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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 possible 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 abiotic and biotic 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 [9,10]. There are diverse and widespread insect pest and pathogens that are challenge for the sustainable agriculture [11]. During the recent years, due to outbreak of a single insect pest, fall armyworm (*Spodoptera frugiperda* L.) across sub-Saharan Africa, India, Bangladesh, Sri Lanka, Thailand, and China, 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 loss in the affected regions [12-15]. 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 [16]. In addition, chemical pesticides are a serious hazard to human health and the environment [17]. Pest resistance to chemical pesticides is another problem. In a recent report, herbicide resistance was reported in 262 weed species globally [18] Novel approaches for pest control are required for sustainable agricultural production, in order to minimize the dependence on pesticides, and to protect the environment and beneficial micro fauna [19]. Contrary to chemical control, the development of pest-resistant crop cultivars has become popular idea that is durable, economical, and environment friendly [20]. It is of key significance to achieve durable and environmentally-friendly biotic stress tolerance in crops to ensure food security on sustainable basis [21,22].

Most crop breeding strategies for abiotic and biotic 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 [23,24]. Therefore, the development of genotypes with resistance against several stresses by pyramiding multiple genes from different sources into a single plant is now emphasized [25-28]. Crop stress tolerance development has been elucidated in several studies by the pyramiding of multiple resistance genes [29,30] (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 [28,31-33].

Abiotic stresses also adversely affect the growth and yield of crops [34]. These can even affect the plant species survival [35,36]. Every year considerable crop losses occur due to floods [37]. Salinity is another problem for crop production and most crop plants are sensitive to salts throughout their life cycle, but seedling stage is more crucial [38]. There are also some salt-resistant crops varieties that express salt-responsive genes to tolerate the excessive salts, and the quantitative trait locus (QTLs) linked with these genes may be mapped through microsatellite markers for breeding of the salt-tolerant lines [39,40]. The cultivar, NonaBokra, which is tolerant to salt, was successfully mapped with SKC1, and this was a good achievement that conserved K^+ homeostasis under salinity [41,42]. Similarly, many goals have been achieved in the development of drought-tolerant traits in other crops through marker assisted breeding methods [43]. Many drought-tolerant genes have been explored and successfully engineered in many crops for the development of drought resistance [44,45]. 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 [46-48]. 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 [5,49]. 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 attributing to 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 many crop species. 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 (*Lysopersicon esculentum* L.**)**, rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and many other cereals [28].

Resistance developed through a single gene can be overcome by pests after a few years [50-53], so it is needed to develop unique and efficient strategies to enhance crop resistance against stresses to improve yield and quality on a sustainable basis [54-56]. Gene pyramiding may be one of the superior techniques to accomplish durable resistance against various stresses in crop production [57-59]. Sustainable improvement of crops by integrating multiple resistance genes is essential to ensure agricultural production across a range of climatic conditions [60]. In most cases, more than one gene control some 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 [61]. 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 [59,62-65]. Marker assisted breeding could make it possible to effectively combine resistant genes into a single genetic background in the shortest possible time [66,67]. In this review, we highlight the advances made in gene pyramiding to address crop production challenges and also to identify the limitations that need to be addressed in utilizing gene pyramiding techniques.

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 [68-70]. 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 [45,71]. The inherited traits and resistant genes are transferred from donor parents into recipient lines by crossing and backcrossing, and are identified via the selection process. The backcrossing method is also used for resistance gene pyramiding [72]. (ii) Pedigree breeding; 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 [71], and (iii) recurrent selection; 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 [73] (Figure 1; A, B, C, D).

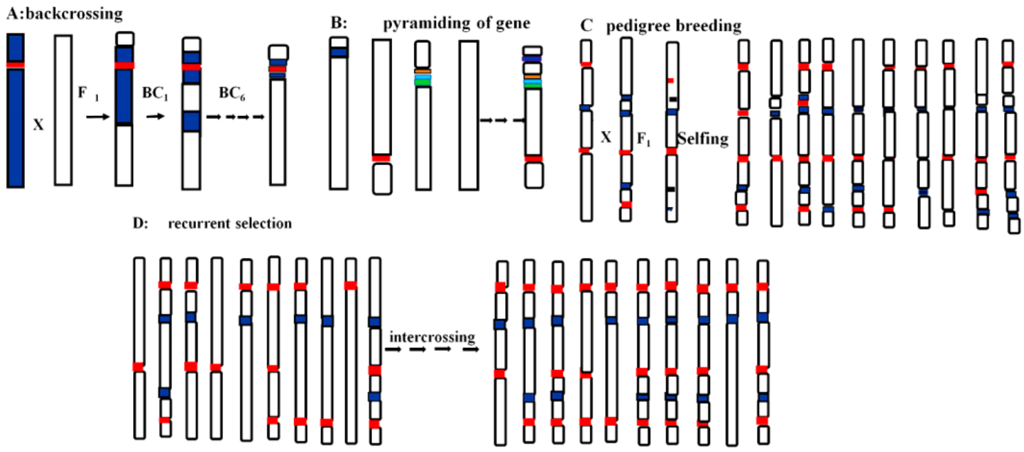

Figure 1. Conventional methods of plant breeding[74].

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 a 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 prove a good source for MAS in breeding programs [76]. Easy accessibility of polymorphic markers, linkage maps, and quantitative trait loci (QTLs) for different quantitative and qualitative traits have facilitated for construction of inter- and intra-specific maps [77,78]. Quantitative trait loci, have been identified and linked with the 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 omic regions identification, which play role in the expression of desirable genes [11]. Marker assisted selection is involved in genotype identification, diversity and purity analysis, selection of parent lines, and the study of hybrid vigor [84] and 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]. In many crop plants MAS 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 (insect pests, resistance) [84,88]. 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 [90]. These traits may be monogenic or polygenic [85,90], and their locations are referred to as QTL, which may have a greater effect than others as major genes. Quantitative traits locus essentially refers to those genes that might be good option for selection; however, it is difficult to find inheritance at QTL only, but it helps to locate marker sequence inheritance, close to or within the QTL. Selection of markers is done on the basis of closeness to QTL, so sophisticated and well organized procedures are adopted for the identification of marker sequences closely linked with major genes on the chromosomes [91]. The reliability of a phenotype should be accurately predicted by the markers, and these are commonly applied to confirm the trueness of first filial generation ($F_{1)}$, genetic purity testing of seeds, cultivar, linkages construction, gene, and QTLs mapping associated with other biological and physiological functions [86]. The attributes of DNA-based markers for effective applications are, their reliability and close link to the trait of interest (< 5 cM genetic distance) [92]. It is also desirable that markers applications should be convenient for other researchers to handle with good reproducibility, and should also be simple and fast. Hence, a high output and quick methods of efficiency are important factors to be taken into account. These should also possess high polymorphism and should have co-dominance inheritance for homo and hetero zygotes in segregating offspring and should be cost effective [93-95].

3.2. Marker Assisted Backcrossing

Marker-assisted backcrossing (MABC), a simple form of marker-assisted selection, is currently being widely applied in molecular breeding [96]. Marker-assisted backcrossing targets one or more genes or QTLs transfer from one donor parent into some other 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.

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As a result, these have a vital importance in backcross breeding for the pyramiding of two or more genes linked with desired stresses tolerance [97]. MABC gene pyramiding involves three levels of selection (Figure 2) [66,98]. Crossing is done between the recurrent parent donor parents for the F_1 hybrid, which is then backcrossed up to three generations to obtain 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 satisfactory and be time consuming, its precision for gene pyramiding is good [99,100]. In the other breeding technique(Figure 2B), the recurrent parent is crossed with donor parents to obtain F_1 hybrids, and these are intercrossed to get improved F_1 and this 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 2C), the first two schemes (Figure 2 A&B) are combined; it involves simultaneous crossing of the recurrent parent with multiple donor parents, and then backcrossing up to the BC3 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, and 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 [28,101-104].

