Review

Gene Pyramiding for Sustainable Crop Improvement against Biotic and Abiotic Stresses

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Abstract: Sustainable agricultural production is endangered by several ecological factors, such as drought, extreme temperatures, excessive salts, parasitic ailments, and insect pest infestation. These challenging environmental factors may have adverse effects on future agriculture production in many countries. In modern agriculture, conventional crop-breeding techniques alone are inadequate for achieving the increasing population's food demand on a sustainable basis. The advancement of molecular genetics and related technologies are promising tools for the selection of new crop species. Gene pyramiding through marker-assisted selection (MAS) and other techniques have accelerated the development of durable resistant/tolerant lines with high accuracy in the shortest period of time for agricultural sustainability. Gene stacking has not been fully utilized for biotic stress resistance development and quality improvement in most of the major cultivated crops. This review emphasizes on gene pyramiding techniques that are being successfully deployed in modern agriculture for improving crop tolerance to biotic and abiotic stresses for sustainable crop improvement.

Keywords: gene pyramiding; marker-assisted selection; stress resistance; crop improvement

1. Introduction

The green revolution has resulted in substantial progress in yield and quality improvement of important food crops globally [1]. However, the conventional crop-breeding method is insufficient for advancing crop improvement at the rate that is necessary to meet the food requirement for the steadily increasing human population [2,3]. The Food and Agriculture Organization estimated that worldwide food production must be increased by 70% by 2050 in order to meet the food demand of the expanding population [4]. Thus, to meet the increasing food demand, smart and rapid crop-breeding tools are required to simultaneously improve multiple agronomic and nutritional traits [5].

There are several yield-reducing factors in food grains, and it is estimated that annual losses are about 25% and 6–25% globally due to biotic and abiotic stresses, respectively [6,7]. In many developing countries, environmental stresses are severely affecting agricultural production [8]. The most important biotic stressors of food crops are plant diseases and insect pests [9]. There are diverse and widespread insect pests and pathogens that are a challenge for sustainable agriculture [10]. During the recent years, an outbreak of a single insect pest, fall armyworm (*Spodoptera frugiperda* L.), across sub-Saharan

Africa, India, Bangladesh, Sri Lanka, Thailand, and China has damaged more than 80 plant species, including fruits, vegetables, staple foods (maize, rice, sorghum, and millet), and cash crops (cotton and sugarcane) in these regions and has threatened food security and caused huge economic losses [11–14]. Therefore, appropriate and effective control strategies are required for the control of these biological threats to avoid food and economic losses across the globe.

Chemical pesticides have been used to reduce biotic damage of crops for several years, but recently the use of chemical pesticides has been discouraged due to many adverse factors, such as high product cost and the need for multiple applications, which is unaffordable by the majority of small-scale farmers [15]. In addition, chemical pesticides are a serious hazard to human health and the environment [16]. Pest resistance to chemical pesticides is another problem. In a recent report, herbicide resistance was documented in 262 weed species globally [17]. Novel approaches for pest control are required for sustainable agricultural production in order to minimize the dependence on pesticides and protect the environment and beneficial micro fauna [18]. Contrary to chemical control, the development of pest-resistant crop cultivars has become a popular idea that is durable, economical, and environment friendly [19]. It is of key significance to achieve durable and environmentally-friendly biotic stress tolerance in crops to ensure food security on a sustainable basis [20,21].

Most crop-breeding strategies for biotic and abiotic stress resistance are based on the insertion of a single resistant gene into plants, and thus crop resistance only lasts for a short period of time [22,23]. Therefore, the development of genotypes with resistance against several stresses by pyramiding multiple genes from different sources into a single plant is now emphasized [24–27]. Crop stress tolerance development has been elucidated in several studies by the pyramiding of multiple resistance genes [28,29] (Table 1). However, this technique has not been fully utilized for biotic stress resistance and crop quality enhancement in most of the major cultivated crops [27,30–32].

