Chapter 3
Resistance to Biotic Stress: Theory and Applications in Maize Breeding

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Abstract By virtue of its higher genetic diversity, maize has better adaptability to various climatic situations and has high yield potential than other cereals. However, the incidence of pests and diseases at different stages of the crop can reduce the yield drastically. Several strategies have been adopted to manage biotic stresses in maize to maintain the yielding ability. Apart from the chemical method of disease

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C. Kole (ed.), *Genomic Designing for Biotic Stress Resistant Cereal Crops*,
https://doi.org/10.1007/978-3-030-75879-0_3

management, improving the crop for natural resistance has paid much dividend for sustainable maize production. With the advent of high throughput phenotyping method followed by genotyping, targeted trait improvement has become easy. Molecular marker technology—a non-destructive method—enables indirect selection of genotypes without exposing them to epiphytotic condition. This has been found to be efficient over existing traditional methods of screening followed by selection. The information on QTLs, novel genomic resources have provided better understanding of tolerance traits. Although GE technologies have been successful in development of genotypes to combat pathogens in important crops, they are not yet fully exploited for the management of insect pests. The most important limitation has been the lack availability of target genes at present against the insect pests. Genome editing is becoming powerful tool which enables the possibilities of developing resistant gene by targeted gene modification. Though maize is recalcitrant to regeneration, protoplast transient assay made easy the utilization of CRISPR technology in developing disease resistant maize. Institutional support followed by policy intervention makes new technological interventions finding way for improving crops. Social beliefs and ethical issues should be taken care while targeting next generation breeding approaches to develop insect or disease resistant maize.

Keywords Biotic stresses · Genetic diversity · Breeding approaches · Molecular mapping · New biotechnological tools · Transgenics · Social issues

3.1 Introduction

Maize (*Zea mays* L.) is the most important cereal crop worldwide after wheat and rice with the global production of 1147 Mt. Among the top corn producing countries, United States hold the first position with the yield of 11.86 tha^{-1}, followed by European Union, Ukraine, China, Argentina and India. Generally, maize is grown for grain or fodder and silage production. It is having direct economic value on mankind as grain is primarily grown for human consumption, especially in tropics. In Asia, compared to human food, the demand for maize as an animal feed will have more impact on the production scenario. More than doubling of production is expected from the present level of 165 Mt to almost 400 Mt in 2030 (Paliwal et al. 2000). The Food and Agriculture Organisation (FAO) predicts the requirement of an additional 60 Mt of maize grain to meet the demand by 2030. Maize is a versatile crop, having wider adaptability to different climatic situations, from temperate to tropical conditions. Being a C4 crop, maize has the highest yield potentiality compared to other cereals, but due to the damage by insect and pest attack, global maize production is under threat. One of the main deterrents to achieving grain yield in maize is its susceptibility to many pests and diseases (Devi and Thakur 2018).

3.2 Description on Different Biotic Stresses

3.2.1 Maize Diseases

Among the maize diseases caused by fungi, bacteria and viruses, fungal diseases like banded leaf and sheath blight (BLSB), turcicum leaf blight (TLB), maydis leaf blight (MLB), post-flowering stalk rots (PFSR) complex, downy mildews (DM), rust, smut, seed rots and seedling blights, leaf spots and blights etc. are of major concern (Saxena et al. 2008). Under favourable conditions, these diseases cause immense losses to both quantity and quality of grain produced. World maize trade in 2019–20 is now forecast to reach nearly 167 Mt, almost unchanged from the previous season despite experiencing annual global yield loss recorded up to 20–41% in maize (FAO 2020).

Maydis leaf blight (MLB) disease or southern corn leaf blight (SCLB), caused by *Bipolaris maydis* (Nishik. and Miyake) Shoemaker [*Cochliobolus heterostrophus* (Drech.) Drech.] is one of the impending threats to global maize production. The pathogen *B. maydis* possesses three physiological races viz. race O, race T (Hooker 1972; Ullstrup 1972), and race C (Wei et al. 1988). The race-T is more prevalent in the United States of America (USA). In USA, it resulted in an epidemic during 1970 by the extensive usage of CMS-T cytoplasm based maize lines to develop commercial maize hybrids. The race C is prevalent in China and is pathogenic on maize inbred lines having CMS-C cytoplasm (Wei et al. 1988). On the other hand, the race 'O' is predominantly prevalent in the southern Atlantic coast of the USA, India, Africa, and Western Europe (Balint Kurti et al. 2007), which can infect all types of susceptible maize cultivars, irrespective of the cytoplasm (Smith 1975) and can reduce the grain yield up to 41% (Sharma et al. 2005).

Banded leaf and sheath blight (BLSB) disease is caused by a versatile soil borne fungus *Rhizoctonia solani* f. sp. *sasakii* (Kuhn) Exner [teleomorph: *Corticium sasakii*, syn. *Thanatephorus cucumeris* Frank (Donk)] which is not producing any spores. Generally, this pathogen is identified by characteristics of the mycelium and sclerotia. The pathogen is an imperfect fungus (Deutermycetes) belonging to AG 1-1A anastomosis group of *R. solani* isolates (Yang and Li 2012; Hooda et al. 2015).

Post-flowering stalk rots (PFSR) are the world's most destructive diseases of corn. Diseases such as Fusarium Stalk Rot (*Fusarium verticillioides* (Sacc) Nirenberg, Syn *F. moniliformae*), Charcoal Rot (*Macrophomina phaseolina* (Tassi) Goid.) and Late Wilt (*Cephalosporium maydis* Samra, Sabet. and Hingorani) are commonly associated with PFSR. Among them, charcoal rot (*M. phaseolina*) is dominant one and occurs as a complex along with *F. verticilloides* in some locations. *M. phaseolina* is an anamorphic ascomycete of the family Botryosphaeriaceae and causes the disease charcoal rot on a broad range of plants in many areas of the world. The lack of a known teleomorph has hindered its proper taxonomy (Crous et al. 2006).

Turicum leaf blight (TLB) or northern corn leaf blight (NCLB) is another important disease caused by an Ascomycete *Exserohilum turcicum* (Pass.) Leonard and Suggs [*Setosphaeria turcica* (Luttr.) K. J. Leonard and Suggs, formerly known as *Helminthosporium turcicum*] which belongs to family Pleosporacae (Leonard et al. 1989). In the United States various races of the pathogen exist, of which race 'O' was predominant in the mid-1970s, Race 1 was the most prevalent race in the region by the mid-1990s (Ferguson and Carson 2007). The Indian scenario of the races of *S. turcica* is blurred so far.

Downy mildews (DM) are caused by a group of Oomycetes like *Perenosclerospora sorghi* Weston &Uppal (Sorghum downy mildew), *Sclerophthora rayssiae* Kenneth, Koltin & Wahl (Brown stripe downy mildew), *Peronosclerospora sacchari* Miyake and Shaw (Sugarcane downy mildew) and *Pernosclespora heteropogoni* (Rajashan downy mildew). All these genera cause both external and systemic infection. As a result, the severely affected plants do not produce any ear or tassel or in most cases deformed ears are developed that directly affect the grain yield (Kenneth 1970; Bock et al. 2000; Isakeit and Jaster 2005).

Rusts in maize are of two types. The common rust is caused by *Puccinia sorghi* Schwein (also known as *Puccinia maydis*). The second one is polysora rust or tropical rust or southern rust caused by *Puccinia polysora* Underw. The physiological races of *P. polysora* were reported long back by Ryland and Storey (1955). Seventeen virulence patterns were identified among the 60 isolates tested (Casela and Ferreira 2002). *Puccinia sorghi* can cause severe damage to susceptible maize varieties and limit production mainly in tropical countries. However, the threat has largely been overcome by resistant varieties. *Puccinia sorghi* is no longer a serious problem on maize although late season plantings are severely affected. Commonly the hosts of *P. sorghi* are maize and *Oxalis* species (wood sorrel). Different spore-producing stages *of P. sorghi* occur on each host, but the sexual stages occur on *Oxalis.*

3.2.2 Maize Insects

About two dozen insect species cause economic damage to maize globally (Ortega and de Leon 1974; Guthrie 1989). The most damaging and difficult to manage among them are the stalk borers. They feed on the foliage in the beginning and later bore into the stalk, where it kills plants or drastically reduce the yield by stalk tunneling which affects xylem and phloem transportation, leading to stunted plant growth. Since maize has high foliage compensation ability, yield reduction is mainly caused by stalk damage. The pests coming under this category are European corn borer [*Ostrinia nubilalis* (Hübner)] in North America, Europe and North Africa, Asian corn borer or Oriental corn borer [*Ostrinia furnacalis* (Guenee)], spotted stemborer [*Chilo partellus* (Swinhoe)], Mediterranean corn borer or pink stem borer [*Sesamia nonagrioides* (Lefebvre)] or pink borer [*Sesamia cretica* (Led)], African maize borer [*Sesamia calamistis* (Hmps)], pink stem borer

[*Sesamia inferens* (Walker)], African maize stalk borer [*Busseola fusca* (Fuller)], African sugarcane borer [*Eldana saccharina* (Walker)], Southwestern corn borer [*Diatraea grandiosella* (Dyar)], American sugarcane borer [*Diatraea saccharalis* (Fabricius)], neotropical corn borer [*Diatraea lineolata* (Walker)].

The only foliage feeder which cause economic loss because of its voraceous feeding habit is fall armyworm [*Spodoptera frugiperda* (J. E. Smith)]; causes direct damage to corn ears too. This pest is currently posing a global challenge since its invasion in Africa in 2016, Asia in 2018 and Australia in 2020. The pests directly causing aesthetic and economic damage to corn ears are corn earworms [*Helicoverpa zea* (Boddie)] and *Helicoverpa armigera* (Hübner), where the former is more damaging and restricted to Americas. The economically damaging corn rootworm complex, [*Diabrotica* spp. viz., the western corn rootworm (*Diabrotica virgifera virgifera* LeConte), the northern corn rootworm (*Diabrotica barberi* Smith and Lawrence) and the southern corn rootworm (*Diabrotica undecimpunctata* howardi Barber)] cause damage to roots, where 15% yield loss per each damaged node is predicted (Tinsley et al. 2013). Corn rootworm species are native to the western hemisphere, however, WCR, the most damaging among all, invaded Europe (Berger 2001).

Corn leaf aphid [*Rhopalosiphum maidis* (Fitch) is the globally distributed sucking pest of maize, which usually attack pre-tasseling stage to grain filling stage and occasionally cause economic damage. Average density of 818 aphids at V10–VT stage corn can cause 28% yield reduction (Al Eryan and El Tabbakh 2004). Plant hoppers *Cicadulina mbila* (Naude) and *Peregrinus maidis* (Ashmead), cause damage primarily by acting as vector of viral diseases like maize streak virus (MSV) and maize stripe virus (MStV) respectively in maize (Roca De Doyle and Autrey 1992).