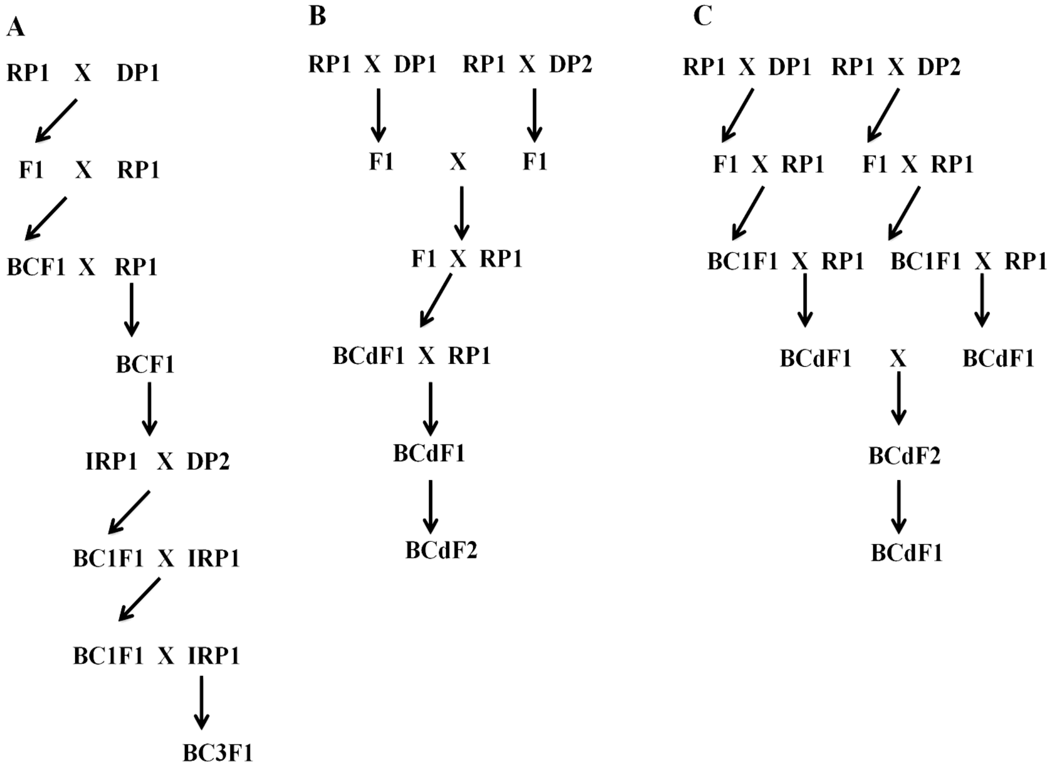

Figure 2. Schematic presentation of different backcrossing strategies for gene pyramiding. RP, recurrent parent; DP, donor parent; BC, backcross; IRP, improved recurrent parent; **A**, stepwise transfer; **B**, simultaneous transfer; **C**, simultaneous and stepwise transfer combined [98].

3.3. Marker Assisted Recurrent Selection

Recurrent selection is considered an efficient approach for pyramiding multiple traits in plants; however, its efficacy of selection is not satisfactory because phenotypic selection depends on environments while genotypic selection takes much time (two to three cropping seasons for a cycle of selection) [105]. Marker assisted recurrent selection is an improved system that enables genotype selection and intercrossing in one cropping season (Figure 3), which can facilitate the efficacy of recurrent selection and expedite the selection process [106], 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 [83,107-109]. Many researchers have reported that the genetic advance obtained through MARS in maize was almost double as compared with phenotypic selection [108,110,111]. Bankole [112] also reported effectiveness of MARS for improvement of yield and related traits in maize through MARS. The basic procedure of MARS comprises some steps, such as selection of the parent lines from a similar and non-similar population[113]. Maker assisted recurrent selection F3-derived individuals are generally satisfactory and multiplied through a single-seed decent strategy for increasing the 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 3, four genotypes are crossed to generate two pair of offspring with one each resulting in F1. At each cycle, genotyping is performed to identify the best F1 individuals, which could be used again in the next cycle of recombination[114].

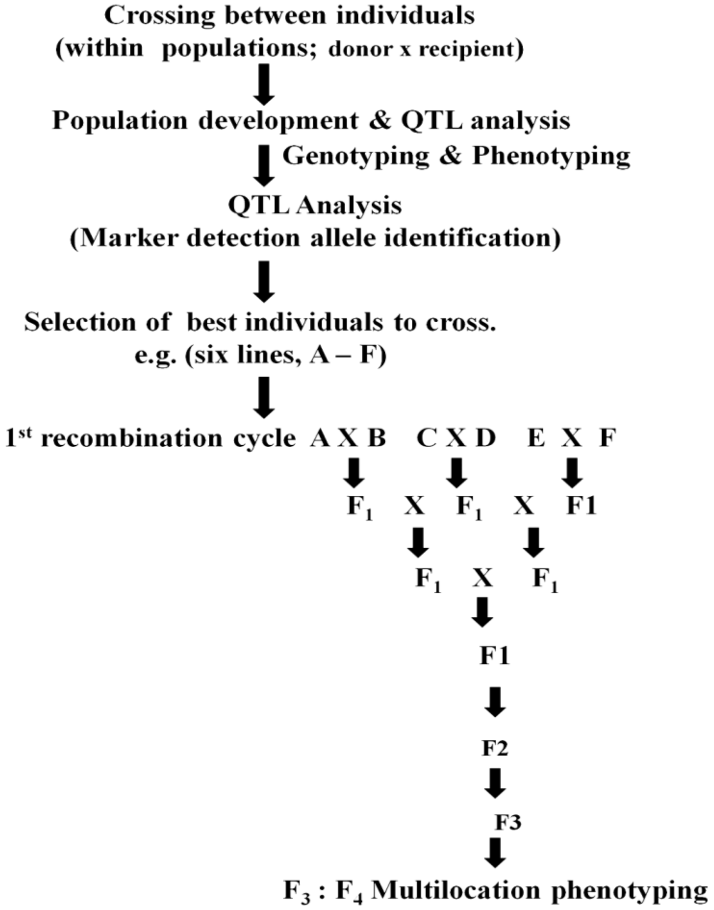

Figure 3. Schematic representation of marker assisted recurrent selection in crops [28]. .

3.4. 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 [25,49,84]. It aims at accumulating several resistance genes with known effect on a trait of target and confers durable resistance against different stresses [88], and became possible with recent advances in molecular markers techniques [115-117]. 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, 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 [85,118,119].

MAS facilitates the monitoring several traits at a time, while separate field trials are required to screen for individual traits with traditional breeding method [45]. Moreover, MAS also 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 costs and agronomic inputs for field trials. When several genes conferring resistance to similar stresses are assembled, the markers are potent and effective to classify those plants bearing genes of interest from the undesirable ones[120]. 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 tolerance in crops [120-122].

3.5. 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 [123-125]. MAS 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 [126]. 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 [127]. Advances made in 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	Reference
Biotic stress tolerance			
Potato	Late blight resistance	*Rpi-phu 1*, *Rpi-rzc*	[128]
Cotton	Bacterial blight/sheath resistance	*Chi11*, *t1p*, *Xa21*	[129]
	Bollworm resistance	*Cry1Ac*, *Cry2Ab*	[130]
	Weed and pathoge resistance	*ptxD/Phi*	[6]
	Insect pest resistance	*Cry1Ac*, *Cry2Ac*	[131]
Wheat	Leaf and stem rust resistance	*SrCad*, *Sr33*, *Lr34*, *Fhb*	[132]
	Cereal cyst nematode resistance	*CreX*, *CreY*, CRISPR-Cas9	[133]

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	Aphid resistance	*Gn2, Gn4*	[134]
Rice	Gall midge resistance	*Gm1, Gm2, Gm4*	[75]
	Blast resistance	*Pi(2)t, Pi25, Pi(t)a, Xa4, Xa5, Xa13, Xa21*	[135,136]
	BPH resistance	*Bph1, Bph2*	[137]
	Blight resistance	*Xa5, Xa13, Xa21*	[138]
	Bacterial , Sheath blight, stem borer	*Xa12, Rc7, Cry1AB1, Cry14c*	[139]
Soybean	Mosaic virus resistance	*Rsv1, Rsv3, Rsv4*	[31]
Tomato	Leaf curl/spotted virus	*Ty-1, Ty-3, Sw-5*	[140]
Barley	Mosaic virus resistance	*rym4, rym5, rym9, rym11*	[141]
	Strip rust resistance	*3 QTL*	[142]
Com	Com borer resistance	*Cry1le, Cry1Ac*	[143]
Chickpea	Lepidopteran resistance	*Cry1Ac, Cry1Ab*	[144]
Pepper	Root-knot nematode resistance	*Me1, Me2*	[145]
Abioic stress tolerance			
Rice	Cold tolerance	*9PssT-3,9PssT-7, 9PssT9,*	[146]
	Cold tolerance	*9SCT1a, 9SCT2*	[147]
	Drought tolerance	*Soltol*	[75]
	Drought tolerance	*QTLs*	[148]
	Cold tolerance	*qPSST-3, qPSST-7, qPSST-9, qSCT1a, TSF4-1*	[149]
	Heat, drought, salt, and cold resistance	*OsHSP18.6*	[150]
Quantitative and qualitative traits			
Cereal	High yield	*Gn1a/OsCKX2, APO1, WFP/OsSPL 14*	[151]
	Seed shape	*GW2, GS 3, 9SW5*	[151]

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 limitation in marker use in many developing countries for crop improvement[152]. Constant electric supply to preserve the markers at 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 [153,154]. 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, or allele 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 distance between a gene or QTL and the linked marker. It is undesirable for recombination with higher distance between a maker and a major gene [155-157].

