## Chapter 6

# Genomic Designing for Resistance to Biotic Stresses in Sesame

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**Abstract** Sesame (*Sesamum indicum* L.) is an important oilseed crop unique with high contents of unsaturated fatty acids and antioxidants. Sesame originated from tropical regions and possesses high tolerance to drought and infertility conditions, while the resistance level to biotic stresses (especially fungal diseases) is relatively low. We describe here the major diseases and pests causing significant damage to sesame production and the progresses made in the genetics and breeding research for disease resistance in sesame. The application of genomics-assisted breeding in sesame is also deliberated.

**Keywords** Sesame · *Sesamum* · Biotic stress · Disease resistance · Breeding · MAS · Genomics-assisted selection

## 6.1 Description on Different Biotic Stresses in Sesame Production

### *6.1.1 Economic Important of Sesame*

Sesame (S*esamum indicum* L., $2n = 26$) is an annual oilseed crop (Fig. 6.1) and is widely cultivated in tropical and subtropical regions in the world. Its cultivation history could be traced back to the Early Bronze Age (3,000 BC) (Bedigian 2004). Sesame belongs to the *Sesasum* genus, Pedaliaceae family, and is the sole cultivated

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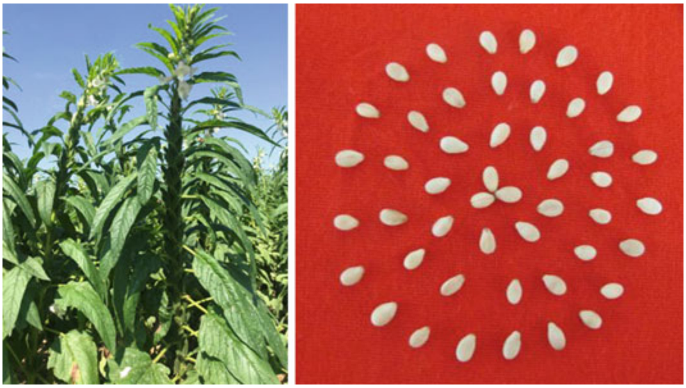

**Fig. 6.1 Sesame plant and mature seeds**. Left image is a Chinese sesame variety Yuzhi Dw609 cultivated in field. Right side image is sesame seeds with white seed coat color (Provided by H. Zhang)

species of the *Sesamum* genus (Zhang et al. 2013a; b). Sesame seed contains abundant oil (50–55%), proteins (18–20%), carbohydrate (13–25%), and antioxidants (0.3–1.5%) (Zhang et al. 2019), and is named as the queen of the oilseed crops for the specific and high seed quality. Especially, in China, sesame is also used as a medicinal ingredient for health care.

At present, sesame is widely cultivated in about 75 countries. The total harvested area of the world reaches to 10.5 million hectare. The total annual production is about 6 million tons. Sudan, India, Myanmar, Tanzania, and China are the leading countries for sesame production in the world. However, the average yield of the world sesame keeps low to 577.9 kg per hectare according to FAO statistics data. Besides the traditional and extensive cultivation styles, biotic stresses specially diseases are the main factors affecting the yield and seed quality of sesame (Zhang et al. 2019).

### 6.1.2 Reduction in Yield and Quality Due to Biotic Stresses

There are tens of pests and diseases which make huge damages to sesame growth and development and the final seed yield. At least eight fungal diseases cause economic losses to sesame in the world production areas (Kolte 1996; Khalifa 2003; Didwania 2019). Especially, the top three fungi diseases including Fusarium wilt, charcoal rot, and Alternaria leaf spot can cause a loss of about 25–40% of seed harvest (El-Bramawy and Wahid 2006; El-Bramawy et al. 2008). Prathuangwong and Yowabutra (1997) explored the relationship between the severity of bacterial leaf spot disease caused by *Pseudomonas syringae* pv. *sesami* and the yield loss under both greenhouse and field conditions. As the severity of disease increased by 1% for all sesame seeds, the average yield of the 14 sesame varieties reduced by 1.30% and 0.92%, respectively.

To reveal the damage of pathogens on sesame yield and quality, Li et al. (2017) systematically investigated on the yield related traits including plant height, capsule zone length, capsule node number, the capsule number per plant, seed yield, and seed quality traits of the three sesame varieties TP4816-0, JDP12-0, and JDP21 cultivated in the artificial Fusarium wilt disease nursery. Five *Fusarium* strains with high pathogenicity were cultured and evenly applied into nursery soil. For the samples with disease grade 3 syndrome, the value of seed yield and seed quality traits significantly changed ($p < 0.05$). The seed quality related traits including seed appearance traits, seed nutrition components, and oil quality were affected accordingly. For grade 4 plants, no mature seeds could be harvested (Li 2017; Yuan et al. 2018). Especially, three indicators (L, a*, and b*) of the seed coat color traits varied with the change of grade level from 0 to 3, and could be used to indicate the occurrence of the Fusarium wilt disease. Meanwhile, the results showed that the polysaccharide content is significantly affected by pathogen invasion and disease occurrence. For grade 3 samples of the tested three varieties, the acid values increased by 36.48–68.27% and the peroxide value decreased by 15.04–51.92%, which finally reduced the oil quality and taste.