Abiotic stresses also adversely affect the growth and yield of crops [33], and these can even affect plant survival [34,35]. Every year, considerable crop losses occur due to floods [36]. Salinity is another problem for crop production and most crop plants are sensitive to salts throughout their life cycle and especially at the seedling stage [37]. There are also some salt-resistant crop varieties that express salt-responsive genes to tolerate excessive salts, and the quantitative trait locus (QTLs) linked with these genes may be mapped through microsatellite markers for breeding of salt-tolerant lines [38,39]. The cultivar, NonaBokra, which is tolerant to salt, was successfully mapped with SKC1, and conserves K⁺ homeostasis under salinity [40,41]. Similarly, many goals have been achieved in the development of drought-tolerant traits in other crops through marker-assisted breeding methods [42]. Many drought-tolerant genes have been explored and successfully engineered in many crops for the development of drought resistance [43,44]. Low-temperature tolerant genes (*OsRAN1*) and QTLs have also been identified in many plants for further use in cold-tolerance development in crop varieties through molecular marker breeding tools [45–47]. Yet, gene pyramiding has not been fully utilized in the area of abiotic stress improvement for durable resistance.

The incidence of crop stresses has necessitated the adoption of superior and innovative technologies to protect yield potential of various crops under stressful growing conditions. However, a problem in the improvement of crops through traditional breeding methods is that not only the desired genes, but some unwanted genes, continue to exist even after several backcross generations and the ability to screen for these is not easy. Compared to traditional breeding, advances in molecular technologies have led to precise, sophisticated, and rapid breeding through molecular markers [5,48]. Marker-assisted selection (MAS) comprises indirect selection of traits with the marker linked with the desired gene for the tagging of some important agronomic trait that otherwise is not easy to mark for resistance against pathogens, diseases, and abiotic stresses and therefore protects against losses in yield and quality characteristics. Marker studies, especially on near isogenic lines (NILs) and bulk segregate or recombinant inbred lines (RILs), have hastened gene mapping in crops. Through the use of random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and microsatellite markers, sequence-tagged sites have been developed successfully in tomato

(*Lycopersicum esculentum* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and many other cereals [27].

Resistance developed through a single gene can be overcome by pests after a few years [49–52], so it is necessary to develop unique and efficient strategies to enhance crop resistance against stresses to improve yield and quality on a sustainable basis [53–55]. Gene pyramiding may be one of the superior techniques to accomplish durable resistance against various stresses in crop production [56–58]. Sustainable improvement of crops by integrating multiple resistance genes is essential to ensure agricultural production across a range of climatic conditions [59]. In most cases, more than one gene controls a specific trait, so it is necessary to manipulate multiple genes for evolving resistance against biological and non-biological agents, such as chemicals, diseases, pests, and weeds [60]. For long-term and durable resistance development, the pyramiding of diverse resistance genes against a single pathogen or pest in a single genotype can help for long-term resistance development [20,58,61–63]. Marker-assisted breeding could make it possible to effectively combine resistant genes into a single genetic background in the shortest possible time [64,65]. In this review, we highlight the advances made in gene pyramiding to address crop production challenges and also identify the limitations that need to be addressed in utilizing gene pyramiding techniques. A graphical presentation to combine different genes (gene pyramiding) from different parents into a single genotype is shown in Figure 1.

Figure 1. A graphical representation of gene pyramiding for sustainable crop improvement against biotic and abiotic stresses.

2. Types of Gene Pyramiding in Plant Breeding

Gene pyramiding is a crop-breeding technique that can be applied in conventional and advanced molecular breeding programs to introduce novel lines. The conventional technique of crop breeding develops new crop varieties by employing traditional techniques and routine natural processes, as compared to modern and sophisticated tools of the current era [66–68]. The technique involves sequential gene pyramiding deployed in the same plant. The conventional pyramiding technique involves backcross breeding: crossing a hybrid with one of the parental lines, followed by selection for the desired characteristic [44,69]. The inherited traits and resistant genes are transferred from donor parents into recipient lines by backcrossing, pedigree breeding, or recurrent selection. With backcrossing, the traits of interest are identified via the selection process. The backcrossing method is also used for resistance gene pyramiding [70]. Pedigree breeding is a method of genetic improvement of self-pollinated species in which superior genotypes are selected from segregating generations and proper records of the ancestry of selected plants are maintained at each stage of selection [69]. Recurrent selection is an efficient and modified form of progeny selection, where selection for some specific trait(s) is conducted within consecutive segregating progeny generations on the basis of phenotypic characteristics [71] (Figure 2A–D).