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. DNA molecular markers have made it possible for quick and precise plant breeding, and early generation selection for significant traits without extensive field work. Gene pyramiding, through MAS, has enabled integration of multiple genes into a single plant to achieve the goals of biotic or abiotic stress resistance and higher yield with desired nutritional quality. Durability of abiotic and biotic tolerance is crucial for sustainably achieving global food requirements. Many researchers have been reported for the successful pyramiding and function of the resistance genes into crops to increase the durable resistance against a different insect pests and pathogens. An overview of gene pyramiding in major important crops species has been presented in Table 1. The utilization of host plant resistance is the best package, economically viable, durable, and environment friendly for stress tolerance. 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 single genotype to insure long-lasting resistance for sustainable crop production [51,96,158,159].

6. Conclusion

There are several stresses that cause devastating effects on 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 loss. 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, and 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 technique should be cost effective and technical collaboration, financial support may be extended to the developing countries for the improvement of their local germplasm. Successful accumulation of resistant genes has been accomplished in the areas of a biotic 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 of food 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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References

1. Davis, K.F.; Chhatre, A.; Rao, N.D.; Singh, D.; Ghosh-Jerath, S.; Mridul, A.; Poblete-Cazenave, M.; Pradhan, N.; DeFries, R. Assessing the sustainability of post-Green Revolution cereals in India. *Proceedings of the National Academy of Sciences* **2019**, *116*, 25034-25041. doi:10.1073/pnas.1910935116.
2. Barrett, C.B. Measuring Food Insecurity. *Science* **2010**, *327*, 825-828. doi:10.1126/science.1182768.
3. Atique ur, R.; Farooq, M.; Rashid, A.; Nadeem, F.; Stuerz, S.; Asch, F.; Bell, R.W.; Siddique, K.H.M. Boron nutrition of rice in different production systems. A review. *Agronomy for Sustainable Development* **2018**, *38*, 25. doi:10.1007/s13593-018-0504-8.
4. Mwobobia, E.G.; Sichangi, A.W.; Thiong'o, K.B. Characterization of wheat production using earth-based observations: a case study of Meru County, Kenya. *Modeling Earth Systems and Environment* **2020**, *6*, 13-25. doi:10.1007/s40808-019-00699-4.
5. Kage, U.; Kumar, A.; Dhokane, D.; Karre, S.; Kushalappa, A.C. Functional molecular markers for crop improvement. *Critical reviews in biotechnology* **2016**, *36*, 917-930. doi:10.3109/07388551.2015.1062743.
6. Pandey, P.; Irulappan, V.; Bagavathiannan, M.V.; Senthil-Kumar, M. Impact of Combined Abiotic and Biotic Stresses on Plant Growth and Avenues for Crop Improvement by Exploiting Physio-morphological Traits. *Front. Plant Sci* **2017**, *8*. doi:10.3389/fpls.2017.00537.
7. Waddington, S.R.; Li, X.; Dixon, J.; Hyman, G.; de Vicente, M.C. Getting the focus right: production constraints for six major food crops in Asian and African farming systems. *Food Security* **2010**, *2*, 27-48. doi:10.1007/s12571-010-0053-8.
8. Bowman, M.S.; Zilberman, D. Economic Factors Affecting Diversified Farming Systems. *Ecology and Society* **2013**, *18*. doi:10.5751/ES-05574-180133.
9. Belsky, J.; Joshi, N.K. Impact of Biotic and Abiotic Stressors on Managed and Feral Bees. *Insects* **2019**, *10*, 233. doi:10.3390/insects10080233.
10. Trueman, S. Plant Stresses: Abiotic and Biotic Stresses. *ThoughtCo, thoughtco.com/plant-stresses-abiotic-and-biotic-stresses-419223.* **2020**.
11. Choudhary, K.; Choudhary, O.P.; Shekhawat, N.S. Marker assisted selection: a novel approach for crop improvement. *Am Eurasian J Agron* **2008**, *1*, 26-30.
12. Womack, E.D.; Williams, W.P.; Smith, J.S.; Warburton, M.L.; Bhattramakki, D. Mapping Quantitative Trait Loci for Resistance to Fall Armyworm (Lepidoptera: Noctuidae) Leaf-Feeding Damage in Maize Inbred Mp705. *Journal of Economic Entomology* **2020**, *113*, 956-963. doi:10.1093/jee/toz357 %J Journal of Economic Entomology.
13. Kansiime, M.K.; Mugambi, I.; Rwomushana, I.; Nunda, W.; Lamontagne-Godwin, J.; Rware, H.; Phiri, N.A.; Chipabika, G.; Ndlovu, M.; Day, R. Farmer perception of fall armyworm (Spodoptera frugiderda J.E. Smith) and farm-level management practices in Zambia. *Pest manag Sci* **2019**, *75*, 2840-2850. doi:10.1002/ps.5504.
14. Assefa, F. Status of Fall Armyworm (Spodoptera frugiperda), Biology and Control Measures on Maize Crop in Ethiopia: A Review. *International Journal of Entomological Research* **2018**, *6*. doi:10.33687/entomol.006.02.2498.
15. Nagoshi, R.N.; Goergen, G.; Tounou, K.A.; Agboka, K.; Koffi, D.; Meagher, R.L. Analysis of strain distribution, migratory potential, and invasion history of fall armyworm populations in northern Sub-Saharan Africa. *Scientific reports* **2018**, *8*, 3710. doi:10.1038/s41598-018-21954-1.
16. Sharifzadeh, M.S.; Abdollahzadeh, G.; Damalas, C.A.; Rezaei, R. Farmers' Criteria for Pesticide Selection and Use in the Pest Control Process. *Agriculture* **2018**, 8-24.

17. Vincelli, P. Genetic Engineering and Sustainable Crop Disease Management: Opportunities for Case-by-Case Decision-Making. *Sustainability* **2016**, *8*, 495.
18. Federico, A.C.; Darci, A.G.; Tranel, P.J. Empirical investigation of mutation rate for herbicide resistance. *Weed Science* **2019**, *67*, 361-368.
19. Pandeya, D.; López-Arredondo, D.L.; Janga, M.R.; Campbell, L.M.; Estrella-Hernández, P.; Bagavathiannan, M.V.; Herrera-Estrella, L.; Rathore, K.S. Selective fertilization with phosphite allows unhindered growth of cotton plants expressing the ptxD gene while suppressing weeds. *Proc Natl Acad Sci U S A.* **2018**, *115*, E6946-E6955. doi:10.1073/pnas.1804862115 %J Proceedings of the National Academy of Sciences.
20. Hanson, P.; Lu, S.-F.; Wang, J.-F.; Chen, W.; Kenyon, L.; Tan, C.-W.; Tee, K.L.; Wang, Y.-Y.; Hsu, Y.-C.; Schafleitner, R., et al. Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. *Scientia Horticulturae* **2016**, *201*, 346-354. doi:https://doi.org/10.1016/j.scienta.2016.02.020.
21. Mundt, C.C. Durable resistance: a key to sustainable management of pathogens and pests. *Infect Genet Evol* **2014**, *27*, 446-455. doi:10.1016/j.meegid.2014.01.011.
22. Johnson, R. A Critical Analysis of Durable Resistance. *Annual Review of Phytopathology* **1984**, *22*, 309-330. doi:10.1146/annurev.py.22.090184.001521.
23. Kottapalli, K.R.; Lakshmi Narasu, M.; Jena, K.K. Effective strategy for pyramiding three bacterial blight resistance genes into fine grain rice cultivar, Samba Mahsuri, using sequence tagged site markers. *Biotechnology Letters* **2010**, *32*, 989-996. doi:10.1007/s10529-010-0249-1.
24. Steiner, B.; Michel, S.; Maccaferri, M.; Lemmens, M.; Tuberosa, R.; Buerstmayr, H. Exploring and exploiting the genetic variation of Fusarium head blight resistance for genomic-assisted breeding in the elite durum wheat gene pool. *Theoretical and Applied Genetics* **2019**, *132*, 969-988. doi:10.1007/s00122-018-3253-9.
25. Suresh, S.; Malathi, D. Gene Pyramiding For Biotic Stress Tolerance In Crop plants. *Weekly Science Research Journal* **2013**, *1*.
26. Muthurajan, R.; Balasubramanian, P. Pyramiding Genes for Enhancing Tolerance to Abiotic and Biotic Stresses. *In: Jain S., Brar D. (eds) Molecular Techniques in Crop Improvement. Springer, Dordrecht* **2010**.
27. Pancaldi, F.; Trindade, L.M. Marginal Lands to Grow Novel Bio-Based Crops: A Plant Breeding Perspective. *Front. Plant Sci* **2020**, *11*. doi:10.3389/fpls.2020.00227.
28. Rana, M.; Sood, A.; Hussain, W.; Kaldate, R.; Sharma, T.R.; Gill, R.K.; Kumar, S.; Singh, S. Chapter 6 - Gene Pyramiding and Multiple Character Breeding. In *Lentils*, Singh, M., Ed. Academic Press: 2019; https://doi.org/10.1016/B978-0-12-813522-8.00006-6pp. 83-124.
29. Bai, Y.; Kissoudis, C.; Yan, Z.; Visser, R.G.F.; van der Linden, G. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. **2018**, *93*, 781-793. doi:10.1111/tpj.13800.
30. Ruengphayak, S.; Chaichumpoo, E.; Phromphan, S.; Kamolsukyunyong, W.; Sukhaket, W.; Phuvanartnarubal, E.; Korinsak, S.; Korinsak, S.; Vanavichit, A. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. *Rice (New York, N.Y.)* **2015**, *8*, 7-7. doi:10.1186/s12284-014-0035-0.
31. Zhu, S.; Li, Y.; Vossen, J.H.; Visser, R.G.F.; Jacobsen, E. Functional stacking of three resistance genes against Phytophthora infestans in potato. *Transgenic Res* **2012**, *21*, 89-99. doi:10.1007/s11248-011-9510-1.
32. Piquerez, S.J.M.; Harvey, S.E.; Beynon, J.L.; Ntoukakis, V. Improving crop disease resistance: lessons from research on Arabidopsis and tomato. *Frontiers in plant science* **2014**, *5*, 671-671. doi:10.3389/fpls.2014.00671.

