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# Sunflower Breeding for Resistance to Abiotic and Biotic Stresses

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## Abstract

Due to a specific structure of its main organs (root, stem, leaves, and head), sunflower can be successfully grown on marginal soils and in semiarid conditions, and it is more resistant to abiotic stresses, than other field crops. Unfortunately, it is very sensitive to biotic stresses.

In sunflower breeding for resistance to abiotic stresses, the greatest progress has been made in selection for drought resistance. Breeders use over 30 different parameters in sunflower screening for drought resistance, with physiological ones being the predominant type. The best breeding results have been achieved using the phenomenon of stay-green, with the added bonus that this method incorporates into the cultivated sunflower not only drought resistance but resistance to *Macrophomina* and *Phomopsis* as well. The diversity of the wild *Helianthus* species offers great possibilities for increasing the genetic resistance of the cultivated sunflower toward abiotic stresses. In using wild sunflower species in sunflower breeding for drought resistance and resistance to salinity, best results have so far been achieved with *H. argophyllus* and *H. paradoxus*, respectively. In addition to the use of wild *Helianthus* species, sunflower breeding for abiotic stress resistance should also make more use of molecular breeding techniques. More progress has been made in sunflower breeding for heat resistance than in that for cold resistance. Specific breeding programs dealing with sunflower resistance to mineral deficiency and mineral toxicity have yet to be established.

Concerning biotic stresses, the main problem in sunflower cultivation is caused by fungal diseases. Genetic variability of cultivated sunflower is very low and deficient in disease-resistance genes. Due to wild sunflower species of the *Helianthus* genus, genes that confer resistance to certain diseases were discovered and incorporated into the genotypes of the cultivated sunflower. Based on the wild species, genes were found that confer resistance to *Plasmopara halstedii*, *Puccinia helianthi*, *Verticillium dahliae*, *V. albo-atrum*, and *Erysiphe cichoracearum*. Furthermore, wild sunflower species provide a high level of tolerance (field resistance) to *Phomopsis/Diaporthe helianthi*, *Macrophomina phaseolina*, *Albugo eragopognis*, and *Alternaria* ssp. Sources of resistance to other harmful diseases are sought after within wild sunflower species.

With the use of one wild species of *H. annuus* from Kansas (USA.), genes conferring resistance to a group of imidazolinone (IMI) or sulfonylurea herbicides were discovered. Moreover, similar genes were found through induced mutations. These sources of resistance provide successful control over a broad spectrum of weeds, which infest sunflower crops, including broomrape.

The growth of the parasitic weed sunflower broomrape (*Orobanche cumana* Wallr) is a major issue in sunflower production, especially in Central and Eastern Europe, as well as in Spain. Six races of broomrape have been detected (A, B, C, D, E, and F) and dominant resistance genes ( $Or_1$ ,  $Or_2$ ,  $Or_3$ ,  $Or_4$ ,  $Or_5$ , and  $Or_6$ ) were found in wild sunflower species. During the last 4–10 years, new virulent races of broomrape emerged in several European countries. Geneticists and breeders work on finding the sources of resistance to the new broomrape races in wild sunflower species.

Numerous insect species cause economic damages during sunflower production, especially in North America (the homeland of sunflower). *Homoeosoma* species are the most widespread insects that infest sunflower. *Homoeosoma nebulella* infests sunflower in Europe and Asia, while infestation with *H. electellum* poses a major problem in USA, Canada, and Mexico. Based on the use of wild sunflower species *H. tuberosus*, genes conferring resistance to *Homoeosoma* species were incorporated. Sunflower has an armored layer in the hull, which provides resistance to this insect. Sources of resistance to other economically harmful insects are sought after.

New methods in biotechnology, particularly marker genes, have been frequently used in breeding for abiotic and biotic stresses.

**Keywords:** Abiotic and biotic stresses, breeding, interspecies hybridization, resistance, sunflower, wild species

## 1. Introduction

### 1.1. Sunflower breeding for resistance to abiotic stresses

Abiotic stresses not only determine the geographical and regional distribution of crops but also dictate if a potentially arable piece of land can actually be used for cultivation. According to an estimate, 24.2% of the world's geographic area is potentially arable. However, only 10.6% of the geographic area is under actual cultivation, while the rest is not available for cultivation due to one or more abiotic stresses [1]. According to the same author, drought is the main abiotic factor, as it affects 26% of the arable area. Mineral toxicities/deficiencies are second in importance, while frost stands third. Drought is the most limiting of all abiotic stresses, and it affects well over one-third of the soils worldwide. Plants that manage to survive the effects of drought stress show a decrease in fertility, yield, and product quality [2].

Characterization of drought tolerance is very complex and interrelated to many factors. Drought is a multidimensional stress affecting plants at various levels of their organization. Sunflower is grown in a number of countries on so-called marginal soils, often in semiarid conditions where almost every year an abiotic stress of one kind or another is present acting

as a limiting factor on crop production. However, of all field crops, sunflower is best able to withstand drought conditions, primarily on account of the structure of its organs [3].

Drought is the main cause not only of differences between mean yield and potential yield but also of yield variations from year to year and therefore of yield instability [2].

Using the results of our own studies and those of other authors, the present chapter discusses the progress that has so far been made in sunflower breeding for resistance to abiotic stresses and indicates possible future directions in this area of sunflower research.

## 1.2. Sunflower breeding for resistance to drought

Previous experiences in sunflower cultivation have shown that drought can be a limiting factor in realizing the potential of a variety or a hybrid.

In sunflower breeding for resistance to drought, just like in the other crops, a number of physical and morphological parameters are at play. The accumulation of genes for these parameters in a single genotype makes it possible to increase resistance to drought [4].

Škorić [5] states that sunflowers must be resistant to both soil and air drought, that is, to high temperatures during flowering (pollination) and the oil synthesis stage. The ways to achieve this desired goal are as follows: a more efficient root system, a certain systemic composition of the main organs, and resistance to certain diseases (*Macrophomina phaseoli*). In addition to efficient water use, the root system must have the ability for efficient nutrient use under stress conditions.

On the one hand, resistance depends on the selection of genotypes whose flowering and maturity end before the occurrence of stress (early maturity).

On the other hand, mechanism of drought resistance incorporates the modification of certain physiological and morphological parameters, which enables a more efficient use of water reserves during the period of stress. The mechanism manifests itself through a more aggressive root system or water use reduction via a more efficient stomatal apparatus plus the interaction of these factors.

The inheritance of tolerance of drought based on high osmotic pressure was found to be controlled by partial dominance and overdominance. The inheritance of drought tolerance measured by temperature shock was found to be based on nonallelic interaction of genes contained in the system of partial dominance [6].

Soil drought limits water uptake and consumption by plants. Transpiration intensity decreases strongly, which, in combination with high air temperature, leads to overheating of plants. The protective reaction of plants against water shortage is the increased ability of cells to retain water. Respiration intensity typically increases under the influence of drought. Prolonged drought forces the plants to reduce the energy efficiency of respiration [22].

Fulda *et al.* [8] used their own results and those of other authors to conclude as follows. Obviously, water stress acclimation is a multigene acclimation, in which many different physiological processes and many drought stress-inducible genes are involved. Functionally,

these gene products can be distinguished into osmolyte synthesis, protection factors for macromolecules (chaperons, LEA/dehydrin type genes), proteases, membrane proteins (aquaporins, transporters, detoxification enzymes (glutathione-S-transferase (GST) and superoxide dismutase (SOD)), and genes of regulatory proteins such as transcription factors (TFs), protein kinases, and protein phosphatases. Although the alterations in all of these processes related to drought stress have been widely investigated in many model species and a few crop species, reports on sunflower are limited.

Studying the influence of water deficit and canopy senescence pattern on sunflower root functionality during the grain-filling phase, Lisanti *et al.* [9] have concluded that both water deficit and intrinsic canopy senescence dynamics can profoundly affect root functionality during grain-filling. The effects of these factors and their interactions, especially under drought, on yield merit focused attention in future research

According to Singh [1], drought seems rather difficult to define and more difficult to quantify. For example, the common criteria used in the various definitions are precipitation, air temperature, relative humidity, evaporation from free water surface, transpiration, wind, air flow, soil moisture, and plant conditions. A working definition of drought may be "the inadequacy of water availability, including precipitation and soil moisture storage capacity, in quantity and distribution during the life cycle of a crop to restrict the expression of its full genetic yield potential".

Therefore, under conditions of drought, water stress develops in the plants as the demand exceeds water supply; this may occur due to atmospheric or soil conditions and is reflected in a gradient of water potentials developed in the soil/soil-root interface and the leaf, the transpiring organ. Thus, moisture stress may be defined as the inability of plants to meet the evapotranspirational demand. Moisture stress is likely to develop to a different rate in different plant organs along this gradient [10].

Drought resistance may be defined as mechanism(s) causing minimal loss of yield in a drought environment relative to the maximum yield in a constraint-free, that is, optimal environment for the crop. However, it does not exist as a unique heritable plant attribute. The various mechanisms by which a crop can minimize yield loss due to drought are grouped into the following three categories:

1. drought escape
2. dehydration avoidance, and
3. dehydration tolerance [1]

Drought escape describes the situation where an otherwise drought-susceptible variety performs well in a drought environment simply by avoiding the period of drought. Early maturity is an important vehicle for drought escape, suitable for environments subjected to late-season drought stress [1].

Early sunflower hybrids generally have lower leaf area index (LAI), lower total evapotranspiration, and lower yield potential than the later ones. According to Škorić [11], early sunflower

hybrids are most often susceptible to *Macrophomina*, and thus in cases where there is an early occurrence of drought such hybrids may become affected, thus nullifying any positive effect early maturity may bring.

Dehydration avoidance is the ability of a plant "to retain a relatively higher level of hydration under conditions of soil or atmospheric water stress." Therefore, the various physiological, biochemical, and metabolic processes involved in plant growth and yield production are not internally exposed to stress, but they are protected from water stress [10]. The common measure of dehydration avoidance is the tissue water status as expressed by water or turgor potential under conditions of water stress. This can be achieved by either reducing transpiration (such plants are often called water savers) or increasing water uptake (such plants are often termed as water spenders). Wild species are readily classifiable as water savers and water spenders, but crop plants ordinarily exhibit a combination of both features, probably as a result of selection by man.

Drought not only reduces the rate of photosynthesis but also directs the photosynthetic metabolism toward increased formation of low-molecular weight compounds such as alanine, hexoses, and malic acid [12]. When the drought ends, sunflower plants are capable of again having a high rate of photosynthesis, thus compensating for the negative effects of water deficiency.

As sunflower plants respond to drought, the free proline content of their leaves increases, because proline, due to its structure, increases the water retention capacity of the cell [13].

When breeding for dehydration avoidance, it is highly important that a considerable attention is paid to parameters such as reduced transpiration, osmotic adjustment, abscisic acid (ABA), cuticular wax, and leaf characteristics (leaf pubescence, altering the leaf angle, and leaf rolling). It is also especially important to find ways to increase water uptake by creating a more powerful, deeper, and well-branched root system [14].

#### 1.2.1. Sources of drought resistance

Several types of germplasms are used in sunflower breeding for drought resistance:

1. landraces;
2. cultivated hybrids and varieties;
3. wild species of the genus *Helianthus*; [15]; and
4. genetically engineered germplasm.