The main storage pests of maize, which cause loss of quality and quantity of maize grains across the world, are maize weevil [*Sitophilus zeamais* (Motschulsky)], angoumois grain moth, [*Sitotroga cerealella* (Olivier)], the lesser grain weevil [*Sitophilus oryzae* (Linnaeus)] and the larger grain borer [*Prostephanus truncatus* (Horn)]. *P. truncatus* the most damaging among all, is restricted to Americas and Africa. Grain weight losses due to *S. zeamais* and *P. truncatus* can go up to 20 and 35% respectively (Tefera et al. 2016). In addition, several minor and potential pests attack maize around the world causing less frequent economic damage.

Occurrence and severity of insect pests of maize vary by geographical location and vary by season within a geographical area. Since insects are cold-blooded animals, they generally prefer a temperature range of 25–35 °C. They undergo diapause in harsh summer periods, influencing their number of generations produced in a year; so is the severity of damage. For example, two generations of ECB are observed in maize crop of United States, whereas only one generation occurs in central Europe (Bohn et al. 1999).

Similarly, *C. partellus*, the most destructive native pest of maize in India, is more prevalent in *kharif* crop, where the extent of crop loss was about 27–80% (Jalali et al. 2014). Whereas in Nepal, a country with less geographic and climatic

variability, a narrow range of yield reduction (27–30%) was reported (Sharma and Gautam 2010). Severity of infestation and yield losses caused by maize stemborers varies in African continent, where geography, season, cultivars and cultivation practices are the contributory variables. In East Africa, *C. partellus*, *C. orichalcociliellus*, *E. saccharina*, *B. fusca*, and *S. calamistis* are major maize stemborers where, the later three occurs as major pests in West Africa also. In South Africa, *B. fusca* and *C. partellus* are the only major pests (Kfir et al. 2002; Sharma and Gautam 2010).

3.3 Stages and Extent of Damage

Among the various biotic factors causing damage to the maize crop, diseases viz., maydis leaf blight (MLB), banded leaf and sheath blight (BLSB), downy mildews (DM), rust, smut, and post flowering stalk rots (PFSR) etc. are most important (Singh and Shahi 2012). Under ideal circumstances, these diseases inflict immense losses both in quantity and quality of grain produced (Yadav et al. 2015). Annually around one percent of the total grain yield is reduced by BLSB alone in India (Sharma et al. 2005). But premature death of plants by diseases can cause drastic reduction in grain yield near to 97% (Sagar and Bhusal 2019). Similarly, MLB causes considerable yield losses even up to 70% (Kumar et al. 2009). Losses due to the downy mildews from India and several SE Asian countries have been accounted as high as 40–60%. In southern India especially Tamil Nadu and Karnataka have been reported downy mildew epidemics at various times. The projected losses resulted by major diseases of maize in India is nearer to 13.2% of which foliar diseases (5%), stalk rots, root rots and ear rots (5%) are accountable for substantial yield reduction. A wide range of crop yield losses caused by maize diseases has been tabulated in (Table 3.1). Similarly, occurrence and severity of insect pests of maize vary by geographical location and season within a geographical area.

Most vulnerable stages of maize to these pests are three leaf stage to flowering stages. However, European corn borer (ECB), the most destructive among all, also damages at reproductive stage where stalk breakage; tassel, ear and kernel damage, and ear/cob drop are common (Chiang and Hodson 1950). ECB had been causing crop losses of about one billion US$ annually in United States alone prior to the introduction of Bt corn hybrids (Hutchison et al. 2010). All hybrids were susceptible to ECB in Europe, where 0.28% and 6.05% grain yield reduction with every one percent damaged plant and one ECB larva per plant respectively was reported (Bohn et al. 1999). The only foliage feeder which cause economic loss because of its voracious feeding habit is fall armyworm [*Spodoptera frugiperda* (J. E. Smith)], which cause direct damage to corn ears too. The pests directly causing aesthetic and economic damage to corn ears are corn earworm [*Helicoverpa zea* (Boddie)], and less frequently by *Helicoverpa armigera*. The sucking pests of maize viz., maize leafhopper [*Cicadulina mbila* (Naude)], corn leaf aphid [*Rhopalosiphum maidis* (Fitch)], cause more indirect damage by acting as vectors of viral diseases in maize.

Table 3.1 Important maize diseases along with their causal agents and yield losses

S. No.	Disease	Causal agent	Losses (%)	Reference
1	Turcicum blight/Northern corn leaf blight	*Helminthosporium turcicum* (*Exerohilum turcicum*)	20–90	Razzaq et al. (2019)
2	Maydis blight/Southern corn leaf blight	*Bipolaris maydis* (*Cochliobolus heterotropus*)	9.7–11.7	Manjunatha et al. (2019)
3	Gray leaf spot	*Cercospora zeae*	5–30	Ward et al. (1999)
4	Curvularia leaf spot	*Cochliobolus lunatus*	10–60	Akinbode 2010
5	Brown spot	*Physoderma maydis*	6–20	Lal and Chakravarti (1976)
6	Southern corn/Polysora rust	*Puccinia polysora*	50–100	Liu et al. (2016)
7	Common corn rust	*Puccinia sorghi*	18–49	Groth et al. (1983)
8	Eye spot	*Aureobasidium zeae*	14–44	Chang et al. (1990)
9	Head smut	*Sporisorium reilianum*	Up to 30	Njuguna 2001
10	Common smut	*Ustilago zeae*	40–100	Pope and McCarter (1992)
11	Ear rot	*Fusarium verticillioides*	5–15	Ako et al. (2003)
12	Sorghum downy mildew and Rajasthan downy mildew	*Peronosclerospora sorghi* and *P. heteropogoni*	30	Singh and Kaur (2018)
13	Banded leaf and sheath blight	*Rhizoctonia cerealis* or *solani* f. sp. *sasakii*	10–90	Sagar and Bhusal (2019)
14	Various stalk rot	*Macrophomina phaseolina, Pythium inflatum*	30–35	Costa et al. (2019)
15	Fusarium stalk rot	*Fusarium verticillioides*	10	Archana et al. (2019)
16	Root rot	*Fusarium graminearum*	25–30	Hebbar et al. (1992)
17	Maize dwarf mosaic	Maize dwarf mosaic virus (MDMV)	0–90	Goldberg and Brakke (1987)
18	Maize rough dwarf	Maize rough dwarf virus (MRDV)	10–70	Dovas et al. 2004
19	Bacterial stalk rot	*Dickeya zeae*	85–90	Kaur et al. (2014)

Source Dey et al. (2015)

A. Damage by *Chilo partellus* B. Damage by Fall armyworm

Fig. 3.1 Insect damage in maize (Photo courtesy, Suby S. B, IIMR, New Delhi). **a** Damage by *Chilo partellus* **b** damage by Fall armyworm

The storage pests of maize which cause loss of quality and quantity of maize grains across the worlds are, greater rice weevil or maize weevil [*Sitophilus zeamais* (Motschulsky)], angoumois grain moth, [*Sitotroga cerealella* (Olivier)]. In addition to these, other insects also damage maize crop significantly under favourable conditions (described in the previous section) (Figs. 3.1 and 3.2).

3.3.1 Disease Management

The disease management strategy by cultural methods is reported to be effective in the major diseases. In case of BLSB, stripping of the lower leaves can restrict the occurrence and spread of the disease (Sharma and Hembram 1990; Kaur et al. 2020). Management of crop debris, deep tillage, crop rotation with non-host species, decreasing plant density and timely showing can help reduce the incidence of MLB disease (Kaur et al. 2014). Ridge planting and paired row planting methods were successful in minimizing MLB disease severity. The PFSR disease can also be managed by crop rotation with non-cereal crops, deep summer ploughing in April and May, burning of crop residues. In addition, avoidance of the water stress condition at the time of flowering by providing irrigation till grain filling stage significantly reduces PFSR disease occurrence. Various cultural practices such as soil solarization, balanced soil fertility, crop rotation with non-host crop and flooding as well as fallowing can reduce late wilt disease (*Cephalosporium maydis* Samra, Sabet and Hingorani) severity and losses (Degani et al. 2018). However, all these cultural practices will only be successful if all farmers in the vicinity harmonize their activities.

Management of crop diseases using chemicals is the mainstay till date. The wider use of chemical pesticides is due to their more effectiveness, ease of

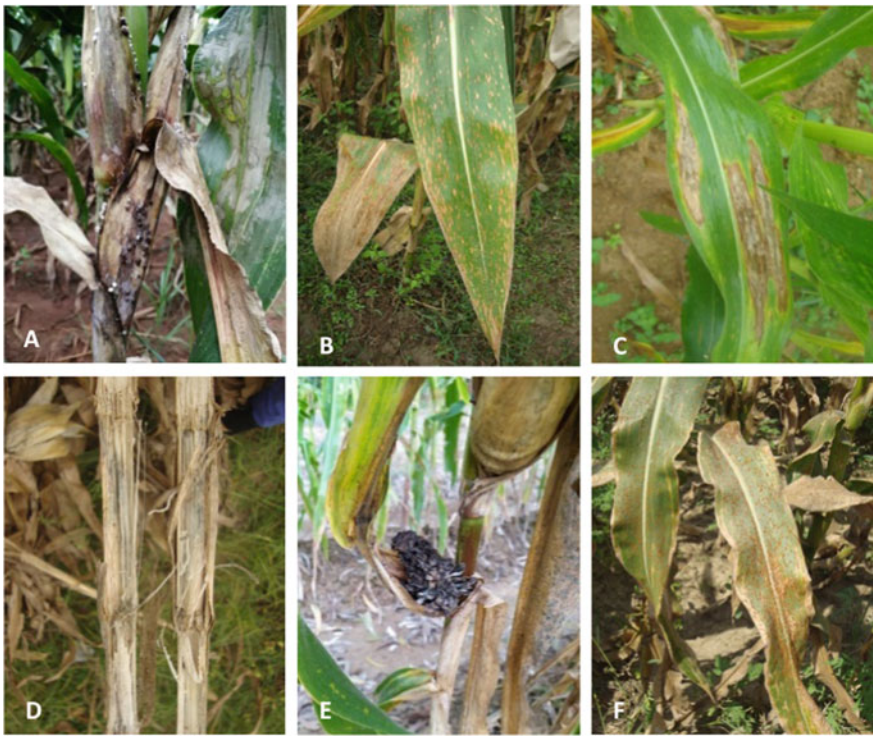

Fig. 3.2 Fungal disease of maize (Photo courtesy, Robin Gogoi, IARI, New Delhi). **a** Banded leaf and sheath blight; **b** maydis leaf blight; **c** turcicum leaf blight; **d** charcoal rot (Post flowering stalk rot); **e** smut; **f** common rust

application, availability and stability. Chemical pesticides are generally fast-acting, may damage the crop less than those caused by the diseases. The fungicides have long been recommended are Mancozeb @2.5 g/L against common rust, Polysora rust, MLB and TLB; Propiconozole 25% EC (Tilt) @1 ml/Lagainst rusts; Metalaxyl MZ @2 g/L against downy mildews; Carbendazim, Tebuconazole, Hexaconazole @1 gm or ml/L, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC against BLSB disease. The pre-flowering stalk rot disease can be minimized using bleaching powder containing 33% chlorine @10 kg/ha as soil drench at pre-flowering in standing crop. Foliar spray with the combination of Carbendazim 12% + Mancozeb 62.7% was reported to be as effective against Fusarium stalk rot disease.