Figure 2. Conventional methods of plant breeding and selection. Each vertical bar is a graphical representation of an individual's genome within a breeding population, and the colored segments specify genes and/or QTLs that affect the selected traits. Genes associated with various behaviors are portrayed in different colors (i.e., red, blue). "X" denotes a cross between parents, and the arrows represent the same form of successive crosses. An asterisk underneath an individual implies a desirable genotype. **(A)** Backcrossing. A donor genotype (blue bar) harboring a particular desirable gene (red) is crossed with an elite genotype targeted for enhancement (white bar), with offspring regularly crossed with the elite genotype. Each process of backcrossing includes selection for the interest gene and recovery of an increased proportion of the genome of the elite line. **(B)** Pyramiding of genes. By crossing and selection, genes/QTLs linked with different beneficial traits (blue, red, turquoise) are mixed into the same genotype. **(C)** Breeding the pedigree. Two individuals with suitable and compatible phenotypes are crossed; the F_1 offspring is self-pollinated to correct new, improved variations of the genotype. **(D)** Recurrent selection. An individual population (10 in each case) separates for two traits (red, blue), each of which is influenced by two major favorable QTLs. Individual intermingling and selection for suitable phenotypes/genotypes increases the frequencies of favorable alleles at each locus. No individual in the initial population had all desirable alleles in this example, but after recurrent selection, half the population possessed the desired genotype. Recurrent selection can be conducted in parallel for hybridized crops within two complementary populations to extract lines, which are then crossed to form hybrids; this process is called reciprocal recurrent selection [69,72].

The pyramiding scheme of the genes can be divided into two sections. The first element is called a pedigree, which is structured to cumulate all target genes in a single genotype called the root genotype. The second component is called the fixation stage to fix the target genes in a homozygous state to extract the ideal genotype [73]. A potential technique for the fixation steps is the generation of a population of doubled haploids from the root genotype. Genetically complex improvement of the germplasm traits requires the genome to be reshuffled to produce new favorable gene combinations in the progeny [72]. The pedigree breeding method produces such innovation for improved performance by crossing and recombining amongst superior complementary parents and selection among segregating progeny. Here, the genotypes receive a population of gametes, and their genetic material is multiplied. This leads to a population of fully homozygous individuals, which can include the ideotype. Using this technique, the ideal genotype can be obtained after the root genotype is obtained in just one additional generation, and there are chances of recovering transgressive segregants by the pedigree method. Moreover, this approach is useful for highly inherited traits, such as seed size, form, disease and insect resistance, height, and maturity. It is commonly used for disease resistant variety production, if it is controlled by major genes [74].

3. Molecular Techniques in Breeding Programs

Crop breeding has been improved to a great extent in recent years and now precision breeding has become possible in the shortest possible time with the advent of modern molecular tools. Innovative molecular breeding tools are being used to improve crop varieties, which mostly involve MAS and gene transformation. Single nucleotide polymorphisms and insertion deletions polymorphisms are abundantly found all over the genome of plants [75], and these are a good source for MAS in breeding programs [76]. Easy accessibility of polymorphic markers, linkage maps, and QTLs for different quantitative and qualitative traits have facilitated construction of inter- and intra-specific maps [77,78]. Quantitative trait loci have been identified and linked with resistance to many plant diseases [79]. The association of QTLs with yield-related traits has not been widely applied [80–82]. These can be used as a marker's linkage with key important genes for the selection and improvement of crops by stacking multiple traits into a variety through modern breeding tools [83].