Use of landraces and cultivated hybrids and varieties has produced some positive results, but not to the extent that would secure stable sunflower production under drought conditions. The best results in increasing the drought resistance of cultivated sunflower have been achieved using wild species of the genus *Helianthus*.

Over the last 10–14 years, highly drought-tolerant germplasms based on *H. argophyllus*, which have a commercial value, have been created in various breeding centers.



Research and characterization of physiological mechanisms in wild sunflower are just beginning. Škorić [16] suggests that in breeding for drought tolerance, there should be a greater effort to expand the use of other wild species such as *H. deserticola*, *H. hirsutus*, *H. maximiliani*, *H. Tuberosus*, and others.

### 1.2.2. Using different traits in sunflower breeding for drought resistance

Škorić [7] reported that over 30 different parameters were used in the study of drought resistance and breeding for drought resistance in sunflower. Among these, the most frequently used were physiological parameters.

Chimenti *et al.* [17] reported that high osmotic families extracted more water from the profile during the stress period and had greater grain yield and leaf area duration than families with a low degree of osmotic adjustment. The same authors concluded that osmotic adjustment can contribute to post-anthesis drought tolerance in sunflower through increased water uptake, reduced impact on grain number, grain size, and greater leaf area duration.

Andrei [18] concluded that high self-fertility (24–49%) in some hybrids ensured a greater stability in sunflower yield under stress conditions.

Studying the influence of drought stress on growth, protein expression, and osmolyte accumulation in sunflower, Fulda *et al.* [8] reported that osmolyte analysis revealed an accumulation of glucose (24–30-fold), inositol (20–30-fold), proline (10–20-fold), fructose (3–6-fold), and sucrose (4–4-fold) in extracts from leaves of drought-stressed plants. Changes in protein expression of drought-stressed versus control plants were detected in colloidal Coomassie-stained 2D-polyacrylamide gel electrophoresis (PAGE).

Sato *et al.* [19] studied the correlation between the responses of leaf expansion and hypocotyl elongation to water deficit in sunflower genotypes. Based on the results obtained, they reported that the response of hypocotyl growth to water deficit ranged between 31 and 48%, while that of leaf growth ranged between 40 and 63%. There was a significant positive correlation ( $p < 0.01$   $R^2 = 0.61$ ) between both responses. The correlation was also significant using Pearson's correlation test ( $p < 0.04$ ,  $r = 0.78$ ).

Petcu *et al.* [20] studied physiological traits for the quantification of drought tolerance in sunflower and determined as follows. The reduction in leaf area, shoot size, and biomass accumulation of sunflower seedlings under water stress conditions determined the increase in root/shoot ratio. This suggests that for young plants the main sink was survival. In a late stage of vegetation, the root/shoot ratio decreased under drought stress in some hybrids but increased in others, suggesting that for mature plants the main sink was the yield. The physiology work has focused on morpho-physiological traits induced by drought and associated with drought tolerance of plants and the elaboration of screening methods for rapidly measuring drought tolerance using plants in an early stage of vegetation.

Based on the results of Škorić [7, 11], practical results in sunflower breeding for drought resistance have been achieved by using the stay-green phenomenon. Here, we should warn

that in the selection of lines on the basis of stay-green criteria, only lines with a high degree of self-fertility should be looked for, otherwise a wrong choice of genotypes will be made.

The use of the stay-green criterion involves the selection of not only genotypes resistant to drought but also those resistant to *Macrophomina*, which tends to be a problem under stress conditions. Also, genotypes resistant to *Phomopsis* may be simultaneously selected, as confirmed by the inbred lines Ha-48, Ha-22, CMS-1-40, PH-BC-2-91, PR-ST-3, RHA-SES, RHA-483, etc. as well as the hybrids made from these lines, which combine several resistance systems. Vrânceanu [21] confirmed the validity of using the stay-green criterion in the selection for drought resistance [22].

Petrović *et al.* [23] concluded that nitrate reductase activity and free-proline accumulation rate, which underwent large modifications in plants under water stress, may serve as parameters for the evaluation of sunflower genotypes for drought tolerance.

Working on the determination of water stress index in sunflower, Orta *et al.* [24] found statistically significant correlations between CWSI (crop water stress index) calculated from single leaf temperatures on the one hand and stomatal resistance, leaf area index, and available water in the root on the other.

Early sunflower hybrids generally have lower leaf area index, total evapotranspiration, and yield potential than the later hybrids. However, according to Škorić [11], early hybrids are typically sensitive to *Macrophomina*, so in the case of an early manifestation of drought they become infected and thus the advantage of earliness is nullified.

Some breeders believe that drought avoidance can be achieved by developing very early sunflower hybrids or by moving the sowing date (early or late sowing) in order to avoid the dry period. Dehydration avoidance can be achieved in several ways, for example, by selecting genotypes with reduced transpiration (water savers) or by increasing the uptake of available water from the soil by a powerful root system (water spenders).

Characteristics that appear to be correlated with drought tolerance include deeper rooting depth and more efficient root uptake of water, tolerance to high osmotic pressure, low transpiration rates, and plant ability to recover after wilting under heat stress.

The genetics of sunflower resistance to drought has not been studied sufficiently, despite numerous attempts and use of different plant characteristics. It appears safe to say that the drought resistance (tolerance) is controlled by a set of genes.

### 1.3. Sunflower breeding for resistance to salinity

Abiotic stress can be generated by mineral salts, which affect a considerable portion of the global arable land. Salinity ranks second after moisture stress. This stress may occur in the form of a specific mineral deficiency or toxicity, or as accumulation of an excess amount of soluble salts in the root zone [1].

Sunflowers are grown on low-to-medium-saline soils in many countries. These countries face soil salinity as a serious limiting factor in sunflower production. However, it should be



remembered that there are several wild *Helianthus* species that naturally grow on saline soils. These species are important sources of genes for resistance to salinity. Breeders should apply effective screening methods in order to identify the wild species that possess genes useful in breeding for salinity resistance and equally effective breeding methods to transfer these genes into cultivated sunflower genotypes [22].

Seiler [25] stated that several wild species of *Helianthus* are native to salt-impacted habitats and may possess genes for salt tolerance. The same author reports that Chandler and Jan [26] evaluated three wild *Helianthus* species for salt tolerance, namely *H. paradoxus*, *H. Debilis*, and *H. annuus* population native to salty desert areas, and obtained the following results. *Helianthus debilis* tolerated a salt concentration about the same as cultivated sunflower, wilting at an NaCl concentration of 240–400 mM. The wild ecotype of *H. annuus* had a higher tolerance, with some plants surviving the NaCl concentration of 800 mM. *Helianthus paradoxus* was highly salt tolerant, with some plants surviving at 1300 mM of NaCl. Salt tolerance was a dominant trait in hybrids between *H. paradoxus* and cultivated *H. annuus*, which did as well as the wild parent.

The emergence percentage, emergence index, shoot length, and shoot fresh weight can be used as selection criteria for salt tolerance in sunflower at the seedling stage [27].

Tolerance of sunflower genotypes to salinity has been investigated by a number of researchers. Prakash *et al.* [28] found that turgor is not correlated with salt tolerance. The accumulation of proline shows a higher impact on tolerance to salinity. Since callus development, seed germination, and vigor are associated, the former could be a more reliable index of salt tolerance.

The involvement of turgor and proline in salt tolerance seems to be doubtful [29]. Prakash *et al.* [28] stated that turgor cannot be related to salt tolerance. However, proline accumulation seems to be more due to the effect of salinity.

Evidently, using *H. paradoxus* and possibly some other wild *Helianthus* species, sunflower breeders can successfully achieve high resistance to salinity. It is important to determine the selection criteria that can be applied in the breeding program, and these can be cell survival, seed germination, dry matter accumulation, leaf death or senescence, leaf ion content, leaf necrosis, root growth, osmoregulation, etc. [1].

#### **1.4. Sunflower breeding for resistance to mineral deficiency and mineral toxicity**

Sunflowers require only 10 macroelements (C, O, H, N, P, K, S, Ca, Fe, and Mg) and 6 microelements (B, Mn, Cu, Zn, Mo, and Co) for their growth and development. Air and water are the sources of carbon, oxygen, and hydrogen. The rest of the elements are taken up from the soil or fertilizers and are divided into primary elements, secondary elements, and microelements [14]. Sunflower nutrition has been the subject of many books and scientific papers, which have established optimum levels of each individual macro- and microelement needed for the normal growth and development of sunflower on different types of soil. There is also voluminous literature on the deficiencies or excess levels (toxicity) of individual elements and how they affect sunflower growth and development.

Studying the diversity of elements in sunflower inbred lines, Sarić *et al.* [30] came to the conclusion that the genetic specificity for mineral nutrition is manifested not only through different contents of mineral elements but also through their distribution into individual plant organs.

As there are unfortunately no major breeding programs anywhere in the world that deal specifically with sunflower resistance to mineral deficiency and mineral toxicity, sunflower breeders should consider a possibility of establishing one or more such programs. They would have to choose appropriate breeding methods and targets, define selection criteria, and select potential resistance sources (most likely wild *Helianthus* species) [16].

### 1.5. Sunflower breeding for heat resistance

Singh [1] made a very good definition of the heat and cold resistance, which reads: "Each plant species, more particularly genotype, has an *optimum range of temperatures* for its normal growth and development: the specific temperatures would depend not only on the genotype but also on the stage of growth and development of a given genotype. When temperature moves beyond this optimal range, it generates *temperature stress*, i.e., temperature interferes with the performance. Temperature stress may be grouped into the following three categories: (1) heat stress, (2) chilling stress and (3) freezing stress."

Sunflower is characterized by high adaptability to high temperatures. At high temperatures, sunflower intensifies the process of transpiration so that its leaves remain relatively cool. Transpiration rate can be increased only if sufficient water is supplied and this calls for a deep and well-developed root system. Therefore, the choice of genotypes with a deep and powerful root system is an important criterion in the selection for sunflower tolerance to high temperatures [22].

Another important criterion is the tolerance to intensive transpiration. For the environments in which high air temperatures frequently occur at the flowering stage, breeders should select genotypes capable of producing large quantities of pollen and maintain pollen viability under such conditions. It is also important for the pistil and its stigma, or for the disk flowers on the whole, to be tolerant to high temperatures, which ensures pollination and seed formation [22].

Yet another criterion for the selection of genotypes adapted to climates with high temperatures and air and soil drought is the capacity for high seed (formation) filling rate and rapid synthesis of oil in response to stress conditions.

In order for sunflower breeders to be able to determine the right breeding methods, targets, and selection criteria and to choose their breeding materials for selection for heat resistance, they must have a detailed knowledge of how sunflower organs respond to high temperatures. Sunflower is exposed to high temperatures in arid and semiarid conditions, which have been prevalent in much of Europe in 2007. High temperatures may be accompanied by high, but also low humidity levels.

The present knowledge on sunflower heat resistance allows sunflower breeders to define their selection criteria more easily and to search for sources of heat resistance in wild *Helianthus* species.

Breeding for resistance to high temperatures should be combined with selection for drought resistance. Intensive breeding programs on sunflower heat resistance should be organized in countries where excessive temperatures are a regular occurrence. Selection for heat resistance is an integral part of many breeding programs and is often combined with breeding for increased productivity and resistance to dominant diseases and drought [16].

### 1.6. Sunflower breeding for resistance to low temperatures (cold)

In many environments, crop productivity is limited by low temperatures. When temperatures remain above the freezing level, that is,  $>0^{\circ}\text{C}$ , it is called chilling, while freezing describes temperatures below this level, that is,  $<0^{\circ}\text{C}$ .