Biocontrol approach is an important measure for plant disease control without posing adverse effect on the environment (Gogoi et al. 2018). Mechanisms such as antibiosis, siderophore production, induced resistance, and competition are the modes of action of the bioagents (Yobo et al. 2004). Several micro-organisms are known to parasitize *Rhizoctonia* species and those are mainly fungi like

Trichoderma, *Gliocladium*, and *Laetisaria*, bacteria (*Pseudomonas fluorescence*) and nematodes (*Aphelenchus avenae*) (Singh and Shahi 2012). BLSB disease incidence could be drastically reduced by applying *P. fluorescens* and *T. harzianum* in the field and it improves plant growth as well (Sivakumar et al. 2000; Meena et al. 2003). Combined use of seed and foliar treatment with fluorescent Pseudomonas from maize rhizoplane was most effective against BLSB (Gamliel and Katan 1993) and the result was on par with the systemic fungicide carbendazim. In case of Fusarium stalk rot, seed treatment with *T. harzianum* (4 g/kg seed) along with soil application of castor or neem cake (250 kg/ha), 15 days prior to sowing helps in disease management (Saravanakumar et al. 2017). Application of *Trichoderma* formulations in furrows after mixing with FYM @1 kg/100 kg FYM at least 10 days before its use in the field in moist condition (Hussain et al. 1990) and seed treatment with talc-based powder formulation of *T. viride* (*T. asperellum*) @12 g/kg seed can check the appearance of charcoal rot disease (Shekhar and Kumar 2010). Thus, the ultimate goal of reducing fungicide use in maize production could be achieved by using different bio-origin fungicides in rotations with traditional fungicides. But successful biological control of the diseases requires more knowledge-intensive strategies.

Resistance of the host plant plays a significantly important role in integrated disease management approach. Therefore, identification of resistance genes against the aggressive pathogens and combining them with high grain yield is a priority. Crop diseases, especially the BLSB of maize, can be managed by using different management strategies at some level. It includes cultural practices, chemical management, host resistance and biological control. But the studies revealed that none of the disease managerial measures alone is absolutely effective. Hence, identification of climate resilient components and their combination for integrated disease management (IDM) modules development are expected to provide best management of the diseases like BLSB (Hooda et al. 2015). Use of fungicides and bio-control agents viz., *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* as seed and soil treatment can also restrict the BLSB disease to some extent. Seed treatment with *Trichoderma harzianum* (6 g/kg of seeds) and 2 sprays of 0.25% Mancozeb at 40 and 50 DAS are found effective for the management of turcicum leaf blight disease (Khedekar et al. 2010). Seed treatment with a combination of carbendazim + *T. viride* revealed maximum increase in seed germination (89.4%) followed by reduction in disease severity (83.8%) of Fusarium stalk rot (*F. verticillioides*) in maize (Khokhar et al. 2014). In Nepal, IDM approach was reported to be the most appropriate technology for management of stalk rot complex which is an exclusively soil borne nature (Subedi et al. 2016).

3.3.2 Insect Management

The losses caused by the insect pests can be managed by adapting several strategies. Among the various strategies, the use of chemical insecticides is the major one across the globe, but, it has negative impacts viz., ecological damage, environmental pollution, human health hazards and development of resistance in the insect pests. The host plant resistance (HPR) is a most effective alternative and economical approach to control insect pests. Breeding for resistant cultivar is a sustainable approach. In USA, the efforts towards breeding insect resistant maize cultivars has started after the discovery of European corn borer in 1917 (Guthrie 1989).

The success of breeding program to develop resistant cultivars depends on availability of broad germplasm base, knowledge of resistance mechanism, efficient and reliable screening techniques, mode of inheritance, selection of right breeding procedure, etc. In the recent past, new molecular techniques have facilitated plant breeding and brought improvements in cultivars resistance against insect pests (Guthrie 1989). Identification, development and utilization of sources of resistance against different insect pests of maize play important role in designing management strategies (Mihm 1997).

Historically, many cultivars with insect resistance have been developed utilizing conventional breeding methods. In CIMMYT, sub-tropical source populations were developed with multiple borer resistance (MBR population) by following recombination and recurrent selection under artificial infestation with southwestern corn borer (SWCB, *Diatraea grandiosella*), sugarcane borer (SCB, *Diatraea saccharalis*), European corn borer (ECB, *Ostrinia nubilalis*) and fall armyworm (FAW, *Spodoptera frugiperda*) (Mihm 1985). From the different organizations diverse source populations were obtained and used for development of MBR population.

3.4 Traditional Breeding Approaches

Traditional breeding comprises all those breeding methods that have been developed since the origin of agriculture and are still commonly used even today. Conventional breeding can be defined as the development or improvement of crop cultivars with the help of natural processes and conservative tools for manipulating plant genome within the natural genetic boundaries of the species (Acquaah 2015), in contrast to molecular plant breeding, which utilizes modern, sophisticated and sometimes radical tools.

In any breeding programme involving incorporation of a new a trait, including disease resistance, breeder has to consider the phenomenon of 'trait compensation' by which the gains in other desired characters may suffer (like yield potential) due to addition of a new trait (Badu Apraku and Fakorede 2017). Therefore, breeder has to consider the economic sustainability of incorporation of biotic stress resistance. For this, breeder has to consider the frequency and extent of biotic stress in the

target area and extent of economic damage caused. Breeder may opt for major-gene resistance (qualitative resistance conferred by R-genes) or minor gene resistance (quantitative resistance conferred by QTLs). The major gene resistance has complete expression with high levels of resistance, simple inheritance and is usually race specific. But, this type of resistance may be quickly defeated by co-evolving parasites. However, some cases of durable major-gene resistance have been reported (Badu Apraku and Fakorede 2017). The durable resistance is defined as "the resistance that remains effective when a cultivar is grown widely in environments favouring disease development" (review by Michelmore 2003). The concept of durable resistance has proved a very useful concept in disease resistance breeding. The example of such durable resistance has been seen in Indian inbreds. The maize inbred lines CM104 and CM105 have shown resistance to turcicum leaf blight (TLB) as well as maydis leaf blight (MLB) at 19 diverse locations in India for more than 14 years. Furthermore, these lines registered resistant reaction also in countries like Hawaii, Nigeria and Kenya for TLB, and Cameroon, Mexico, Hawaii and Korea for MLB (Sharma et al. 1993a, b).

Quantitative resistance provides intermediate or partial resistance to the parasite in contrast to qualitative resistance and is thought to be controlled by a set of genes that are distinct from, or showing partial similarity with those involved in qualitative resistance (Wisser et al. 2005; Fu et al. 2009). Quantitative resistance is expected to be more durable as many minor genes with small effects exert lower selection pressure and presents greater hurdles to overcome by the parasite (Parlevliet 2002). Even though a large number of quantitative resistance sources have been reported, especially for disease resistance in plants (Young 1996), there is no clear understanding of genetic basis or the mechanisms of defense involved in quantitative resistance.

Once the decision on the type of resistance to be used in breeding is made, the next step is to identify suitable sources of resistance. The resistance may be found in the primary gene pool of the crop and often within the related species. Sources of resistance have been reported in related taxonomic groups, viz., landraces, commercial cultivars, wild progenitors, related species and genera. Further, breeder has to bear in mind that use of germplasm with common genetic base should be minimized or avoided in disease breeding programmes. Devastating epidemics have been observed, as with the southern leaf blight in USA, when the genetic or cytoplasmic homogeneity was achieved. In general, breeding for biotic stress resistance in maize can be depicted as follows (Fig. 3.3).

Conventional approaches for biotic stress in brief are discussed for diseases and insects separately in the following paragraphs.

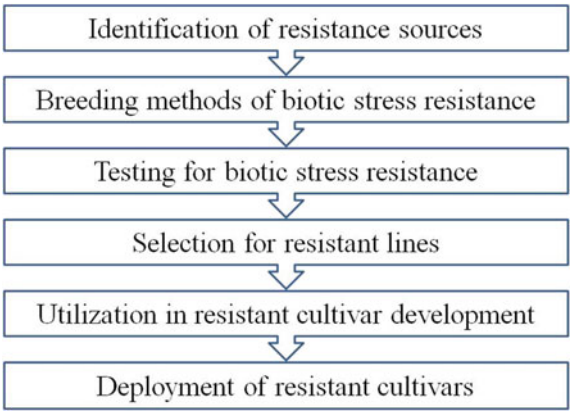

Fig. 3.3 Flow diagram for breeding for biotic stress resistance in maize

3.4.1 Conventional Approaches in Breeding for Disease Resistance

The systematic efforts on conventional approaches for disease resistance began after Biffen's (1905) demonstration in wheat that disease resistance in plants is under genetic control. This was further strengthened by works of Flor (1946) on flax rust and understanding of the genetics of pathogenicity, and Van der Planck (Plank 1963), who suggested two types of resistance, viz., vertical (qualitative) and horizontal (quantitative) resistance.

Breeding for disease resistance in maize, as in other crops, begins with the screening germplasm to identify resistant sources (donors). Precision phenotyping for disease resistance using disease hot spots or artificial epiphytotic or disease screening hubs is the most important step into identify stable sources of resistance. In the next step, backcross breeding scheme is used to introgress resistance gene from the donor parent into an agronomically superior line or inbred (Fig. 3.3). To achieve this, knowledge on the genetic architecture of disease resistance genes in maize need to be explored to assess the nature of resistance (qualitative or quantitative) in the donor parent (Ali and Yan 2012).