3.1. Molecular Marker-Assisted Selection

Selection of a trait in plants through molecular markers normally involves identification of omic regions, which play a role in the expression of desirable genes [10]. Marker-assisted selection is involved in genotype identification, diversity and purity analysis, selection of parent lines, and the study of hybrid vigor [84]. It utilizes DNA-based markers that are directly linked with the targeted gene to help phenotypic evaluation and improvement of breeding efficiency by selecting the target genes within the germplasm, but not genetic engineering, which involves transfer of foreign gene sequences [85–87]. Marker-assisted selection has been effectively applied for stress resistance development and quality improvement in many important crops, such as rice (resistance to bacterial blight, blast, flood, and salinity and improvement in grain quality), wheat (resistance to leaf rust and powdery mildew), and cotton (resistance to insect pests) [88]. Previously, crop breeders used performance of plants and information obtained from them to conclude about their genetic makeup, but it was time consuming, strenuous, and not so efficient [85,89]. Thus, direct handling of genes that are involved in controlling specific traits can speed up the breeding process [74]. These traits may be monogenic or polygenic [74,85], and their locations are referred to as QTL, which may have a greater effect than others as major genes. Quantitative trait loci essentially refers to those genes that might be a good option for selection; however, it is difficult to find inheritance at QTLs only. Instead, QTLs help to locate marker sequence inheritance, close to or within the QTL. Selection of markers is done on the basis of closeness to QTLs, so sophisticated and well-organized procedures are adopted for the identification of marker sequences closely linked with major genes on the chromosomes [90]. The reliability of a phenotype should be accurately predicted by the markers, and these are commonly applied to confirm the trueness of the first filial generation (F_1), genetic purity testing of seeds, cultivars, linkage construction, genes, and QTL mapping associated with other biological and physiological functions [86]. The attributes of DNA-based markers for effective applications are their reliability and closeness to the trait of interest (<5 cM genetic distance) [91]. It is also desirable that marker applications be convenient for researchers to handle with good reproducibility, and also simply and quickly. Hence, high output and quick methods of efficiency are important factors to be taken into account. These should also possess high polymorphism, have co-dominance inheritance for homo and hetero zygotes in segregating offspring, and be cost effective [92–94].

3.2. Marker-Assisted Backcrossing

Marker-assisted backcrossing (MABC), a simple form of marker-assisted selection, is currently being widely applied in molecular breeding [95]. Marker-assisted backcrossing targets one or more genes or QTLs transferred from one donor parent into another superior cultivar or genotype to improve a targeted trait. Contrary to conventional backcrossing, MABC depends on the alleles of a marker linked with desirable genes or QTLs instead of phenotypic performance. Through MABC, the outcomes

can be obtained within a shorter period of time (about two years) [85]. In principle, MABC can be utilized in any crop-breeding program. Markers are helpful in the backcross selection for the desired alleles or genes, which are difficult to select based on phenotypic observations, such as pyramiding of disease and pest resistance genes in a specific genotype, where these may overlap each other's effect epistatically. Markers may be used for the selection of some progeny where recombination occurs near the targeted gene containing the allele with some DNA of the donor plant. These have a vital role in backcross breeding for the pyramiding of two or more genes linked with desired stresses tolerance [96]. Marker-assisted backcrossing gene pyramiding involves three levels of selection (Figure 3) [64,73]. Crossing is done between the recurrent parent and donor parents for the F_1 hybrid, which is then backcrossed up to three generations to obtain the best parent. It is further crossed with another donor parent for pyramiding two or more genes of interest. Although this technique is considered to have low satisfaction and also time consuming but, its precision for gene pyramiding, is considered good (Figure 3A) [97,98]. In other breeding techniques (Figure 3B), the recurrent parent is crossed with donor parents to obtain F_1 hybrids, and these are intercrossed to get improved F_1 , which is further backcrossed with the recurrent parent to produce an improved recurrent parent. Thus, pyramiding is done in the pedigree itself. In the third strategy (Figure 3C), the first two schemes (Figure 3A,B) are combined; it involves simultaneous crossing of the recurrent parent with multiple donor parents, and then backcrossing up to the BC_3 generation. The backcrossed populations are then intercrossed with each other to achieve gene pyramiding. This technique is considered most suitable, because it is less time consuming and fixation of genes is certain. However, success or failure of this technique depends on many factors, such as distance between the closest markers and the target gene, the number of target genes, the genetic base, the genetic background in which the target gene is manipulated, and the marker type. If proper selection criteria are maintained, then MABC-based gene pyramiding can produce durable and sustainable crop improvement [27,99–102].