33. Alconi, C.; Stevanato, P.; Motto, M.; Biancardi, E. Breeding for Biotic Stress Resistance/Tolerance in Plants. In: Ashraf M., Öztürk M., Ahmad M., Aksoy A. (eds) Crop Production for Agricultural Improvement. Springer, Dordrecht. **2012**.
34. Ramegowda, V.; Da Costa, M.V.J.; Harihar, S.; Karaba, N.N.; Sreeman, S.M. Chapter 17 - Abiotic and biotic stress interactions in plants: A cross-tolerance perspective. In *Priming-Mediated Stress and Cross-Stress Tolerance in Crop Plants*, Hossain, M.A., Liu, F., Burritt, D.J., Fujita, M., Huang, B., Eds. Academic Press: 2020; https://doi.org/10.1016/B978-0-12-817892-8.00017-9pp. 267-302.
35. Yu, Y.; Yang, Z. The isolation and characterization of a putative dehydrin gene in Triticum aestivum L. *Biochemical Systematics and Ecology* **2016**, *66*, 173-180. doi:https://doi.org/10.1016/j.bse.2016.04.003.
36. Waqas, M.A.; Kaya, C.; Riaz, A.; Farooq, M.; Nawaz, I.; Wilkes, A.; Li, Y. Potential Mechanisms of Abiotic Stress Tolerance in Crop Plants Induced by Thiourea. **2019**, *10*. doi:10.3389/fpls.2019.01336.
37. Loo, Y.Y.; Billa, L.; Singh, A. Effect of climate change on seasonal monsoon in Asia and its impact on the variability of monsoon rainfall in Southeast Asia. *Geoscience Frontiers* **2015**, *6*, 817-823. doi:https://doi.org/10.1016/j.gsf.2014.02.009.
38. Thitisaksakul, M.; Tananuwong, K.; Shoemaker, C.F.; Chun, A.; Tanadul, O.-u.-m.; Labavitch, J.M.; Beckles, D.M. Effects of Timing and Severity of Salinity Stress on Rice (Oryza sativa L.) Yield, Grain Composition, and Starch Functionality. *Journal of Agricultural and Food Chemistry* **2015**, *63*, 2296-2304. doi:10.1021/jf503948p.
39. Tiwari, S.; Sl, K.; Kumar, V.; Singh, B.; Rao, A.R.; Mithra Sv, A.; Rai, V.; Singh, A.K.; Singh, N.K. Mapping QTLs for Salt Tolerance in Rice (Oryza sativa L.) by Bulked Segregant Analysis of Recombinant Inbred Lines Using 50K SNP Chip. *PLoS One* **2016**, *11*, e0153610. doi:10.1371/journal.pone.0153610.
40. Bimpong, I.K.; Manneh, B.; Sock, M.; Diaw, F.; Amoah, N.K.A.; Ismail, A.M.; Gregorio, G.; Singh, R.K.; Wopereis, M. Improving salt tolerance of lowland rice cultivar 'Rassi' through marker-aided backcross breeding in West Africa. *Plant Science* **2016**, *242*, 288-299. doi:https://doi.org/10.1016/j.plantsci.2015.09.020.
41. Ren, Z.-H.; Gao, J.-P.; Li, L.-G.; Cai, X.-L.; Huang, W.; Chao, D.-Y.; Zhu, M.-Z.; Wang, Z.-Y.; Luan, S.; Lin, H.-X. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics* **2005**, *37*, 1141-1146. doi:10.1038/ng1643.
42. Das, P.; Nutan, K.K.; Singla-Pareek, S.L.; Pareek, A. Understanding salinity responses and adopting 'omics-based' approaches to generate salinity tolerant cultivars of rice. *Frontiers in plant science* **2015**, *6*, 712-712. doi:10.3389/fpls.2015.00712.
43. Oladosu, Y.; Rafii, M.Y.; Samuel, C.; Fatai, A.; Magaji, U.; Kareem, I.; Kamarudin, Z.S.; Muhammad, I.i.; Kolapo, K. Drought Resistance in Rice from Conventional to Molecular Breeding: A Review. *Int J Mol Sci* **2019**, *20*, 3519. doi:10.3390/ijms20143519.
44. Sandhu, N.; Dixit, S.; Swamy, B.P.M.; Raman, A.; Kumar, S.; Singh, S.P.; Yadaw, R.B.; Singh, O.N.; Reddy, J.N.; Anandan, A., et al. Marker Assisted Breeding to Develop Multiple Stress Tolerant Varieties for Flood and Drought Prone Areas. *Rice* **2019**, *12*, 8. doi:10.1186/s12284-019-0269-y.
45. Rai, N.; Bellundagi, A.; Kumar, P.K.C.; Kalasapura Thimmappa, R.; Rani, S.; Sinha, N.; krishna, H.; Jain, N.; Singh, G.P.; Singh, P.K., et al. Marker-assisted backcross breeding for improvement of drought tolerance in bread wheat (Triticum aestivum L. em Thell). *Plant Breeding* **2018**, *137*, 514-526. doi:10.1111/pbr.12605.