## *6.1.3 Taxonomy of Causal Agents of the Diseases*

### 6.1.3.1 Fusarium Wilt Disease and Host Resistance Evaluation

Sesame Fusarium wilt (SFW) disease is one of the most important sesame diseases in the world and has been detected and determined since 1920s (Armstrong and Armstrong 1950; El-Shakhess et al. 2007). SFW is caused by *Fusarium oxysporum* f. sp. *sesami* (FOS) (Fig. 6.2) (El-Shazly et al. 1999; Li et al. 2012; Miao et al. 2019;

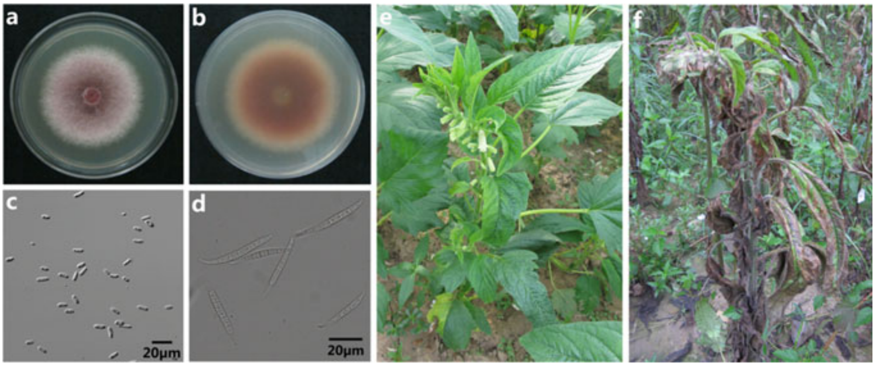

**Fig. 6.2 FOS pathogen and sesame plant inoculated by Fusarium wilt disease**. **a** FOS colony front side; **b** FOS colony back side; **c** FOS conidium. Bar = 20 μm; **d** FOS chlamydospore. Bar = 20 μm; **e** FW seedling with low disease grade; **f** FW sesame plant with high disease grade (Provided by Hongmei Miao)

Duan et al. 2020). SFW is a worldwide disease and usually occurs on 15% plantlets in the ordinary sesame field and causes considerable loss of seed yield (Wang et al. 1993; Elewa et al. 2011; Li et al. 2017; Wei et al. 2018). Inoculation experiment indicated that the sesame plantlets presented the Fusarium wilt symptom in 1–2 weeks later after inoculated with $1 \times 10^6$ microconidia/mL FOS suspension in greenhouse (Miao et al. 2019). However, field investigation indicates that the FOS pathogen commonly infects the root tissue, causes damping-off (such as leaf chlorosis, abscission, stem necrosis, and internal vascular browning), and results in whole plant wilting and death at seedling and adult stages in sesame (Li et al. 2012; Miao et al. 2019).

In the past a few years, hundreds of *Fos* isolates have been identified from wilted sesame plants in China. The morphological and pathogenic characteristics and the genetic diversity of the Fos isolates have been further described (Li et al. 2012; Qiu et al. 2014; Duan et al. 2020). Duan et al. (2020) compared the 69 *Fos* isolates collected from major sesame production regions in China. Based on the inoculation evaluation on the three differential sesame hosts, the 69 isolates were grouped into the three pathogenic groups. Correspondingly, 10 *SIX* (secreted in xylem) genes that translate one family of effectors of FOS were detected and found to reflect the difference of the three pathogenic groups for the first time. Further transcriptome analysis showed that most homologues of *Fos SIX* genes presented the specific expression profiles in sesame during the *Fos* infection. The results supported that most of the *Fos SIX* genes play important roles in the virulent genotypes.

To evaluate the resistance level of sesame accessions to FOS disease, the resistance evaluation indicator with the five ratings under natural infection conditions were used in sesame (El-Bramawy and Wahid 2006; Qiu et al. 2014; Miao et al. 2019). The disease index (DI) was calculated and the five DI value scale was determined for resistance evaluation (Qiu et al. 2014).

Based on the above standards, Miao et al. (2019) established the evaluation method of sesame resistance to Fusarium wilt disease at vegetative stage and evaluated 40 cultivated accessions. The results showed that 57.5% samples were highly susceptible (HS, $DI > 70$), while 27.5% were showing high- or medium-resistance ($15 < DI \leq 55$) and proved presence of low percentage of elite germplasm accessions with high resistance to FOS strains.

To reflect the genetic basis of the resistance to FOS in sesame, some researchers evaluated the Fusarium wilt resistance of sesame populations under the natural or artificial field condition (Wang et al. 1993; El-Bramawy and Wahid 2006; Sìlme and Çarğirgan 2010). Only a few dominant genes should determine the resistance level of sesame to FOS (Wang et al. 1993; Bakheit et al. 2000; El-Bramawy and Shaban 2007). Recently, with the aid of genome data and the above concise evaluation method, Miao et al. (2017) evaluated disease resistance in an $F_2$ population derived from the parent DS899 (DI 6.1%) and JS012 (DI 100%) to FOS pathogen no. HSFO09095 and located one major quantitative trait locus (QTL), which indicated that the resistance of the population to FOS is controlled by single gene pair.

### 6.1.3.2 Charcoal Stem Rot Disease and Host Resistance Evaluation

Charcoal stem rot disease is the most important and soil-borne disease for sesame because of the huge loss of the seed yield. Charcoal stem rot disease in sesame is caused by the fungus *Macrophomina phaseolina* (Tassi.) Goid. (MP) and also widely occurs in the world (Mihail and Taylor 1995; Rajput et al. 1998; El-Bramawy and Wahid 2006). Charcoal rot disease can occur in all stages of life cycle of a sesame plant and the incidence rate reaches to 10–25% with high yield loss (Vyas 1981; Wang et al. 2017). The common symptoms of the charcoal stem rot disease include the sudden wilting and spot blight on plant stem (Fig. 6.3). The stems get brown and black and the syndrome gradually extends upward. Subsequent extension of the black and infected stem results in the death of the plant. The peak stage of the disease occurs at the final flowering stage. After infected by MP pathogen, the root becomes brittle and black. On the infected plants, the capsules change to black and crack prematurely, and the seeds shrivel with low yield and quality.