of selection) [103]. Marker-assisted recurrent selection (MARS) is an improved system that enables genotype selection and intercrossing in one cropping season (Figure 4), which can facilitate the efficacy of recurrent selection and expedite the selection process [104] and help in integration of multiple favorable genes. For complex agronomic traits, such as grain yield and biotic and abiotic stress resistance, the pyramiding of multiple QTLs into crops is recommended, and expression-QTL (eQTL), protein-QTL (pQTL), and metabolite-QTL (mQTL) types of analysis are applied on the multiple traits [83,105–107]. By sequencing the QTL region in several segregating lines, genotyping offers a rapid method for building high-resolution maps in a season to quicken positional cloning. Sequencing of each line within a recombinant inbred line (RIL) population has been used to easily and reliably classify recombinant breakpoints [108]. Therefore, a QTL peak was co-located with a GW5 gene (a major QTL on chromosome 5, qGW5) that regulates grain width [109]. Sequencing could target specific regions to identify the underlying QTL genes that control drought tolerance in wheat [110]. Expression-QTL, pQTL, or mQTL is linked with these traits. These intermediate molecular phenotypes help to reveal the genotypic variation and underlying morphological and physiological characteristics [111]. Mapping of eQTL patterns that regulate complex traits in the main regulatory regions can be defined and used in the molecular breeding of plants. Expression-QTLs reveal regions that affect gene expression in cis (close to the target gene itself, such as a promoter element) or in trans (distant to the gene). High eQTLs are normally cis-regulated; the eQTL position hence generally indicates the actual gene location [112]. The genes displaying differential expression between the recombinants can be genetically mapped to see if their position is related to the targeted QTLs. For instance, 88 genes that are differentially expressed during drought stress were linked with drought tolerance QTLs in maize and further selected as positional cloning candidates [113].

Figure 4. Schematic representation of marker-assisted recurrent selection in crops. Individual parents within the population were selected to cross to generate F_1 offspring. F_1 individuals were allowed selfing to produce 300 F_2 progenies using the single-seed decent method. F_2 individuals were advanced to F_3 , F_4 , and F_5 . Genotyping was carried out on individual progenies. The best seeds were selected and evaluated in multi-location phenotyping. Qualitative trait loci (QTL) analysis was done and modeling was conducted prior to selection of QTLs for recombination. The six best genotypes per offspring of F_3 were selected (A–F). The first recombination cycle generated F_1 individuals, which were selfed to produce; F_2 and F_2 were advanced to F_3 and F_4 for multi-location phenotyping [27].

Many researchers have reported that the genetic advance obtained through MARS in maize was almost double as compared with phenotypic selection [106,114]. Bankole [115] also reported on the effectiveness of MARS for improvement of yield and related traits in maize. The basic procedure of MARS comprises some steps, such as selection of the parent lines from a similar and non-similar population [116]. Marker-assisted recurrent selection of F_3 -derived individuals is generally satisfactory and multiplied through a single-seed decent strategy for increasing seed to conduct multiplication trials. Large plant numbers are preferred to rely on the accuracy of QTL mapping. Further, QTL can be evaluated after geno-phenotypic analysis for the selection of markers and suitable alleles. The best population is selected for recombination, as shown in the example in Figure 4, where four genotypes are crossed to generate two pairs of F_1 offspring. At each cycle, genotyping is performed to identify the best F_1 individuals, which could be used again in the next cycle of recombination [115].

3.4. Omics Techniques for Crop Improvement

Omics involves many techniques used to classify and measure the functions and relationships of broad collections of molecules of various types in an organism [117]. The technology includes transcriptomics (sequencing) [118,119], proteomics (structure and function of the proteins) [120–123], and metabolomics (detection and quantification in various biological organisms of metabolites and chemical signatures of cellular regulatory processes) [124–128] and enables seed profiling of gene transcription, protein expression, and/or small molecule synthesis and genome-level metabolism. Metabolomics is the innovative omics branch of science that identifies/quantifies the metabolites and chemical processes of the cellular regulatory system of the organisms. The metabolome is used to characterize genetic or environmental variations in any species and thus have a significant role in exploring environment and gene interactions, characterization of mutants, phenotyping, and biomarker identification. Thus, metabolomics is an innovative approach to be applied for exploration of various metabolic networks, linked with biotic and abiotic stress tolerance in plants and crop improvement [129]. Omics technology facilitates the rapid identification of nutritionally significant markers that may help to grow new, quality crops [130,131]. A key drawback of metabolomics is the lack of knowledge about several (secondary) metabolic pathways and reference standards in many of the metabolites (especially the myriad of secondary plant metabolites), and the inability to potentially classify the vast majority of synthesized metabolites in the plant kingdom [132]. Other problems associated with Omics technology are the cost of the technology for its direct application [128], and linkage of primary and secondary plant metabolites with very complex metabolic pathways. However, advanced metabolomics tools, such as gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectroscopy (LC-MS), and non-destructive nuclear magnetic resonance spectroscopy (NMR) allow the effective detection, identification, assessment, and evaluation of these metabolites [129]. Use of metabolic QTL-assisted breeding may lead to greater understanding of the function of specific genes of interest and their use for better screening and breeding of elite clones of crops [133–135].