In other major cereal crops like wheat and rice, qualitative disease resistance is extensively used. In contrast, a few major resistance genes (R genes) have been identified and utilized in maize (Ramakrishna et al. 2002), such as *Ht* genes against northern leaf blight (Welz and Geiger 2000) and the *Rp* genes against common rust. This is because, the majority of resistance available against diseases in maize is quantitative disease resistance (QDR). The major reason for the predominance of QDR in maize might be due to its outcrossing nature and hence, it is substantially more genetically diverse than wheat or rice (Buckler et al. 2001). Maize breeders, therefore, have more diversity available to them within adapted germplasm and effective QDR to maize pathogens is available and widely utilized, compared with wheat or rice breeders. This might also be due to the fact that maize is attacked by

fewer commercially important biotrophic pathogens than that of wheat. Furthermore, it is possible to bring together multiple small-effect QTLs to achieve effective levels of QDR in maize through population improvement schemes. Hence, population improvement approaches are more commonly used in maize for improving both agronomic performance and disease resistance. Therefore, it is important to collect and evaluate germplasm continuously to identify new sources of disease-resistant genes, which in turn enables the breeder to incorporate multiple disease resistance into breeding populations before deriving varieties from such populations.

Resistance sources to foliar diseases of maize including maydis leaf blight (MLB, southern corn leaf blight-SCLB), turcicum leaf blight (TLB), northern corn leaf blight-NCLB) (Ayiga Aluba et al. 2015; Bhat et al. 2017; Kurosawa et al. 2018), gray leaf spot (GLS) (Dhami et al. 2015), polysora and common rust, downy mildew (DM), some viral diseases, *Aspergillus* contamination (Hooda et al. 2012; Badu Apraku and Fakorede 2017) have been identified and are incorporated successfully through conventional breeding. In addition, multiple disease resistance (MDR) in maize has been reported (Martins et al. 2019). MDR loci conferring resistance to SCLB, GLS, and NCLB are believed to have relatively small effects individually and the effects may be below the detection threshold to detect them as individual loci (Balint Kurti et al. 2010; Martins et al. 2019). However, there has been a very little progress in resistance against banded leaf and sheath blight, post flowering stalk rots and ear rots (Ali and Yan 2012).

Gene pyramiding and multiline development are not popularly used as incorporation of multiple genes becomes a tedious and lengthy process. These strategies are expected to become much more practical in future. Furthermore, many *R* genes confer resistance against only one or few strains of pathogen and do not provide broad-spectrum resistance as in case QDR. Nonetheless, understanding of the function of R genes at molecular level and of downstream signal transduction pathways might provide strategies to overcome these deficiencies (Balconi et al. 2012).

3.4.2 *Conventional Approaches in Breeding for Insect Resistance*

Breeding for Insect resistance start with screening of a germplasm for variability in the level of resistance of a genotype to target pest by quantifying the effect of insect on plants and the effect of plants on insect (Mihm 1985). The most essential components for successful screening programme for insect resistance are a broad germplasm base, the established population of a target pest and its mass production. Standardization of the most susceptible stage of plant, the dose of insect for infesting plants, and an accurate phenotyping method are to be established before screening. Once the resistance source is found, a suitable breeding scheme is

followed. Compilation and reviews on mechanisms of resistance, its genetics, sources of resistance and conventional breeding for insect resistance in maize for Americas and Africa have been published (Mihm 1985, 1997; Guthrie 1989; Wiseman and Davis 1990; Mugo et al. 2001; Kumar 2002; Brooks et al. 2007).

Breeding for insect resistance in corn began in 1920s for ECB resistance (Guthrie 1989). International Maize and Wheat Improvement Center (CIMMYT) derived a source population (sub-tropical) with multiple borer resistance (MBR) to combat multiple pests in the area of release of a new cultivar and for increased durability of resistance. MBR was developed from germplasm sources resistant to SWCB, SCB, ECB and FAW, through conventional pedigree breeding using resistant germplasm sources of Mississippi State University, CIMMYT population 47, Antigua populations, Cornell University and University of Missouri. MBR population is characterized by tough and fibrous leaf tissue where the cell wall components are reinforced with phenolic acids, which reduces digestibility and nutritional value of the plants to the pests (Bergvinson et al. 1994). Later, MBR was found to possess good amount of resistance to *C. partellus* and *B. fusca* and served as stemborer resistance source around the world. Its success was attributed to additive variation of the polygenes involved in resistance and the genotypes derived from MBR showing general combining ability as the primary source of variation among F_1 for resistance and grain yield (Mugo et al. 2001).

Of this, the landmark populations like Antigua Gpo2 population served the basis of insect resistant lines released around the world. Corn host plant research unit of USDA-ARS extensively worked on this and other resistant sources to derive many insect resistant lines, of which Mp496 (Scott and Davis 1981) was the pioneer, derived from Antigua Gpo2 by direct selection. Subsequently, many superior lines resistant to FAW, SWCB and P. rust such as Mp703 (eight generations of selection by selfing resistant plants of Gpo2 population by Williams and Davis 1980), Mp704 (eight generations of selection by selfing the cross between Mp496 and an S2 population of Republica Dominica Gpo1 by Williams and Davis 1982), Mp701 and Mp702 (selection from bulk populations derived from crosses involving Antigua Gpo. 1 and Antigua Gpo. 2, and Republica Dominica Gpo1 respectively by Scott et al. 1982), Mp705, Mp706, and Mp707 (selfing selections from MpSWCB-4(l) for eight generations by Williams and Davis 1984), Mp708 (in addition to FAW and SWCB this line is resistant to root knot nematode. developed by selfing selections from a cross of Mp704 and Tx601 for eight generations by Williams et al. 1990), Mp713 and Mp714 (Mp713 derived from MBR population and Mp714 from GT-DDSA, a corn earworm resistant population Williams and Davis 2000), and Mp716 (derived from a cross between Mp708 and Mp78:518 by Williams and Davis 2002).

HPR was explored for stored product pests at CIMMYT, where Caribbean germplasm bank accessions like Guadeloupe and Cuba land races served as LGB resistant source (Kumar 2002). Subsequently, "CubaGuard" was derived by recurrent section and selfing under LBG pressure.

Generally, the lines resistant to one pest tended to be resistant to other pests and diseases, indicative of a broad-spectrum resistance. This could be the result of

co-evolution of maize pests with its host plant under different ecologies or man-made evolution by accelerated resistance breeding efforts. For instance, the line Mp496, released in 1981 has good resistance to FAW, and fairly good resistance to ECB, sorghum downy mildew, maize chlorotic dwarf and moderate resistance to maize dwarf mosaic and southern corn rust (Scott and Davis 1981). This suggests that there might be few genetic regions operate in tandem to give broad-spectrum resistance, as observed in the inbred lines, Mp704 and Mp708 with leaf feeding resistance to FAW and SWCB (Brooks et al. 2007).

3.4.3 Limitations of Conventional Approaches in Breeding for Biotic Stress Resistance

Although conventional breeding has achieved tremendous results for many traits and since many years, it also has some serious limitations. First, it takes very long time to achieve desired results. Second, breeding can only be done between two sexually compatible lines. Third, when hybridization is done, many other traits are transferred along with the trait/s of interest—both positive and negative traits resulting in linkage drag. Fourth, the use of distant relative or tertiary gene pool in breeding for resistance poses following problems. (i) failure to get F_1 seed between the crop and the donor species, (ii) sterile interspecific or intergeneric hybrid, and (iii) poor recombination between the chromosomes of crop and the donor species (Harlan and De Wet 1971). When distant hybridization is used, the resistance may be realised after the removal of undesirable genes trough many generations of backcrossing.

3.5 Genetic Resources of Resistant Genes

The array of genetic resources at our disposal, together with new biotechnology techniques, gives us with a healthy measure of optimism for meeting the world's future food requirements (Hoisington et al. 1999). The genepool of maize consists of two genera, *Zea* and *Tripsacum,* of family Poaceae. These species are housing tremendous genetic diversity that is potentially useful in maize improvement either through hybridization or through special techniques, such as embryo rescue. The genepool classification is based on the ease of genetic exchange through sexual reproduction (Harlan and de Wet 1971). The cultivated species of the genus *Zea* (*Z. mays* ssp. *mays*) represents the primary genepool and all other taxa in the genus *Zea* that are popularly known as "teosintes" form the secondary genepool. All the species in the genus *Tripsacum*, not easily crossable with cultivated maize and require special techniques, are classified as tertiary genepool. The genetic resources with biotic stress resistance have been summarized below Table 3.2.

Table 3.2 List of germplasm resources of maize with potential to improve biotic stress tolerance

Sl. No.	Biotic stress	Germplasm	Reference
Primary genepool			
1	Foliar diseases	Tuxpeno crema-land race	Kloeppe et al. (1999)
2	Downy mildew	Suwan-1 (OPV-Thailand)	Dhillon et al. (2002)
3	Multiple diseases	Prabhat (OPV-India)	Dhillon et al. (2002)
4	*Sitophilus zeamais* (maize weevil)	Palomero Toluqueno (Popcorn landrace)	Arnason et al. (1993)
5	*Prostephanus truncates* (Larger grain borer)	Caribbean land races	Kumar (2002)
6	Northern leaf blight (inbred lines of maize)	DMSC 16-1, Gen1858, HKI PC 4B-1, HKI 141-1, HKI 141-2, CML141	Hooda et al. (2012)
7	Southern leaf blight (inbred lines of maize)	DMSC 16-2, V351-1, CM 114, CML 165, CML 167, HKI-139	
8	Brown stripe downy mildew (inbred lines of maize)	CUBA 380, DMSC36, HKI-PC-4B-1, DTPYC9-F46-3-1, ESM-11-3, LM 6, LM 12, LM 16, V 355, V 341-1, CM 123, CM 149, CM 500,	
9	Post flowering stalk rot	WINPOP-1, WINPOP-2, WINPOP-3, WINPOP-21, WINPOP-21, WINPOP-43-1, HKI-2-6-2-4(1-2)-4, HKI 226, HKI 1040-5, CML 451(P2)	
10	Polysora rust	DMSC 16-1, DMSC 16-2, WINPOP-43-1, WINPOP-43-2, HKI-2-6-2, HKI1040-5, PFSR/51016-1, LM 16, CM 105, HKI 141-1,	
11	Rajasthan downymildew	LM15, CM114, HKI C 78, DMHOC 4, PFSR- R9, PFSR-S3, PFSR- R10, JCY3-7-1-2-1	
12	Curvularia leaf spot	LM11, LM 12, LM 16, V 335, V 341, V 351, CM121, CM 123, CM 144, CM 502, HKI 141, CML384, CML 395	
13	Multiple disease resistant (MDR)	LM11, LM 12, LM 16, V 335, V 341, V 351, CM121, CM 123, CM 144, CM 502, HKI 141, HKI 1352-5-8-9, CML384, CML 395	
14	Fall army worm	CMS 23, CMS 24, Zapalote Chico, CMS 45, Amarillo Cristalino, WP 1, RR 060, MG 05, Guatemala 786, NõdzobPrê, Puerto Rico 13	Viana and Guimarães (1997)
Secondary genepool			
1	*Corn Smut disease*	*Teosinte*	Mammadov et al. (2018)
2	*H. turcicum*	*Z. diploperennis*	
3	*H. maydis*		

