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Original article

Unlocking The Genetic Code of the Sunflower Resistance to Downy Mildew and Orobanche: A Review

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Abstract

Sunflower (*Helianthus annuus* L.) is the third most widely grown oilseed worldwide, the production concentrated in Ukraine, Russia, Argentina, Turkiye, Romania, and USA. Due to global demand for sunflowers, there is an increase in cultivation of sunflowers. Nonetheless, sunflower production is affected by abiotic and biotic stress factors. Particularly, the parasite plant Orobanche and fungus downy mildew are the two major biotic stress factors that could cause serious losses in sunflower production. Various control methods such as chemical, mechanical, biological, cultural, etc. are used to cope with these two problems. Due to the speed proliferation of the two, the traditional methods are insufficient to provide sustainable and environmentally friendly solutions. Therefore, it is necessary to use combinations of classical breeding and genomic approaches to identify genetic bases of resistance and incorporate these into breeding programs to achieve long-term resistance to Orobanche and downy mildew. In this article, the classical methods used in the development of varieties resistant to Orobanche and downy mildew as well as GWAS approach to identification of the genomic regions controlling resistance to both.

Keywords: Downy mildew; GWAS; Orobanche; Resistance; Sunflower (*Helianthus annuus* L.)

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INTRODUCTION

Sunflower

Helianthus annuus L., commonly known as the sunflower, is a plant species classified taxonomically within the family Asteraceae and the genus Helianthus. There are 51 known species of the genus Helianthus, including 14 annuals and 37 perennials. Sunflower is a diploid (2n=34) plant that is widely cultivated as an oilseed crop and confectionery plant (Akpınar et al., 2019; Hladni et al., 2017; Meral, 2019). There are varieties of sunflowers that can be open pollinated or cross pollinated, but it is predominantly an open-pollinated plant.

Although the origin of sunflower is North America, nowadays it is produced worldwide. The production of sunflower is sizable in Pakistan, India, Turkiye, Ukraine, USA, China, Russia, Argentina, Canada, Spain, Iran, and Romania. It is known that sunflowers can grow at sea level and in many different regions up to an altitude of 3,000 m and is a medium drought resistant plant. The optimal temperature conditions for the development of sunflower are in the range of 15-35℃. It has been reported that high temperatures, extremely cold and very irrigated soils stress the sunflower and negatively affect the yield. The sunflower is adapted to clay loam or silty clay loam soils. It has been reported that sandy loam soil conditions are the most effective in sunflower development. It is known that there are optimal conditions for growing sunflowers in slightly acidic to slightly alkaline soils such as pH 6.8-7.5. (Adeleke & Babalola, 2020; Bashir, 2015; Hladni et al., 2017; Meral, 2019; Puttha et al., 2023).

Sunflowers are primarily cultivated for their oil and seeds (Figure 1). In addition to the two main uses, sunflowers or byproducts are also utilized in biofuels, feed industry and textile industry albeit in relatively lower proportions (Adeleke and Babalola, 2020). Sunflowers have rich biochemical content with vitamins (A, D, E and K), manganese, dietary fiber, carotenoids, alkaloids, caffeic acid, carbohydrates, tannins, saponins, steroids, high protein content, mineral, flavonoids, and phenol (Adeleke & Babalola, 2020; Bashir, 2015). Although the nutritional content of sunflower varies due to numerous factors such as genotype, soil type, agricultural practices, climate and processing conditions, in general, the composition of sunflower content can be summarized as Figure 2 (Meral, 2019; Petraru et al., 2021).

Basic plant biology

Sunflowers are propagated by seed. Sunflowers are affected by various abiotic and biotic stresses. Depending on the conditions of soil and climate, differences in the morphological properties of sunflower may occur. In addition, the morphological characteristics of sunflowers vary depending on the geographical location where they are found and different varieties. The morphological properties of sunflower in general are as follows: Sunflower has branched or unbranched stems that can grow up to from 150 to over 200 cm tall. Although the length of the sunflower plant varies between 1.6 m and 1.8 m, it is a plant that mostly grows vertically at a length of 1.6 m and has a strong stem structure. The stem diameter is usually between 3-6 cm, but it can vary from 1 to 10 cm. The inner part of the stem is filled with spongy parenchyma and has a gnarled structure. The stem of the sunflower can be smooth or pubescent structure that acts as a defense mechanism. There are leaves and nodes on the stem of sunflower (Bayraktaroğlu, 2024; Seiler, 1997).

The leaf consists of the blade (lamina) and the petiole, which connects it to the stem. Petiole length varies between 10-30 cm. Sunflower leaves are usually heart-shaped (cordate), but they can also be lance-shaped, inverted lance-shape. The surface of the leaf can be flat, concave, or convex in cross-section. The leaf surface has pubescent. Sunflower leaves are large with serrated edges and light green. Thanks to the large leaves that the sunflower has, it can perform photosynthesis by using sunlight in the maximum way. This adaptation increases the productivity of the plant (Bayraktaroğlu, 2024; Seiler, 1997).

Sunflowers have a taproot structure as a root system that can penetrate the soil to a depth of 150 to 270 cm. This taproot allows the plant to get water and nutrients more efficiently. Root development in sunflowers is faster than leaf development. The lateral roots, which reach a large soil area and allow the plant to take in water and nutrients from the surface, begin to spread from the taproot 10-15 cm upper part of the soil. The developing lateral roots are usually concentrated in the upper 30 cm of soil surface. While the roots of sunflower are denser in the layers near the soil surface, the root density decreases in the depths of the soil. Adventitious roots, consisting of the main roots or stem, provide an important support for the plant to adapt to water stress and physiological difficulties. Root development may vary depending on environmental conditions. The formation of adventitious roots is activated in situations such as flooding (Bayraktaroğlu, 2024; Seiler, 1997).