3.5. Marker-Assisted Gene Pyramiding in Developing Resistant Crop Varieties

Gene stacking or pyramiding is a useful technique for transferring several desired genes or QTLs from different parents into a single genotype in the shortest possible time (two to three generations), as compared with conventional breeding, which takes a minimum of six generations to recuperate 99.2% of the recurrent parent genome [24,48]. It aims at accumulating several resistance genes with known effect on a trait of target and confers durable resistance against different stresses [88], and it became possible with recent advances in molecular markers techniques [136–138]. Plant scientists have successfully utilized this technique to pyramid resistant genes or QTLs through the help of closely associated markers against biotic stresses, such as late blight (*Phytophthora infestans* L.), bacterial blight (*Xanthomonas campestris* L.), gall midge (*Contarinia quinquenotata* L.), mosaic viruses, powdery mildew (*Podosphaera xanthii* L.), and many abiotic stresses, such as salinity, drought, heat, and cold, as well as quality improvement in many major crops, as shown in Table 1. Marker-assisted gene pyramiding

also provides ease in selection of QTL allele-linked markers with similar phenotypic expression. Thus, pyramiding of several genes or QTLs is endorsed as a possible approach to improve quantitative as well as qualitative traits in plants [72,85,139].

Marker-assisted selection facilitates the monitoring of several traits at a time, while separate field trials are required to screen for individual traits with traditional breeding method [44]. Moreover, MAS facilitates cost-effective gene stacking by selecting desirable plants at the initial stage of growth, which greatly reduces field space, maintenance cost of germplasm, and cost of agronomic inputs for field trials. When several genes conferring resistance to similar stresses are assembled, the markers are potent and effective for classifying those plants bearing genes of interest from undesirable ones [140]. It could be inferred undoubtedly that marker-assisted gene pyramiding is quick, proficient, economical, and a simple technique for application in plant breeding to pyramid genes of interest to build up multiple stress tolerances in crops [72,84,140,141].

3.6. Gene Pyramiding Involving Polygenic Applications

Many quantitative traits, such as yield and quality, along with tolerance to biotic and abiotic stresses have great economic value. Genetic expression affecting these traits is normally regulated by a large number of loci that have some impact on the development of phenotypic traits. These loci (QTLs) can be manipulated by molecular markers as Mendelian entities to harbor resistance against stresses and to improve many quantitative and qualitative traits [142–144]. Marker-assisted selection has been used successfully in polygenic trait development in many crop plants with a high level of success through genotype and pedigree selection and introgression of alien genes in elite lines using advanced backcrossed inbred selection [145]. However, polygenic traits related with yield improvement pose some complications, because yield trait selection is done by crossing between the best lines from a pool. Hence, QTLs mapped in one population may have low significance for other populations. Genetic improvement using the advanced backcross quantitative trait loci (AB-QTL) technique has been utilized for many important traits, such as fruit quality improvement and fungus (mold) resistance in tomato [146]. The advances made in the utilization of gene pyramiding techniques to improve the yield, quality, and stress tolerance in many crops are summarized in Table 1.

Table 1. Gene pyramiding for biotic, abiotic stresses, and quality improvements in crops.