46. Zhang, H.; Zhang, J.; Wei, P.; Zhang, B.; Gou, F.; Feng, Z.; Mao, Y.; Yang, L.; Zhang, H.; Xu, N., et al. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. *Plant biotechnology journal* **2014**, *12*, 797-807. doi:10.1111/pbi.12200.
47. Shinada, H.; Iwata, N.; Sato, T.; Fujino, K. QTL pyramiding for improving of cold tolerance at fertilization stage in rice. *Breed Sci* **2014**, *63*, 483-488. doi:10.1270/jsbbs.63.483.
48. Almeida, D.M.; Almadanim, M.C.; Lourenço, T.; Abreu, I.A.; Saibo, N.J.; Oliveira, M.M. Screening for Abiotic Stress Tolerance in Rice: Salt, Cold, and Drought. *Methods in molecular biology (Clifton, N.J.)* **2016**, *1398*, 155-182. doi:10.1007/978-1-4939-3356-3_14.
49. Hasan, M.M.; Rafii, M.Y.; Ismail, M.R.; Mahmood, M.; Rahim, H.A.; Alam, M.A.; Ashkani, S.; Malek, M.A.; Latif, M.A. Marker-assisted backcrossing: a useful method for rice improvement. *Biotechnol Biotechnol Equip* **2015**, *29*, 237-254. doi:10.1080/13102818.2014.995920.
50. Sanghera, G.S.; Wani, S.H.; Hussain, W.; Singh, N.B. Engineering cold stress tolerance in crop plants. *Current genomics* **2011**, *12*, 30-43. doi:10.2174/138920211794520178.
51. Lv, H.; Fang, Z.; Yang, L.; Zhang, Y.; Wang, Y. An update on the arsenal: mining resistance genes for disease management of Brassica crops in the genomic era. *Hortic Res* **2020**, *7*, 34. doi:10.1038/s41438-020-0257-9.
52. van Esse, H.P.; Reuber, T.L.; van der Does, D. Genetic modification to improve disease resistance in crops. *The New phytologist* **2020**, *225*, 70-86. doi:10.1111/nph.15967.
53. Borrelli, V.M.G.; Brambilla, V.; Rogowsky, P.; Marocco, A.; Lanubile, A. The Enhancement of Plant Disease Resistance Using CRISPR/Cas9 Technology. *Frontiers in plant science* **2018**, *9*, 1245-1245. doi:10.3389/fpls.2018.01245.
54. Sedeek, K.E.M.; Mahas, A.; Mahfouz, M. Plant Genome Engineering for Targeted Improvement of Crop Traits. *Front. Plant Sci.* **2019**, *10*. doi:10.3389/fpls.2019.00114.
55. Kah, M.; Tufenkji, N.; White, J.C. Nano-enabled strategies to enhance crop nutrition and protection. *Nature Nanotechnology* **2019**, *14*, 532-540. doi:10.1038/s41565-019-0439-5.
56. Bailey-Serres, J.; Parker, J.E.; Ainsworth, E.A.; Oldroyd, G.E.D.; Schroeder, J.I. Genetic strategies for improving crop yields. *Nature* **2019**, *575*, 109-118. doi:10.1038/s41586-019-1679-0.
57. Fuchs, M. Pyramiding resistance-conferring gene sequences in crops. *Current Opinion in Virology* **2017**, *26*, 36-42. doi:https://doi.org/10.1016/j.coviro.2017.07.004.
58. Liu, R.; Lu, J.; Zhou, M.; Zheng, S.; Liu, Z.; Zhang, C.; Du, M.; Wang, M.; Li, Y.; Wu, Y., et al. Developing stripe rust resistant wheat (Triticum aestivum L.) lines with gene pyramiding strategy and marker-assisted selection. *Genetic Resources and Crop Evolution* **2020**, *67*, 381-391. doi:10.1007/s10722-019-00868-5.
59. Ashkani, S.; Rafii, M.Y.; Shabanimofrad, M.; Miah, G.; Sahebi, M.; Azizi, P.; Tanweer, F.A.; Akhtar, M.S.; Nasehi, A. Molecular Breeding Strategy and Challenges Towards Improvement of Blast Disease Resistance in Rice Crop. *Frontiers in plant science* **2015**, *6*, 886-886. doi:10.3389/fpls.2015.00886.
60. Srivastava, V.; Thomson, J. Gene stacking by recombinases. *Plant biotechnology journal* **2016**, *14*, 471-482. doi:10.1111/pbi.12459.
61. Gupta, S.K.; Pandey, M.K. Chapter 19 - Breeding for Disease and Insect-Pest Resistance. In *Integrated Pest Management*, Abrol, D.P., Ed. Academic Press: San Diego, 2014; https://doi.org/10.1016/B978-0-12-398529-3.00021-Xpp. 401-417.
62. Mago, R.; Lawrence, G.J.; Ellis, J.G. The application of DNA marker and doubled-haploid technology for stacking multiple stem rust resistance genes in wheat. *Molecular Breeding* **2011**, *27*, 329-335. doi:10.1007/s11032-010-9434-0.

Peer-reviewed version available at *Agronomy* **2020**, *10*, 1255; doi:10.3390/agronomy10091255

63. Liu, J.; Liu, D.; Tao, W.; Li, W.; Wang, S.; Chen, P.; Cheng, S.; Gao, D. Molecular marker-facilitated pyramiding of different genes for powdery mildew resistance in wheat. *Plant Breeding* **2000**, *119*, 21-24. doi:10.1046/j.1439-0523.2000.00431.x.
64. Mundt, C.C. Durable resistance: A key to sustainable management of pathogens and pests. *Infection, Genetics and Evolution* **2014**, *27*, 446-455. doi:https://doi.org/10.1016/j.meegid.2014.01.011.
65. Nelson, R.; Wiesner-Hanks, T.; Wisser, R.; Balint-Kurti, P. Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics* **2018**, *19*, 21-33. doi:10.1038/nrg.2017.82.
66. Pathania, A.; Rialch, N.; Sharma, P.N. 9 - Marker-Assisted Selection in Disease Resistance Breeding: A Boon to Enhance Agriculture Production. In *Current Developments in Biotechnology and Bioengineering*, Dubey, S.K., Pandey, A., Sangwan, R.S., Eds. Elsevier: 2017; https://doi.org/10.1016/B978-0-444-63661-4.00009-8pp. 187-213.
67. Dixit, S.; Singh, U.M.; Singh, A.K.; Alam, S.; Venkateshwarlu, C.; Nachimuthu, V.V.; Yadav, S.; Abbai, R.; Selvaraj, R.; Devi, M.N., et al. Marker Assisted Forward Breeding to Combine Multiple Biotic-Abiotic Stress Resistance/Tolerance in Rice. *Rice* **2020**, *13*, 29. doi:10.1186/s12284-020-00391-7.
68. Floros, J.D.; Newsome, R.; Fisher, W.; Barbosa-Cánovas, G.V.; Chen, H.; Dunne, C.P.; German, J.B.; Hall, R.L.; Heldman, D.R.; Karwe, M.V., et al. Feeding the World Today and Tomorrow: The Importance of Food Science and Technology. *Comprehensive Reviews in Food Science and Food safety* **2010**, *9*, 572-599. doi:10.1111/j.1541-4337.2010.00127.x.
69. Su, J.; Jiang, J.; Zhang, F.; Liu, Y.; Ding, L.; Chen, S.; Chen, F. Current achievements and future prospects in the genetic breeding of chrysanthemum: a review. *Hortic Res* **2019**, *6*, 109-109. doi:10.1038/s41438-019-0193-8.
70. Zhang, Y.; Massel, K.; Godwin, I.D.; Gao, C. Applications and potential of genome editing in crop improvement. *Genome biology* **2018**, *19*, 210. doi:10.1186/s13059-018-1586-y.
71. Malav, A.K.; Indu; Chandrawat, K.S. Gene pyramiding: An overview. *International Journal of Current Research in Biosciences and Plant Biology* **2016**, *3*, 22-28.
72. Hall, L.M.; Booker, H.; Siloto, R.M.P.; Jhala, A.J.; Weselake, R.J. Chapter 6 - Flax (Linum usitatissimum L.). In *Industrial Oil Crops*, McKeon, T.A., Hayes, D.G., Hildebrand, D.F., Weselake, R.J., Eds. AOCS Press: 2016; https://doi.org/10.1016/B978-1-893997-98-1.00006-3pp. 157-194.
73. Cui, Y.; Patel, J.; Zou, J.; Keller, W.A. *Oilseed Brassicas. In Compendium of Transgenic Crop Plants (eds C. Kole and T.C. Hall). doi:10.1002/9781405181099.k0202*; 2009; doi:10.1002/9781405181099.k0202.
74. Moose, S.P.; Mumm, R.H. Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. *Plant physiology* **2008**, *147*, 969-977. doi:10.1104/pp.108.118232.
75. Das, G.; Rao, G.J.N. Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. *Frontiers in plant science* **2015**, *6*, 698-698. doi:10.3389/fpls.2015.00698.
76. Hayashi, K.; Hashimoto, N.; Daigen, M.; Ashikawa, I. Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. *Theoretical and Applied Genetics* **2004**, *108*, 1212-1220. doi:10.1007/s00122-003-1553-0.
77. Liu, W.; Maurer, H.P.; Li, G.; Tucker, M.R.; Gowda, M.; Weissmann, E.A.; Hahn, V.; Würschum, T. Genetic architecture of winter hardiness and frost tolerance in triticale. *PLoS One* **2014**, *9*, e99848-e99848. doi:10.1371/journal.pone.0099848.
78. Tullu, A.; Tar'an, B.; Warkentin, T.; Vandenberg, A. Construction of an Intraspecific Linkage Map and QTL Analysis for Earliness and Plant Height in Lentil. *Crop Science* **2008**, *48*, 2254-2264. doi:10.2135/cropsci2007.11.0628.

79. Taylor, P.W.J.; Ades, P.K.; Ford, R. QTL mapping of resistance in lentil (Lens culinaris ssp. culinaris) to ascochyta blight (Ascochyta lentis). *Plant Breeding* **2006**, *125*, 506-512. doi:10.1111/j.1439-0523.2006.01259.x.

80. Fedoruk, M.J.; Vandenberg, A.; Bett, K.E. Quantitative Trait Loci Analysis of Seed Quality Characteristics in Lentil using Single Nucleotide Polymorphism Markers. *The Plant Genome* **2013**, *6*, plantgenome2013.2005.0012. doi:10.3835/plantgenome2013.05.0012.

81. van Eeuwijk, F.A.; Bustos-Korts, D.; Millet, E.J.; Boer, M.P.; Kruijer, W.; Thompson, A.; Malosetti, M.; Iwata, H.; Quiroz, R.; Kuppe, C., et al. Modelling strategies for assessing and increasing the effectiveness of new phenotyping techniques in plant breeding. *Plant Science* **2019**, *282*, 23-39. doi:https://doi.org/10.1016/j.plantsci.2018.06.018.