(continued)

Table 3.2 (continued)

Sl. No.	Biotic stress	Germplasm	Reference
4	Maize chlorotic dwarf virus	*Z. diploperennis*	Findley et al. (1982)
5	*Fusarium spp.*	*Z. spp. mexicana*	Pásztor and Borsos (1990)
6	Downey mildew	*Z. spp. mexicana*	Mammadov et al. (2018)
7	Corn borer	*Z. mays spp. mexicana*	Pásztor and Borsos (1990)
8	Asiatic corn borer	*Z. mays spp. mexicana,*	Ramirez (1997)
9	Asiatic corn borer	*Z. mays spp. diploperennis,*	
10	Asiatic corn borer	*Z. mays spp. perennis*	
11	Corn rootworm	*T. dactyloides*	Prischmann et al. 2009
12	*S. frugiferda*	*Z. diploperennis*	Farias Rivera et al. (2003)
13	*H.turcicum*, *H.maydis*	*Z. diploperennis*	Wei et al. (2003)
14	Northern leaf blight	*Teosinte*	Ott (2009)
15	*Ustilagomaydis*	*Teosinte*	Chavan and Smith (2014)
Tertiary genepool			
1	*Colletotricum graminicola*	*T. dactyloides*	Bergquist (1979)
2	*Rust disease*	*T. dactyloides*	Mammadov et al. (2018)
3	*P. sorghi* (RpTd gene)	*T. dactyloides*	Bergquist (1981)
4	*Helminthosporimturcicum*	*T. dactyloides*	Bergquist (1979)
5	*H. maydis*		
6	*Erwinia stewartii*		
7	*Puccinia sorghi*		

3.5.1 *Utilization of Identified Novel Genes in Maize Improvement*

Despite the importance of maize as a major staple crop globally, only a few biotic stress resistance genes have been identified and validated through mutagenesis or transgenic approaches. The resistance genes so far identified and cloned against disease resistance include two qualitative resistance genes, *Rp1-D* and *Hm1*, and four quantitative resistant genes with relatively large effects, *ZmHtn1, ZmWAK, ZmTrx*, and *Rcg1*. Besides, some genes which are strongly implicated in disease resistance and several QTLs against different diseases have been reported. Insect resistance is largely quantitative in maize and few QTLs have been identified. In addition, Cry protein genes have been used to develop maize transgenics resistant against lepidopteran insects. These genes are summarized as follows (Table 3.3).

3.6 Diversity Analysis

The genetic diversity analysis in a crop germplasm provides breeders with valuable information to select parents for hybridization and for diverse inbred development (Ertiro et al. 2017). This in turn helps in classifying and describing inbreds into distinct heterotic groups and help in determining the genetic variability in the selected accessions/lines for target traits (Semagn et al. 2012). Several authors have documented the extent of genetic diversity in maize. The genetic diversity analyses in maize germplasm collection have been carried out in maize by both morphological and molecular approaches. Even though diversity analysis using morphological traits has many disadvantages (Botha and Venter 2000), it provides an excellent analysis of variation at phenotypic level coupled with the information on Genotype × Environment interaction. The characterization of accessions through phenotypic descriptors is the first step to classify, describe and assess the potential of available germplasm. Such an exercise will enhance the value of these germplasm in maize breeding (Prasanna and Sharma 2005; Wasala et al. 2013). The inbred lines of tropical and subtropical regions have more alleles and greater gene diversity than temperate inbred lines. Hence, tropical germplasm may be useful in temperate regions as well. It is observed that only 80% alleles present in land races are present in improved inbred lines of maize, implying that substantial additional genetic diversity can be found in landraces. Moreover, compared to the progenitor (teosinte), maize has fewer alleles and hence alleles present in teosinte can provide additional source of genetic diversity for use in maize improvement (Vigouroux et al. 2005). In India, well characterized landraces through SSR marker analysis led to the better understanding of population structure (Prasanna et al. 2010). Molecular marker-based study involving progenitor and wild relatives provided insights into the domestication events in maize (Matsuoka et al. 2002).

Table 3.3 Gens/QTLs identified conferring resistance to maize diseases

Biotic stress	Gene/QTL	Method	Mechanism	Reference
Genes implicated in biotic stress resistance in maize				
Aspergillus ear rot	*ZmLOX3* (dQTL)	Implicated by Mutant analysis	Lipoxygenase	Gao et al. (2009)
Downey mildew	*GRMZM2G028643, GRMZM2G128315, & GRMZM2G330907*	Candidate gene	LRR (leucine rich repeat)	Kim et al. (2020)
Downey mildew	*AC210003.2_FG004*	Candidate gene	Peroxidase (POX) gene	Kim et al. (2020)
Northern leaf blight and Stewart's wilt	*pan1* (dQTL)	Implicated by fine-mapping and mutant analysis	Receptor-like kinase	Jamann et al. (2014)
Northern leaf blight	*Remorin* (dQTL)	Implicated by fine-mapping and mutant analysis	Remorin_C domain (PFAM 03763)	Jamann et al. (2016)
Northern leaf blight, southern leaf blight, and grey leaf spot	*GST* (dQTL)	Implicated by association analysis	Glutathione S-transferase	Wisser et al. (2011)
Shoot fly (*Atherigonana spp.*)	*qDH9.1* (and *qEC9.1*)	QTL mapping	NA	Vikal et al. (2020)
Fall army worm	*gl15* (*glossy 15*)	Candidate gene	NA	Brooks et al. (2007)
Maize leaf feeding insects	*Mir* cysteine proteinase gene family	Candidate gene	NA	Cordero et al. (1994)
Helicoverpazeae	Maize *Rip3:1*	Ribisomal inactivating protein	Impair susceptible ribosomes	Dowd et al. (2003)
European corn borer	QTLs on chromosomes 1, 2, 4, 5, 6, 8	$F_{2:3}$ mapping population derived from a cross B73Ht (susceptible) × Mo47 (resistant)	Leaf feeding	Jampatong et al. (2002)
Cloned genes of disease resistance in maize				
Maize leaf blight and ear mold	*Hm1*	Transposon tagging	NADPH-dependent HC-toxin reductase	Johal and Briggs 1992

(continued)

Table 3.3 (continued)

Biotic stress	Gene/QTL	Method	Mechanism	Reference
Common rust	*Rp1-D*	Transposon tagging	NB-LRR	Collins et al. 1999
Exerohilum turcicum	*Htn1* (dQTL)	Fine-mapping, mutant analysis	Wall-associated receptor-like protein	Hurni et al. 2015
Sugarcane mosaic virus disease	*ZmTrxh* (dQTL)	Transposon tagging	Atypical h-type thioredoxin	Liu et al. 2017
Anthracnose stalk rot	*Rcg1* (dQTL)	Transposon tagging	NB-LRR	Frey2005
Head smut	*ZmWAK*(dQTL)	Sequential fine-mapping, and RNAi	Wall-associated kinase	Zuo et al. (2015)
Transgenes for insect resistance in maize				
Corn rootworm	*Cry34Ab1, Cry35Ab1*	Transgenic	Bt toxin-Cry protein	Hellmich and Hellmich 2012
Lepidopteran insects	*cry1A.105*, *cry2Ab, CryIF, Cry3Bb1, mCry3A, Vip3A*	Transgenic	Bt toxin-Cry protein	Castagnola and Jurat-Fuentes (2012)

3.7 Glimpse on Classical Mapping in Maize

The morphological marker is a genetic trait detectable by a naked eye and that aids to identify, predict, or characterize the trait linked to it. For instance, the traits such as seed colour, seed shape, flower colour, leaf pigmentation, leaf shape, flower color, pubescence color, awn type and length, fruit shape, stem length, and such other agronomic traits. These markers are easy to identify without any special instrument or modern technique. Use of markers as an assisting tool to select the plants with desired traits had started in breeding long time ago. Since ancient times, various morphological markers have been used to investigate the variation for utilization in plant breeding (Karaköy et al. 2014) and in construction of linkage maps by classical two- and/or three-point tests. Some of these markers are linked with other agronomic traits and thus can be used in indirect selection. Markers of this type have been used in resistance breeding. For instance, the tomato *Tm-2* gene for resistance to tobacco mosaic virus (TMV) is linked to an anthocyaninless seedling marker (Robinson et al. 1970) and a peach mildew resistance gene is linked to the size of foliar glands (Connors 1922).

In maize, insect resistance is significantly correlated with morphological features. For instance, dense waxes on stem and leaf surface against southwestern corn borer (Hedin et al. 1993) and fall armyworm (Yang et al. 1993), low trichome density against corn earworm (Widstrom et al. 1979), silica against European corn borer (Rojanaridpiched et al. 1984), and tight husks against corn earworm (Wiseman et al. 1977). These plant characteristics have been considered while breeding for insect resistance in maize through conventional plant breeding approaches. However main disadvantages of morphological markers are, they are limited in number, influenced by the plant growth stages, various environmental factors (Eagles et al. 2001), and some have deleterious effects, pleiotropy, epistasis, and rare polymorphism.

Traditional method of identification of disease/insect resistance gene is time consuming and affect much by environmental condition prevailed. Hence markers linked to the trait of interest came as an improvement over traditional method of identification and mapping of genes. Before mapping a gene of interest, understanding the inherence of particular trait is at most important. In maize, one recessive major gene, *rhm*1, found to confers resistance to race O of *Cochliobolus heterostrophus* (Zaitlin et al. 1993). Resistance is associated with relatively few changes in gene expression or protein levels (Simmons et al. 2001). Monogenic resistance was reported in case of MLB (Faluyi and Olorede 1984) initially followed by the role of QTL in its expression later. It was established that in the adult plant, *rhm1* confers a level of quantitative resistance (Thompson et al. 1987) and *rhm* was mapped to the short arm of chromosome 6 with two restriction fragment length polymorphism (RFLP) marker loci (UMC85 and p144). The gene *Hm2* and *Hm1A* confer adult plant resistance to *C. carbonum* race (Balint Kurti et al. 2007, 2008). MLB resistance QTL are found in the same bin in populations derived from two or more different crosses (McMullen and Simcox 1995; Wisser et al. 2006).