The sunflower flower is an inflorescence structure called the capitulum, which usually forms a large flower head. The head diameter varies between 6-75 cm depending on the growing conditions and the variety, but it is mostly measured in the range of 18-25 cm. The angle of the head in sunflower is an important adaptation parameter that varies to protect against bird damage and sun damage. It has been stated that the most suitable head angle for yield in sunflower cultivation is 135-180°. Sunflowers are usually pollinated by insects, although not as often as insects, pollination by winds also occurred. The ray flowers are located on the outer ring of the head and are usually large, golden yellow, can be pale yellow, orange-yellow or reddish in color, which attracts insects. Disk flowers, each of which is tube-shaped and hermaphrodite (containing both male and female reproductive organs), are in the center of the flower head. Pollination and fertilization take place in disk flowers. There are between 1000-2000 disk flowers at the head of the sunflower. At the base of the capitulum there are leaflets called bracts that protect the flower head. It is known that the sunflower reaches the flowering stage within 60-70 days. Although it varies depending on the genetic structure of the plant and environmental conditions, the time for sunflowers to reach harvest maturity is in the range of 125-130 days (Bayraktaroğlu, 2024; Seiler, 1997).

Sunflower seeds structurally consist of three main parts: the seed coat, which protects the seed from physical damage and environmental disadvantages, the endosperm, which provides energy and nutrients for the embryo during germination, and the embryo, which contains the genetic material needed to start the development of a new plant. Inside the embryo there are Palisade parenchyma cells containing large fat-rich aleurone particles and protein crystals (Seiler, 1997).

Significance

Sunflower is an economically important oilseed plant worldwide, which has diverse uses in various industries, especially the food industry. Sunflower is the third most widely used oilseed across the world. Although it is usually grown as an oilseed in the world, seeds are also used as a snack and feed for birds as well as planted as an ornamental plant. The world sunflower seed production data between 2017-2023 is shown Figure 3 (USDA, 2024).

Ukraine and Russia are the two largest global sunflower producers. In addition to this, countries such as the European Union, Argentina, China, Turkiye, and the United States supply for most of the world's sunflower production. In 2024, the world sunflower production reached 50,512,000 metric tons. According to the data for 2024, Turkiye ranks in the top five in sunflower production with a production of 1,375,000 metric tons (3% of the world production). In 2024, sunflower production in Turkiye is intensively carried out in the Marmara region (42%). While 21% of the production in the region is in the Central Anatolia region, it is followed by the Southeast (14%) and the Black Sea region (11%) (USDA, 2024). Although it is mostly grown as an oilseed in Turkiye, it is also consumed and imported as a snack. In addition, the protein-rich pulp that remains after the oil is removed is used as feed for birds and chickens. Sunflower production and import statistics of Turkiye between 2018 and 2023 are indicated in Figure 4 (TÜİK, 2023, 2022, 2021, 2020, 2019, 2018). According to data, sunflower production in Turkiye does not satisfy domestic consumption, and the resulting deficit is compensated for through imports. For this reason, sunflowers are strategically important for Turkiye both from an industrial and economic perspective.

According to these data, there has been a general increase in the amount of sunflower production from 2018/2019 to 2022/2023. However, this increase in the amount of production is not enough to cover imports. In other words, the supply demand balance cannot be established. Because domestic sunflower production is insufficient to satisfy demand, imports are at their highest values in all the years and are following a steady increase. In addition, the export quantities are limited because domestic production is insufficient to satisfy demand. To increase production, the cultivation areas can be expanded, or studies can be carried out to further increase productivity in the existing areas (TÜİK, 2018, 2019, 2020, 2021, 2022, 2023).

Sunflower production problems related to downy mildew and Orobanche

The demand for sunflowers is increasing every year (Figures 3 and 4). Sunflower grain and oil yields vary depending on the growing conditions and the varieties used and agricultural practices. Despite providing suitable soil, water, and climatic conditions for high yield, biotic factors can reduce sunflower yield. Biotic factors that significantly affect sunflower yield can generally be classified as downy mildew and Orobanche. Downy mildew is caused by the fungi species, *Plasmopara halstedii* (Farlow) Berlese & de Toni. Downy mildew primarily forms a gray structure on the lower part of the sunflower leaves and is observed as yellow lesions on the upper surface of the leaf, while stunting occurs on the sunflower plant in the later stages of the disease. This disease occurs at high relative humidity and temperatures approximately 20℃ and spreads rapidly. With the use of resistant varieties in production and the correct fungicide applications, the effects of this problem can be reduced and partially controlled (Bayraktaroğlu, 2024; Goossen and Sackston, 1968; Tülek et al., 2022).

Broomrape (*Orobanche cumana*) is a parasitic plant that grips to sunflower roots, absorbs water and nutrients, deteriorating the plant health and leading to losses of up to 100% in sunflower yield. According to the degree of virulence of Orobanche, there are different races from A to H. The most virulent race known is race H. The development of more aggressive races around the Black Sea in Turkiye has increased the importance of this problem. The most effective solution against this parasitic plant is the development of resistant sunflower varieties. In particular, the transfer of resistance genes obtained from wild species such as *Helianthus debilis* Nutt. is known to give successful results in breeding studies. It is aimed to overcome these two problems encountered in sunflower agriculture by using modern agricultural techniques and genetic breeding studies (Cvejić et al., 2020; Humann et al., 2019; Louarn et al., 2016).

Basic information related to downy mildew

Plasmopara halstedii (Farl.) Berl. et de Toni, is an obligate biotrophic oomycete pathogen belonging to the family Peronosporaceae, popularly known as downy mildew or sunflower mildew. *P. halstedii* attacks flowering plants of the Asteraceae family to complete their life cycle and infects the *Helianthus*, *Bidens*, *Artemisia,* and *Xanthium* genera. There are about 80 known species of downy mildews. Downy mildew has its origin in North America, but it is observed everywhere sunflower agriculture is carried out, such as Albania, Austria, Bulgaria, Czech Republic, Egypt, Estonia, France, Germany, Hungary, Italy, Moldova, Morocco, Romania, Slovakia, Spain, Switzerland, Turkiye, Russia, Ukraine and Yugoslavia (Gascuel et al., 2014; Goossen & Sackston, 1968; Gulya et al., 1997).