For sunflower, it is important to increase its resistance to cold in the early stages of growth and development, that is, at germination, emergence, and the stage of two to three leaf pairs, so as to enable successful early sowing. Cold resistance at maturation should be increased as well in order to enable sunflower growing at higher altitudes and in colder regions. Sources of cold resistance should be sought exclusively in the wild *Helianthus* species that are found growing wild in the mountains where winters are harsh and springs are cold [16].

Apart from wild *Helianthus* species, induced mutations can also be successfully used as sources of resistance to low temperatures.

Excellent results in the development of sunflower genotypes resistant to cold were achieved by Kalaydzhyan *et al.* [31, 32], who applied induced mutations by chemical mutagens, first of all DMS. Resistance to low temperatures was tested in 44,000 seeds of about 2,000 mutagenic progenies by planting them in late fall/early winter. Some 499 plants from 72 mutagenic progenies (0.91%) survived the harsh winter and low temperatures (down to  $-20^{\circ}\text{C}$ ). The following mutants showed highest resistance to low temperatures:

- in the case of M-1248 (progenies of 40–43), the overwintering rate was 63%;
- in the case of M-1976 (progenies of 14–20), the overwintering rate was 48%;
- in the case of M-2002 (progenies of 44–64), the overwintering rate was 42%;
- in the case of the cultivar Radnik (control), the freezing rate (death) was 100%.
- These mutants should be subjected to the cold test in the climatic chamber in order to obtain more reliable results.

In any case, Kalaydzhyan *et al.* [31, 32] evidently developed a unique germplasm, which can be used for the development of winter genotypes and genotypes tolerant to low temperatures. Unfortunately, sunflower geneticists and breeders around the world seem to be unaware of these outstanding results.

## 1.7. Sunflower breeding for tolerance to herbicides

In the past decade or so, significant results were achieved in sunflower breeding for resistance (tolerance) to herbicides from the class of imidazolinones and some herbicides from the class of sulfonylureas (SU).

Acetolactate synthase (ALS), also called acetohydroxyacid synthase (AHAS), is the first enzyme in the biosynthesis of three vital amino acids in plants: valine, leucine, and isoleucine. Four different classes of herbicides inhibit ALS, thus causing the herbicidal effect. The most common are imidazolinones and sulfonylureas. They have been widely used since their introduction in the early 1980s, and now they constitute one of the major weed control mode-of-action classes for many crops. Resistant (tolerant) plants rapidly metabolize the herbicide in herbicidally inactive form. Sensitivity is likewise due to the lack of metabolic detoxification (Stoenescu, personal communication).

Advantages of ALS-inhibiting herbicides are as follows: very low application rate, broad spectrum of weed control (broad leaf and grassy weed species), broad range of crop, selectivity, etc.

### 1.7.1. Development of IMI-resistant sunflower hybrids

A wild population of annual *H. annuus* from a soybean field in Kansas that had been repeatedly treated with imazethapyr for 7 consecutive years developed resistance to the imidazolinone and sulfonylurea herbicides [33]. Resistance to imazethapyr and imazamox herbicides has great potential for producers in all regions of the world for controlling several broad-leaved weeds.

Miller and Al-Khatib [34] reported that the USDA-ARS (NDSU) research team quickly transferred this genetic resistance into cultivated sunflowers and released public “IMISUN” lines in 1998. At the same time, Alonso *et al.* [35], IFVC research team, Novi Sad, and several private companies in Argentina incorporated IMI resistance from the wild population of *H. annuus* L. from Kansas into their elite lines and developed the first IMI-resistant hybrids [22]. Genetic stocks IMISUN-1 (oil maintainer), IMISUN-2 (oil restorer), and IMISUN-3 (confection maintainer) have been developed and released [36]. Miller and Al-Khatib [34] also released one oilseed maintainer and two fertility restorer breeding lines with imidazolinone herbicide resistance.

Malidža *et al.* [37] reported having transferred resistance to imidazolinones from the wild *H. annuus* L. from Kansas into the elite line HA-26 using three generations per year (one in the field and two in the greenhouse). They stated that the resistance was controlled by a single partially dominant gene. Alonso *et al.* [35] were among the first in the world to transfer genes from the wild *H. annuus* L population collected in Kansas into a cultivated sunflower genotypes resistant to the herbicide imazethapyr, which also 100% controlled (destroyed) broomrape in sunflowers.

Studying the mode of inheritance of resistance to imidazolinone herbicides by using F<sub>2</sub> and test-cross population, Bruniard and Miller [38] concluded that the resistance was controlled

by two genes, a major gene having a semidominant type of gene action (*Imr1*) and a second gene (*Imr2*) with a modifier effect when the major gene is present.

Resistance in sunflower can only be achieved with homozygosity (*Imr1 Imr1*, *Imr2 Imr2*) of both resistance genes in inbred line or in a hybrid [38].

Sala *et al.* [39] reported having obtained a new source of IMI resistance, CLHA-PLUS, developed by means of induced mutations. The line was obtained through ethyl methanesulfonate mutagenesis and selection for the herbicide imazapyr. Also, the authors proved at the molecular level that CLHA-PLUS is different from *Imr1* and that both of them are allelic variants of the locus AHASL1 [40].

It has been shown experimentally that the gene CHLA-PLUS has a higher degree of IMI resistance than the gene *Imr 1 Imr 2*. Breeding centers wishing to use the CHLA-PLUS gene for breeding purposes have to sign a contract on its use with the company BASF. At the same time, BASF provides a protocol for screening for resistance at the molecular level (CLEARFIELD® Protocol SF30).

The recently established CLEARFIELD® (a BASF trademark) Production System for Sunflower provides growers with a new technology, which ensures broad-spectrum postemergence grass and broad-leaved weed control combined with high-performing sunflower hybrids from leading seed companies or public institutions.

BASF Corp. has also established two testing systems which serve to approve IMI-resistant sunflower hybrids as CLEARFIELD®, based mainly on relative tolerance compared with a standard resistant hybrid: Global and Country Qualification System.

Over the last 5 years, there has been a rapid spread of IMI (CLEARFIELD®)-resistant hybrids in the USA, Argentina, and especially central and eastern Europe, where new races of broomrape, which can be successfully controlled by this technology, have emerged.

#### 1.7.2. Development of hybrids resistant to sulfonylurea (tribenuron-methyl)

Simultaneously with sunflower breeding for IMI resistance, work has been started on the development of hybrids resistant to herbicides from the tribenuron-methyl group of sulfonylureas. To date, two resistance sources have been discovered:

The first one was derived from SU-resistant wild *Helianthus annuus* plants collected from the same area in Kansas where IMI resistance was found. The USDA-ARS (NDSU) research group incorporated this genetic resistance into cultivated sunflower and released public lines SURES in 2001 [41].

At the same time, sunflower breeders in various breeding centers (public and private) in the world introduced the sulfonylurea resistance gene into their elite lines, and thus created resistant hybrids.

The second SU resistance was detected by DuPont within an artificial mutagenesis project conducted in the early 1990s. This material was reselected, purified, and tested by Pioneer/



DuPont during 1998–2000. Several mutation events were evaluated and selectivity to the sunflower mutation event SU7 was confirmed for a narrow range of SU herbicides.

Also, in SU-resistant hybrids, it is necessary that both parent lines possess resistance, because of the partial domination in inheritance of this trait.

### 1.7.3. *The use of molecular techniques in sunflower breeding for resistance to abiotic stress*

Molecular studies as part of sunflower breeding for resistance to abiotic stress should be focused on the recognition of chromosomal segments carrying genes that contribute to the determination of tolerance, provide the possibility to partition the character, and can be used as a tool for an efficient manipulation of the breeding material. For this purpose, genetic maps of neutral molecular markers, such as isozyme and restriction fragment length polymorphism loci, can be an efficient tool for the determination of useful genes [42].

Belhassen *et al.* [43] and Cellier *et al.* [44] were among the first to use molecular techniques in sunflower breeding for resistance to abiotic stress.

Belhassen *et al.* [43] started breeding for drought tolerance from an interspecific cross with *H. argophyllus*. Four cycles of divergent selection using the physiological criterion of leaf cuticular transpiration (relative water loss) allowed the production of two contrasting genotypes: T– (low level of leaf cuticular transpiration) and T+ (high level of leaf cuticular transpiration). Field experiments showed better yield tolerance index combined with good potential yield for T– hybrids in some locations. Physiological analyses conducted in the field and in controlled conditions allowed to distinguish the two genotypes for only one parameter – osmotic adjustment. Molecular comparison revealed the existence of a cDNA differentiating T– from T+. This cDNA has high homology with an amino acid transporter. A quantification of the amino acid concentrations during water deficit in T– and T+ lines showed that the T– plants accumulate significantly more proline than T+ ones. Using this cDNA, RFLP and STS analysis allowed the differentiation of the two lines.

Cellier *et al.* [44] studied a sunflower genotype showing drought tolerance in field conditions (R1 genotype) and another exhibiting drought sensitivity (S1 genotype). They found that R1 tolerance was characterized by a delay of both wilting and decrease of leaf water potential. To analyze R1 tolerance at a molecular level, they isolated different cDNAs (named SDI for Sunflower Drought Induced) corresponding to transcripts accumulated in water-stressed R1 leaves by subtractive hybridization. The analysis of transcript accumulation in both genotypes upon drought stress suggested a differential expression in the *sdi* genes. Absciscic acid-mediated induction in the tolerant genotype was observed for four of the *sdi* genes and was found to differ among them. Sequence analysis of SDI clones showed high identity with known proteins, including nonspecific lipid transfer proteins (nsLTPs), early light-inducible proteins (ELIPs), or dehydrin, predicted to be involved in various physiological processes.

Arce *et al.* [45] studied sunflower atypical transcription factors and miRNAs playing a key role in responses to abiotic stresses. In order to achieve the desired results, they used a series of molecular biology techniques. These techniques and strategies include database analysis, phylogenetic tree construction, screening of genomic DNA libraries, isolation of cDNA clones,

expression studies using northern blots, western blots, and quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), functional analyses using plant transformation, both stable and transient, confocal microscopy, and microarrays.

Among their findings was the conclusion that transcription factors are proteins able to recognize and bind specific DNA sequences present in the regulatory regions of their target genes. Upon binding, entire signalization cascades are induced or repressed and the plant can adapt itself, at least temporarily, to the adverse conditions to which it is subjected.

Based on the copious results, Arce *et al.* [45] made the following conclusions.

The most amazing results obtained during these studies and other current studies are related to the divergence in structure and function of TFs and miRNAs found in sunflower, apparently conserved in some cases in other *Asteraceae* species but not in model plants. The release of the genomic sequence together with the advance in transformation techniques will certainly help to better understand how sunflower evolved to be adapted to abiotic stress factors and which novel regulating molecules are playing key roles in such an adaptation.

Alberdi *et al.* [46] studied the relationship between a set of molecular markers (amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR)) and leaf expansion parameters under water-deficit conditions in a cross of two public sunflower lines of contrasting response, in its  $F_2$  and  $F_{2.3}$  progenies, and in an independent  $F_8$  recombinant inbred line (RIL) population.

Based on phenotypic trials (two in growth chambers –  $F_3$  and  $F_{2-3}$ ) and experiments in a greenhouse (RIL population), certain leaves collected during these experiments were used for DNA extraction. Using a set of 60 SSR and 41 AFLP markers, they achieved significant results, which may be useful for the development of molecular markers for assisted selection in breeding programs oriented to generate new cultivars with improved adaptation to water stress conditions.

