotype has a geographically widespread distribution and is found at a relatively high frequency (27%) in the wild. In sunflower, the length of the genome contains 3.6 billion A, T, C and G nucleotides distributed among 17 chromosomes, corresponding to an area of 3.6 gigabases. The situation reported as the biggest problem of the sunflower plant is that more than four-thirds of the total genome length consists of long repetitive DNA segments called long terminal repetitive retrotransposons. These pieces of DNA are very similar to each other, making it difficult to determine which pieces belong where [94]. To better understand the evolutionary history of the sunflower's genome, Badouin et al. [95] compared it to several closely (lettuce, artichoke) and more distantly (coffee, grape) related species. They were able to confirm a large increase in previously known genome size, tripling its size.

The first molecular marker studies against abiotic stress resistance in sunflower was conducted in 1996 [96]. Arce et al. (2012) [97] conducted sunflower uncommon transcription factors and miRNAs playing a key role in responses to abiotic stresses. A number of molecular biology techniques have been used to achieve the desired results. Phylogenetic tree structures, Database analysis, screening of genomic DNA libraries, isolation of cDNA clones, expression studies using Western blot and Northern blot, quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), both functionally consistent and transient plant transformation, concentrated Focused microscopy and microarrays were performed. The findings reveal that transcription factors are proteins that can bind and interact with specific DNA sequences located in the regulatory regions of target genes. To understand the relationship between leaf expansion parameters and the set of AFLP and SSR molecular markers under water stress conditions, the researchers conducted a study in the F2 and F2:3 offspring of two public sunflower lines and an independent F8 recombinant pure line (RIL) population. The findings show that breeding programs may contribute to the development of molecular markers to better adapt new varieties to water stress. [98].

Genomic sequence data for sunflower is now available, allowing the development of new SNP-based markers associated with economically critical traits. These markers must be specifically linked to traits that have been studied in detail in other organisms; such as seed oil content and resistance genes [99, 100]. The biggest goals of sunflower breeding programs, which are expensive and time-consuming, are stated to be the cultivation of early maturing and high-yielding hybrids. Parental selection of potential high-yielding hybrids and their innocence testing can be cited as limitations in breeding heterosis. Advances in molecular technologies have supported this process. Genotyping enables the search for methods of preserving superior genetic resources [101]. A sunflower breeding program has been developed with the use of molecular markers. These markers generally show codominant properties and are known for their highly polymorphic structure. However, it should also be noted that they have a strong association with the trait of interest, can be measured at all growth stages, and have phenotypically neutral properties [102]. Various studies have documented those studies on sunflower highlight the importance of marker-assisted selection (MAS) in the heterosis generation process, estimating genetic diversity, identifying integrated lines, and detecting heterotic patterns [103]. Markers potentially appropriate for MAS have been identified via Quantitative Traits Loci (QTL) mapping of economically important features [103, 104].

Result and Suggestions

Asteraceae family and the sunflower plant have been an important plant group both economically and scientifically for years. For years, studies have been carried out on many parameters such as obtaining the highest yield per unit area, increasing plant nutritional content, and resistance to diseases and pests. Considering the importance of the sunflower plant in terms of both agricultural and biological diversity and soil fertility, it is seen that it is a plant that always maintains its importance. Changing global climate conditions require the application of innovative approaches in plant cultivation. Access to food resources for

the rapidly growing world population is only possible with innovative agricultural practices. For all these reasons, the use of modern breeding methods, biotechnological methods and molecular markers have become indispensable elements in plant breeding. With the development of modern breeding methods, quality and high-yield products can be obtained per unit area and at the same time, our existing resources can be used effectively.

Compliance with ethical standards
Conflict of interest
The author declares no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
The entire work was written by Rabia Vildan Şahin.

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