The life cycle of downy mildew on sunflowers begins with the production of sporangia in the asexual phase of mildew and the release of motile zoospores under appropriate conditions. During the sexual stage of disease, the male (antheridia) and female (oogonia) organs form at the hyphal ends and merge to form oospores, which serve as survival structures under adverse environmental conditions. Upon contact with sunflower roots, *P. halstedii* zoospores encyst and penetrate the epidermal cells. These zoospores penetrate plant tissues using special structures called haustoria, which extract nutrients and facilitate the growth of the pathogen like Orobanche (Gulya et al., 1997).

Although the effects of the type and severity of downy mildew on the host vary depending on the amount of inoculum, soil moisture, temperature, in general, the symptoms observed in the host plant can be summarized as follows. One of the main symptoms observed during the downy mildew infection in sunflower is damping-off of the seedlings. Downy mildew primarily forms a white sporulation on the lower part of the sunflower cotyledons and leaves. Downy mildew infection disrupts the physiological structure of the host plant, leading to a decrease in photosynthesis, and due to insufficient chlorophyll production, it causes the formation of puckered and yellow lesions on the upper leaf surface of the sunflower. In addition, stunting is observed due to shortening of plant internodes. The diameter of the sunflower capitulum is suppressed due to infection which results in a decrease seeds oil yield. The entire disease process takes place within 5-18 days. Downy mildew poses a serious threat to sunflower agriculture with yield losses of up to 100% on the host plant. The oospores can stay dormant under the soil for up to 5-10 years. Mildew spores are highly adaptive and can be dispersed by wind or water, allowing them to quickly infect plants over large areas. Their high reproductive rate and genetic variability make them formidable pathogens capable of evolving resistance to fungicides. Additionally, the ability of downy mildew to develop new races makes it difficult for breeding studies to create resistant sunflower varieties (Bán et al., 2023; Gascuel et al., 2014; Gulya et al., 1997; Tör et al., 2023; Tülek et al., 2022). As a result, *P. halstedii* in agriculture is an important problem that needs to be dealt with. To cope with the problem more effectively, there is a need for integrated management that combines genetic resistance and cultural practices (Viranyi & Spring, 2011).

Basic information regarding Orobanche

Orobanche cumana Wallr. popularly known by the name broomrape, is a genus of parasitic plants with diploid chromosomes belonging to the family Orobanchaceae. There are about 170 known species of Orobanche. Orobanche is especially common in areas with a mild climate such as mediterranean climate, but it is also found in some regions in Europe, Asia, and parts of Africa. Because Orobanche does not have chlorophyll, it binds to the roots of plants such as sunflowers, tomatoes, beans, tobacco, wheat, cotton, potato, eggplant corn and legumes to satisfy the needs of water, minerals, nutrients, and carbon from the plant of which it is a host. Because Orobanche are obligate holoparasites, they are completely dependent on the host plant to complete their entire life cycle. Therefore, it causes serious yield losses in host plants (Cvejić et al., 2020; Goldwasser et al., 2001; Schneeweiss et al., 2004).

Orobanche has a fleshy stem, usually leafless. In addition, it is a plant with a characteristic appearance, having a tubular structure, flowers in bright colors such as yellow, brown, purple or reddish, which allows pollinator insects to attract it. Orobanche has a high mutation capacity. Although Orobanche is predominantly self-pollinating, it has a high genetic diversity among populations due to its high mutation capacity. Orobanche has the capacity to produce 50,000-500,000 of seeds. Orobanche has seeds of very small size weighing 1.0-2.5 µg and their seeds can stay dormant under the soil for up to 15-20 years (Cvejić et al., 2020; Goldwasser et al., 2001; Schneeweiss et al., 2004). Orobanche seeds can be easily distributed to the environment through wind, agricultural equipment, human and animal movements, and pollinators such as insects and bumblebee. Different host plants can secrete different chemical stimulants in their roots, which makes their species of Orobanche specific to their host. Brooms do not germinate when there is no host plant in the environment, and their seeds remain dormant in the soil until they find their hosts. Suitable temperature conditions for germination of Orobanche are in the range of 20-25℃. The germination of Orobanche seeds occurs when the host plant secretes one of the chemical compounds called strigolactone, dehydrocostus lactone (DCL), sesquiterpene lactones, costunolide, tomentosin, or 8-epixanthatin. After germination, the roots of the plant are attached to the root tissue of the host and form a special structure called the haustorium, which allows water and nutrients to be taken from the host. Although the effects of Orobanche on the host vary depending on the host plant type and environmental conditions, in general, it causes regressions in the growth, leaf and root development of the host and, as a result, a decrease in the capacity to perform photosynthesis, this leads to energy losses in the plant, resulting in low yield and quality products (Figure 5). This parasitic relationship suppresses the growth of the host, reduces the product efficiency by 50% and above, and in some cases can lead to the death of the host (Cartry et al., 2021; Fernández-Martínez et al., 2015; Louarn et al., 2012; Ueno et al., 2014).

There are a total of 8 known races of Orobanche, these are A, B, C, D, E, F, G, and H. At first, there were only A and B races, and the distinction of this was divided depending on whether it was seen in different species. In later times, more races began to appear, and racial distinctions were made depending on the degree of virulence. Accordingly, the G and H races are the most virulent races. Although Orobanche is widely found in Turkiye, Spain, Israel, Kazakhstan, China, etc., in recent years, it has been observed that virulent races have spread in countries such as Tunisia, Spain, Russia, America. There is not a certain order of distribution of Orobanche races according to location. Even in different parts of the same country, different races can be observed (Table 1). There are Or-resistance genes against Orobanche races (Cvejić et al., 2020; Miladinović et al., 2014; Shindrova, 2006; Škorić and Pacureanu, 2010) .

For A-E races, the Or1-Or5 dominant genes control endurance. But the Orobanche races can overcome these resistance genes by rapidly mutating. Therefore, recessive genes Or6 and Or7 have been identified for races that are more virulent. In this case, it requires the sustainability of research of resistance genes for new virulent races. Orobanche produces many seeds, and these seeds can remain intact underground for a long time until they find their host, and most importantly, its high mutational ability and easy, wide propagation ability make it difficult to control. In addition, since the hosts of the Orobanche are important agricultural crops such as sunflowers, cotton, corn and wheat etc. people are trying to develop more resistant and sustainable plants using genetic and genomic tools against the Orobanche and its new more virulent races (Bayraktaroğlu, 2024; Cvejić et al., 2020).