For charcoal rot disease, the diversity of host species and the geographic range are wide (Jana et al. 2005; Babu et al. 2010; Saleh et al. 2010). Zhang and Feng (2006) made the charcoal rot resistance standard with five grades (Zhang and Feng

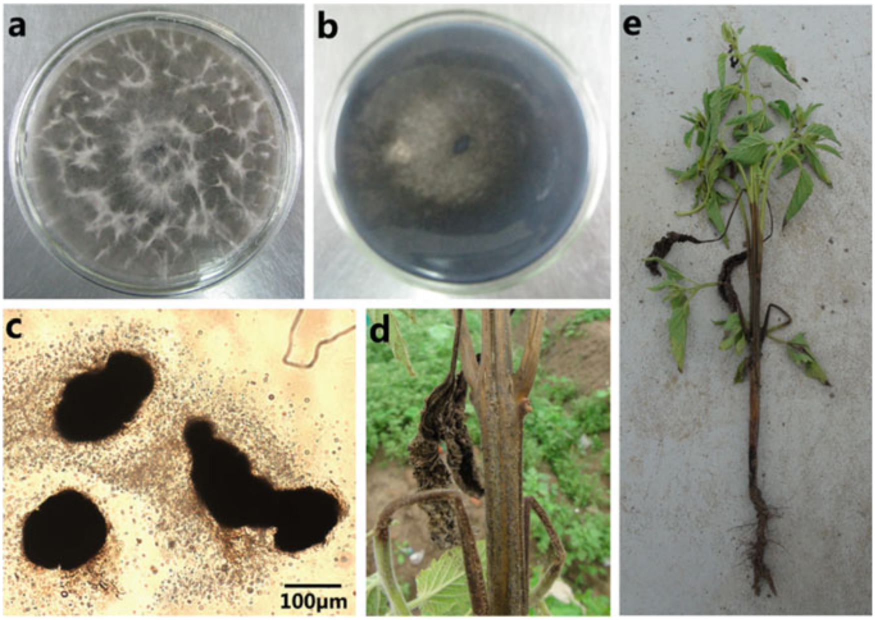

**Fig. 6.3 Symptoms of sesame charcoal rot disease in sesame**. **a** *Macrophomina phaseolina* strain colony front side; **b** *Macrophomina phaseolina* strain colony back side; **c** Sclerotia of *Macrophomina phaseolina* strain. Bar = 100 μm; **d** dark-colored sclerotia mass of *Macrophomina phaseolina* strain on the stem of diseased plant; **f** Symptom of charcoal rot disease in sesame (Provided by Hongyan Liu)

2006; Wang et al. 2017). The resistance level to charcoal rot disease is calculated based on the relative resistance indicator. Meanwhile, Thiyagu et al. (2007) used the five grades with different infection percentage (Dinakaran and Mohammed 2001) and established the artificial screening and sick plot methods to assay the resistance to the charcoal rot disease. Similar to the resistance to FOS, the charcoal rot disease resistance in sesame was controlled by a few dominant genes (El-Bramawy and Shaban 2007).

#### 6.1.3.3 Leaf Diseases and Host Resistance Evaluation

For sesame, leaf diseases are also common diseases (Fig. 6.4), as the main leaf diseases such as leaf blight can occur on 30–40% of the plants and cause the loss of above 30% seed yield (Zhao et al. 2014). There are many types of leaf diseases in sesame caused by different pathogens. For instance, leaf blight disease is caused by Fungus *Helminthosporium sesami* Miyake (Poole 1956; El-Fawy et al. 2018); Alternaria leaf spot (or black spot) disease is caused by fungus *Alternaria sesami* (Kawamura) Mohanty et Behera (Dolle and Hegde 1984b); Nigrospora leaf blight disease is caused by fungus *Nigrospora sphaerica* (Dutta et al. 2015); brown spot

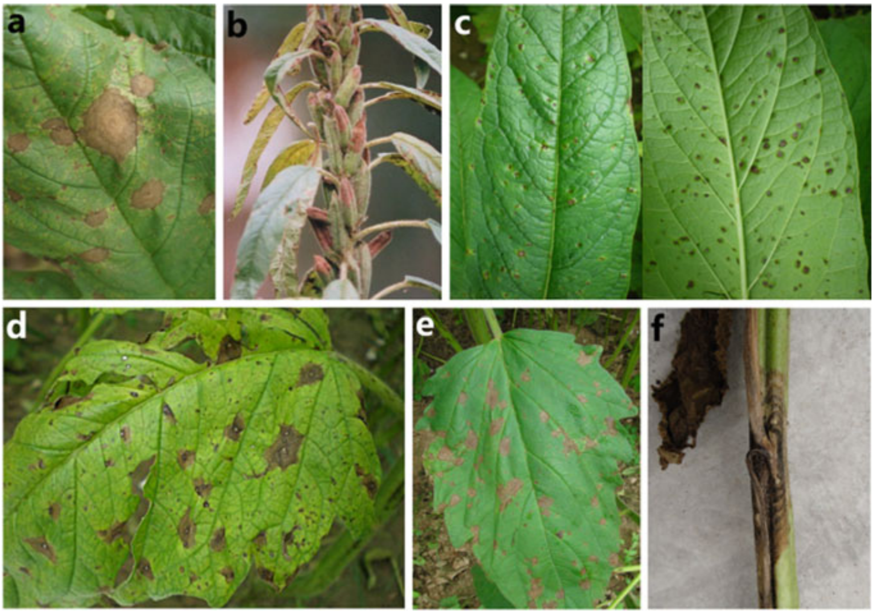

**Fig. 6.4 Symptoms of the main leaf diseases in sesame**. **a** Sesame leaf blight in leaf; **b** Sesame leaf blight in capsule; **c** Sesame Alternaria leaf spot in leaf; **d** Sesame brown spot in leaf; **e** Sesame Corynespora leaf spot in leaf; **f** Sesame *Corynespora* leaf spot in stem; **g** Nigrospora leaf blight in leaf (Provided by Hongyan Liu)

disease is caused by fungus *Ascochyta sesami* Miura; and Corynespora blight (or Corynespora blight spot) disease is caused by fungus *Corynespora cassiicola* (Berk and Curt) (Shivas et al. 1996). All the above five diseases are the most common leaf diseases in sesame (Zhao et al. 2014; Zhang et al. 2019).

Alternaria leaf spot disease is very common and could infect some wild species (Mehta and Prasad 1976; Dolle and Hegde 1984a). As to Corynespora blight, the pathogens can infect between plants under suitable conditions, and spread to stem, leaf petiole, and capsule, and cause disease aggravation. At early stage, the disease spots are angular brown with circular speckle lesions (5–15 m in diameter) and scatter on both surfaces of the infected leaf. Subsequently, the diameter of the lesions would expand. Previous report indicated that *Corynespora cassiicola* pathogen can exist in soil for more than two years (Qi et al. 2011). The fungus can be spread through seeds both internally and externally, and can survive in the plant debris. Therefore, the primary spread medium of the pathogens in the field should be seeds and plant residues. The secondary medium may be wind borne conidia.