Crop	Traits	Pyramided Genes	References
Biotic stress tolerance			
Potato	Late blight resistance	<i>Rpi-phu 1, Rpi-rzc</i>	[147]
Cotton	Bacterial blight/sheath resistance	<i>Chi11, t1p, Xa21</i>	[148]
	Bollworm resistance	<i>Cry1Ac, Cry2Ab</i>	[149]
	Weed and pathogen resistance	<i>ptxD/Phi</i>	[6]
	Insect pest resistance	<i>Cry1Ac, Cry2Ac</i>	[150]
Wheat	Leaf and stem rust resistance	<i>SrCad, Sr33, Lr34, Fhb</i>	[151]
	Cereal cyst nematode resistance	<i>CreX, CreY, CRISPR-Cas9</i>	[152]
	Aphid resistance	<i>Gn2, Gn4</i>	[153]
Rice	Gall midge resistance	<i>Gm1, Gm2, Gm4</i>	[75]
	Blast resistance	<i>Pi(2)t, Pi25, Pi(t)a, Xa4, Xa5, Xa13, Xa21</i>	[154,155]
	BPH resistance	<i>Bph1, Bph2</i>	[156]
	Blight resistance	<i>Xa5, Xa13, Xa21</i>	[157]
	Bacterial, sheath blight, stem borer	<i>Xa12, Rc7, Cry1AB1, Cry14c</i>	[158]
Soybean	Mosaic virus resistance	<i>Rsv1, Rsv3, Rsv4</i>	[30]
Tomato	Leaf curl/spotted virus	<i>Ty-1, Ty-3, Sw-5</i>	[159]
Barley	Mosaic virus resistance	<i>rym4, rym5, rym9, rym11</i>	[160]
	Strip rust resistance	3 QTL	[161]
Com	Com borer resistance	<i>Cry1le, Cry1Ac</i>	[162]

Table 1. Cont.

Crop	Traits	Pyramided Genes	References
Chickpea	Lepidopteran resistance	<i>Cry1Ac, Cry1Ab</i>	[163]
Pepper	Root-knot nematode resistance	<i>Me1, Me2</i>	[164]
Abiotic stress tolerance			
Rice	Cold tolerance	<i>9PssT-3, 9PssT-7, 9PssT9,</i>	[165]
	Cold tolerance	<i>9SCT1a, 9SCT2</i>	[166]
	Drought tolerance	<i>Soltol</i>	[75]
	Drought tolerance	<i>QTLs</i>	[167]
	Cold tolerance	<i>qPSST-3, qPSST-7, qPSST-9, qSCT1a, TSF4-1</i>	[168]
	Heat, drought, salt, and cold resistance	<i>OsHSP18.6</i>	[169]
Quantitative and qualitative traits			
Cereal	High yield	<i>Gn1a/OsCKX2, APO1, WFP/OsSPL 14</i>	[170]
	Seed shape	<i>GW2, GS 3, 9SW5</i>	[170]

4. Challenges in Molecular Markers Utilization in Plant Breeding

A major challenge of using molecular markers in plant breeding is the high cost to secure and maintain molecular laboratories [88]. There is a huge initial capital cost requirement for marker development, and it is one of the main limitations in marker use in many developing countries for crop improvement [171]. Constant electric supply to preserve the markers at a very low temperature is another major constraint. Additionally, using MAS for line development lacks consistency in determining phenotypes of crops, and bias sampling from a small population may be misleading for specific expression [172,173]. Recombination is another problem, which may occur during DNA replication, because during the recombination process, someone may not be sure about the exact marker option that is linked to an individual gene or allele. Markers are categorized as direct (within major gene) or indirect markers (near major gene), and recombination is the function of the distance between a gene or QTL and the linked marker. It is undesirable for recombination with higher distance between a marker and a major gene [174–176].

Quantitative trait loci mapping is based on the concept of population mapping for the presence or absence of different locus markers and the identification of markers capable of differentiating quantitative traits among the population. The effective recognition of the target hybrid depends on the parents selected and the gene effects involved in the trait expression [177]. Most economic traits of many crops are complex and regulated by multiple genes, each having its own effect; hence, MAS application also has some limitations in the breeding of desired characteristics. Plant breeding is also referred to as applied quantitative genetics, and modern quantitative genetic tools are expediting crop improvement through combining linked and unlinked genes [178–180]. The inclusion of genome-wide markers is needed to overcome these problems. Genomic selection (GS) is an upgraded version of MAS aimed at applying approximate genome-wide markers of all loci and measuring the estimated genomic breeding value (EGBV) to achieve more precise and consistent selection. Genetic selection has opened up a new promising era for research into molecular breeding, and quantitative genetics can be extended to association mapping between markers and phenotypes considering marker and phenotypic knowledge of populations, but in GS models, there is a problem of higher markers than the number of phenotypic observations [178]. Recent advances in breeding for quantitative trait selection, involving high-throughput genotyping and phenotyping events, whole genome sequence (WGS), high recombination ratios, and other advancements, such as speed breeding in quantitative genetics, have allowed scientists to better understand quantitative traits and accelerate the crop improvement process [179]. High-throughput genotyping and phenotyping using spectral reflectance/absorbance, thermography, optical imaging, and platforms/methodologies for root system analysis and trait selection with high accuracy and crop improvement can be accelerated by the new innovation of “speed