Genetic control of resistance to pathogens

Various methods are used to cope with the downy mildew disease (Figure 6). However, *P. halstedii* can rapidly adapt to selective pressure and can quickly break down the resistance of the host. Although attempts are made to partially control the transmission of mildew disease by using chemical methods, chemicals have inverse effects on the environment and cannot provide a sustainable solution. In addition, it has been observed that over time, the disease shows insensitivity to chemical controls. Therefore, various methods such as biological methods, crop rotation, cultural, and the development of resistant varieties are used to cope with mildew. Although wild *Helianthus* species are resistant to disease, new mildew races can overcome these resistance genes. With the recent advances in genomics, studies are underway to develop new hybrids resistant to this disease, which causes serious yield losses in sunflower agriculture (Gulya et al., 1997; Tör et al., 2023).

Like control of downy mildew, various methods such as the cultural, biological, physical, and development of resistant hybrids are used to cope with the Orobanche. These methods are summarized in Figure 7. (Cvejić et al., 2020; Habimana et al., 2014). To identify resistant alleles, improve breeding strategies, and increase long-term resistance to the Orobanche and *P. halstedii* threat to sunflower cultivation, it is necessary to use combinations of classical breeding and biotechnological approaches (Cvejić et al., 2020).

Resistance to downy mildew

The control of downy mildew caused by *Plasmopara halstedii* is a critical problem in sunflowers globally with the rapid emergence of new physiological races of the pathogen. Even though people use classical and chemical methods to control, these methods have not been highly efficient. Therefore, to prevent yield losses and wasting resources, emerging genomic methods could be employed for increasing resistance and reduce environmentally problematic chemical use (Gontcharov & Goloschapova, 2021; Molinero-Ruiz, 2022; Pecrix et al., 2018).

Resistance to downy mildew in sunflower is mainly qualitative and is controlled by individual major resistance genes commonly named *Pl* genes (e.g., Pl_1, Pl_2, Pl_6). *Pl* genes are responsible for race-specific defense mechanisms against *P. halstedii*, which have various pathotypes in different *Helianthus* species and different countries (Gascuel et al., 2016; Molinero-Ruiz, 2022). About thirty-six pathotypes of this pathogen have been identified worldwide in the last 50 years and more than 40 *Pl* genes, including the Pl_1-Pl_{35}, Pl_v-Pl_z, and Pl_{Arg} genes, have been mapped (Gascuel et al., 2016; Pecrix et al., 2018). Some wild *Helianthus* species such as *H. annuus*, *H. argophyllus* etc., and hybrids obtained by crossing with these species are the main sources of resistance. It has been reported that the Pl_{15}, Pl_{17}-Pl_{20}, Pl_{33}-Pl_{35}, and Pl_{Arg} genes provide universal resistance to several local and new virulent pathotypes and have been widely utilized in breeding programs (Ma et al., 2019; Molinero-Ruiz, 2022). QTL mapping used in the identification and mapping of resistance mechanisms has become increasingly popular in sunflower research as a powerful tool for studying complex characteristics such as GWAS. Eventually, the combination of GWAS with traditional breeding and molecular techniques offers promising potential toward the development of downy mildew resistant sunflower cultivars with long-term resistance (Filippi et al., 2022; Korte and Farlow, 2013; Talukder et al., 2019).

Resistance to Orobanche

Due to limited success of traditional control strategies, including chemical and cultural applications, in coping with Orobanche, the new research focus is increasing efficacy through genetic resistance (Škorić and Pacureanu, 2010). Even if varieties resistant to the Orobanche are developed, this full parasitic plant continues to be a problem for sunflower production in Turkiye and around the world since it creates new virulent races in different environmental and climatic conditions. In recent years, it has been observed that more virulent Orobanche races have emerged in parallel with the increasing sunflower production and its spread over large areas. This situation requires that breeding for resistance should be a continuous and comprehensive study. Orobanche resistance is primarily controlled by major resistance genes (*Or* genes) (Bayraktaroğlu, 2024; Cvejić et al., 2020). These genes prevent the parasite from successfully attaching or penetrating host roots, thereby providing race-specific (vertical) resistance (Lebedeva et al., 2023). For A-E races, the *Or*1-*Or*5 dominant genes control endurance. But the Orobanche races can overcome these resistance genes by rapidly mutating. Therefore, recessive genes *Or*6 and *Or*7 have been identified for races that are more virulent (Cvejić et al., 2020; Lebedeva et al., 2023). The resistance mechanism is generally governed by gene-for-gene interactions, with the resistance gene product of the host plant recognizing and responding to specific avirulence factors produced by the parasitic plant (Lebedeva et al., 2023).

Genetic control of the partial resistance has been identifies as quantitative inheritance and expression of this resistance is in the form of lower germination stimulation, reduced haustorium penetration or increasing immune capability of the host plant through elevating physical and chemical capability of the root systems (Fernández-Martínez et al., 2012; Lebedeva et al., 2023). Studies indicated that molecular developments have significantly increased the detection of resistance mechanisms in sunflowers. Marker-assisted selection (MAS) has been used to minimize the time used in the breeding program and to facilitate the introgression of resistance genes into the host plant. MAS allows determining the resistance status of sunflower genotypes against Orobanche by using markers related to Or genes (Cvejić et al., 2020; Kucherenko, 2024). The method of combining different resistance genes (e.g. pyramid), which allows increasing the plant’s resistance to the emergence of new races of the parasite, can provide broader-spectrum and permanent resistance. To cope with the more virulent breeds of Orobanche, it will be necessary to take advantage of high-throughput genotyping and GWAS studies for the development of durable resistance strategies in sunflower cultivation. GWAS is a powerful tool for identifying loci associated with resistance to Orobanche, which ensures higher resolution than classic QTL mapping in determining the resistances of the Orobanche and allows the identification of both major-effect and minor-effect loci that contribute to resistance (Calderón-González et al., 2023; Cvejić et al., 2020).