82. Ahmed, M.M.; Huang, C.; Shen, C.; Khan, A.Q.; Lin, Z. Map-based cloning of qBWT-c12 discovered brassinosteroid-mediated control of organ size in cotton. *Plant Science* **2020**, *291*, 110315. doi:https://doi.org/10.1016/j.plantsci.2019.110315.

83. Ribaut, J.M.; de Vicente, M.C.; Delannay, X. Molecular breeding in developing countries: challenges and perspectives. *Current opinion in plant biology* **2010**, *13*, 213-218. doi:https://doi.org/10.1016/j.pbi.2009.12.011.

84. Collard, B.C.Y.; Mackill, D.J. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philos Trans R Soc Lond B Biol Sci* **2008**, *363*, 557-572. doi:10.1098/rstb.2007.2170.

85. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Hasan, M.M.; Oladosu, Y.A.; Magaji, U.G.; Akos, I.; Olalekan, K.K. Bacterial leaf blight resistance in rice: a review of conventional breeding to molecular approach. *Molecular biology reports* **2019**, *46*, 1519-1532. doi:10.1007/s11033-019-04584-2.

86. Oladosu, Y.; Rafii, M.Y.; Magaji, U.; Abdullah, N.; Miah, G.; Chukwu, S.C.; Hussin, G.; Ramli, A.; Kareem, I. Genotypic and Phenotypic Relationship among Yield Components in Rice under Tropical Conditions. *BioMed Research International* **2018**, *2018*, 8936767. doi:10.1155/2018/8936767.

87. Quibod, I.L.; Perez-Quintero, A.; Booher, N.J.; Dossa, G.S.; Grande, G.; Szurek, B.; Vera Cruz, C.; Bogdanove, A.J.; Oliva, R. Effector Diversification Contributes to Xanthomonas oryzae pv. oryzae Phenotypic Adaptation in a Semi-Isolated Environment. *Scientific reports* **2016**, *6*, 34137-34137. doi:10.1038/srep34137.

88. Das, G.; Patra, J.K.; Baek, K.-H. Insight into MAS: A Molecular Tool for Development of Stress Resistant and Quality of Rice through Gene Stacking. *Frontiers in plant science* **2017**, *8*. doi:10.3389/fpls.2017.00985.

89. Poczai, P.; Varga, I.; Laos, M.; Cseh, A.; Bell, N.; Valkonen, J.P.T.; Hyvönen, J. Advances in plant gene-targeted and functional markers: a review. *Plant Methods* **2013**, *9*, 6. doi:10.1186/1746-4811-9-6.

90. Miah, G.; Rafii, M.Y.; Ismail, M.R.; Puteh, A.B.; Rahim, H.A.; Islam, K.N.; Latif, M.A. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *Int J Mol Sci* **2013**, *14*, 22499-22528. doi:10.3390/ijms141122499.

91. Wang, Z.; Baulcombe, D.C. Transposon age and non-CG methylation. *Nature communications* **2020**, *11*, 1221. doi:10.1038/s41467-020-14995-6.

92. Shirasawa, K.; Hirakawa, H. DNA marker applications to molecular genetics and genomics in tomato. *Breed Sci* **2013**, *63*, 21-30. doi:10.1270/jsbbs.63.21.

93. Bohle, H.M.; Gabaldón, T. Selection of marker genes using whole-genome DNA polymorphism analysis. *Evol Bioinform Online* **2012**, *8*, 161-169. doi:10.4137/EBO.S8989.

94. Nadeem, M.A.; Nawaz, M.A.; Shahid, M.Q.; Doğan, Y.; Comertpay, G.; Yıldız, M.; Hatipoğlu, R.; Ahmad, F.; Alsaleh, A.; Labhane, N., et al. DNA molecular markers in plant breeding: current status

and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment* **2018**, *32*, 261-285. doi:10.1080/13102818.2017.1400401.

95. Tong, H.; Küken, A.; Nikoloski, Z. Integrating molecular markers into metabolic models improves genomic selection for Arabidopsis growth. *Nature communications* **2020**, *11*, 2410. doi:10.1038/s41467-020-16279-5.

96. Pilet-Nayel, M.-L.; Moury, B.; Caffier, V.; Montarry, J.; Kerlan, M.-C.; Fournet, S.; Durel, C.-E.; Delourme, R. Quantitative Resistance to Plant Pathogens in Pyramiding Strategies for Durable Crop Protection. *Frontiers in plant science* **2017**, *8*, 1838-1838. doi:10.3389/fpls.2017.01838.

97. Ye, Y.; Cai, M.; Ju, Y.; Jiao, Y.; Feng, L.; Pan, H.; Cheng, T.; Zhang, Q. Identification and Validation of SNP Markers Linked to Dwarf Traits Using SLAF-Seq Technology in Lagerstroemia. *PLoS One* **2016**, *11*, e0158970. doi:10.1371/journal.pone.0158970.

98. Joshi, R.; Nayak, S. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. *Biotechnology and Molecular Biology Review* **2010**, *5*, 51-60.

99. Kushwah, A.; Gupta, S.; Bindra, S.; Johal, N.; Singh, I.; Bharadwaj, C.; Dixit, G.P.; Gaur, P.M.; Nayyar, H.; Singh, S. Chapter 6 - Gene pyramiding and multiple character breeding. In *Chickpea: Crop Wild Relatives for Enhancing Genetic Gains*, Singh, M., Ed. Academic Press: 2020; https://doi.org/10.1016/B978-0-12-818299-4.00006-3pp. 131-165.

100. Pazhamala, L.; Saxena, R.K.; Singh, V.K.; Sameerkumar, C.V.; Kumar, V.; Sinha, P.; Patel, K.; Obala, J.; Kaoneka, S.R.; Tongoona, P., et al. Genomics-assisted breeding for boosting crop improvement in pigeonpea (Cajanus cajan). *Frontiers in plant science* **2015**, *6*, 50-50. doi:10.3389/fpls.2015.00050.

101. Hu, J.; Xiao, C.; He, Y. Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. *Rice* **2016**, *9*, 30. doi:10.1186/s12284-016-0099-0.

102. GL, J. Molecular Marker-Assisted Breeding: A Plant Breeder's Review. *In: Al-Khayri J., Jain S., Johnson D. (eds) Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Springer, Cham* **2015**.

103. J, P.; R, M.R.; R, S.A.; C, R.S.; R, K.C. Marker-Assisted Breeding for Stress Resistance in Crop Plants. *In: Rout G., Das A. (eds) Molecular Stress Physiology of Plants. Springer, India* **2013**.

104. Y, R.; K, T.; C, M.; P, G. Marker-Assisted Pyramiding Resistance Genes Against Angular Leaf Spot and Common Bacterial Blight Disease into Preferred Common Bean Cultivar "REDWOLAITA". *Adv Crop Sci Tech* **2019**, *7*, 416. doi:10.4172/2329-8863.1000416.

105. Jiang, W.; Zhou, H.; Bi, H.; Fromm, M.; Yang, B.; Weeks, D.P. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. *Nucleic acids research* **2013**, *41*, e188. doi:10.1093/nar/gkt780.

106. Cai, J.; Wang, S.; Su, Z.; Li, T.; Zhang, X.; Bai, G. Meta-analysis of QTL for Fusarium head blight resistance in Chinese wheat landraces. *The Crop Journal* **2019**, *7*, 784-798. doi:https://doi.org/10.1016/j.cj.2019.05.003.

107. Almeida, G.D.; Makumbi, D.; Magorokosho, C.; Nair, S.; Borém, A.; Ribaut, J.-M.; Bänziger, M.; Prasanna, B.M.; Crossa, J.; Babu, R. QTL mapping in three tropical maize populations reveals a set of constitutive and adaptive genomic regions for drought tolerance. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **2013**, *126*, 583-600. doi:10.1007/s00122-012-2003-7.

108. Crosbie, T.M.; Eathington, S.R.; Johnson Sr, G.R.; Edwards, M.; Reiter, R.; Stark, S.; Mohanty, R.G.; Oyervides, M.; Buehler, R.E.; Walker, A.K., et al. *Plant Breeding: Past, Present, and Future*; In Plant Breeding: The Arnel R. Hallauer International Symposium (eds K.R. Lamkey and M. Lee). doi:10.1002/9780470752708.ch1: 2006; doi:10.1002/9780470752708.ch1.