Liu and Jan [47] closely studied the results of molecular studies about abiotic stresses in light of their own as well as other authors' research. They concluded that approaches using molecular biology, functional genomics, transcriptome, and proteomics have been used to identify genes or quantitative trait loci (QTLs) and proteins correlated with the network of the response to such stresses, which will provide knowledge for the development of hybrids with resistance or tolerance to them. Some wild species grow in locally extreme environments providing an opportunity to study species from these habitats.

Studying the phenomenon of salt tolerance in sunflower, Lexer *et al.* [48] identified an EST that codes for the Ca-dependent protein kinase with maps to a salt-tolerance QTL in sunflower.

## 1.8. Conclusions

Due to the basic structure of its main organs (root, stem, and leaves), sunflower is more resistant to abiotic stresses than other field crops. Therefore, it is usually grown on soils of lower quality ("marginal soils") and in semiarid and arid conditions, where it is often exposed to abiotic stresses.

When it comes to sunflower breeding for resistance to abiotic stresses, the greatest progress has been made in selection for drought resistance. The progress was achieved by using various criteria and parameters, but the most headway was made by using physiological parameters.

The best and the most affordable method for testing sunflower for drought resistance is the use of “stay-green” character. By using “stay-green” in sunflower selection for drought resistance, the selection for *Macrophomina* and *Phomopsis* resistance is made at the same time.

Wild sunflower species of *Helianthus* are successfully used in selection for drought resistance. *Helianthus argophyllus* is most commonly used in selection for drought resistance via interspecies hybridization. Thus, new germplasms have been developed in a number of breeding centers. Moreover, several more wild species deserve to be used in selection for drought resistance. The use of molecular breeding techniques enables faster and more efficient achievement of desired results in sunflower resistance to drought.

Significant results in sunflower selection for salinity resistance have been obtained by the use of *H. paradoxus* via interspecies hybridization.

Cold resistance can be increased by using certain wild species of sunflower, but especially induced mutations.

Wild species of sunflower are insufficiently used in selection for high temperature resistance, that is, heat resistance, as well as mineral deficiency and mineral toxicity resistance.

By using a population of wild *H. annuus* L. and induced mutations, great headway in sunflower selection for resistance to herbicides from the imidazolinones and sulfonylureas (tribenuron-methyl) group has been made. Sunflower resistance to broomrape (*Orobancha* spp.) has also been achieved.

## 2. Sunflower breeding for resistance to biotic stresses

Concerning biotic stresses in sunflowers, it can be safely concluded that diseases caused by different fungi present the most serious problem. Broomrape, the parasitic angiosperm, is in the second place, viruses and bacteria in third and fourth [22].

### 2.1. Sunflower diseases

The original variability of the cultivated sunflower is very narrow and different in genes applicable in selection for the improvement of different agronomic traits, especially those conferring resistance to diseases.

Diseases are a limiting factor in the production of sunflower in all continents where it is grown. Different diseases are dominant in different growing regions, depending on the prevailing environmental conditions. Some diseases cause economic damage to sunflower in all sunflower-growing regions of the world. More than 30 different pathogens that attack sunflowers and cause economic loss in production have been identified so far (Table 1). Sunflower breeders

have achieved significant results in finding genes for resistance or high tolerance to certain diseases in wild species and incorporating them into cultivated sunflower genotypes possessing high combining ability [22].

Disease	Pathogen
Downy mildew	<i>Plasmopara halstedii</i>
Broomrape	<i>Orobanche cumana</i>
White rot	<i>Sclerotinia sclerotiorum</i>
Stem canker	<i>Diaporthe helianthi</i>
Alternaria blight	<i>Alternaria helianthi</i> , <i>A. helianthinificiens</i>
Rust	<i>Puccinia helianthi</i>
Phoma black stem	<i>Phoma macdonaldii</i>
Virus	<i>Sunflower chlorotic mottle virus</i>
Verticillium wilt	<i>Verticillium dahliae</i>
Charcoal rot	<i>Macrophomina phaseolina</i>
White blister rust	<i>Albugo tragopogonis</i>
Fusarium wilt	<i>Fusarium</i> spp.
Rhizopus head rot	<i>Rhizopus</i> spp.

**Table 1.** The most common sunflower diseases

Wild sunflower species have been a valuable source of resistance genes for many of the common pathogens of the cultivated sunflower. The relative severity of individual diseases varies widely, depending on climate and host cultivars. Breeding for resistance often is the most effective means of control. Sources of resistance or improved levels of tolerance for most diseases are available among the cultivated sunflower and the wild species of *Helianthus* [49].

Changes in the racial composition of certain pathogens have also been caused by the introduction of hybrids in commercial production, which are substantially more homogeneous with respect to the previous period when genetically heterogeneous open-pollinating varieties were grown.

Vear [50] recommended for efficient disease control in future breeding programs to combine vertical and horizontal resistance if available. If not, marker-assisted selection should be used to combine QTLs with different additive defense mechanisms [22].

Galina Pustovoit [51] evaluated new cultivars based on interspecific hybridization (*H. tuberosus* × cultivated sunflower) – Progress, October, Yubileyniy 60, and Novinka. Based on the results achieved in the field and by inoculation, the author concluded that the new cultivars possess group immunity, that is, resistance to downy mildew, rust, *Macrophomina*, *Phoma*, and broomrape.

To be successful in breeding for disease resistance, the sunflower breeder must be thoroughly acquainted with general principles of resistance breeding, major approaches to management of resistance genes, stability of sunflower resistance to certain pathogens, monitoring of interactions between the host (sunflower), pathogen and the environment, and resistance types (vertical and horizontal). Finally, he has to have an adequate germplasm at his disposal, select a method of breeding, and develop a strategy for achieving the desired goal [22].

The aim of this research is to review biotic stresses in sunflower, indicate their significance, and reveal the sources of resistance and methods of selection in order to achieve the desired goal.

#### 2.1.1. Downy mildew [*Plasmopara halstedii* (Farl.) Berl. et de Toni)]

Downy mildew [*Plasmopara halstedii* (Farl.) Berl. et de Toni)] occurs in all regions around the world in which sunflower is grown as a major oil crop. Downy mildew occurs with light intensity in years with a wet spring.

Downy mildew control was successfully maintained with dominant genes for a long period. This period roughly corresponds to the presence of only two races of downy mildew, the European race, controlled by the dominant gene *Pl<sub>1</sub>*, and North American, controlled by the *Pl<sub>2</sub>* gene. Unfortunately, changes took place in the past 14 years and there occurred a number of new races. These new races of downy mildew were registered in France, Hungary, USA, Argentina, and several other countries [22].

Viranyi [52] reported that the most detailed and up-to-date list of global distribution of *P. halstedii* pathogens has been compiled by Gulya [54] in a paper presented at the 2nd International Downy Mildew Symposium, Kostelec, Czech Republic. In the accurate overview, he comprised as many as 34 pathotypes (races), an unbelievably high number considering the fact that in most sunflower-producing countries from just a few to 12 well-distinguished virulence phenotypes exist. Europe, France, Germany, and Spain reported the highest numbers but the pathogen is rather diverse in the USA, Canada, and in South Africa as well. Furthermore, there are five *P. halstedii* pathotypes (300, 330, 710, 730, and 770) that are universally distributed globally, recorded from North and South America, Europe and Africa. Apart from the quantitative aspect of virulence, it is interesting to consider the dynamics of diversity as well, that is, the changes in a given region over time. In this respect, France leads with the highest number of new pathotypes arisen in the last 6–7 years [53].

Here, it should be mentioned that genes for resistance to the new races were quickly found in wild species and promptly transferred into genotypes of the cultivated sunflower [55]. An international set of differential lines has been made which makes it possible to determine which downy mildew races are present in a certain region. The set of differentials is supplemented with new lines as new downy mildew races occur [50].

The dynamism of changes in downy mildew races may be illustrated by the fact that, conclusive, with 2011, at least 18 downy mildew races have been determined in the world (100, 300, 304, 307, 314, 330, 700, 703, 704, 710, 711, 714, 717, 721, 730, 731, 770,...).



The testing of breeding materials by inoculation methods is in constant improvement and continuous progress. These issues have been dealt with by a large number of researchers, including Gulya *et al.* [56], Gulya *et al.* [57], Jouffret *et al.* [58], Tourvieille de Labrouhe *et al.* [59], Molinero-Demilly *et al.* [60], and others.

Tourvieille de Labrouhe *et al.* [61] reported that, in addition to major genes, nonrace-specific resistance contributes to the expression of resistance to downy mildew as well. The study also showed that the nonrace-specific resistance is inherited independently of major genes. Furthermore, Vear *et al.* [62] concluded that the inheritance of nonrace-specific resistance is under additive control. The authors reported that two QTLs may explain 42% variation in field reaction to downy mildew. This form of resistance was mapped as belonging to linkage groups 8 and 10. At the same time, they argued that this quantitative resistance is not related to any of the known major resistance gene clusters.

Also, Vear *et al.* [63] have developed a procedure for the development of new B-lines and parallel conversion into the *cms* form from source population. We should also mention here the procedure (scheme) of Vear *et al.* [64] for introgressing *Pl* genes into elite B-lines by backcrossing and simultaneous conversion into the *cms* form while performing resistance screening at the molecular level. This method significantly shortens the cycle of *Pl* gene introgression into elite lines.

According to Tourvieille de Labrouhe *et al.* [65] and Vear *et al.* [62], breeders should develop a strategy of simultaneous selection for nonspecific resistance and major gene resistance along with requisite use of molecular markers.

According to Seiler [49], complete resistance to the downy mildew pathogen was found in annual species *H. annuus*, *H. argophyllus*, *H. debilis*, and *H. petiolaris* and perennial *H. decapetalus*, *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H. xlaetiflorus*, *H. mollis*, *H. nuttallii*, *H. scaberrimus*, *H. pauciflorus*, *H. salicifolius*, and *H. tuberosus* [66].

Diploid perennial species *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. nuttallii*, and *H. smithii* and their interspecific hybrids were resistant to downy mildew [67].

With the rapid improvement of molecular techniques and their use in plant pathology, new developments have opened new insights into research on fungal biology, detection technology, and genetics and host–pathogen interactions. For example, Hammer *et al.* [68] in Germany, using different approaches, were successful in detecting fungal structure from sunflower host tissues.

### 2.1.2. White rot [*Sclerotinia sclerotiorum* (Lib.) de Bary]

White rot is a major problem in countries with a humid climate or in years with an extremely wet summer. The fungus itself is polyphagous. It attacks over 360 plant species, which increases its variability and makes the selection for resistance difficult [69]. The major problem in the selection are the three types of the diseases (on the root, stem, and head) controlled by different mechanisms of resistance [11].

Sunflower stalk and head rot incident by *Sclerotinia sclerotiorum* (Lib.) de Bary is considered the most important disease of the crop in many parts of the world. Since cultural practices or fungicides are insufficient to control the disease, efforts are being made by breeders to develop resistant or tolerant cultivars. This may explain the dominance of publications dealing with various aspects of resistance [52].

When breeding sunflower for resistance to all three forms of *Sclerotinia* attack, it is necessary to combine two or three different tests [70].