Genotyping SNP markers general information

In recent years, the emergence of high-throughput genotyping methods such as genotyping by sequencing (GBS) and whole genome sequencing allowed the identification and genotyping a large number of SNPs in virtually any crop, accelerating genome-wide association studies (Cebeci et al., 2024; Şakiroğlu, 2022).

Shallow-pass high-throughput sequencing is a cost-effective approach used in genetic variations across large populations by capturing a limited portion of the genome. Low coverage sequencing can allow genome-wide data to be obtained in species with large genome sizes, such as sunflowers. Also, prior SNP discovery is not necessary for shallow-pass sequencing. In this way, the cheap but genome-wide sequence data obtained can provide an advantage in the identification of genetic markers associated with resistance traits to diseases such as Orobanche and downy mildew (Ma et al., 2018; Nishtha et al., 2025).

GWAS

General information

Genome wide association studies (GWAS) are experimental designs used to understand the relationship between genetic variations and phenotypic traits in a mapping population (Visscher et al., 2017). GWAS was initially used to understand complex human traits and genetic variations associated with the phenotypes of diseases such as asthma, diabetes, coronary heart disease, some types of cancer, and psychiatric disorders, to studies personalized medicine, advances in clinical care and was gradually adapted to crop species to identify true causative polymorphism in crop genomes to improve economically important traits (Beck et al., 2014; McCarthy et al., 2008; Tam et al., 2019; Uffelmann et al., 2021). With the emerge of next generation sequencing (NGS) technology, it has become widespread for identifying genetic factors that control various traits in plants and animals that are important and of economic value to humans. The first GWAS study conducted outside the medical field was conducted on the model organism *Arabidopsis thaliana* in 2005 (Rogers, 2025; Tibbs Cortes et al., 2021).

It analyzes markers (largely single nucleotide polymorphisms (SNPs)) across the genome to find statistical correlations with phenotypic traits. In GWAS, detection of alleles associated with the target phenotypic trait is based on linkage disequilibrium (LD) between the two. LD is a condition in which certain SNPs are inherited with the genes controlling the phenotypic traits more often than expected in a population. In other words, LD refers to the non-independent relationship between SNPs and the trait of interest. Thus, LD can be used in GWAS studies to determine the regions associated with diseases or desired characteristics (Cebeci et al., 2024; Tibbs Cortes et al., 2021).

One of the most important problems in association mapping studies is that a significant part of the SNPs associated with phenotype are the result of population structure and unknown kinship relationship, called false positives in literature, rather than real physical proximity. In association studies, different statistical models are used only for the purpose of establishing a real relationship between phenotype and genotype (Sakiroglu & Brummer, 2017).

Models

In GWAS, genotype and phenotype association can be analyzed using different statistical models and the variation of the models reflects the diversity in the aims of the studies, population structure, computational power available, and the nature of the phenotype. The most common statistical models used in GWAS are summarized in Figure 8 (Cebeci et al., 2024; Şakiroğlu, 2022).

The *general linear model* (GLM) does not include the kinship matrix in the model while determining the relationship between markers and phenotypic traits. This model is generally suitable for binary phenotypes (e.g. disease/healthy), but it cannot adequately control environmental factors. Therefore, false positive results are high ((Şakiroğlu, 2022; Zhang et al., 2010). The *mixed linear model* (MLM) adds both population structure and kinship matrix to the model and corrects the effects of population structure in the model and reduces false positives. Although this model is successful in reducing false positives, it has the disadvantages of being computationally expensive and slow on large datasets (Lipka et al., 2012; Şakiroğlu, 2022; Zhang et al., 2010). *Bayesian models* take a probabilistic approach when determining the effect of each SNP on the phenotype. The *Bayesian model* employes Markov Chain Monte Carlo (MCMC) methods while analyzing genetic effects based on certain prior knowledge and provides and advantage especially in the detection of rare variants or small effective SNPs (Wolc and Dekkers, 2022). However, these complex to implement models require high computational power in large data sets (Guan and Stephens, 2011).

Multi-locus mixed models (MLMM) allow for a more exhaustive analysis of genetic effects by evaluating multiple SNPs simultaneously. These models have potential to elevate statistical power compared to single-locus models. However, there are some limitations to this model such as the risk of overfitting in large data sets and the complexity of interpreting the analysis results (Wang et al., 2014). *Compressed mixed linear models* (CMLM) are models used when quantitative traits are related to the population structure and the standard MLM gives false negative results and is weak in detecting true associations. This model divides the sample into groups and adds the genetic values of these groups to the model as random effects (Cebeci et al., 2024). *FarmCPU* (*Fixed and random model Circulating Probability Unification*), a modified MLMM algorithm, was developed to minimize false-positive and false-negative results deriving from population structure in the genome-wide analysis of quantitative traits. This model uses two stage process. In the first stage, genetic markers are added to the model as covariates, while in the second stage, random effects are evaluated to prevent spurious associations. This approach increases statistical power and allows for improved accuracy of association analysis (Cebeci et al., 2024; Liu et al., 2016).

GWAS in sunflower for downy mildew

In recent years, GWAS has increasingly been used in sunflower breeding programs to identify SNP markers associated with resistance to downy mildew. Notably, evidence from the GWAS analysis that two new resistance genes that were successfully identified, Pl_{37} and Pl_{38}, are located on chromosomes 4 and 2, respectively. The study was conducted using a panel of 768 diverse inbred sunflower lines that were genotyped with 65,398 SNPs obtained through genotyping-by-sequencing (GBS). These genes were derived from the wild sunflower species *H. annuus* and *H. praecox*, both of which exhibit resilience to the most prevalent and virulent *P. halstedii* races (Qi et al., 2023). Similarly, key QTL regions were identified associated with the *Rf*7 and Pl_{34} genes, which are important for fertility restoration and resistance to downy mildew, respectively. The study was used the GWAS approach on a panel of 333 sunflower lines genotyped with 8,723 SNP markers (Talukder et al., 2019).

In conclusion, all these studies show that GWAS is a powerful tool to identify resistance-associated loci in sunflowers and to help develop long-term sustainable resistant sunflower cultivars.