Peer-reviewed version available at *Agronomy* **2020**, *10*, 1255; doi:10.3390/agronomy10091255

109. Gu, R.; Chen, F.; Long, L.; Cai, H.; Liu, Z.; Yang, J.; Wang, L.; Li, H.; Li, J.; Liu, W., et al. Enhancing phosphorus uptake efficiency through QTL-based selection for root system architecture in maize. *Journal of Genetics and Genomics* **2016**, *43*, 663-672. doi:https://doi.org/10.1016/j.jgg.2016.11.002.
110. Ribaut, J.-M.; Ragot, M. Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. *Journal of experimental botany* **2006**, *58*, 351-360. doi:10.1093/jxb/erl214 %J Journal of Experimental Botany.
111. Eathington, S. Practical applications of molecular technology in the development of commercial maize hybrids. In Proceedings of Proceedings of the 60th annual corn and sorghum seed research conferences.
112. Bankole, F.; Menkir, A.; Olaoye, G.; Crossa, J.; Hearne, S.; Unachukwu, N.; Gedil, M. Genetic Gains in Yield and Yield Related Traits under Drought Stress and Favorable Environments in a Maize Population Improved Using Marker Assisted Recurrent Selection. **2017**, *8*. doi:10.3389/fpls.2017.00808.
113. Ali, M.; Zhang, L.; DeLacy, I.; Arief, V.; Dieters, M.; Pfeiffer, W.H.; Wang, J.; Li, H. Modeling and simulation of recurrent phenotypic and genomic selections in plant breeding under the presence of epistasis. *The Crop Journal* **2020**, https://doi.org/10.1016/j.cj.2020.04.002. doi:https://doi.org/10.1016/j.cj.2020.04.002.
114. Bankole, F.; Menkir, A.; Olaoye, G.; Crossa, J.; Hearne, S.; Unachukwu, N.; Gedil, M. Genetic Gains in Yield and Yield Related Traits under Drought Stress and Favorable Environments in a Maize Population Improved Using Marker Assisted Recurrent Selection. *Frontiers in plant science* **2017**, *8*, 808-808. doi:10.3389/fpls.2017.00808.
115. Perumalsamy, S.; Bharani, M.; Sudha, M.; Nagarajan, P.; Arul, L.; Saraswathi, R.; Balasubramanian, P.; Ramalingam, J. Functional marker-assisted selection for bacterial leaf blight resistance genes in rice (Oryza sativa L.). *Plant Breeding* **2010**, *129*, 400-406. doi:10.1111/j.1439-0523.2009.01705.x.
116. Arunakumari, K.; Durgarani, C.V.; Satturu, V.; Sarikonda, K.R.; Chittoor, P.D.R.; Vutukuri, B.; Laha, G.S.; Nelli, A.P.K.; Gattu, S.; Jamal, M., et al. Marker-Assisted Pyramiding of Genes Conferring Resistance Against Bacterial Blight and Blast Diseases into Indian Rice Variety MTU1010. *Rice Science* **2016**, *23*, 306-316. doi:https://doi.org/10.1016/j.rsci.2016.04.005.
117. Van Ooijen, J.W. JoinMap®4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma BV, Wageningen. **2006**.
118. Richards, E.J. Inherited epigenetic variation — revisiting soft inheritance. *Nature Reviews Genetics* **2006**, *7*, 395-301. doi:10.1038/nrg1834.
119. Moose, S.P.; Mumm, R.H. Molecular plant breeding as the foundation for 21st century crop improvement. *Plant physiology* **2008**, *147*, 969-977. doi:10.1104/pp.108.118232.
120. Kumar, A.; Sandhu, N.; Dixit, S.; Yadav, S.; Swamy, B.P.M.; Shamsudin, N.A.A. Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grain yield under drought. *Rice* **2018**, *11*, 35. doi:10.1186/s12284-018-0227-0.
121. Angeles-Shim, R.B.; Reyes, V.P.; del Valle, M.M.; Lapis, R.S.; Shim, J.; Sunohara, H.; Jena, K.K.; Ashikari, M.; Doi, K. Marker-Assisted Introgression of Quantitative Resistance Gene pi21 Confers Broad Spectrum Resistance to Rice Blast. *Rice Science* **2020**, *27*, 113-123. doi:https://doi.org/10.1016/j.rsci.2020.01.002.
122. Collard, B.C.Y.; Mackill, D.J. Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. **2008**, *363*, 557-572. doi:doi:10.1098/rstb.2007.2170.
123. Zorrilla-Fontanesi, Y.; Cabeza, A.; Domínguez, P.; Medina, J.J.; Valpuesta, V.; Denoyes-Rothan, B.; Sánchez-Sevilla, J.F.; Amaya, I. Quantitative trait loci and underlying candidate genes controlling

Peer-reviewed version available at *Agronomy* **2020**, *10*, 1255; doi:10.3390/agronomy10091255

agronomical and fruit quality traits in octoploid strawberry (Fragaria × ananassa). *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **2011**, *123*, 755-778. doi:10.1007/s00122-011-1624-6.

124. Verma, S.; Zurn, J.D.; Salinas, N.; Mathey, M.M.; Denoyes, B.; Hancock, J.F.; Finn, C.E.; Bassil, N.V.; Whitaker, V.M. Clarifying sub-genomic positions of QTLs for flowering habit and fruit quality in U.S. strawberry (Fragaria×ananassa) breeding populations using pedigree-based QTL analysis. *Hortic Res* **2017**, *4*, 17062. doi:10.1038/hortres.2017.62.

125. Xin, F.; Zhu, T.; Wei, S.; Han, Y.; Zhao, Y.; Zhang, D.; Ma, L.; Ding, Q. QTL Mapping of Kernel Traits and Validation of a Major QTL for Kernel Length-Width Ratio Using SNP and Bulked Segregant Analysis in Wheat. *Scientific reports* **2020**, *10*, 25. doi:10.1038/s41598-019-56979-7.

126. Yousef, G.G.; Juvik, J.A. Comparison of Phenotypic and Marker-Assisted Selection for Quantitative Traits in Sweet Corn. **2001**, *41*, 645-655. doi:10.2135/cropsci2001.413645x.

127. Robert, V.J.M.; West, M.A.L.; Inai, S.; Caines, A.; Arntzen, L.; Smith, J.K.; St.Clair, D.A. Marker-assisted introgression of blackmold resistance QTL alleles from wild Lycopersicon cheesmanii to cultivated tomato (L. esculentum) and evaluation of QTL phenotypic effects. *Molecular Breeding* **2001**, *8*, 217-233. doi:10.1023/A:1013734024200.

128. Śliwka, J.; Jakuczun, H.; Chmielarz, M.; Hara-Skrzypiec, A.; Tomczyńska, I.; Kilian, A.; Zimnoch-Guzowska, E. Late blight resistance gene from Solanum ruiz-ceballosii is located on potato chromosome X and linked to violet flower colour. *BMC genetics* **2012**, *13*, 11. doi:10.1186/1471-2156-13-11.

129. Maruthasalam, S.; Kalpana, K.; Kumar, K.K.; Loganathan, M.; Poovannan, K.; Raja, J.A.J.; Kokiladevi, E.; Samiyappan, R.; Sudhakar, D.; Balasubramanian, P. Pyramiding transgenic resistance in elite indica rice cultivars against the sheath blight and bacterial blight. *Plant cell reports* **2007**, *26*, 791-804. doi:10.1007/s00299-006-0292-5.

130. Jackson, R.E.; Bradley, J.R., Jr.; Van Duyn, J.W. Performance of Feral and Cry1Ac-Selected Helicoverpa zea (Lepidoptera: Noctuidae) Strains on Transgenic Cottons Expressing One or Two Bacillus thuringiensis ssp. kurstaki Proteins Under Greenhouse Conditions. *Journal of Entomological Science* **2004**, *39*, 46-55. doi:10.18474/0749-8004-39.1.46 %J Journal of Entomological Science.

131. Puspito, A.N.; Rao, A.Q.; Hafeez, M.N.; Iqbal, M.S.; Bajwa, K.S.; Ali, Q.; Rashid, B.; Abbas, M.A.; Latif, A.; Shahid, A.A., et al. Transformation and Evaluation of Cry1Ac+Cry2A and GTGene in Gossypium hirsutum L. *Frontiers in plant science* **2015**, *6*, 943-943. doi:10.3389/fpls.2015.00943.

132. Zhang, B.; Chi, D.; Hiebert, C.; Fetch, T.; McCallum, B.; Xue, A.; Cao, W.; Depauw, R.; Fedak, G. Pyramiding stem rust resistance genes to race TTKSK (Ug99) in wheat. *Canadian Journal of Plant Pathology* **2019**, *41*, 443-449. doi:10.1080/07060661.2019.1596983.

133. Ali, M.A.; Shahzadi, M.; Zahoor, A.; Dababat, A.A.; Toktay, H.; Bakhsh, A.; Nawaz, M.A.; Li, H. Resistance to Cereal Cyst Nematodes in Wheat and Barley: An Emphasis on Classical and Modern Approaches. *Int J Mol Sci* **2019**, *20*, 432. doi:10.3390/ijms20020432.

134. Liu, Y.; Santi, D.V. m<sup>5</sup>C RNA and m<sup>5</sup>C DNA methyl transferases use different cysteine residues as catalysts. *Proceedings of the National Academy of Sciences* **2000**, *97*, 8263. doi:10.1073/pnas.97.15.8263.