Mancel and Shein [71] found that *Sclerotinia* isolates taken from different plant species differed in the degree of virulence. They also found that sunflower isolates that had been repeatedly subcultured in the laboratory were significantly less virulent than isolates recently obtained from sunflower.

When we discuss the three types of sunflower infection by *Sclerotinia*, it is easy to achieve high tolerance to the mid-stalk infection by selecting genotypes resistant to lice [11]. Young leaves of such genotypes are not injured by lice and therefore these plants avoid infections.

Using four different tests for the evaluation of *Sclerotinia* resistance (basal stem infection, ascospore, and oxalic acid injection into the back face of the head), Baldini *et al.* [72] found that the inbred line 28R was most tolerant to the basal stem and white head rot infections and it also showed the best performance in oxalate and culture filtrate tests, which indicated the presence of a specific resistance to oxalate.

Van Becelaere and Miller [73] tried different inoculation procedures for evaluation of resistance to *Sclerotinia* head rot. According to their results, the best method involved the spraying of heads at the beginning of flowering with 4 cm<sup>3</sup> of a suspension of ascospores, which contained 4000 ascospores per milliliter, and covering the heads with brown paper bags immediately after inoculation. Measurements of inoculation could begin as early as 34 days after the inoculation.

Vear *et al.* [50] studied the virulence of 10 *Sclerotinia* isolates. They found differences in in vitro growth rate and sclerotia production as well as some highly significant isolate and genotype effects. They concluded that the available resistance in sunflower genotypes has partial, nonrace-specific, and horizontal characteristics and that it should be durable.

Using an in vitro screening test based on callus induction to evaluate *Sclerotinia* resistance, Drumeva *et al.* [74] found that the test allowed the identification of the breeding material with high to moderate resistance to the pathogen.

When developing inbred lines, sunflower breeders should take note of the results of Van Becelaere [75], who found that the general combining ability (GCA) effects of female lines were relatively larger than the GCA effects of male lines, which indicated that, at least in that particular research, the female lines had a greater influence on the resistance of the hybrids.

When considering the methods of selection for white rot tolerance, recurrent selection and pedigree method were found to produce the best results.

Vear *et al.* [63] used the pedigree method to select sunflower heads resistant to *Sclerotinia*. They applied the ascospore test on F<sub>2</sub> and F<sub>4</sub> plants and the mycelium test on F<sub>3</sub> plants. Their results showed that in all cases there was a variation in the level of resistance among F<sub>3</sub> families. The gains in relation to their parents ranged from 24 to 61%.

Vear *et al.* [76] applied 14 cycles of recurrent selection to a sunflower restorer population developed in 1978 and they obtained significant results. The mycelium test was used in the first three cycles and a combined test with a suspension of ascospores in the subsequent cycles. About 80% reduction of the infected area was achieved in the fourth cycle. In the 12th cycle, the latency index (a measure of incubation period) in the ascospore test was doubled. Simple regression provided the best relation with this cycle, indicating that further increase in the degree of tolerance was possible.

Christov [66] and Christov *et al.* [77] reported that higher-ploidy perennial species (hexaploid and tetraploid species) exhibited greater susceptibility than the diploids, with *H. glaucophyllus*, *H. divaricatus*, *H. salicifolius*, and *H. mollis* having the highest frequency of healthy plants. Tolerance to *Sclerotinia* was observed in the perennials *H. eggertii*, *H. pauciflorus*, and *H. smithii* and annuals *H. annuus*, *H. argophyllus*, *H. petiolaris*, and *H. praecox* [78].

Interspecific hybrids based on *H. nuttallii*, *H. giganteus*, and *H. maximiliani* were reported to show resistance against stem infection by Henn *et al.* [79]. Miller and Gulya [80] developed four maintainer and four restorer oilseed lines with improved tolerance to *Sclerotinia* stalk rot. The inbred line HA 410 released by Miller and Gulya [80] derived from a wild perennial hexaploid, *H. pauciflorus* (= *rigidus*), had a moderate tolerance to stalk rot. *Sclerotinia* root rot tolerance was observed in perennials *H. mollis*, *H. nuttallii*, *H. resinosus*, and *H. tuberosus* [81].

Among the perennial species, resistance to *Sclerotinia* was observed in population of *H. tuberosus*, *H. divaricatus*, *H. hirsutus*, *H. maximiliani*, *H. mollis*, *H. nuttallii*, *H. occidentalis*, and *H. rigidus* (= *pauciflorus*) grown under natural infection conditions [82].

*Sclerotinia* head rot tolerance was observed in perennials *H. resinosus*, *H. tuberosus*, *H. decapetalus*, *H. grosseserratus*, *H. nuttallii*, and *H. pauciflorus* [83–85].

In the past decade, advances were made in the research of *Sclerotinia* resistance at the molecular level, particularly in the marker-assisted selection [86, 50, 62, and many others]. The new methods are expected to provide significant help to sunflower breeders [86].

### 2.1.3. Sunflower rust (*Puccinia helianthi* Schw.)

Rust is the second most important sunflower disease considering its global distribution. The disease causes economic losses in sunflower production in North and South America, Australia and Africa. Based on our own observations, rust is present in several countries in Asia (China, India, Iran, Kazakhstan, and others), but its racial composition has not been determined yet. Fortunately for Europe, the local rust population is fairly stable. Rust races were studied most extensively in North America. Sackston [87] determined four North American races, 1, 2, 3, and 4. Race 4 was identified by Yang [88] and race 6 by Lambrides and Miller [89].

Antonelli [90] and Senetinner *et al.* [91] studied sunflower resistance to an Argentinean rust isolate, clone 340, and found that the lines MP 447, MP 444, and LC 74/74-20620 were resistant to it and that the resistance was controlled by a single dominant gene.

Hugues *et al.* [92] studied the occurrence and distribution of rust in Argentina in the period 1982–2008. Their results indicated that resistant cultivars were stable in terms of rust resistance. They also concluded that a single rust pathotype existed in central and southern sunflower-growing regions of Argentina, which was in contrast to previous studies.

In Africa, the determination of rust races in sunflower was done only in Mozambique. Using differential lines from Canada and USA, Vicente and Zazzerini [93] found that the rust race 4 was present in Mozambique.

In Europe, rust has been studied on a limited scale. Most of the work had been done at VNIIMK, Krasnodar. Studying various methods of inoculation by rust, Galina Pustovoi and Slyusar [94] concluded that growing a mixture of resistant genotypes in spatial isolation completed by selection of resistant plants was the most appropriate method.

Miller *et al.* [95] tested 343 genotypes for resistance to rust and found that 12 genotypes were resistant to race 4. The authors also found that the lines HA-R1, HA-R3, HA-R4, HA-R34, and 647-1 shared the same locus,  $R_4$ , while the line HA-R2 had a different one that was named R4.

Kochman and Goulter [96] proposed a system for identification of rust races in sunflower, and examined the slow-rusting phenomenon and resistance gene pyramiding to control sunflower rust.

Sendall *et al.* [97] studied the diversity of *Puccinia helianthi* pathosystem in sunflower in Australia at the molecular level and found a set of 24 lines and determined putative resistance genes.

Regarding the methods of artificial inoculation, Gulya and Maširević [98] provided a detailed description of inoculation techniques for evaluating sunflower resistance to rust under laboratory conditions (greenhouse experiments) as well as under field conditions. They also ranked the differential lines in three sets: set one (S-37-388, CM90RR, and MC29), set two (P-386, HA-R1, and HA-R2), and set three (R3-HA, HA-R4, and R4-HA).

Wild species of the genus *Helianthus* are a rich gene pool for further identification of resistance genes and their use to forestall the emergence of new races of *Puccinia helianthi*.

#### 2.1.4. Stem canker (*Phomopsis*) *Diaporthe helianthi*

In the past three decades, *Phomopsis* has become the most destructive disease on the global scale. Its large-scale occurrence was first registered in the Vojvodina Province (Serbia) and Romania in 1980, when it caused large economic damage to sunflower production. Soon afterwards, it was registered in most sunflower-growing countries in Europe (France, Hungary, Slovakia, Bulgaria, Ukraine, Russia, and Italy). In the early 1980s, its presence was reported in the USA, Canada, Argentina, Uruguay, Australia, Iran, and some other countries [22].



The first significant results in sunflower breeding for resistance to *Phomopsis* were achieved in Serbia and Romania.

Škorić [99] reported that of 4000 inbred lines and 2000 experimental hybrids, only four lines exhibited field resistance to *Phomopsis*. Two of these lines had been derived from interspecific hybrids (cultivated sunflower  $\times$  *H. tuberosus*): one was obtained from a cross of *H. argophyllus*  $\times$  Armavirski 9344 and the restorer line SNRF-69 was derived from a local population from Hungary.

Based on extensive research, Vrânceanu *et al.* [100] found that the sunflower resistance to *Phomopsis* is of the horizontal type and that it is positively correlated with the stay-green phenomenon. The authors reported that, of all Romanian hybrids, Select has the highest degree of tolerance to *Phomopsis*.

Škorić [99] found that three female lines (Ha-22, Ha-74, and Ha-BCPL) and the restorer line SNRF-6 are field resistant to *Phomopsis*. Resistance was transferred to the hybrids NS-H-43, NS-H-44, and H-NS-44 developed from these lines. The same author also reported that *Phomopsis* resistance is positively correlated with *Macrophomina* and *Phoma* resistance as well as with drought tolerance.

Vrânceanu *et al.* [101] concluded that partial dominance is expressed in the inheritance of *Phomopsis* resistance in some cases, while additive inheritance is much more frequent. The same authors found that the stay-green stem at the ripening stage is positively correlated with *Phomopsis* resistance.

Much work has been done lately on the use of molecular markers in breeding for *Phomopsis* resistance.

Studying recombinant inbred lines derived by crossing LR4-17 (resistant) with HA89 (susceptible) at the molecular level, Langar *et al.* [102, 103] concluded that unlinked segments carried major QTLs for different components of resistance, and that the resistances of leaves and stems could be pyramided with a marker-assisted selection.

Molecular studies on the intraspecific diversity of this fungus using intergenic spacer sequence analysis revealed a high homology among French/Yugoslavian and among Italian isolates [104]. The phylogenetic tree obtained from the aligned data revealed three separate groups. The analysis also showed that all isolates originating from countries with regular and severe outbreaks of the disease (e.g., France, Yugoslavia, etc.) formed a well-defined taxon with relatively low variability compared with isolates from Italy where the disease seldom occurs. In another paper, Rekab *et al.* [105] pointed out a polyphyletic nature of this fungus.

Škorić [99] and Dozet [106] reported high levels of resistance to *Phomopsis* in *H. maximiliani*, *H. hirsutus*, *H. pauciflorus*, *H. mollis*, *H. resinosus*, and *H. tuberosus*.

Interspecific hybrids based on *H. eggertii* and *H. smithii* showed high tolerance to *Phomopsis* in Bulgaria [107].



Christov [78] identified annuals *H. annuus*, *H. argophyllus*, and *H. debilis* and perennials *H. pauciflorus*, *H. glaucophyllus*, and *H. eggertii* as potential sources of *Phomopsis* brown stem canker resistance, based on field screening in Bulgaria.

Nikolova *et al.* [108] reported resistance to stem canker in progenies of interspecific hybrids of perennial *H. pumilus*. Resistance to *Phomopsis* was reported in interspecific hybrids derived from *H. argophyllus*, *H. deserticola*, *H. tuberosus*, and *H. xlaetiflorus* [109].