GWAS in sunflower for Orobanche

A considerable number of studies in the literature aim to identify the polymorphisms responsible for resistance to Orobanche in sunflowers. Developing Orobanche resistant sunflower varieties remains a significant challenge because of the complex dynamics between the host and the parasite, along with the continuous emergence of new Orobanche races capable of overcoming existing resistance mechanisms. In this context, GWAS is proven to be an effective approach for uncovering genomic regions associated with the resistance through the analysis of natural genetic variation in sunflower (Calderón-González et al., 2023).

Although there are only a few studies conducted in the literature to determine the genetic regions associated with Orobanche resistance traits in sunflower. To the best of authors knowledge, the first GWAS study was conducted by Calderón-González et al., (2023) that used diverse germplasm panel of 104 sunflower accessions using 600K AXIOM® genome-wide SNP array. The study successfully identified 14 significant SNPs located on chromosomes 3,5,10,13, and 16 associated with resistance. In addition, the role of certain QTLs previously reported in sunflower Orobanche resistance has been confirmed (Calderón-González et al., 2023). The whole genome sequencing based genotyping coupled with the GWAS techniques provides a powerful frame for advancing resistance in sunflower breeding in a rather challenging continuous evolution of Orobanche races and the limitations of traditional breeding. Although GWAS has been used to traits such as abiotic stress tolerance, flowering time and flower morphology, there are no previous studies focusing on resistance to Orobanche in sunflower (Calderón-González et al., 2023). Therefore, in future research, GWAS studies could be devoted to Orobanche resistance (Dimitrijevic and Horn, 2018; Lebedeva et al., 2023).

The second study applied GWAS for Orobanche resistance in sunflower was the GWAS mapping of the *HaOr5* gene locus that was initially roughly mapped by QTL mapping as a resistant to race E of Orobanche (Pubert et al., 2024). The study was conducted using a set of 544 sunflowers that were genotyped with 49,499 SNPs using the 50k AXIOM® array. Using the GWAS approach in combination with QTL, the *HaOr5* gene was located in a 300 kb interval on chromosome 3 containing a cluster of putative resistance genes (Pubert et al., 2024).

Conclusion and Future Perspectives

As Orobanche and Mildew are among the top biotic stress factors that have detrimental effects on sunflower production across the globe, effective and sustainable methods to control these two factors are of paramount importance. Identification of the natural resistance to these two and incorporating them to breeding germplasm is one the most effective combat strategies. Identification of such resistance and mapping genetic control in the sunflower genome is the key challenge for all the breeding programs across the globe. GWAS studies are proven to be effective methods to pinpoint genetic control of many traits in a wide array of crops and sunflower is no exception. The studies reported here indicate that resistance genes to various races of Orobanche and mildew could in fact be mapped using GWAS. The success of such studies depend on the adding a wide range of germplasm in mapping population to increase likelihood of the containing true causative polymorphism in the mapping panels and should be performed in many locations. The identified resistance can significantly accelerate MAS strategies and contribute to the development of Orobanche and Mildew resistant varieties.

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Tables

Tablo 1. Orobanche races are designated in countries.

Country	Orobanche Races	References
Turkiye	D, E, F, G, and H	(Bayraktaroğlu, 2024; Cvejić et al., 2020)
Russian	A, B, C, D, E, F, G, and H	(Cvejić et al., 2020; Škorić & Pacureanu, 2010)
Ukraine	A, B, C, D, E, F, G, and H	(Cvejić et al., 2020; Škorić & Pacureanu, 2010)
Moldova	B, C, E, and F	(Cvejić et al., 2020)
China	A, B, C, D, E, F, and G	(Cvejić et al., 2020)
Kazakhstan	C, and G	(Cvejić et al., 2020)
Serbia	B, E, and F	(Cvejić et al., 2020; Miladinović et al., 2014)
Romania	A, B, C, D, F, G, and H	(Cvejić et al., 2020; Shindrova, 2006)
Bulgaria	A, B, C, D, E, F, G, and H	(Cvejić et al., 2020; Shindrova, 2006)
Spain	B, C, D, E, F, and G	(Cvejić et al., 2020; Škorić & Pacureanu, 2010)
Tunisia	E, and G	(Cvejić et al., 2020)

Figure legends

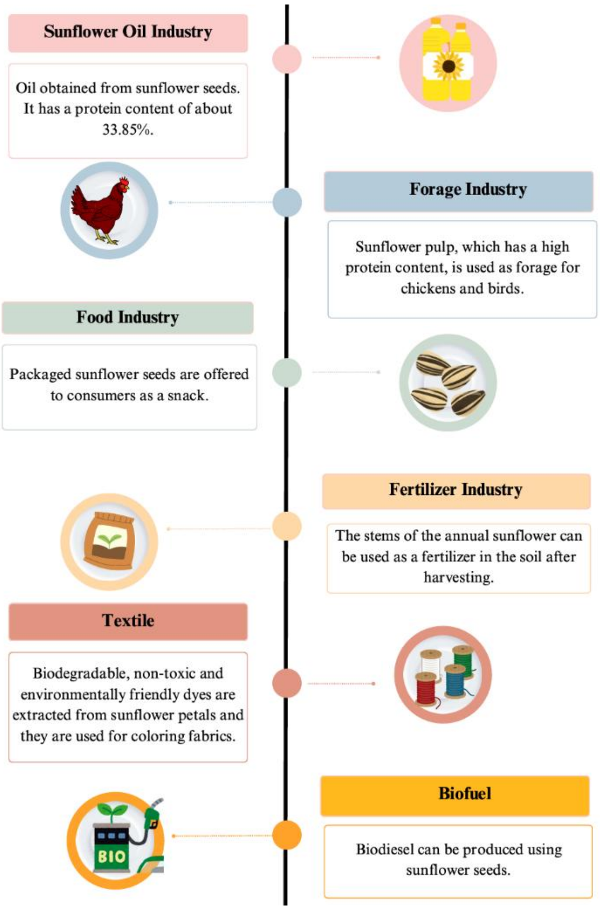


Figure 1. Sunflowers can be used in various fields of industry.

