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Advances in Developing Multigene Abiotic and Biotic Stress-Tolerant Rice Varieties

Nitika Sandhu, Shailesh Yadav and Arvind Kumar

Abstract

Increasing incidences of multiple abiotic stresses together with increasing population are the major constraints to attain the global food security. Rice, the major staple food crop is very much prone to various abiotic and biotic stresses, which can occur one at a time or two or more together in a single crop growing season and adversely affects the rice production and productivity. The devastating effect of multiple stresses on rice crop is much more erratic and complex leading to higher losses in the crop grain yield. The concurrent occurrence of multiple stresses can destroy rice production in many of the rainfed areas of South and Southeast-Asia. Genomics-assisted breeding strategies have been instrumental in introgression of various major effect QTLs/genes into rice mega varieties and have proven successful in achieving the desired level of tolerance/resistance to various abiotic stresses in different crop species. Keeping the present scenario of changing climate in mind, the chapter discusses the recent past success in combining tolerance to two or more abiotic stresses in mega rice varieties applying genomics-assisted breeding and development of high-yielding climate resilient rice through stacking of multiple genes/QTLs, which can withstand in a cascade of multiple stresses occurring regularly in rainfed environments.

Keywords: abiotic stress, biotic stress, genomic-assisted breeding, pyramiding, QTLs, rice, yield

1. Introduction

Global warming and the changing climatic conditions lead to the concurrence of multiple abiotic and biotic stresses individually/or in combination [1, 2] thus adversely affecting the rice crop growth and yield [3]. The changing climate, more and more extreme weather events are increasing the probability of simultaneous multiple abiotic stresses, including extra pressure from biotic stresses. Abiotic and biotic stresses reported to have significant negative impact on rice crop survival, growth, development and yield in most parts of the world, especially the Asia and Africa [4, 5]. The abiotic stresses such as drought, salinity, cold, high temperature and heavy metals are known to influence the occurrence of biotic stresses [6–8]. The combined effect of multiple stresses may resulted the minor pests to become the potential threats in the coming future [1, 9, 10].

The rice farming is practiced in various diverse ecological zones. The rice cultivation system in diiferent growing areas are mainly depends upon various factors such as available water, soil type, and the prevailing monsoon. Rice produc- tion faces various constraints in various ecology of rice cultivation (**Table 1**). Rice crop faces multiple stresses during different stages of its growth and development and around 70% reduction in yield was reported due to the occurance of abiotic stresses at different stages of growth and development [5]. Similarly, the major biotic stresses such as bacterial leaf blight, blast, brown plant hopper, brown spot, sheath blight and gall midge reported to impart severe crop yield losses or even complete crop failure during infestation [4]. The growth of rice yield has dete- riorated from 2.3% per year during 1970s–1980s to about 1.5% during 1990s, and to <1% in the first decads of this present century [11]. Although the rice produc- tion has improved considerably over time but it is not sufficient to cope with the increasing demand globally [12]. The annual shortage of rice is expected to rise from 400,000 tons in 2016 to around 800,000 tons by 2030 [13].

As crop plants are immobile, they have to respond to the different abiotic and biotic stresses in the field itself. Breeding efforts in developing tolerance for single stress such as drought, heat, salinity, cold, insect and pathogen or a single stress type viz. abiotic or biotic may be tricky because plants may respond differentially to different or simultaneous occurance of stresses. The increase in resistance/tolerance to one type of stress may be at the cost of resistance/tolerance to another stress [14]. Breeding of high yielding multiple stress tolerant/resistant rice varieties with better grain quality is the urgent need of the hour since many decades [15]. Improvement of germplasm involving improved donors free from undersirable linkage, identification and introgression of genomic regions after validation involving recent advances in genomics-assisted breeding has provided opportunity to combat the challenges arising due the occurance of multiple stresses [16]. An integrated genomics-assisted breeding approach to introgress desirable genes/QTLs conferring tolerance/resistance to major abiotic and biotic stresses in addition to improved yield and quality will help to combat the present situation [16–20]. The commercial use of QTLs/genes-conferred multiple stress tolerant/resistant rice varieties provides an effective, economical and environment friendly approach to protect the crop yield and productivity. In past few years, the identification of genomic regions associated with drought, submergence and heat tolerance and introgression and pyramiding of these regions applying markers assisted selection/backcross approach have successfully led to the development of