In sesame field, some of the leaf diseases often occur simultaneously in population and are affected by both sesame genotype and environments. The complication of the disease occurrence limits the genetic inheritance analysis of the resistance to leaf diseases in sesame. Some studies reported that the seed color trait was related with the disease resistance (El-Bramawy et al. 2008; Li et al. 2014). As to the morphological characters of the sesame resistance to leaf disease, El-Bramawy and Shaban (2007) investigated the disease syndrome of 45d old sesame plants infected by *Alternaria* leaf spore suspension ($2 \times 10^3$ spore per mL) under greenhouse conditions according to Karunanithi (1996). After inoculated by *Alternaria* leaf spore suspension for 1 week, the leaf spot percent of the samples ranged from 1.32 to 18.54% and presented the mostly additive variance. Inheritance analysis results of the crosses of RT-273 (resistant) and Gulbarga Local Black (susceptible) showed that the resistance to Alternaria blight in sesame was controlled by single dominant gene pair (Eshwarappa 2010). For the advanced generations $F_3$ and $F_4$ under field condition, the resistance to Alternaria blight disease was controlled by the single dominance of gene action (Goudappagoudar et al. 2014).

#### 6.1.3.4 Other Diseases in Sesame

Besides the above diseases, powdery mildew (*Erysiphe cichoracearum*) and phyllody are also common and worldwide diseases in sesame (Venkata et al. 2013). Powdery mildew occurs in the season with low temperature. In addition, viral diseases are caused by virus ions and are transmitted by insects (mainly aphids). Viral diseases mostly occur in the seedling stage, with symptoms such as plant dwarfing, mosaic leaves, narrowing leaves, yellow-green spots, and deformed stems and leaves which finally result in fewer capsules and lower yield.

#### 6.1.3.5 Disease Control

Plant diseases are the interaction results between susceptible host plants and virulent pathogens under different environments. To control disease occurrence, some methods including agronomic management, pharmaceutical treatment, and cultivation management have been widely applied (Elad and Pertot 2014). Agronomic management is a traditional management method which could be traced back to the ancient times. The main management solutions include seed disinfection, reasonable close planting, crop rotation, water and fertilizer management, and soil preparation. Of which seed treatment such as seed priming can reduce the early infection of pathogens and is regarded as an effective and key method to control the disease spread. Moreover, harvesting crop on time is also an effective solution to reduce seed loss and control the fungal diseases.

As to the soil-borne pathogens such as *F. oxysporum* isolates, to realize the disease prevention prior to infection is the best choice (Lievens et al. 2008; Ghini et al. 2011). Cleaning up the infected left over debris and soil in time is necessary. Especially for susceptible crops, planting in the fields infected by pathogens should be avoided. In addition, giving the balanced nutrition with abundant potassium, nitrogen, and other vital nutrients is effective.

At present, spraying chemical fungicides is still the first choice for farmers to prevent and control crop diseases for the easy adaptability, convenient use, and immediate therapy. Powdery mildew occurs frequently in sesame disease, Kabiet al. (2019) reported that powdery mildew disease could be prevented by dusting sulfur and other chemicals. Moreover, biological control is another safe and effective tool for sesame disease control (Sunita et al. 2019). Till now, several strains with high biological control function to FOS and MP pathogens have been found and are being tested on sesame (Unpublished data, Haiyang Zhang). Therefore, studies on the effective control of fungal infection provide the promising methods for biological control in sesame. As far as the use of pesticides is concerned, in the case of spraying pesticides on a single leaf surface, 50 EC 0.1% profenofos with foliar spraying method has the better effects than 5% NSKE plant-derived insecticides on capsule stem borer, leafhoppers, and larvae control (Nayak et al. 2019).

Biocontrol techniques with the natural products and biological agents are ecofriendly and important for improving the seed yield and quality of sesame (Didwania 2019). Especially for the soil-borne pathogens, the use of biological agents is a promising method to realize the disease control (Deacon and Berry 2010). In the past few decades, some potential biological organisms have been isolated, identified, and gradually commercialized. Lubaina and Murugan (2015) evaluated the efficiency of *Trichoderma* species as biocontrol against *Alternaria sesami* in sesame. The results showed that *T. harzianum* can colonize and inhibit the growth of *Alternaria* pathogen which exerted an obvious biological control effect. Moreover, applying microorganisms is also a biological control method to inhibit the phytopathogens (Baker and Paulitz 1996). *VA mycorrhizal* fungi can protect plants from damage via increasing the activity of the antifungal chitinase enzymes in roots (Zeng 2006). Ziedan et al. (2011) developed biotic agents such as *Trichoderma* and *VA mycorrhiza* to control sesame wilt and root rot diseases. The results showed that the protection effects were great and the seed yield significantly increased. The mixed inoculation using *mycorrhizal* symbiotic bacteria and biological control agent was more effective than applying single strain (Ziedan et al. 2011).In order to understand the role of silicon in the prevention and treatment of sesame charcoal rot, the effects of different silicon sources on the mycelial growth of *M. phaseolina* were performed in vitro (Siddiqet al. 2019).

At present, biological control is attracting more and more attention for agricultural practices. Integration of biological control agents, plant botanicals, and organic amendments can reduce the use of farmland fungicides and ensure the safety of agricultural ecology (Nayan et al. 2019). Considering the demand for green and healthy seed products, safe alternatives to chemical fungicides should be emphasized and applied in the future.

### *6.1.4 Insect-Pests and Their Management*

Sesame yield is easily affected by insect-pests. Insect-pests are one of the main factors restricting sesame production. Twenty nine pests attacking sesame in India (Delhi) have been reported (Baskaran et al. 1997). Especially, leaf webber and capsule borer (*Antigastra catalaunalis*), gall midge (*Asphondylia sesami*), pod sucking bug (*Elasmolomus sordidus*), sphingid moth (*Acherontia styx*), leaf hopper (*Orosius albicinctus*), and white fly (*Bemisia tabaci*) are the main pests for sesame (Baskaran et al. 1997). Of which sesame leaf webber and capsule borer (Lepidoptera: Pyralidae) are the most harmful pest for sesame and can cause the loss up to 90% and 80.42%, respectively (Gnanasekaran et al. 2010; Wazire and Patel 2016).