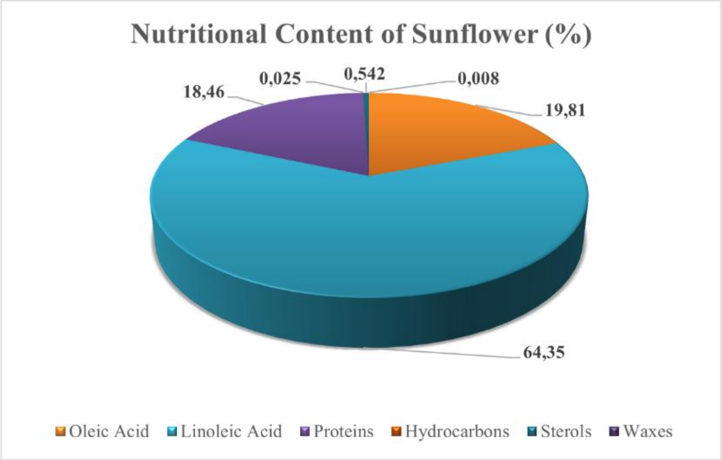


Figure 2. Nutritional content of sunflower (%).

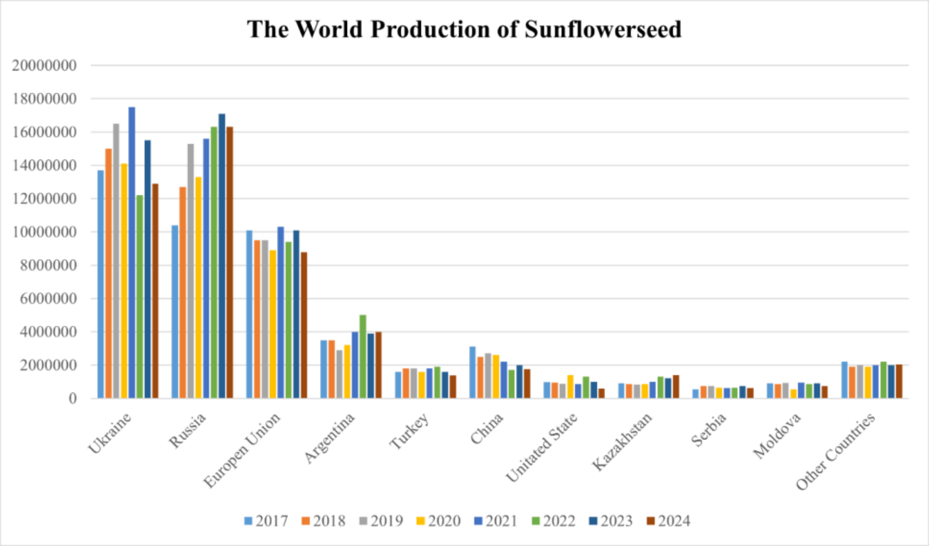


Figure 3. The world sunflower seed production data between 2017-2023 (USDA, 2024).

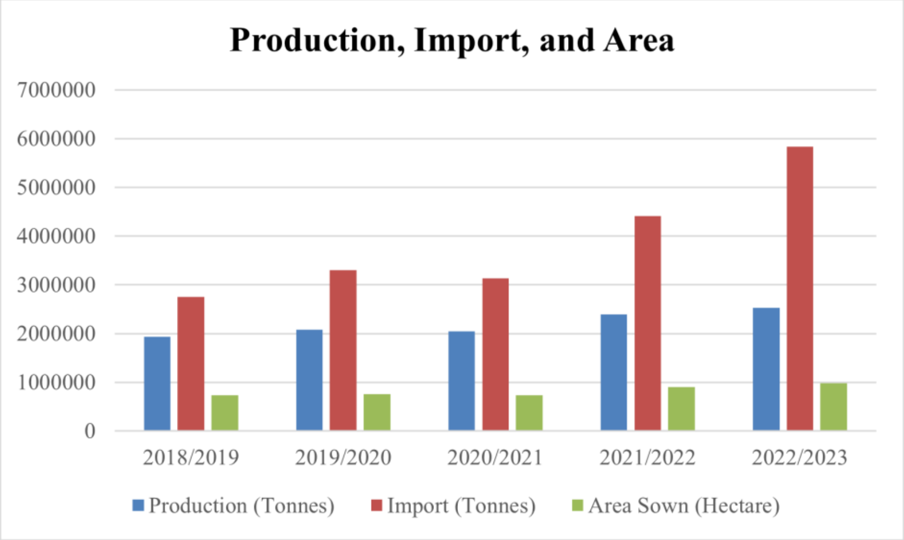


Figure 4. The quantities of sunflowers produced, area sown and imported in Turkiye in 2018-2023 (TÜİK, 2018, 2019, 2020, 2021, 2022, 2023).

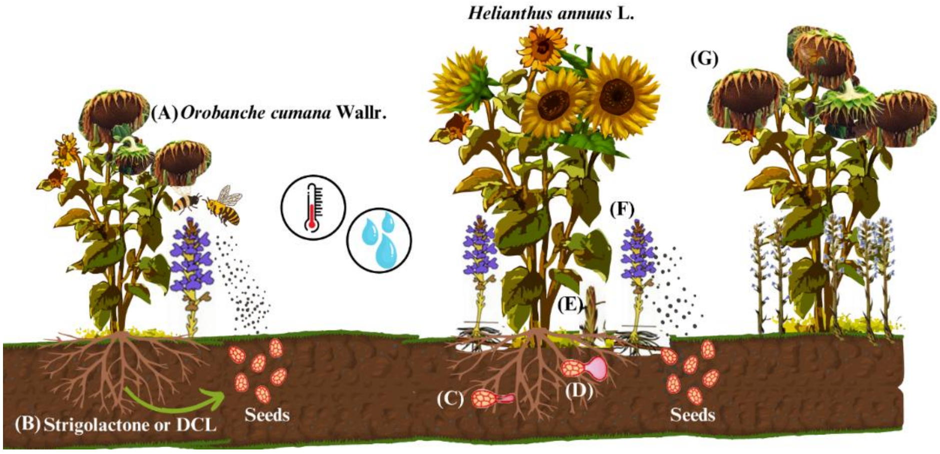


Figure 5. Shown here is the life cycle of the Orobanche on a sunflower. (A) Orobanche seeds in suitable temperature and humidity; (B) Incoming strigolactone or dehydrocostus lactone (DCL) signal from the host plant (sunflower); (C) Germination of the Orobanche and attachment of the haustorium to the host root; (D) The Orobanche thickens and forms suckers at the point where it attaches to the host; (E) tubercle development; (F) Later, the Orobanche grows and flowering again, creates new seeds; (G) Eventually, the Orobanche spreads through the sunflower roots, causing serious yield losses to the sunflower.