Complete resistance to *Phomopsis* was reported in interspecific hybrids of *H. salicifolius* by Encheva *et al.* [110] and Škorić [22].

State research and private companies have developed a rich germplasm for *Phomopsis* resistance.

#### 2.1.5. *Verticillium* wilt (*Verticillium dahliae* Kleb.)

In addition to *Verticillium dahliae* Kleb., sunflowers are attacked by *Verticillium albo-atrum* R. et B. and *Verticillium lateritium* Bertk. *Verticillium dahliae* Kleb. is the most harmful of these three fungi and it is also the most widespread globally. It causes economic damage to sunflower production in North and South America, Europe, North Africa, Australia, and some countries in Asia [22].

Sunflower breeding for *Verticillium* wilt resistance has been extensive in the USA, Canada, and Argentina. Putt [111] discovered the first sources of resistance to *Verticillium* wilt. His discovery was confirmed by Fick and Zimmer [112]. Resistance to the American race was found in the line HA-89 derived from the Russian cultivar VNIIMK 8931. It is controlled by a single dominant gene.

Bruniard *et al.* [113] and Bertero de Romano [114] found a *Verticillium* race in Argentina that could not be controlled by the gene V<sub>1</sub> (HA89).

Bruniard *et al.* [113] reported to have developed the lines V144, V99, V134, and V196 resistant to the Argentine race of *Verticillium*.

Gulya [54] reported that in 2002 he had found a new strain of *V. dahliae*, which was able to overcome the simple, V-1 dominant resistant gene used in oilseed and confection hybrids. The author tested a diverse germplasm and found that the Russian variety VNIIMK 8883 had genes for resistance to the new strain of *Verticillium dahliae*.

Several researchers used wild sunflower species in order to identify the source of resistance to *Verticillium* wilt.

Assessing the resistance of interspecific hybrids (cultivated sunflower × *H. tuberosus*) to *Verticillium* wilt, Galina Pustovoit and Krokhn [115] found a different mode of inheritance of resistance (two or three recessive genes or two complementary dominant genes), which hinders the development of resistant genotypes.

Putt [111] discovered a source of resistance in line CM144, which was derived from an interspecific hybrid of wild *H. annuus*. Škorić [116] determined high tolerance to *Verticillium dahliae* in *H. occidentalis*, *H. hirsutus*, and *H. tuberosus*.

### 2.1.6. Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid]

Synonyms for this fungus are *Sclerotium bataticola* Taub., *Macrophomina phaseoli* (Maubl.) Ashby and *Rhizoctonia bataticola* (Taub.) Butler.

Charcoal rot causes economic damage to sunflower production in arid regions. It is widespread in most sunflower-growing countries.

Charcoal rot may cause premature death of sunflowers grown on light, sandy soil under hot and dry climate. The disease is well known in the southern part of Europe [52].

Manici *et al.* [117] concluded that the great variability in pathogenicity in all the climatic areas of Italy suggests good adaptation of *Macrophomina* to the host.

This pathogen has been studied by many authors. Iliescu [118] and Ionita and Iliescu [119] published a detailed review of charcoal rot symptomatology, taxonomy, epidemiology, pathogenesis, and control of *Macrophomina* in sunflowers. To our knowledge, a most detailed description of charcoal rot has been provided by Aćimović [120].

Walcz and Piszkev [121] have developed an inoculation method for screening sunflower lines for resistance to this pathogen.

Mihaljčević [122, 123] conducted the most detailed studies on the effectiveness of inoculation methods with *Macrophomina*. According to his results, the method of Hsi (1961) was the best of the four inoculation methods tested. Hsi developed this method for sorghum testing and Mihaljčević [122] adapted it for sunflower testing.

Ahmad *et al.* [124] examined 13 exotic sunflower inbred lines and eight *Macrophomina* isolates. The tested inbred lines differed significantly in agronomic characteristics (head diameter, head weight, number of seeds per head, 1000-seed weight, and yield per unit area). The inbred lines HAR 1 and HAR 2 were resistant/tolerant across all charcoal rot isolates, while HA 822 was susceptible to the disease development and two charcoal rot isolates (MP9 and MP21) were virulent in affecting the head weight.

Mihaljčević [122] also found high resistance levels in lines derived from the Argentine cultivars Pehuan INTA, Ciro, and Klein as well as in the lines GVP-1 and GVP-2, derived from varietal populations (VNIIMK, Krasnodar) developed by interspecific hybridization with *H. tuberosus*.

Galina Pustovoit and Gubin [83] found the sources of resistance to *Macrophomina* in the F<sub>14</sub> progenies of the interspecific hybrid VNIIMK8931 × *H. tuberosus*. A radical inoculation method (injecting fungus suspension into the head tissue) confirmed a complete resistance in 62 lines.

Studies of wild sunflower species have been insufficient to enable the identification of resistance genes as the sources of resistance against charcoal rot. Seiler [49] concluded that interspecific hybrids based on *H. tuberosus* have resistance to charcoal rot. Wild species *H. mollis*, *H. maximiliani*, *H. resinosus*, *H. tuberosus*, and *H. pauciflorus* have also shown resistance.

#### 2.1.7. *Phoma black stem (Phoma macdonaldii Boerema)*

According to Ćimović [120], the synonym for this fungus is *Phoma oleracea* var. *helianthi-tuberosi* Sacc.

*Phoma black stem* is in large expansion in several countries in the world. It causes premature drying of plants (forced ripening) resulting in economic damage that increases from 1 year to another [22].

Viranyi [52] points out that *Phoma black stem* is extremely severe in France where basal stem lesions often result in lodging.

The inoculation method described by Maširević [125] is recommended to sunflower breeders. For efficiency, molecular markers should be used when screening breeding material.

Fayzalla [126] examined in detail the resistance to *Phoma macdonaldii* in a large set of Novi Sad genotypes of cultivated sunflower and several wild species. Using an inoculation method, he found that there was no satisfactory tolerance to *Phoma macdonaldii* in the genotypes of the cultivated sunflower. Among the wild species, however, high tolerance was registered in *H. maximiliani*, *H. argophyllus*, *H. tuberosus*, and *H. pauciflorus*.

*Phoma black stem* resistance has been reported in several perennial species: *H. eggertii*, *H. hirsutus*, *H. resinosus*, and *H. tuberosus* [99].

Encheva *et al.* [110] stated that interspecies of hybrids based on *H. salicifolius* are highly resistant to *Phoma black stem*.

Christov [78] also confirms that interspecies hybrids based on *H. eggertii*, *H. debilis*, and *H. argophyllus* exhibit high levels of resistance to *Phoma*.

Darwishzadeh *et al.* [127] undertook experiments to determine the partial resistance of sunflower genotypes to seven isolates and highly significant differences were observed among genotypes, isolates, and their interactions. Two genotypes exhibited specific resistance with a wide range of isolate-nonspecific partial resistance appearing as well. In addition, QTLs were also found associated with isolate-specific and nonspecific resistance [128]. Alignan *et al.* [129] developed a 1000-element cDNA microarray-containing genes putatively involved in primary metabolic pathways in order to identify genes responsible for partial resistance. They were successful in identifying 38 genes differently expressed among genotypes, treatments, and times.

According to Škorić [99], resistance to *Phoma black stem* is positively correlated with resistance to *Phomopsis* stem canker and charcoal rot.

#### 2.1.8. *Alternaria blight (Alternaria helianthi Tub. et Nish.)*

Ćimović [120] cited the following synonyms for *Alternaria blight*: *Helminthosporium helianthi* Hansf., *Alternaria leucanthemum* Nelen et Vas. and *Embellisia helianthi* (Hansf.). The same author stated that sunflowers are also attacked by *Alternaria zinniae* Pape, *Alternaria alternata* (Fr.) Keiss (synonym *Alternaria tenuis* Ness.) and *Alternaria helianthinificiens* Simmons, Walcz,

and Roberts. Of these species, *Alternaria helianthi* is the most common on sunflowers and the best studied from the point of view of sunflower resistance. It was found to attack sunflowers in all continents where this oilseed crop is grown. In the previous decade, it caused the most extensive economic damage on sunflowers in India and Brazil. According to Aćimović [120], most of the cultivated sunflower genotypes are sensitive to *Alternaria* blight.

Regina *et al.* [130] concluded that the occurrence of *Alternaria helianthi* in southern Brazil depended on the pathogen race and sunflower cultivar to a large extent. Attack is most intensive on crops sown in December and least intensive in October-sown crops. Dudienas *et al.* [131] claimed that *Alternaria* causes economic damage in Brazil, especially in humid conditions.

Aćimović [132] tested 1389 inbred lines for 4 years under field conditions and found that only six lines possessed satisfactory tolerance to *Alternaria* blight.

Madhavi *et al.* [133] found the sources of resistance to *Alternaria* blight in *H. tuberosus* and *H. occidentalis*.

Lipps and Herr [134] examined 496 sunflower genotypes for resistance to *Alternaria* for 3 years and found tolerance in eight genotypes only. A different situation was encountered when the *H. tuberosus* population was inoculated in the greenhouse. Based on the obtained results, the authors concluded that *H. tuberosus* can be used as a source of resistance to *Alternaria helianthi*.

Morris *et al.* [135] confirmed that all 21 annual taxa and 18 of 21 perennial species evaluated were susceptible to *A. helianthi* using applied spore suspensions, while perennial species *H. hirsutus*, *H. pauciflorus* ssp. *subrhomboides*, and *H. tuberosus* appear to resist infection by *A. helianthi*.

Sujatha *et al.* [136] determined that nine perennial *Helianthus* species, *H. maximiliani*, *H. mollis*, *H. divaricatus*, *H. simulans*, *H. occidentalis*, *H. pauciflorus* and *H. decapetalus*, *H. resinosus*, and *H. tuberosus* were highly resistant to *Alternaria* leaf spot; all annuals were susceptible.

Christov [78] reported that perennial *H. decapetalus*, *H. laevigatus*, *H. glaucophyllus*, and *H. ciliaris* were potential sources of genes for *Alternaria* resistance.

Complete resistance to *Alternaria* leaf spot was reported in interspecific hybrids of *H. salicifolius* by Encheva *et al.* [110]. Škorić [81] obtained similar results.

## 2.2. Other fungal diseases

There is a large number of other fungal diseases of sunflower that cause economic damage to sunflower production in some regions and in some years. Unfortunately, most of them have not been included in breeding programs yet [22].

### 2.2.1. *Fusarium* wilt (*Fusarium* spp.)

According to Aćimović [120], several species of the genus *Fusarium* attack sunflowers: *Fusarium solani*, *Fusarium solani* var. *minus*, *Fusarium oxysporum*, *Fusarium oxysporum*, *F.*

*helianthi*, *Fusarium moniliforme* (syn. *Gibberella fujikuroi*), *Fusarium equiseti*, *Fusarium tabacum*, *Fusarium culmorum*, *Fusarium* sp. and *Fusarium* spp.

Viranyi [52] states that *Fusarium wilt* (*Fusarium* spp.) has been reported as a pathogen of concern only from Russia [137] where it appeared to be harmful for sunflower production. Based on the extent of necrosis incited by the fungus on the main root and the root–hypocotyl transition zone of sunflower seedlings, some tolerance to pathogen attack could be detected among the genotypes [138]. In a breeding program, a number of new breeding lines were developed exhibiting relatively good field tolerance [139].