Carson et al. (2004) identified a total of 11 QTLs governing resistance against MLB. Another six significant QTLs (LOD > 3.1) were identified for resistance to MLB which were located on the chromosome 1, 2, 3, 6, 7 and 8 (Balint Kurti and Carson 2006). Seven potential QTLs, and the two strongest among them being located on chromosome 3 (bin 3.04) and 9 (bin 9.04), were reported by recently, Kump et al. (2011) identified 32 QTLs using nested association mapping population. As pointed out earlier, disease resistance in maize is mostly quantitative in nature. It can be noted that many dQTLs (disease QTLS) and only few R genes (qualitative resistance) have been reported in maize. Wisser et al. (2006) compiled the information from 50 publications on mapping of disease resistance pertaining to 11 different diseases in maize. In all, these papers reported the locations of 437 dQTLs, 17 R-genes, and 25 R gene analogs. The analysis of the distribution of resistance loci indicated that the dQTLs are distributed over all 10 chromosomes and covered 89% of the genetic map. Further, it indicated the presence of clusters of dQTLs for multiple diseases. There is an evidence for the association of dQTL with maturity related QTL. On the dQTL consensus map, each maize chromosome had co-localizing dQTL for at least two different diseases. Also, MDR was found to be associated with many common chromosomal segments. These distinct dQTL distributions for the different diseases imply that certain breeding schemes may be more suitable for some diseases (Wisser et al. 2006).

3.7.1 Map-Based Cloning of Genes for Resistance

Northern corn leaf blight (NCLB) is one of the most devastating foliar diseases caused by the fungus *Exserohilum turcicum* (teleomorph *Setosphaeria turcica*) and result in huge economic loss in maize. *Htn1* locus has been reported to confer quantitative and partial resistance against NCLB (Gevers HO 1975) and mapped at the locus to a 23.1-cM interval of chromosome 8. Inclusion of additional marker within the interval narrowed down the interval to a 4.7-cM with the flanking markers MA0003 (SNP) and bnlg1782. This distance represented 1.3-Mb on physical map which was sequenced in resistant parent RP4Htn1 using a BAC library. Further using sequence-based approaches narrowed down *Htn1* between newly designed SNP markers MA0024 and MA0013 representing a 131.7-kb distance carrying three putative candidate genes *ZmWAK-RLK1*, *ZmWAK-RLK2* and *ZmWAK-RLP1*. Later, Jamann et al. (2016) fine mapped the maize *remorin* (*ZmREM6.3*) locus and demonstrated its role in conferring quantitative resistance against NCLB.

Resistance to BLSB has been reported to be governed by multiple genes, and till now genes with major effect has not been reported. Further, maize varieties with complete resistance are not available. Hence, unravelling the genetic mechanisms and mining resistance genes can be a boon for BLSB resistance breeding. Li et al. (2019) performed GWAS for BLSB using 542,438 SNPs (MAF $\geq$ 0.05) in the association panel of 318 maize inbred lines consisted of 133 tropical or subtropical,

78 temperate and 71 of mixed origin. Wide phenotypic variation for lesion length was observed with average lesion length 0.8–14.13 cm in the panel. GWAS analysis using the general linear model (GLM) could identify 28 SNPs ($P < 1 \times 10^{-5}$) corresponding to nine loci and distributed on four (1, 4, 7 and 8) linkage group. Out of 28 SNPs, the most significant SNP chr4.S_180199219 ($P < 1.84 \times 10^{-6}$) at chromosome 4 was present in second exon of the gene *GRMZM2G109140*. The gene was designated as *ZmFBL41* as the predicted F-box protein (41 kDa) shares 79% sequence similarity with rice *OsFBX61*. Resequencing of *ZmFBL41* and comparative analysis of susceptible (28) and resistant (23) lines identified four SNPs in the second exon in strong LD along with the lead SNP 2867 ($r^2 > 0.8$). These five SNPs could be assigned to two haplotypes, viz., resistant (haplotype 1) and susceptible (haplotype 2). However, these haplotypes did not affect the *ZmFBL41* expression level. To confirm the role of *ZmFBL41* in BLSB resistance, disease incidence and expression level of zmfbl41 carrying Mutator insertion in the 5′ UTR was compared with inbred line W22 which showed 28% reduced expression as well as disease index in *zmfbl41*. Further, transgenic rice cultivar Zhonghua 11 overexpressing the susceptible ZmFBL41^{B73} allele developed longer lesions. Hence, *ZmFBL41*^{B73} was found to be a negative regulator of BLSB resistance and degrade a target protein, cinnamyl alcohol dehydrogenase (ZmCAD).

3.8 Association Studies in Maize

Importance of discovering durable pest and disease resistance necessitates additional genetic mapping of diseases tolerant genes. Genome wide association mapping identifies regions of the genome associated with different biotic stresses and gives clue for directional selection to accelerated crop improvement. Majority of the biotic stress resistance in maize are governed by many genes and its inheritance is quantitative in nature. In order to analyse quantitative characteristics, association mapping utilizes ancestral recombination and natural genetic variation within a population and is based on the linkage disequilibrium principle (Geiringer 1944; Lewontin and Kojima 1960). The non-random co-segregation of alleles into two loci is one of the functional concepts of linkage disequilibrium.For association mapping research design, this observation is important as it can be used to calculate the marker density desired for scanning relatively undiscovered regions of the genome as well as the maximum resolution that can be obtained in the target population for genotype-phenotype associations (Ersoz et al. 2009).

The first association study at genome wide scale was reported in maize, in 2018, in which 8590 loci, in 553 elite maize inbred lines were used. Large scale Genome wide analysis provides new opportunity to understand the genetic architecture of complex quantitative traits such as biotic stress tolerance. More than 40 QTLs map for phenologic traits and kernel related traits in maize which are indirectly responsible for stress tolerance (Li et al. 2013). There exist successful and practical examples of association mapping in maize which give a new avenue for

identification and/or introgression of rare alleles into elite maize germplasm via a molecular marker assisted breeding. In a wide range of African agro-ecologies, Genome wide association mapping (GWAS) was used in maize inbred and double haploid lines to map several complex traits including disease and insect resistance, for example, resistance to maize chlorotic mottle virus and response to the Mediterranean corn borer (MCB) (Awata et al. 2019) (Table 3.4).

In the past few decades understanding of disease tolerance has been improved by the inclusion of GWAS techniques in the identification of marker trait association and trait specific identification of genotypes. However, relatively small portion of phenotypic variation for a trait can be explained in any given GWAS. So further, genomic studies to uncover this missing part can be explore in future.

Table 3.4 Some of the biotic stress tolerant traits dissected via a GWAS in maize are given below

Traits category	Phenotype	Population	Sample size	Number of markers	Reference
Stress resistance	Disease resistance	IAP	1487	8.2 K	Van Inghelandt et al. (2012)
		IAP	527	557 K	Chen et al. (2015)
		IAP	1687	201 K	Zila et al. (2014)
		IAP	999	56 K	Ding et al. (2015)
		IAP	890	56 K	Mahuku et al. (2016)
		IAP	818	43.4 K	Chen et al. (2016)
		IAP	274	426 K	Mammadov et al. (2015)
		IAP	287	461 K	Tang et al. (2015), Warburton et al. (2015)
		IAP	280	459 K	Gowda et al. (2015)
		IAP	267	47 K	Zila et al. (2013)
		IAP	346	60 K	Farfan et al. (2015)
		IAP	267	287 K	Horn et al. (2014)
		USNAM	4892	1.6 M	Poland et al. (2011), Kump et al. (2011)
	Insect resistance	IAP	302	246 K	Samayoa et al. (2015)
	Hyper sensitive response	IAP	231	47 K	Olukolu et al. (2013)
		USNAM	3381	26.5 M	Olukolu et al. (2014)

Source Xiao et al. (2017)

3.9 Genomics-Aided Breeding for Traits Conferring Resistance

3.9.1 Structural and Functional Genomic Resources

Mutant Libraries

Mutants are one of the most important functional genomics resources in plants and transposon tagging is the widely used approach for gene cloning in maize. Transposon tagging has been used to clone many important genes in maize including the well-known domestication gene (*tb1*) (Doebley and Wang 1997). Maize genes have been tagged using active Mu in different research programmes including Uniform Mu (McCarty et al. 2005) which is widely used by the maize researchers and have uniform Mu-insertion for 30% of maize genes. Some of the other programmes are Maize Targeted Mutagenesis database, Trait Utility System for Corn, Mu array, RescueMu, Photosynthetic Mutant Screen (Brutnell 2002). Maize mutant libraries have also been constructed through targeting induced local lesions in genomes (TILLING) (Till et al. 2004; Lu et al. 2018) and much higher number (80%) of genes have been reported to cover using this approach (Lu et al. 2018).

High Resolution Mapping Populations

Number of high-resolution mapping populations have been developed by maize researchers and are freely available for genetic mapping (https://maizegdb.org/stock_catalog). Intermated B73-Mo17 (IBM) is one of such intermated RIL (IRIL) population which was derived through initial intermating among F_2 (B73 $\times$ Mo17) individuals for four generations and thereafter selfing through single-seed descent (SSD) method. The additional four generation of recombination supported higher (2.7-fold) recombination fraction and longer (3.86-fold) map length (Lee et al. 2002). The another most important available resource is nested association mapping (NAM) population generated by crossing 25 founder lines with the common parent (B73) (Yu et al. 2008). This population has the advantage of both linkage and association mapping (McMullen et al. 2009) and captured approximately three recombination event per gene including total $\sim$136,000 recombination events. These populations have been used to dissect the genetic basis of different traits including trait like disease resistance to southern leaf blight caused by *Cochliobolus heterostrophus* (Balint Kurti et al. 2007; Kump et al. 2011). Further, the "Goodman" maize panel representing the diversity of public breeding programs consists of 302 inbred lines have been characterized using high throughput sequencing and used to dissect the genetic basis of different disease resistance traits including resistance to ear rot resistance (Zila et al. 2013), aflatoxin (Farfan et al. 2015), *Fusarium verticillioides* infection (Stagnati et al. 2019) etc. MaizeGo panel (http://www.maizego.org/Resources.html) consisting of 540 maize lines is another association panel representing the largest AMP panel ever assembled for maize

(Yang et al. 2011), which has also been used to explore disease resistance traits including other traits (Ding et al. 2015; Li et al. 2019).