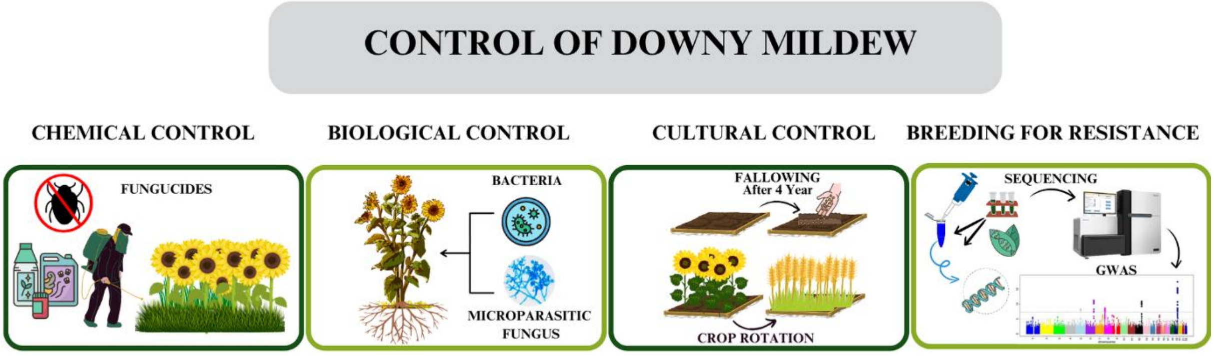
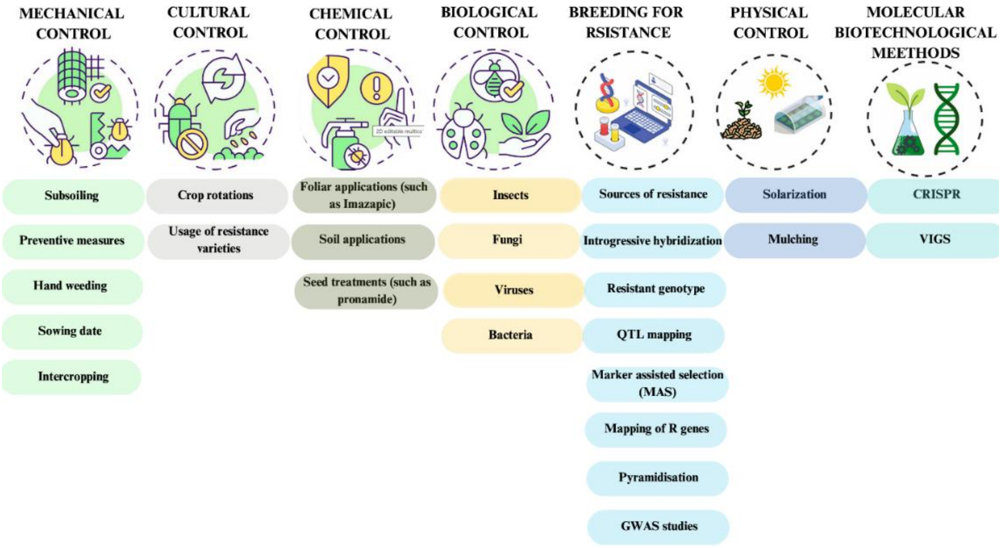


Figure 6. Methods used to cope with *P. halstedii*.



MECHANICAL CONTROL	CULTURAL CONTROL	CHEMICAL CONTROL	BIOLOGICAL CONTROL	BREEDING FOR RSISTANCE	PHYSICAL CONTROL	MOLECULAR BIOTECHNOLOGICAL MEETHODS
Subsoiling	Crop rotations	Foliar applications (such as Imazapic)	Insects	Sources of resistance	Solarization	CRISPR
Preventive measures	Usage of resistance varieties	Soil applications	Fungi	Introgressive hybridization	Mulching	VIGS
Hand weeding		Seed treatments (such as pronamide)	Viruses	Resistant genotype		
Sowing date			Bacteria	QTL mapping		
Intercropping				Marker assisted selection (MAS)		
				Mapping of R genes		
				Pyramidisation		
				GWAS studies		

Figure 7. Control methods of Orobanche.

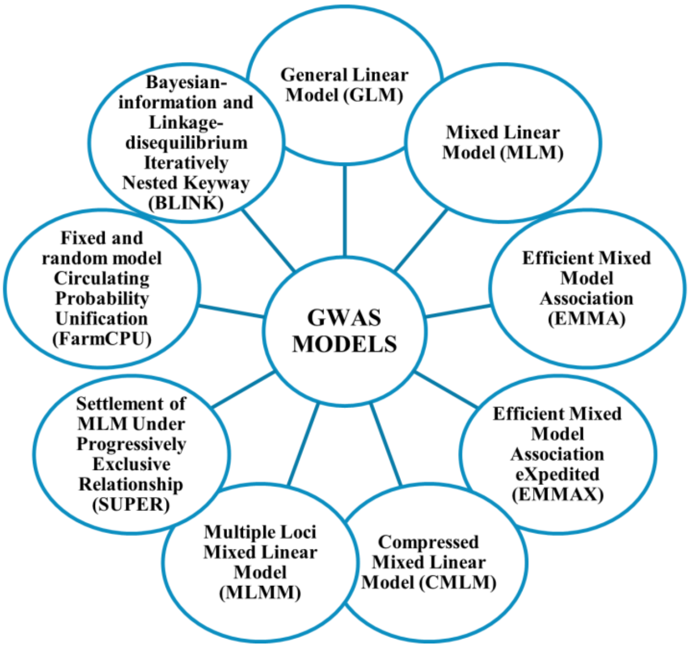


Figure 8. The most used GWAS models.