Many insect-pests have a negative impact on crop yield and quality. Insect-pests in the field are difficult to control. In agricultural production, pesticides are often used to control insect-pests. However, the hidden dangers of drug residues often exist. Ecofriendly pest control methods have been widely used and present the privileges in protecting the agricultural product quality and the surrounding ecosystem. For sesame, the diseases and insect-pests control solutions are being changed from the traditional chemical method to the biological control method now. Some research results have confirmed that salicylic acid can promote plant growth and inhibits the infestation of *M. persicae*, *B. tabaci*, *E. lybica*, *Creontiades* sp., *N. viridula*, and phyllody pathogens (Mahmoud 2013). Liu et al. (2017) integrated a set of comprehensive prevention and control technology system for sesame diseases and insect-pests, based on the individual prevention and control techniques in Northwest China. The control system presented the high effects on insect-pests, diseases, and weeds in sesame field (Liu et al. 2017).

## 6.2 Disease Resistance Breeding in Sesame

### *6.2.1 Traditional Breeding for Disease Resistance*

Crop varieties with high disease resistance inherit the resistance to diseases and enhance the protection from pathogen infection. In the past 50 years, sesame scientists in the world used the simple hybridization, physical and chemical mutagenesis, and heterosis breeding methods in sesame breeding and bred hundreds of new sesame varieties. The resistance of the varieties to pathogens is improved accordingly. With the development of agriculture and crop production, aggregating more elite genes with high resistance to pathogens using new modern breeding techniques seems more necessary.

In the previous studies, some researchers proposed to create male sterile lines and to breed hybrid varieties using the heterosis character of sesame (Pal 1945; Tu et al. 1995; Zhao and Liu 2008). The first sesame male sterile line was found by an Indian scientist and was introduced to the world (Tu et al. 1995). In 2003, the first sesame hybrid ‘Yuzhi 9’ was bred by Chinese scientists and the two-type sterile lines hybrid technique was formed accordingly (Zheng et al. 2003). Meanwhile, mutagenesis technique was also applied to obtain a wide range of male sterile mutants (Li and Chen 1998; Zhao and Liu 2008). However, the male sterile lines currently used in China still have some disadvantages including environmental sensitivity, incomplete sterility, and the high manual cost for 50% of male fertile seedlings removal during producing hybrid seeds (Zheng et al. 2003).

### *6.2.2 Rationale for Molecular Breeding*

Traditional breeding methods are relatively time-consuming and labor-intensive, and require high personal work experience for breeders. In recent years, several modern biological techniques such as genome sequencing and genetic modification rapidly developed. The application of the modern biological techniques, especially the next generation sequencing technique reinforces the modern plant breeding techniques. Modern molecular breeding technology represented by molecular marker breeding, genetically engineering, and molecular design breeding is gradually becoming the mainstream of crop breeding methods around the world. Compared with the traditional breeding techniques, molecular breeding has the advantage of increasing yield and saving labor. With the support of big data, molecular breeding techniques can significantly improve breeding efficiency. The breeding cycle is significantly shortened accordingly.

To further increase the yield level and the total sesame production, and to narrow the gap between demand and supply, new elite varieties with high and stable yield level are more requisite. In a recent few years, the Chinese scientists bred a series of sesame varieties such as Wanzhi, Yuzhi, and Zhongzhi. For example, Wanzhi

No. 10 is a new hybrid bred by the Crop Research Institute, Anhui Academy of Agricultural Sciences, China. The disease index of the variety to stem spot blight and Fusarium wilt diseases was 5.07 and 0.85, respectively. The average yield reached to 1261.65 kg/hm$^2$. The oil and protein content of the seeds were 58.18 and 20.97% (Li et al. 2018). Gnanasekaran et al. (2010) comprehensively considered the genetic effects of the disease resistance, combining ability, and the yield contributing traits and selected more excellent lines with high yield and resistance potential. In 1984, a new variety 'Ahnsankkae' was bred via 20 krad X-ray irradiation, and has become the main sesame variety in Korea for the high yield and strong resistance. Meanwhile, 'Sunwonkkae' is a hybrid derived from an X-ray mutants and Korean local varieties in 1991 (Kang et al. 1994). The goal of traditional crop breeding is clear and mainly focuses on the yield, seed quality or disease resistance of the varieties through various breeding techniques to maximize the advantages of breeding improvement.

Modern biological technology improves the development of molecular breeding techniques including screening disease resistance related genes and molecular markers and genetic transformation or gene editing, and provides an effective method to realize the high breeding efficiency with more new varieties with high disease resistance (Zhang et al. 2012). Marker assisted selection is being applied in breeding projects and for aggregating more disease resistance genes. For example, a new dwarf sesame variety 'Yuzhi Dw609' (Fig. 6.1) was bred from the first dwarf variety Yuhzi Dw607 with short internode length trait by the Henan Sesame Research Center, Henan Academy of Agricultural Sciences (HAAS) in the past a few years. With the aid of hybridization technique and molecular marker assisted selection, Yuzhi Dw609 inherits both the short internode length trait from the parent Yuzhi Dw607 and the high resistance character from the other parent Ganzhi 9 (Unpublished data, Haiyang Zhang).

### *6.2.3 Breeding Objectives*

Breeding disease-resistant varieties requires the knowledge of genetics and inheritance of the disease resistance. The success of any plant breeding program largely depends on the selection of appropriate parents. A few wild *Sesamum* species such as *S. malabaricum* and *S. mulayanum* seem to possess high tolerance to powdery mildew (Venkata et al. 2013). However, elite genes introgressing into the cultivars from wild relatives is still difficult for sesame because of lacking the effective interspecific hybridization techniques. Developing innovative breeding techniques needs long-term work in the future.