3.9.2 Details of Genome Sequencing

Schnable et al. (2009) released the first reference genome (B73 RefGen_v1) of maize based on the sequencing of bacterial artificial chromosomes (BAC) and phasmids. Subsequently, the reference genome has been improved (B73 RefGen_v4) using single-molecule real-time (SMRT) sequencing and high-resolution optical mapping with rapid increase (52-fold) in contig length than previous version with notable progresses in intergenic spaces and centromeres assembly. Comparison of inbred lines with B73 reference genome revealed millions of SNPs and InDels along with many presence/absence variation (PAV), structural variations (SVs), copy-number variation expression presence/absence variation (ePAV) etc. (Springer et al. 2009; Lai et al. 2010; Fu et al. 2013; Hirsch et al. 2014; Jin et al. 2016; Bukowski et al. 2018; Sun et al. 2018). However, identification and mapping of new SNPs has been limited by the use of single reference genome only, which restrict the use of genome data, detection of SVs, and exploration of genetic diversity in real sense. Since 2016, multiple genomes, viz., PH207 (Hirsch et al. 2016), mexicana (Yang et al. 2017a), Mo17 (Yang et al. 2017a, b; Sun et al. 2018), W22 (Springer et al. 2018), HZS (Li et al. 2019), and SK (Yang et al. 2019) have been sequenced, which can be used as representative genomes. Moreover, B73 Ref_V4, Mo17 and SK genome assemblies are of much high quality which can be advantageous for genome annotation, identification of promoters and TEs (Yang et al. 2019).

3.10 Genetic Engineering for Biotic Stress Resistance in Maize

3.10.1 Disease Resistance

Over expression of *Mcchit1* gene in maize significantly reduced frequency and size of lesions compared to the control plants after 5 days inoculation of *Exserohilum turcicum* (Zhu et al. 2011). Transgenic maize expressing an enhanced green fluorescent protein fused to a ZEN-degrading enzyme (zhd101) was evaluated against *F. graminearum* infection. When the seeds were artificially contaminated by immersion in a ZEN solution for 48 h at 28 °C, the total amount of the mycotoxin in the transgenic seeds was consistently reduced to less than 1/10 of that in the wild type (Igawa et al. 2007). Overexpression of *ZmRACK1* in maize enhanced the expression levels of the pathogenesis-related protein genes, *PR-1* and *PR-5* by 2.5–

3 folds, and production of reactive oxygen species production and reduced the symptoms caused by *Exserohilum turcicum* (Wang et al. 2014a, b).Transgenic maize developed by constitutively expressing the Totivirus antifungal protein KP4 exhibited the robust resistance to *U. maydis* and expressed high levels of KP4 without any apparent negative impact on plant development (Allen et al. 2011). Transgenic maize developed by expressing the sorghum *y1* gene encoding a MYB transcription factor yellow seed1 (y1), an orthologue of the maize gene pericarp color1 (p1). LC-MS profiling of fungus-challenged transgenic maize leaves exhibited the increase in luteolinidin and flavonoids content in leaves which facilitated resistance to *Colletotrichum graminicola* infection (Ibraheem et al. 2015). Heterologous expression (under control of the constitutive CaMV 35S promoter) of a *Lablab purpureus* L. α-amylase inhibitor-like protein (*AILP*) in maize was performed and tested against *A. flavus.* Fungal growth has been observed to reduce from 35 to 72% in transgenic maize kernels which, in turn, facilitated into a 62–88% reduction in aflatoxin content (Rajasekaran et al, 2019). Expression of siRNAs (targeting *amy1, aflR* and *aflM* genes) in maize has been reported to provide excellent protection against *A. flavus* (Gilbert et al. 2018; Masanga et al. 2015 and Raruang et al. 2020). Up to 72% reduction in growth of *A. flavus* has been reported in maize expressing Tachyplesin1-derived synthetic peptide AGM182 (Rajasekaran et al. 2018).

An hpRNA targeting P1 protein (protease) gene of *Maize dwarf mosaic virus* (MDMV) was transformed in maize and the transgenic lines were showing excellent protection against MDMV disease (Zhang et al. 2010). Transgenic maize expressing *Maize dwarf mosaic virus* strain B (MDMV-B) coat protein provided resistance to inoculations with MDMV-A or MDMV-B and to mixed inoculations of MDMV and maize chlorotic mottle virus (Murry et al. 1993).To overcome the low efficiency of agronomic protection from maize dwarf mosaic disease, susceptible maize inbred line was transformed with *Agrobacterium* harbouring hpRNA expression vectors containing inverted-repeat sequences of different lengths targeting coat protein (*cp*) gene of MDMV. The MDMV resistance mediated by RNA interference was observed to be relative to the length of the inverted-repeat sequence, the copy number of T-DNA integration and the repeatability of integration sites. A longer hpRNA expression construct shows more efficiency than a shorter one (Zhang et al. 2011). Transgenic maize expressing mutated *Maize streak virus* replication-associated protein provided a higher survival rates than non-transgenic control plants after MSV inoculation. Similar results exhibited by transgenic hybrid developed by crossing T_2 Hi-II with the widely grown, commercial, highly MSV-susceptible, white maize genotype WM3 (Shepherd et al. 2007). Transgenic maize plants expressing dsRNAof *Sugarcane mosaic virus* (SCMV)-*NIb* gene provided 60–85% resistance to SCMV inoculums in field. For silencing of *Rice black-streaked dwarf virus* (RBSDV) coding gene with gene silencing suppressor, amiRNA were constructed and transformed in maize inbred lines Z31. The disease resistance of transgenic homozygous maize with the anti-rough dwarf virus amiRNA has been enhanced as compared to wild type.

3.10.2 Insect Resistance

Crystal toxin protein encoding genes i.e. *Cry1Ab, Cry1Ah, mCry3A,* etc. derived from bacterium *Bacillus thuringiensis* have been cloned downstream to CMV35S or maize ubiquitin promoter and transformed in maize individually or in combinations through micro projectile bombardment or *Agrobacterium* mediated gene transfer. Foreign genes integration into maize genome and their stability was confirmed through PCR and Southern blot analysis. A synthetic gene encoding a truncated version of the Cry1Ab protein was introduced into immature embryos of an elite line of maize. Hybrid plants obtained through crossing of transgenic elite inbred lines with commercial inbred lines were showing excellent resistance against corn borer infestation (Koziel et al. 1993). The gene *Cry1Ab* also deployed commercially for control of pyralid stem borers of maize (Baumgarte and Tebbe 2005).

The *cry1Ah* gene from *B. thuringiensis* isolate BT8 was cloned in two plant expression vectors. In the first construct, intron of maize *ubiqutin1* gene was inserted between the maize Ubiquitin promoter and *cry1Ah* gene (pUUOAH) and the second construct contained Ubiquitin promoter and *cry1Ah* gene without intron (pUOAH). Both the constructs were introduced into maize and stable transgenic plants were obtained. The ELISA results of T_1 and T_2 generation plants exhibited that the expression of Cry1Ah protein in the construct containing the *ubi1* intron (pUUOAH) was 20% higher than that of the intronless construct (pUOAH). Bioassay results showed that the transgenic maize harbouring *cry1Ah* with *ubi1* intron had high resistance to the Asian corn borers than that of the harbouring intronless construct. MIR604 transgenic corn, expressing them Cry3A protein were evaluated for survivorship of western corn rootworm, *Diabrotica virgifera virgifera* LeConte, larvae and compared with the isoline corn at three Missouri sites during 2005 and 2006. The mortality of *D. v. virgifera* due to the mCry3A protein was recorded an average of 94.88% across all seasons. The emergence of beetles was delayed 5.5 days by 50% (Hibbard et al. 2010). Transgenic crops producing insecticidal toxins from the bacterium *B. thuringiensis* are widely planted to manage agricultural insect pests. However, widespread adoption of Bt crops has led to the evolution of Bt resistance among insects. The western corn rootworm, *Diabrotica v. virgifera*, is among the most serious pests of maize in the mid-western United States and is currently managing with Bt maize. While the genes such as *Cry3Bb1*, and the closely related *mCry3A* and *eCry3.1Ab* conferring resistance against western corn rootworm are widely distributed within the Midwest, fewer cases of *Cry34/35Ab1* resistance have been observed and planting of *Cry34/35Ab1* maize is one of the methods used to manage Cry3-resistant rootworm. It has been found that fields with high levels of root injury in *Cry34/35Ab1* maize by western corn rootworm were associated with Cry34/35Ab1-resistant western corn rootworm (Gassmann et al. 2020).

3.11 Bioinformatics as a Tool for Studying Biotic Stress Tolerance in Maize

As whole genome information is rapidly becoming available for various pests and pathogens afflicting maize crop, it has opened up a new avenue for designing rational management strategies against these biotic stresses. The most successful biotic stress resistance deployment in maize during last two decades has been that of commercialization and widespread adoption of herbicide tolerance and insect resistance transgenic traits in maize hybrids. The GM Approval Database developed by International Service for the Acquisition of Agri-biotech Applications (ISAAA) provides the most comprehensive and updated information on approved transgenic events for managing biotic stresses. So far 108 herbicide tolerant events have been approved for cultivation; while 117 events have been approved for insect resistance. The initial sequencing of maize genome (Schnable et al. 2009) and subsequent deluge in sequencing data for various maize inbred lines provided a new and powerful tool for resistance breeding. Over last several years, extensive germplasm screening work had been conducted to identify natural genetic variation in maize germplasm for resistance against various biotic stresses. A number of unique resistant lines have been reported. But, deployment of these resistance sources in elite maize hybrids becomes difficult in absence of information on genomic regions controlling those resistance phenotypes. To address this challenge and hasten the mapping work of biotic stress tolerant genes, a number of bioinformatics resources have been developed. Some of the bioinformatics resources relevant for biotic stress research in maize are listed in Table 3.5. Extensive genomic, transcriptomic, proteomic and metabolomic data of maize with respect to inoculation/infection/infestation with various maize insect pests, pathogens etc. are available in general bioinformatics resources, like National Centre for Biotechnology Information (NCBI) portal. NCBI also hosts similar data for various maize insect pests, pathogens species per se.