135. Joseph, M.; Gopalakrishnan, S.; Sharma, R.K.; Singh, V.P.; Singh, A.K.; Singh, N.K.; Mohapatra, T. Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice. *Molecular Breeding* **2004**, *13*, 377-387. doi:10.1023/B:MOLB.0000034093.63593.4c.

136. Singh, S.; Sidhu, J.S.; Huang, N.; Vikal, Y.; Li, Z.; Brar, D.S.; Dhaliwal, H.S.; Khush, G.S. Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. *Theoretical and Applied Genetics* **2001**, *102*, 1011-1015. doi:10.1007/s001220000495.

137. Liu, Y.; Chen, L.; Liu, Y.; Dai, H.; He, J.; Kang, H.; Pan, G.; Huang, J.; Qiu, Z.; Wang, Q., et al. Marker assisted pyramiding of two brown planthopper resistance genes, Bph3 and Bph27 (t), into elite rice Cultivars. *Rice (New York, N.Y.)* **2016**, *9*, 27-27. doi:10.1186/s12284-016-0096-3.

138. Narayanan, N.N.; Baisakh, N.; Vera Cruz, C.M.; Gnanamanickam, S.S.; Datta, K.; Datta, S.K. Molecular Breeding for the Development of Blast and Bacterial Blight Resistance in Rice cv. IR50. *Crop Science* **2002**, *42*, 2072-2079. doi:10.2135/cropsci2002.2072.

139. Datta, K.; Baisakh, N.; Thet, K.M.; Tu, J.; Datta, S.K. Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **2002**, *106*, 1-8. doi:10.1007/s00122-002-1014-1.

140. Verlaan, M.G.; Hutton, S.F.; Ibrahem, R.M.; Kormelink, R.; Visser, R.G.F.; Scott, J.W.; Edwards, J.D.; Bai, Y. The Tomato Yellow Leaf Curl Virus resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS genetics* **2013**, *9*, e1003399-e1003399. doi:10.1371/journal.pgen.1003399.

141. Werner, K.; Friedt, W.; Ordon, F. Strategies for Pyramiding Resistance Genes Against the Barley Yellow Mosaic Virus Complex (BaMMV, BaYMV, BaYMV-2). *Molecular Breeding* **2005**, *16*, 45-55. doi:10.1007/s11032-005-3445-2.

142. Castro, A.J.; Capettini, F.; Corey, A.E.; Filichkina, T.; Hayes, P.M.; Kleinhofs, A.; Kudrna, D.; Richardson, K.; Sandoval-Islas, S.; Rossi, C., et al. Mapping and pyramiding of qualitative and quantitative resistance to stripe rust in barley. *Theoretical and Applied Genetics* **2003**, *107*, 922-930. doi:10.1007/s00122-003-1329-6.

143. Jiang, F.; Zhang, T.; Bai, S.; Wang, Z.; He, K. Evaluation of Bt Corn with Pyramided Genes on Efficacy and Insect Resistance Management for the Asian Corn Borer in China. *PLoS One* **2016**, *11*, e0168442-e0168442. doi:10.1371/journal.pone.0168442.

144. Mehrotra, M.; Singh, A.K.; Sanyal, I.; Altosaar, I.; Amla, D.V. Pyramiding of modified cry1Ab and cry1Ac genes of Bacillus thuringiensis in transgenic chickpea (Cicer arietinum L.) for improved resistance to pod borer insect Helicoverpa armigera. *Euphytica* **2011**, *182*, 87. doi:10.1007/s10681-011-0501-3.

145. Djian, C.; Palloix, A.; Fazari, A.; Marteu, N.; Barbary, A.; Abad, P.; Sage-Palloix, A.-M.; Mateille, T.; Risso, S.; Lanza, R., et al. Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. *BMC plant biology* **2014**, *14*, 53-53. doi:10.1186/1471-2229-14-53.

146. Yang, Z.; Huang, D.; Tang, W.; Zheng, Y.; Liang, K.; Cutler, A.J.; Wu, W. Mapping of quantitative trait loci underlying cold tolerance in rice seedlings via high-throughput sequencing of pooled extremes. *PLoS One* **2013**, *8*, e68433-e68433. doi:10.1371/journal.pone.0068433.

147. Fujino, K.; Hirayama, Y.; Kaji, R. Marker-assisted selection in rice breeding programs in Hokkaido. *Breed Sci* **2019**, *69*, 383-392. doi:10.1270/jsbbs.19062.

148. Lin, R.; Zhao, W.; Meng, X.; Wang, M.; Peng, Y. Rice gene OsNAC19 encodes a novel NAC-domain transcription factor and responds to infection by Magnaporthe grisea. *Plant Science* **2007**, *172*, 120-130. doi:https://doi.org/10.1016/j.plantsci.2006.07.019.

149. Kim, Y.S.; Kim, I.S.; Shin, S.Y.; Park, T.H.; Park, H.M.; Kim, Y.H.; Lee, G.S.; Kang, H.G.; Lee, S.H.; Yoon, H.S.J.J.o.A., et al. Overexpression of Dehydroascorbate Reductase Confers Enhanced Tolerance to Salt Stress in Rice Plants (Oryza sativa L. japonica). *Journal of Agronomy and Crop Science* **2014**, *200*, 444-456.

150. Wang, A.; Yu, X.; Mao, Y.; Liu, Y.; Liu, G.; Liu, Y.; Niu, X. Overexpression of a small heat-shock-protein gene enhances tolerance to abiotic stresses in rice. **2015**, *134*, 384-393. doi:10.1111/pbr.12289.

151. Kurokawa, Y.; Noda, T.; Yamagata, Y.; Angeles-Shim, R.; Sunohara, H.; Uehara, K.; Furuta, T.; Nagai, K.; Jena, K.K.; Yasui, H., et al. Construction of a versatile SNP array for pyramiding useful genes of rice. *Plant Science* **2016**, *242*, 131-139. doi:https://doi.org/10.1016/j.plantsci.2015.09.008.

152. Jain, A.; Roorkiwal, M.; Kale, S.; Garg, V.; Yadala, R.; Varshney, R.K. InDel markers: An extended marker resource for molecular breeding in chickpea. *PLoS One* **2019**, *14*, e0213999. doi:10.1371/journal.pone.0213999.

153. Gelli, M.; Konda, A.R.; Liu, K.; Zhang, C.; Clemente, T.E.; Holding, D.R.; Dweikat, I.M. Validation of QTL mapping and transcriptome profiling for identification of candidate genes associated with nitrogen stress tolerance in sorghum. *BMC plant biology* **2017**, *17*, 123-123. doi:10.1186/s12870-017-1064-9.

154. Forster, B.P.; Ellis, R.P.; Thomas, W.T.B.; Newton, A.C.; Tuberosa, R.; This, D.; El-Enein, R.A.; Bahri, M.H.; Ben Salem, M. The development and application of molecular markers for abiotic stress tolerance in barley. *Journal of experimental botany* **2000**, *51*, 19-27. doi:10.1093/jexbot/51.342.19 %J Journal of Experimental Botany.

155. Collard, B.C.Y.; Jahufer, M.Z.Z.; Brouwer, J.B.; Pang, E.C.K. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* **2005**, *142*, 169-196. doi:10.1007/s10681-005-1681-5.

156. Nevame, A.Y.M.; Xia, L.; Nchongboh, C.G.; Hasan, M.M.; Alam, M.A.; Yongbo, L.; Wenting, Z.; Yafei, H.; Emon, R.M.; Ismail, M.R., et al. Development of a New Molecular Marker for the Resistance to Tomato Yellow Leaf Curl Virus. *BioMed Research International* **2018**, *2018*, 8120281. doi:10.1155/2018/8120281.

157. Beukeboom, L.W.; Niehuis, O.; Pannebakker, B.A.; Koevoets, T.; Gibson, J.D.; Shuker, D.M.; van de Zande, L.; Gadau, J. A comparison of recombination frequencies in intraspecific versus interspecific mapping populations of Nasonia. *Heredity* **2010**, *104*, 302-309. doi:10.1038/hdy.2009.185.

158. Devi, E.L.; Devi, C.P.; Kumar, S.; Sharma, S.K.; Beemrote, A.; Chongtham, S.K.; Singh, C.H.; Tania, C.; Singh, T.B.; Ningombam, A., et al. Marker assisted selection (MAS) towards generating stress tolerant crop plants. *Plant Gene* **2017**, *11*, 205-218. doi:https://doi.org/10.1016/j.plgene.2017.05.014.

159. Das, G.; Patra, J.K.; Baek, K.-H. Insight into MAS: A Molecular Tool for Development of Stress Resistant and Quality of Rice through Gene Stacking. **2017**, *8*. doi:10.3389/fpls.2017.00985.