## 6.3 Genetic Diversity Related to Disease Resistance in Sesame

### *6.3.1 Phenotype-Based Diversity Analysis*

Sesame has a long cultivation history in China, India, Near East, and Central Asia. These regions are diversity centers for sesame germplasm. Germplasm exchange and selection provides a huge breeding space for sesame scientists (Laurentin and Petr 2006; Mahajan et al. 2007). Thus, assessing the morphological and the genetic diversity of the cultivated accessions is meaningful for genetic base expansion and genetic resources protection during breeding new varieties (Amini et al. 2007). For sesame, the morphological and agronomic traits of a great deal of germplasm accessions have been investigated and the genetic diversity is evaluated based on various morphological and agronomic characteristics, isozyme analysis, and molecular marker polymorphism (Liu et al. 1997). In early stage, the expressed sequence tag-simple sequence repeat (EST-SSR) markers were used to evaluate the level of genetic variation among different sesame genotypes. However, the number of SSR markers used for research on diversity analysis and sesame linkage map construction was very limited before the initiation of the sesame genome project (Zhang et al. 2019).

### *6.3.2 Genetic Background of the Resistance to Diseases*

The primary aim of breeding for sesame scientists is to improve the seed yield. In the breeding genotypes, the resistance to sesame blight, charcoal rot and black spot differs greatly (Kavak and Boydak 2006; El-Shakhess and Sammar 1998). Breeding methods depend to a large extent on the nature and number of the genes that control the genetic behavior of most research traits. In addition, understanding the nature and extent of the gene effects on yield and yield components, as well as the resistance to major diseases (such as Fusarium wilt, charcoal rot, and black spot), is useful for formulating effective breeding strategies for genetic improvement in sesame (Zhang et al. 2019).

### *6.3.3 Relationship with Geographical Distribution*

In the worldwide sesame production regions, at least eight economically important fungal diseases commonly occur (Kolte 1996). In Iran, *Macrophomina phaseolina* is an important soil pathogen, causing charcoal rot in many important crops including sesame (Salahlou et al. 2016). In the history of sesame cultivation, some genotypes from different geographical regions were clustered in the same group. Thus, Pissard et al. (2008) proposed that geographic distribution is an important parameter for germplasm collection, even though the geographic origin character is not always able to predict the genetic difference. Selecting genotypes with suitable genetic distance and excellent agronomic traits in crosses can help breed excellent varieties (Parsaeian et al. 2011). Therefore, in order to improve the disease resistance of sesame varieties, both plant materials exchange and selective crossing between the parents with different geographic distributions and disease resistance levels should be encouraged.

### 6.3.4 Relationship with Other Cultivated Species and Wild Relatives

As we all know, the use of the host plants with high resistance to biotic stresses is a more practical strategy to realize the high yield and seed quality. During breeding new varieties with high disease resistance, understanding the genetic patterns of the disease resistance or drug resistance in hosts is necessary. Even though the wild sources such as *S. malabaricum*, *S. mulayanum*, and *S. prostratum* always have high resistance to pests and diseases, no significant research progress in interspecific hybridization breeding has been achieved so far. The application of the advanced technologies such as molecular markers assisted breeding with resistance genes is a prerequisite for the success of breeding programs (Venkata et al. 2013). Mehetre et al. (1994) reported that *S. mulayanum*, a wild relative of sesame has the high resistance to powdery mildew as well as phyllody (Mehetre et al. 1994). Krishnaswami et al. (1983) studied the heredity of powdery mildew resistance in $F_2$ offspring derived from the susceptible and the resistant parents. The results showed that the resistance to powdery mildew is controlled by the action of two major genes and complementary genes, which provide the foundation for the improvement of powdery mildew-resistant varieties (Krishnaswami et al. 1983).

### 6.3.5 Artificial Induction and Incorporation of Resistance Traits

The morphological and agronomic traits are greatly influenced by environmental factors. However, to change the genetic character and collect the elite traits is the basic solution to improve the agronomic traits in crops. For sesame, breeding is done by using mutagenesis by physical and chemical mutation, genetic transformation, and interspecific hybridization (Zhang et al. 2019). For example, South Korean scientists have bred 14 sesame varieties since 1955. Of these two varieties were bred through mutation breeding. From 1989 to 1992, 2625 mutant pedigree lines and 89,200 plants were planted and investigated (Kang et al. 1994). In addition, sodium azide (NaN)-treatment was also used to create new lines. A dwarf mutant line, ‘Suwon 128’, which was unique in the dwarf shape and strong lodging resistance, was obtained and used for variety breeding (Kang et al. 1994).

In addition, the development of high-throughput sequencing technology greatly promotes the detection of a great amount of sequence-based molecular markers and the application in genetic variation research in sesame.

Root rot is the most harmful disease for the sesame industry. At present, there is no effective prevention and management approach available for root rot resistance. Study of host resistance is the key to solving this disease. In addition, due to the lack of understanding of the molecular mechanisms of *M. phaseolina* interacting with the host, it is not feasible to develop resistant genotypes through genetic engineering. On the basis of genome-wide research, cultivating varieties with inherent resistance is one of the most effective and economical means to control sesame root rot. However, there are currently little data on the genetic analysis of sesame root rot resistance (El-Bramawy and Shaban 2007). Recently, Yu et al. (2013) compared the NBS (nucleotide binding site) type resistance gene analogs (RGAs) and EST sequences and cloned 16 full-length RGAs from 10 different resistant cultivars against *Macrophomina phaseolina* (GenBank accession number: KC477692-KC477707). All these RGA gene sequences belong to the non-TIR-NBS type R genes and contain the specific NBS domains. This result laid the foundation for further screening of resistance genes for *Macrophomina phaseolina* in sesame (Yu et al. 2013).Wang et al. (2017) developed new molecular markers with the aid of genome data and constructed molecular genetic maps. As a result, the genetic maps were applied to determine the QTLs related to the resistance to charcoal rot disease.