There are few research papers dealing with sunflower resistance to *Fusarium*. In one of these earlier papers, Orellana [140] reported that out of 49 inbred lines tested, 23 were resistant to *Fusarium moniliforme*. In recent years, Goncharov [139] produced plants tolerant to *Fusarium* on the basis of laboratory tests and individual selection of plants from three double-cross combinations and an F<sub>3</sub> cross (UV.680 × o.p. cv. Leader).

#### 2.2.2. Rhizopus head rot (*Rhizopus* spp.)

Dry rot of sunflower is caused by the following fungi from the genus *Rhizopus*: *Rhizopus arrhizus* Fisch. (syn. *Rhizopus nodosus* Namysl.), *Rhizopus nigricans* Ehr. (syn. *Rhizopus stolonifer* Eh. et Fr.) and *Rhizopus oryzae* Nent et Geer [120].

Dry rot occurs typically in regions with dry climate and high temperatures. It often causes a significant yield reduction and it particularly reduces the oil content in seeds [22].

It has become an important disease of sunflower in the USA [141]. The disease reduced oil quality and quantity in oilseed sunflower [142]. Infection of sunflower with *Rhizopus* head rot is enhanced by larval feeding of sunflower moth, *Homoeosoma electellum* (Hulst), which contributes to a secondary infection [141].

Yang *et al.* [143] reported that 4 out of 32 wild species and subspecies were resistant when inoculated with *R. arrhizus* and *R. oryzae* Went. The resistant sources were perennial *H. divaricatus*, *H. hirsutus*, *H. xlaetiflorus*, and *H. resinosus*.

One of the pioneer works was that of Agrawat *et al.* [144], who studied the resistance to *Rhizopus nodosus* in 91 sunflower cultivars. Their results based on an inoculation test indicated that resistance existed only in cultivars – Armavirec, Armavirskiy 3497, EC 40277, and K-2217, all from Krasnodar.

*Rhizopus* head rot brings great economic damage in many countries, by decreasing seed yield, seed oil content, and seed development. Unfortunately, few researchers in the world work on the examination of this pathogen.

#### 2.2.3. Powdery mildew (*Erysiphe cichoracearum* DC)

Sunflower is a host to three fungal genera that cause powdery mildew [120, 145]: *Erysiphe cichoracearum* DC, which is widespread in all continents where sunflower are grown, *Leveillula compositarum* Golow., *Leveillula taurica* (Lev.) Arn., and *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.



Since *Erysiphe cichoracearum* DC is common on sunflowers around the world, resistance to this pathogen has been studied most extensively. Saliman *et al.* [146] was among the first to identify wild species from the genus *Helianthus* resistant to *Erysiphe* [120].

Jan and Chandler [147] transferred the resistance from *H. debilis* Nutt. into the line P21. The mode of inheritance in this resistance source was partially dominant. According to unpublished results of Škorić, resistance to powdery mildew exists in several inbred lines, especially those that incorporate genes from *H. tuberosus*.

Breeding programs conducted in Argentina, Australia, and the Republic of South Africa have been targeted on Albugo resistance and several highly tolerant hybrids were obtained.

Seiler [49] indicates that *Helianthus debilis* ssp. *praecox*, and *H. bolanderi*, and 14 perennial species were tolerant of powdery mildew in both field and greenhouse tests [146]. Not all population of perennial species are resistant: population of *H. grosseserratus* and *H. maximiliani* showed differential reactions. Škorić [116] reported that interspecific hybrids with *H. giganteus*, *H. hirsutus*, *H. divaricatus*, and *H. salicifolius* had no powdery mildew symptoms.

Jan and Chandler [147] transferred the resistance from *H. debilis* Nutt. into the line P21. The mode of inheritance in this resistance source was partially dominant. According to unpublished results of Škorić, resistance to powdery mildew exists in several inbred lines, especially those that incorporate genes from *H. tuberosus*.

#### 2.2.4. *Botrytis cinerea* Pers.

Sunflower geneticists and breeders have unjustly neglected the polyphagous fungus *Botrytis cinerea* Pers., although it causes economic damage in sunflower production in some regions.

Prats [148] was the first to discover a source of resistance to *Botrytis cinerea* in the cultivar INRA 64-01.

Burlov and Artemenko [149] found the line Od-2624 to be resistant to *Botrytis*.

Kostyuk [150] studied some 1400 sunflower genotypes and found that none of them were resistant and only some were tolerant to *Botrytis* under natural and inoculation conditions.

#### 2.2.5. White rust (*Albugo tragopogonis* Schr.)

According to Aćimović [120] the synonym for this fungus is *Albugo tragopogonis* (Pers.) Schr. White rust has been registered on sunflowers in several countries and is particularly aggressive in South America (Argentina), Africa (Republic of South Africa), some Asian countries, several countries from the former Soviet Union, and Australia.

Breeding programs conducted in Argentina, Australia, and the Republic of South Africa have been targeted on *Albugo* resistance and several highly tolerant hybrids were obtained [22].

An established breeding centre, which focuses its research on identifying sources of resistance to white rust in wild species of genus *Helianthus*, unfortunately does not exist anywhere in the world.

### 2.3. Viruses and Bacteria

Some viruses are capable of causing disease in sunflowers. The number of viruses that are specific and attack only sunflowers is very limited. In most cases, the main host is another agricultural crop and sunflower is only a secondary host [120]. According to Gulya *et al.* [145], several viruses attack sunflower: aster yellows virus, cucumber mosaic virus, sunflower mosaic virus, sunflower ringspot virus, sunflower yellow blotch virus, leaf crinkle virus, tobacco ringspot virus, tobacco streak virus, tomato spotted wilt virus, potyvirus, etc.

Viruses are typically transmitted by vectors, the most important among which are aphids.

Srechari *et al.* [151] reported three aphid species, *Aphis gossypii* Glove., *Aphis craccivora* Koch, and *Rhopalosiphum maidis* (Fitch), as virus vectors. Among them, *A. gossypii* is best known as the vector that transmits the sunflower mosaic disease.

Lenardon *et al.* [152] detected the sunflower chlorotic mottle virus (SuCMoV) in several regions of Argentina.

Lenardon *et al.* [153] tested 232 lines in the greenhouse using an inoculation method. Only three lines exhibited partial resistance (L33, L74, and L42) to the sunflower chlorotic mottle virus. Of these three lines, L33 was the most resistant. Screening F<sub>2</sub> population of crosses between resistant and sensitive lines in the greenhouse and in field, the authors concluded that the resistance is controlled by a single dominant gene (Remo-1).

In recent years, the sunflower chlorotic mottle virus has been studied intensively at the molecular level.

Dujovny *et al.* [153] conducted a molecular characterization of a new potyvirus (SuCMoV). Arias *et al.* [155] described the effect of SuCMoV on some aspects of carbon metabolism in sunflower plants.

Mailo *et al.* [156] mechanically inoculated one sensitive (20 016) and one tolerant line (B-133) with SuCMoV. Total RNA was isolated from infected leaf tissue for study at the molecular level. The achieved results indicated that the gene expression profiles in the inoculated plants (of the sensitive and the tolerant line) were statistically significant compared with leaves of plants that were not inoculated. Eighty-eight genes were differentially expressed in the tolerant line.

#### 2.3.1. Bacterial diseases

Bacterial diseases of sunflower are caused by pathogenic bacteria. They can be found on sunflowers in most countries where this oil crop is grown. In addition to sunflower, most of these bacteria also attack other crops. The sunflower is typically a secondary host and quite rarely the main host [120].

The most widespread bacteria on sunflowers are *Agrobacterium tumefaciens* (E. F. Sm. and Town.) Conn, *Pseudomonas syringae* pv. *tabaci* (Wolf and Foster 1917) Young, Dye and Wilkie 1978 (synonyms *Pseudomonas tabaci* and *Bacterium tabacum* Wolf and Foster), *Xanthomonas campestris* pv. *phaseoli* (Smith) Due., *Pseudomonas syringae* pv. *helianthi* (Kawamura) Dye, Wilkie

and Young, *Pseudomonas solanacearum* (Smith), *Erwinia carotovora* pv. *carotovora* (Jones) Bergey *et al.*, etc. [120].

There are few papers in the literature dealing with sunflower selection for resistance to bacterial diseases.

Among these few, Nemeth and Walcz [157] reported the occurrence of *Erwinia carotovora* on sunflowers in Hungary in the period 1984–1986. Tests of inbred lines and commercial hybrids conducted under natural conditions indicated that there existed significant differences in resistance to this bacterial disease. However, the tests showed that the breeding material can be tested by inoculation methods under field conditions.

#### **2.4. Sunflower breeding for resistance to broomrape (*Orobancha cumana* Wallr.)**

The parasitic angiosperm broomrape (*Orobancha cumana* Wallr. = *Orobancha cernua* Loelf.) is the cause of many economic losses in sunflower production in a number of countries in the world, especially in central and eastern Europe, Spain, Turkey, Israel, Kazakhstan, and China. Its presence has also been established in Australia.

Sunflower breeders have been fighting *Orobancha cumana* Wallr. for almost a century [22].

According to past researches, there have been different mechanisms of sunflower resistance to *Orobancha*. Most often these are genetic mechanisms, but there are also physiological, biochemical, mechanical, and others.

According to Morozov [158], the first reports of broomrape in sunflower came from Saratov Oblast in Russia and date back to the 1890s. The same author mentions that the first sunflower varieties resistant to race A of *Orobancha* were developed by Plachek at the Saratov breeding station.

At the beginning of the 20<sup>th</sup> century, broomrape spread across Russia significantly and endangered the mass production of sunflower. The first cultivar resistant to race A, Saratovskij 169, was created by Plachek. In the years that followed, other cultivars resistant to race A were also produced (Kruglik A/41, Zelenka, and Fuksinka). As the mass production of sunflower spread quickly, it was followed by a relatively fast production of a new race called B. Zhdanov in Rostov on the Don announced that he had produced several cultivars resistant to a new race (B). During the period 1924–1960, Pustovoit in VNIIMK, Krasnodar created highly productive cultivars, which were resistant to race B [22, 158].

In order to attain their breeding goals and identify sources of broomrape resistance, sunflower breeders must develop a breeding strategy, decide on a breeding method, secure the necessary germplasm and differential lines for broomrape race identification, and choose the appropriate inoculation method and molecular marker technique (marker-assisted selection (MAS)) – Škorić [22].

Vrânceanu *et al.* [159] defined a set of differential lines for the evaluation of the composition of broomrape races. Among them was the AD-66 line, which represented a tester line susceptible to all broomrape races. On the other hand, the differential line (cultivar) Kruglik A41 was

used for race A, the Jdanov-8281 cultivar for race B, the Romanian cultivar Record for race C, line S-1348 for race D, and line P-1380-2 for race E. Pâcureanu-Joița *et al.* [160] included the line LC-1093 as a tester for race F. Unfortunately, there have been no tester lines for the latest broomrape races [160].

Genes for resistance to broomrape races A, B, C, and D are present in varietal populations of sunflower developed in breeding programs from Krasnodar, Armavir, Odessa, Fundulea, and several other places [149]. Genes that confer resistance to races E, F, G, and the latest ones, on the other hand, have been identified in certain wild species of the genus *Helianthus* and have been incorporated into cultivated sunflower genotypes by interspecific hybridization [161, 162].

Galina Pustovoit [163] and her team made a great contribution in this area by developing sunflower varieties through interspecific hybridization in which *H. tuberosus* was used as the donor of *Or* genes. These varieties were used in the identification of *Or*<sub>4</sub> and *Or*<sub>6</sub> genes.