breeding”, through shortening the life cycle of plants with a controlled temperature and enhanced photoperiod [181,182]. Successful adoption of quantitative genetic tools, such as doubled haploid growth, MAS, genetic mapping, genomic selection, high-throughput genotyping/phenotyping, reverse, transgenic, shuttle and speed breeding, and genome editing may be used for desired gene pyramiding and early release of crop varieties resistant to biotic and abiotic stress with improved qualitative and quantitative traits [182]. Application of population genomics is based on the identification of outlier loci, screening large numbers of anonymous loci, and comparing statistics between candidate genes and a random sample of unlinked loci without considering the possible selective agent and the ecological context in which selection occurs [183–185]. Some models and algorithms, such as machine learning and genomic best linear unbiased prediction (GBLUP) are being applied for genomic selection to improve prediction accuracy and overcome this problem [185].

5. Contribution of the Gene Pyramiding Technique to Agricultural Sustainability

The gene pyramiding technique has contributed enormously to modern agriculture. It has led to tolerance development in plants for diseases, insect pests, and abiotic stresses and productivity enhancement on a sustainable basis. Molecular markers have made it possible for quick and precise plant breeding and early generation selection for significant traits without extensive field research. Gene pyramiding, through MAS, has enabled the integration of multiple genes into a single plant to achieve the goals of biotic or abiotic stress resistance and a higher yield with the desired nutritional quality. Durability of biotic and abiotic tolerance is crucial for sustainably achieving global food requirements. Many researchers have reported on the successful pyramiding and function of resistance genes into crops to increase the durable resistance against different insect pests and pathogens. An overview of gene pyramiding in major important crop species has been presented in Table 1. The utilization of host plant resistance is economically viable, durable, and environmentally friendly option for overcoming biotic and abiotic stresses. In most of the cases, biotic resistance developed by a single gene is shaken within a short period of time, so modern breeding techniques are focusing at pyramiding of several genes or QTLs into a single genotype to insure long-lasting resistance for sustainable crop production [50,95,186].

6. Conclusions

There are several stresses that cause devastating effects on the productivity and cultivation of crops throughout the globe. To fulfill the needs of the world’s growing population for food, there is an immense need to safeguard crop plants from biotic and abiotic stresses, which cause yield and quality losses. In the present era, conventional plant breeding has improved with the help of molecular markers and MAS strategies. Many stress-tolerant genes have been integrated into crop plants for higher yield, durable stress resistance/tolerance, and enhanced nutritional traits. Molecular marker genotyping has simplified the breeding selection process and reduced the number of generations required for evaluation and gene integration into a desired cultivar. Marker-assisted selection gene pyramiding should be fully explored to minimize the risk of crop yield and quality reduction due to biotic and abiotic stresses. This strategy has already been utilized successfully for accumulation of several resistant/tolerant genes in some varieties of potato, rice, wheat, and barley. The field results are encouraging for stress tolerance and yield performance. Despite the tremendous advancement of gene pyramiding, there are still challenges that need to be addressed to improve its implementation and vast impact. Marker-assisted gene pyramiding should be cost effective, and technical collaboration and financial support make this more viable for developing countries to use for improvement of their local germplasm. Successful accumulation of resistant genes has been accomplished in the areas of abiotic stress and disease resistance; however, there have only been small achievements in pest resistance and nutritional quality enhancement. Any innovation in a breeding program is measured on the contribution made towards improvement in crop production. Hence, breeders should take maximum

advantage of MAS gene pyramiding, and it should be meritoriously implemented in breeding programs to achieve sustainable agricultural goals.

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