## 6.4 Genetic Analysis and Association Mapping Studies

### *6.4.1 Molecular Marker Development in Sesame*

Compared with the phenotypes and isoform proteins, molecular markers have more advantages for germplasm assessment (Pissard et al. 2008). In the early stage, many studies have been performed to develop the universal molecular markers such as random amplified length polymorphism (RAPD), amplified fragment length polymorphism (AFLP), and inter-simple sequence repeat (ISSR) and revealed the application of the molecular markers in the genetic diversity analysis and association mapping of agronomic traits in sesame (Isshiki and Umezake 1997; Bhat et al. 1999; Ercan et al. 2002, 2004; Kim et al. 2002; Hernan and Petr 2006; Ali et al. 2007; Zhang et al. 2010; Shinwari 2011; Li et al. 2014; Daret al. 2017). Parsaeian et al. (2011) studied the genetic variation in 18 sesame genotypes from Iran and the six foreign genotypes from the Asian countries. The results showed that the genotypes of the various agricultural morphological traits were significantly different, and the genetic variation coefficients of the number of capsule branches per plant, capsule number, plant height, and seed yield per plant were relatively high. According to the polymorphism of RAPD markers, large genetic differences existed between the foreign and Chinese domestic germplasm accessions (Zhang et al. 2004).

Subsequently, the specific markers, such as the SSRs and EST-SSRs were developed and applied for genetic map construction, and genetic diversity and trait association analysis in sesame (Powell et al. 1996; Dixit et al. 2005; Wei et al. 2009). Wang et al. (2017) detected a total of 110,495 genomic SSRs from the sesame genome (var. Zhongzhi No. 13) and provided a plenty of polymorphic SSR markers. In the recent years, construction of the fine genome map for sesame and the genomic analysis has significantly improved the molecular breeding techniques in sesame (Laurentin et al. 2008; Zhang et al. 2019). Wei et al. (2014) combined the three RNA-Seq datasets and compared the single nucleotide polymorphism (SNP) and insertion/deletion (InDel) variants in the three transcriptome sets for the first time. With the abundant genome and transcriptome information, Zhang et al. (2016) constructed the first high-solution SNP genetic map with millions of SNPs in the cultivated sesame varieties and located a gene controlling the inflorescence determinacy. Till now, so many SNP and InDel markers have been detected and applied in the genetics research in sesame (Wei et al. 2014; Wu et al. 2014b; Zhang et al. 2016; Zhang et al. 2019).

### 6.4.2 *Molecular Genetic Linkage Maps and QTLs Related to Disease Resistance in Sesame*

For sesame, the first molecular genetic linkage map was constructed by HAAS using an $F_2$ population and eight EST-SSRs markers, 25 AFLP markers, and 187 RSAMPLs (random selective amplification of microsatellite polymorphic loci) markers in 2009 (Wei et al. 2009). Of which the 30 linkage groups carrying the 220 markers were formed. Subsequently, the map was saturated with more SSR markers and using larger $F_2$ population with 260 individuals (Zhang et al. 2013b). Four QTLs linked to the seed coat color trait were detected. Till now, to our knowledge, at least eight molecular genetic linkage maps have been constructed for sesame (Zhang et al. 2013c; Wu et al. 2014a; Wang et al. 2017; Uncu et al. 2016; Zhang et al. 2016; Mei et al. 2017; Du et al. 2019; Liang et al. 2021). Of these two SSR genetic maps constructed using a recombinant inbred lines (RIL) population were used to locate the QTLs for the waterlogging tolerance and charcoal rot disease resistance traits (Zhang et al. 2013b; Wang et al. 2017).

In order to improve the genome map construction, the first ultra-dense SNP genetic map was developed using the Illumina genome re-sequencing data of an $F_2$ population (Zhang et al. 2016). A total of 3,041 bins representing 30,193 SNPs distributed on the 13 linkage groups (LGs) were developed and the even correspondence of LG and chromosome (Zhang et al. 2016) was realized. The ultra-dense SNP genetic linkage map has been utilized for genome assembly and gene location because of the saturated SNP density.

Meanwhile, to explore the genetic inheritance of the key agronomic traits, the above molecular genetic maps have been used to locate the QTLs related to yield, seed quality, and disease resistance traits (Miao et al. 2017; Zhang et al. 2019; Liang et al. 2021). For example, Wang et al. (2017) detected 10 QTLs significantly associated with the resistance to charcoal rot disease on five LGs using an RIL population. The phenotypic variance explained of the 10 QTLs varied from 5 to 14%. With the aid of the first ultra-dense SNP genetic map and the resistance variation of the $F_{2\text{-}3}$ families to Fusarium wilt disease, Miao et al. (2017) determined a QTL location on LG8 (111.5–112.7 cM). The explanation ratio reached to 51.66% ($P < 0.001$). As a result, the first marker *SiFWR2145* associated with the resistance to Fusarium wilt disease was developed and applied for elite SNP selection during disease resistance breeding.

## 6.5 Genomics-Aided Breeding for Disease Resistance

### *6.5.1 Sesame Genome and Resistance Genes*

In order to improve the sesame breeding techniques, the Sesame Genome Working Group (SGWG) initiated the Sesame Genome Project in 2010 (Zhang et al. 2013a). Based on the complicated sequencing and assembly platform, the chromosome scaled genome map was constructed for sesame (Zhang et al. 2016, 2019). The fine genome is 335.19 Mb in size and contains 31,462 genes (Zhang et al. 2013a; Zhang et al. 2019). At present, the fine genome version 3 for *S. indicum* (var. Yuzhi 11) has been uploaded to NCBI public database. The sesame genome information and the genomics results will be published soon.

In the fine genome version 3, a total of 31,462 genes were annotated (Unpublished data, Haiyang Zhang). Based on the GO database (Gene Ontology Consortium 2004) (http://www.geneontology.org/), all the 31,462 genes were classified into three main categories (i.e., ‘Molecular function’, ‘Biological process’, and ‘Cellular component’ categories) (Fig. 6.5). About 41.53% genes were classified into the category ‘Biological process’, of which 21.86% genes belonged to the ‘metabolic process’ group, followed was the ‘cellular process’ (21.50%). In the second category of ‘Molecular function’, 31.81% genes were further classified into the 15 groups. The top group was ‘binding’ (15.32%). Meanwhile, the third category of ‘Biological process’ contained 26.66% genes.