3.12 Rationale of Genome Designing, Limitations and Prospect of Genomic Designing

The advent of genomics assisted breeding and genome manipulation techniques promises a real revolution in plant breeding, biotechnology and genetic engineering. The use of molecular markers and genomic tools has accelerated the process of plant breeding. The emergence of genome and gene editing tools aid in targeted editing of the genomes and allows the investigations into fundamental basis of biological systems and help achieve the goals of higher productivity and quality of crops coupled with biotic and abiotic stress resistance/tolerance (Kamburova et al. 2017; Tyagi et al. 2020). In contrast to conventional plant breeding methods, these enable greater precision with lesser population size to achieve targeted results

Table 3.5 Bioinformatics resources relevant for biotic stress research in maize

S. No.	Name of resource	Main features	Primary developer/ host of database	Reference/URL
1	Bacterial Pesticidal Protein Resource Center	Comprehensive information on Bt/ non-Bt pesticidal proteins for academics, regulators, and research and development personnel	University of Sussex, Cardiff University, and University of Florida	https://www.bpprc.org/
2	BtToxin_Digger	A comprehensive and high-throughput pipeline for mining toxin protein genes from *Bacillus thuringiensis*	Huazhong Agricultural University	Liu et al. (2020)
3	CryProcessor	Open source tool to carry out massive screening for novel 3d-Cry toxins and obtain sequences of specific domains for further comprehensive in silico experiments in constructing artificial toxins	All-Russia Research Institute for Agricultural Microbiology	Shikov et al. (2020)
4	CryGetter	A tool to automate retrieval and analysis of Cry protein data	Instituto Federal de EducaçãoCiência e Tecnologia de São Paulo	Buzatto et al. (2016)
5	Insects in Indian Agro-ecosystems database	A pictorial database of maize insect-pests in India	ICAR-National Bureau of Agricultural Insect Resources	https://www.nbair.res.in/Databases/insectpests/pestsearch.php?cropname=Maize
6	USDA Ag Data Commons	Data from: Datasets for transcriptomic analyses of maize leaves in response to Asian corn borer feeding and/or jasmonic acid and other genomic data	United States Department of Agriculture	Zhang et al. (2016)
7	International Herbicide-Resistant Weed Database	Global and constantly updated database of herbicide tolerant	Global Herbicide Resistance Action Committee and	http://www.weedscience.org/Home.aspx

(continued)

Table 3.5 (continued)

S. No.	Name of resource	Main features	Primary developer/ host of database	Reference/URL
		weeds in maize and other crops	CropLife International	
8	Maize Genetics and Genomics Database (MaizeGDB)	Genome browser; Genome and gene annotation browser; Nested Association Mapping (NAM) founder lines (25) genome browser; qTeller: a *comparative* RNA-seq expression platform; Metabolic pathways; etc.	United States Department of Agriculture-Agricultural Research Service (USDA-ARS)	Portwood et al. (2019) https://www.maizegdb.org/
9	MaizeMine	Gene, Gene expression, Proteins, Homology, Functions, Variations, etc.	University of Missouri	Elsik et al. (2018) http://maizemine.rnet.missouri.edu:8080/maizemine/begin.do

quickly. Mutagenesis can provide variations, but such as undirected mutagenesis may result in unwanted off-target effects. With the genomic and genome editing tools, it is possible to introduce mutations at specific target loci of interest, which can be analysed and tested for resistance to stresses. Additionally, it also allows the introduction of transgenes at a defined chromosomal location. These technologies are powerful, versatile and will greatly facilitate efficient expression and avoid negative side effects caused, usually by integration of transgene into a different gene. These new tools are expected to facilitate breeding of stress-resistant transgenic or transgene free crops in relatively short time, (Borel 2017). The genome editing (GE) with specialized nucleases will aid in introducing targeted and accurate deletions, insertions, and replacement at site-specific genomic locations. Examples of the use of specialized nucleases include, Zinc Finger Nucleases, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), Oligonucleotide-directed mutagenesis, RNA-dependent DNA methylation, and precision breeding for crop plant improvement (Doudna and Charpentier 2014; Gray and Brady 2016).

The GE tools have been successfully used to control diseases caused by fungi, bacteria, and viruses. In general, CRISPR/cas technique has been used in two ways to control pathogens by editing the genes required for infection process; (i) modifying pathogen genes (ii) modifying plant host genes. GE has been successful in controlling the powdery mildew by editing the host susceptibility factor (mildew-resistance locus-*MLO*) in wheat and tomato (Wang et al. 2014a, b;

Nekrasov et al. 2017), developing resistance against rice blast (*Magnaporthe oryzae*) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), and bacterial speck of tomato (Ortigosa et al. 2019). Furthermore, GE has potential in controlling RNA and DNA viruses of plants (Ali et al. 2015). The GE by CRISPR/cas is expected to play important role in development of resistant genotypes in relatively short time (Kamburova et al. 2017). For maize, which is recalcitrant to regeneration, protoplast transient assay is becoming an efficient tool for testing CRISPR target before starting the transformation of embryos or scutellum derived calli by Agrobacterium or particle bombardment (Gao et al. 2010). The only report on development of GE turcicum leaf blight resistant maize implied the potential of GE in maize for development of disease resistant genotypes as well. The above information does not imply that genome-editing technology is the substitute for conventional or GM or molecular breeding techniques; most probably they have to coexist. However, genome editing would apparently deliver certain benefits better, quickly and with high precision (Lassoued et al. 2019).

Although GE technologies have been successful in development of genotypes to combat pathogens in important crops, they are not yet fully exploited for the management of insect pests. The most important limitation has been the lack of availability of target genes at present against the insect pests. Once such genes are available, targeted mutagenesis of host plants through GE will be able to manage their respective pests (Tyagi et al. 2020).

Although the GE system looks straightforward, it too has limitations. It is difficult to practice gene insertion and in vitro regeneration in recalcitrant crops. There is a need for optimization and development of protocols for plant genome editing such as plant compatible set of vector systems, efficient plant transformation protocols and delivery systems, efficient screening of transformation events, which can be streamlined to enable rapid product development (Schenke and Cai 2020). GE requires implementation of proper bioinformatics specific pipelines, setting up workflows and transformation efficiency. Moreover, mutating plant genes may intervene with the normal cellular and development functions and may affect crop performance. In addition, GE for improved disease resistance depends on the availability of genome sequence information of both plant host and pathogen. At present, information on genes involved in host/pathogen interactions is limited. Also, targeting individual pathogen genes may not be efficient, due to the emergence of new strains with altered virulence and host ranges. QTL analysis for Mediterranean corn borer resistance revealed low percentage of phenotypic variance, which makes marker assisted selection for improving resistance less possible. Pleiotropism or linkage between genes would also imbalance resistance and agronomic traits (Ordas et al. 2009). Polygenic nature of maize resistance to *Busseolafusca* and *Chilopartellus*, which involves additive, dominance, and epistatic effects and its low to moderate heritability makes breeding for HPR difficult in maize (Murenga et al. 2018). Thus, clearly quantitative nature of maize resistance to insect pests which involves polygenes, often with low heritability that vary in spatial and temporal expression makes conventional breeding a challenging task. Since the genome diverts its energy for expressing many resistance traits at the cost

of its yield, the negative relationship between insect resistance and yield is the expected normal consequence. Thus, achieving desirable level of genetic gains is nearly an impossible task. Thus, the application of genomic tools for improving insect resistance in maize is not attempted at a practical level. There is also concern regarding the biosafety and regulatory issues on products developed through GE technologies (Khatabi et al. 2019). Hence, forthcoming regulatory protocols will play role in deciding the mode of testing and commercialization of Genome Edited crop varieties.

3.13 Social, Political and Regulatory Issues

In contrast to traditional plant breeding, new biotechnological tools have both pros and cons in crop improvement. Acceptance of the tools and products obtained by new biotechnological tools are debatable. Always there is a counterargument for utilization of NBT in agriculture. Though NBT has scientific potential, they have been, and are being considered as a fundamentally controversial invention in some countries. Any technology will be successful only after its wide acceptance by consumers, regulators, and non-governmental organizations (NGOs) (Hall and Martin 2005). The acceptance of innovation depends on its extent of socio-political legitimacy, where political influences and cultural aspects matter (Aldrich and Fiol 1994).

To develop insect and pest resistant maize genotypes, genetic engineering played major role in recent years. The genetic engineering in maize has provided economic advantages to some marginal farmers/adopters in the early years. Sustained gains will typically be expected in those situations in which farmers are economically able with the institutional support, such as access to credit, extension services, affordable inputs, and markets.

Institutional factor favours economic benefits to small-scale farmers. Yield can be enhanced and stabilized by improving germplasm, environmental conditions, management practices, and socioeconomic and physical infrastructure for which investments in GE crop R&D may be just one potential strategy to solve agricultural-production and food-security problems. Decision of policy-makers determines much and the ways in which resources are distributed among the different categories of farmers to improve production depends on agricultural policies. Though scientist says genetically engineered crops are economically viable option, but because of credit constraints and the money and time spent on redundant insecticide applications especially by small scale farmers made them apparently non-viable at least in some cases. These outcomes indicated an initial lack of familiarity with genetic-engineering technology and strongly suggested the need for extension services for small-scale farmers, especially during initial deployment (Hamburger 2018).

Precision plant breeding plays an important role in accelerated crop improvement. Genome editing enabled next generation biotechnological tools made

breeding/improving crops with site-specific genetic modification a reality. Mutation/change in the DNA sequence leading to the novel genetic architecture is a natural phenomenon that takes several years, but CRISPR technology based base editing techniques can lead to novel beneficial alterations in plants in quick time. However, controversial debate whether at all and how to regulate genome edited plants has essentially led to the formation of two contrasting schools of thought. Possibility of generation of off targets that would lead to abnormal changes in the ecology/plant system needs attention and gained importance as a matter of discussion (Lassoued et al. 2018). There is differential opinion across different countries. New Policy under the single umbrella is required to facilitate the utilization of novel, fast track breeding systems. Institution support for scientific community as well as farming community will make proper utilization of novel ideas, which support targeted breeding to achieve expected goals in plant breeding (Sprink et al. 2020).

3.14 Future Perspective

Maize is a crop of future of the world; having highest yield potential and providing raw material for many agro-based industries. It is having higher adaptability to various agro-climatic conditions than any other cereal crop. However, insect pests and diseases are affecting maize crop. Integration of different breeding methods along with biotechnological tools is must to develop sustainable resistance breeding mechanism against biotic stresses. Application of New Breeding Tools enables breeding against disease and pest in crop in general and maize in particular. Genomic resources developed in maize play important role in identification of novel genes for pest and disease resistance and understanding on their tolerance mechanism. Sequencing and re-sequencing approaches made genomic assisted maize improvement possible. Utilization of next generation tools and techniques surely finds answer to emerging biotic threats to maize in years to come. Although genome editing is one of the potential novel technologies, recalcitrant nature of maize to transformation and/or availability of little information on maize transformation protocols are responsible for slower pace in its successful utilization in maize. Hence, research efforts on these aspects and related to transgenics followed by application of CRISPR technology may provide answer to biotic stress tolerance in maize.

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