Fernandez-Martinez *et al.* [164] tested 44 wild sunflower accessions (representing 27 perennial and 4 annual species) and 44 cultivated sunflower accessions, which they raised in a growth chamber and then transplanted to a greenhouse. The material was inoculated with the virulent race F (population SE 296). Most of the perennial species proved fully resistant to race F.

Among the wild annual species, *H. anomalus* and *H. agrestis* were completely resistant, while *H. debilis* ssp. *cucumerifolius* and *H. exilis* segregated with regard to *Orobanche* resistance [164].

Interspecific hybrids based on *H. eggertii* and *H. smithii* showed total resistance to broomrape in Bulgaria [107]. Broomrape resistance to the local race in Bulgaria was reported in *H. divaricatus*, *H. eggertii*, *H. giganteus*, *H. grosseserratus*, *H. glaucophyllus*, *H. mollis*, *H. nuttallii*, *H. pauciflorus* (=rigidus), *H. resinosus*, and *H. tuberosus* [107, 165].

Diploid perennial species *H. divaricatus*, *H. giganteus*, *H. glaucophyllus*, *H. grosseserratus*, *H. mollis*, *H. nuttallii*, and *H. smithii* and their interspecific hybrids were resistant to broomrape [67]. Christov [78] reported that several perennial *Helianthus* species showed 100% resistance including *H. tuberosus*, *H. eggertii*, *H. smithii*, *H. pauciflorus*, and *H. strumosus*.

Jan *et al.* [166] crossed the wild sunflower species *H. maximiliani* Schrad, *H. grosseserratus* Mart., and *H. divaricatus* L. with cultivated sunflower and developed four populations (BR1-BR4) resistant to race F in Spain.

Numerous authors in public institutions and private companies use wild sunflower species as donors of genes for resistance to broomrape.

The sources of resistance to broomrape, which have been discovered so far, mostly use the gene of resistance taken from the wild species of the genus *Helianthus*. According to the results obtained so far, there are over 20 wild species of the genus *Helianthus*, which contain the gene of resistance to broomrape [22].

When broomrape occurred, breeders used infested fields for testing selection materials for broomrape resistance in many countries. This method is not reliable enough since in natural conditions infestation with broomrape on those fields is not equally spread, which causes large



experimental errors. A much more reliable method, which is applied on testing resistance, is mixing of broomrape seed (a certain amount) with the selection material in the process of cultivation. In order to accelerate the process of testing the resistance of selection material, there are certain containers used in a greenhouse during the period autumn/winter, which are filled with a mixture of soil and sand filled with broomrape seeds and the seed of sunflower genotypes that are being tested [22].

Panchenko [167] developed a method of testing the selection materials in the greenhouse, which enables simultaneous testing of a great number of lines that are being created. The purpose of this method is preparation of a medium (sterilized soil + sand or perlite) on the tables in the medium. Following that, the appropriate amount of broomrape seed is added and the selection material is cultivated. Within 3–4 weeks after germination, it is possible to make the evaluation of resistance.

In Rustica Prograin Genetique, Grezes-Besset [168] developed a fast method of testing the selection material of sunflower in plastic tubes (a mixture of sand and perlite), which enables a reliable testing of a large number of lines (hybrids) in small space and the cycle lasts about 3 weeks.

However, the most reliable and the most easily applied method of screening breeding materials for broomrape resistance is the use of molecular markers, QTL, RFLP, RAPD, TRAOP, and SSR markers which have been used for this purpose.

Increased use of marker-assisted selection, which gives quick and reliable results, is very positive for sunflower breeding.

The best example of that is the production of hybrids resistant to the imidazolinone group of herbicides, which has proven itself in mass production by cultivating IMI-resistant hybrids followed by controlling broomrape. IMI-resistant hybrids are very important in regions where new races of broomrape have occurred [22].

Dominant genes for resistance to races A, B, C, D, E, and F have been found and incorporated into cultivated sunflower genotypes. In the last 2–3 years, new broomrape populations have been discovered in several countries. None of the existing commercial hybrids resistant to races A, B, C, D, E, and F have proven resistant to these new populations.

## 2.5. Sunflower breeding for insect resistance

Several hundred different species of insects cause infestations in sunflower. However, economic losses are caused only by a few insect species [169]. Some insects transmit several sunflower diseases [170]. *Homoeosoma* spp. are a significant problem in cultivated sunflower on four continents. *Homoeosoma nebulella* (Hubner) infests sunflower in Europe and Asia. In South America, sunflower is infested by *H. heinrichi* (Pastrana), whereas *H. electellum* (Hulst) causes damage to sunflower in Mexico, USA, and Canada.

Cultivar resistance to European sunflower moth (*H. nebulella*) was incorporated in USSR 60–70 years ago through interspecies hybridization of cultivated sunflower and *H. tuberosus* spp. *Purpurellus*, Cockerell [171, 172].



The mechanism of resistance to sunflower moth exists due to the phytomelanin (carbon) layer in the husk. Black hull colour is positively correlated with phytomelanin content in the husk.

North-American species of sunflower moth (*H. electellum*) is far more virulent on cultivated sunflower than *H. nebullella*; hence, resistance breeding to this insect is of great importance in North America.

According to the results of Rogers and Kreitner [173], the presence of phytomelanin in sunflower seed pericarp prevents seed infestation with *H. electellum*. By monitoring the formation of pericarp (husk), it was determined that phytomelanin starts to accumulate between the hypodermis and sclerenchyma 3 days after fertilization, whereas its formation is clearly visible 13 days after fertilization.

In the major production area of North America, there are about 14 principal insect pests of cultivated sunflower, and of this total about six are considered potential economic pests [174].

“Two breeding procedures are recommended for identifying lines with improved resistance to insects attacking cultivated sunflower. These procedures are based on the initial evidence that the resistance to the insects is quantitatively inherited, that is, controlled by several genes. Both are based on recurrent selection and random mating, with the main objective to combine as many of the alleles controlling resistance as possible” [175].

“The recurrent phenotypic selection breeding procedure could be utilized for selection against stem and/or foliage infesting insects. An original ( $C_0$ ) source population may be created by random mating cultivars or lines (e.g., Plant introductions, open-pollinated populations), which are then screened for resistance to a particular insect attack sunflower” [175].

“Recurrent phenotypic selection with  $S_1$  line progeny evaluation could be utilized for selection for head and/or seed infesting insects. An original ( $C_0$ ) source population is created similarly as in the recurrent phenotypic selection procedure. The  $C_0$  population is planted in a normal breeding nursery with the most vigorous plants selected for bagging and self-pollination” [175].

According to Seiler [49], the insects causing most economic damage in North America are: sunflower beetle [*Zygogramma exclamatoris* (Fabritius)], the sunflower stem weevil [*Cylindrocopturus adspersus* (LeConte)], the red and gray seed weevils [*Smicronyx fulvus* (LeConte) and *S. sordidus* (LeConte)], the banded sunflower moth (*Cochylis hospes* Walsingham), the sunflower moth [*Homoeosoma electellum* (Hulst)], and the sunflower midge *Contarinia schulzi* Gagne. The sunflower head moth, *Homoeosoma electellum* is the most widespread and damaging sunflower insect pest in North America, while in Europe and Asia it is *Homoeosoma nebullella* (Hubner).

According to the results obtained by Rogers [176, 177], the following sunflower species exhibit significant levels of resistance to sunflower moth: *H. arizonensis*, *H. ciliaris*, *H. pumilus*, *H. resinosus*, *H. rigidus* × *laetiflorus*, *H. silphiodendron* and *H. smithii*.

Among the insects that cause economic losses to sunflower production, the biggest success was achieved in breeding for resistance to sunflower head moth above all in Europe by the development of cultivars in which an armored layer was induced in the husk from some specific wild species. Similar results were obtained in North America.

High level of resistance to *Bothynus gibbosus* was exhibited by the following species: *H. tuberosus*, *H. maximiliani*, *H. niveus*, *H. xlaetiflorus*, *H. salicifolius*, *H. mollis*, *H. grosseseratus*, *H. Argophyllus*, and *H. ciliaris* [178].

The results of Rogers [176, 179, 180], as well as Rogers and Thompson [178, 181, 182], confirm high levels of resistance to *Zygogramma exclamationis*, *Bothynus gibbosus*, *Masonaphis masoni* and *Empoasca abrupta* in wild sunflower species *H. tuberosus* and *H. maximiliani*, and recommend the use of these species in breeding programs.

Results of Rogers and Thompson [183, 184] confirm significant levels of resistance in two annual and 10 perennial wild species (*Masonaphis masoni*). The highest resistance to aphids was seen in *H. carnosus*, *H. exilis*, *H. floridanus* and *H. radula*.

Weak point in sunflower breeding for resistance to insects is that only few sunflower researchers deal with this issue. Insecticides can be used to some extent, more or less successfully, as a solution to this problem in some species.

## 2.6. Conclusions

Biotic stresses cause great economic damages and act as a limiting factor for the production of sunflower.

Diseases are the main problem among biotic stresses. Using wild sunflower species of the *Helianthus* genus, genes conferring resistance to most dominant diseases were discovered and incorporated to the genotypes of the cultivated sunflower.

Regarding the achievements in sunflower breeding for disease resistance, the results can be divided into four different groups.

The first group consists of work that resulted in the discovery of genetic resistance to certain causative agents of sunflower diseases (*Plasmopara halstedii*, *Puccinia helianthi*, *Verticillium dahliae*, *Verticillium albo-atrum*, and *Erysiphe cichoracearum*).

The second group comprises work in which a high level of tolerance (field resistance) was achieved. This group includes the results achieved in breeding for resistance to *Phomopsis/ Diaporthe helianthi*, *Macrophomina phaseolina*, *Albugo tragopogonis*, and *Alternaria* ssp.

The third group consists of results in which a satisfactory level of tolerance was achieved (*Phoma macdonaldii* and to some extent *Sclerotinia sclerotiorum*).

The fourth group consists of results that were partly achieved, where the level of favorable tolerance, that is, resistance, was not reached (*Rhizopus* ssp., *Botrytis cinerea* and other fungal pathogens).

Viruses and bacteria only pose a minor problem in comparison with diseases. Breeding for resistance to viruses and bacteria also includes wild sunflower species.

Broomrape (*Orobanche cumana* Walr.) is a major global issue in sunflower production, particularly in Central and Eastern Europe. Genes conferring resistance to six races of broomrape have been discovered in some wild sunflower species and incorporated into genotypes of the

cultivated sunflower. Research work, which aims at finding genes of resistance to the newest races within specific wild sunflower species, is in progress.

Sources of resistance to imidazolines and sulfonylurea herbicides (tribenuron-methyl) have been found in a population of wild *H. annuus* from Kansas and incorporated in cultivated sunflower genotypes. Moreover, genes conferring resistance to these herbicides were discovered using induced mutations. The newly developed hybrids, resistant to the abovementioned herbicides, provide successful weed and broomrape control through imidazolines.

Insects are a major issue in sunflower production, especially in North America. Significant results have been obtained through breeding for resistance to sunflower head moth. Wild sunflower species are used in research work aimed at finding the sources of resistance to other economically harmful insects.

Different biotechnological methods (tissue culture, embryo culture, protoplast fusion, molecular markers, in vitro screening, and other methods) have been included in breeding for resistance to biotic stresses.

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