Besides the Illumina sequencing platform, Wang et al. (2014) assembled and published a sesame genome draft (var. Zhongzhi No. 13). Uncu et al. (2015) performed the genome sequencing of var. MMuganli 57 using the Roche 454 GS-FLX technique and obtained 65 Mb sequences with the genome coverage of 19.3%. All the sesame genome data supplies precious information for genomics, genetics, and breeding research in sesame and other crops.

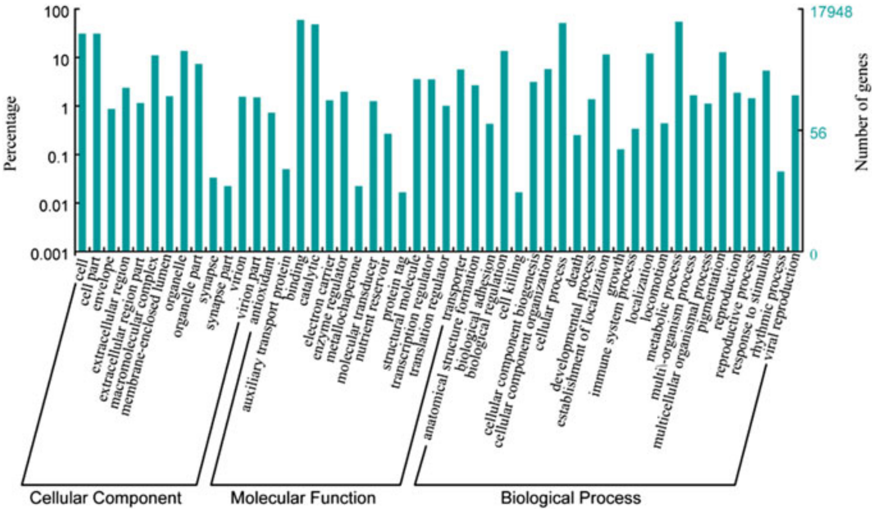

**Fig. 6.5** **GO classification of the sesame genes**. The left vertical axis indicates the gene percentage. The right vertical axis indicates gene number. The horizontal axis indicates gene group type and category (Provided by Haiyang Zhang)

### 6.5.2 *Disease Resistance Gene Digging*

Genome data and the genome re-sequencing techniques stimulated the genome wide association studies (GWAS) of the key agronomic traits in sesame (Wei et al. 2015; Li et al. 2018; Zhang et al. 2019). As to identify molecular markers associated with the resistance to stresses, Wei et al. (2015) performed the GWAS using 705 sesame accessions (Wang et al. 2014). A total of 549 loci associated with the 56 agronomic traits were detected in the population under four environments. Of these 13 loci were related to the susceptibility of phyllody disease. With the GWAS analysis method, one SNP associated with Fusarium wilt resistance using 560 germplasm accessions was determined recently and confirmed the resistance character of dominant gene control in sesame Fusarium disease (Unpublished data, Haiyang Zhang). We believe the genomics research should significantly contribute to the marker assisted breeding in sesame in near future.

In sesame, there are some key orthologous gene families of the AP2/ERF transcription factors and Hsfs (heat shock transcription factors) genes which are probably related with the resistance and tolerance to the biotic and abiotic stresses (Wei et al. 2015; Dossa et al. 2016a, b). Orthologous gene analysis indicated that all these genes probably play the role in regulating drought tolerance and disease resistance in sesame. In sesame, there are about 132 AP2/ERF orthologous genes (Dossa et al. 2016b). The transcription indicated that AP2/ERF gene family was regulated under the drought stress in sesame. In addition, comparative genomics analysis between *S. indicum* and wild *Sesamum* species and other oilseed crops was performed using the fine genome maps. The results indicated that some key disease resistance gene families presented the expansion or constriction characters. The variation of the several R resistance genes or families might be related with the relative low resistance to diseases in the cultivated sesame (data now shown, Haiyang Zhang). Thus, introducing new gene resources from the wild species using modern molecular breeding methods is a prosperous direction for future sesame breeding.

### *6.5.3 Genome Assisted Breeding in Sesame*

In the last two decades, the key objective of sesame breeding was to breed new lines with high and stable yield potential. A few genes involved in regulating the synthesis of fatty acids, seed storage proteins, and secondary metabolites and the tolerance to salt stress have been determined using the gene mapping and homolog analysis (Yukawa et al. 1996; Jin et al. 2001; Lee et al. 2005; Chyan et al. 2005; Hsiao et al. 2006; Kim et al. 2007, 2010; Hata et al. 2010; Zhang et al. 2019). The Sesame Genome Project provides the necessary information for identification of genes and genomics assisted breeding in sesame. With the application of the sequencing technologies and based on the genetics and genomics research in sesame, more than one hundred QTLs, candidate genes, and associated molecular markers were detected (Zhang et al. 2013a, b; Miao and Zhang 2014; Wang et al. 2014; Wei et al. 2015; Dossa et al. 2016a; Miao 2016). We thus believe that the resistance genes to the main diseases, such as Fusarium wilt and charcoal rot should be identified in near future (Zhang et al. 2019).

## 6.6 Future Perspectives and Potential for Boosting Up of Sesame Productivity

In the past two decades, the modern biological technologies and high-throughput sequencing platform accelerated the molecular genetics and breeding research in sesame. However, we have to know that the efficiency of the interspecific hybridization and transgene techniques in sesame is still low and limits the innovation of the breeding techniques. Gene editing technique is immature in sesame. To realize the molecular design with the new varieties with high resistance to environmental conditions is also a huge task for sesame breeders. Before performing the concise molecular breeding technique, more new breeding materials with multiple elite traits should be found or created; more precise molecular markers and genes should be determined and utilized for target screening. Meanwhile, the regulation network of the multiple marker loci and the interaction in sesame genome should be clarified. Therefore, large-scale gene cloning and gene function research are still the main tasks (Zhang H. personal communication). With advance of the sesame genomics research, we believe that the genome designing for sesame resistance breeding and new elite line creation should be carried out in near future.

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