Ecosystem	Source of water	Constraints
Upland	Rainfall	Drought, blast, weeds, low soil fertility, Fe toxicity, soil nematode problem, lodging
Rainfed shallow lowland	Rainfall, water table	Lack of assured irrigation, frequent drought, blast, bacterial leaf blight
Rainfed medium lowland	Rainfall, water table	Lack of assured irrigation, drought, flood, drought and flood in same or different season, bacterial leaf blight, brown plant hopper, gall midge
Rainfed deep lowland	Rainfall, water table, flood water	Lack of assured irrigation, fragile and low productivity, Prevailing abiotic stresses such as flood, salinity, Biotic stresses such as bacterial leaf blight, gall midge, brown plant hopper
Irrigated	Irrigation	Salinity, bacterial leaf blight, brown plant hopper

Table 1.
Rice production constraints in various ecologies of rice cultivation.

drought or flood tolerant version of some of the mega varieties such as Swarna, IR64 and Sambha Mahsuri. Some of these developed genomics-assisted derived breeding lines have been released as varieties in various countries of South Asia and South East Asia for cultivation.

2. Biotic stresses

2.1 Bacterial blight

Rice crop is most vulnerable to bacterial blight (BLB) caused by *Xanthomonas oryzae* pv. *Oryzae* (Xoo). The incidences of BLB reported yield losses of 20–30% and as high 80–100% in moderate and severe infection conditions, respectively [21, 22]. This might be due to the partial grain filling resulted from low photosynthetic activity [23]. Out of already identified 45 BLB resistant (R) genes, 11 genes have been fine mapped and cloned till date applying modern biotechnological [24–27]. The marker-assisted pyramiding approach was applied to pyramid four BLB resistant genes; *Xa4*, *xa5*, *xa13* and *Xa21* in background of high yielding rice varieties to achieve wider and durable resistance [28–31]. The combination of these four BLB genes reported as most stable and showed resistance to most of the pathogen isolates [32, 33].

2.2 Blast

Rice blast (*Magnaporthe oryzae*) is another crucial threat to the rice production caused by fungus *Pyricularia oryzae*. It is affecting leaves, collar, nodes, panicles and panicle neck during vegetative to reproductive stage causing 10–30% under mild infestation [34–36] to 70–80% [37] to 100% [38] yield losses under severe infestation conditions. Till now, more than 100 blast resistant genes have been identified, however only 30 of them has been cloned and functionally characterized [17, 39]. The identified linked markers can be used effectively to provide resistance against this devastating fungal disease. The broad-spectrum blast resistant genes viz. *Pi9* which encodes the NBS–LRR gene clusters and *Pita2* which was mapped on the short arm of chromosome 12 confer resistant to many of the blast races in different countries [40, 41]. Transgenic rice lines carrying *Pi-d2* blast resistance gene transformed involving vectors pCB6.3 kb, pZH01–2.72 kb, and pCB5.3 kb showed various levels of resistance (~92%) against 39 strains of rice blast [42]. The durability of rice blast resistance can be further improved by the hybridization of rice varieties carrying complementary genes to attain multi-genic resistance against broad spectrum pathogen races [43], thereby reducing the selection pressure on a single isolate.

2.3 Brown plant hopper

Brown plant hopper (*Nilaparvata lugens*) is one of the most notorious insect-pest of rice causing large scale destructions across Asia amounting to around 60% crop loss [44]. In addition, it is responsible for the transmission of the virus diseases such as rice grassy stunt virus and rice ragged stunt (RRSV) viruses [45, 46]. To date, 37 BPH resistant genes on six of the total twelve rice chromosomes have been reported from cultivated rice and wild *Oryza* species [17, 47, 48]. Out of these 34 genes, 20 genes were fine mapped and only 8 genes (*Bph3*, *Bph14*, *Bph9*, *Bph17*, *Bph26*, *Bph18*, *Bph29* and *Bph32*) have been cloned and functionally characterized [49–56]. These resistance genes encode NBS-LRR protein family that are being widely used

in marker-assisted breeding programs to develop rice varieties resistance to BPH. However, notable achievements have been made in identification and introgression of BPH resistance genes, rapid evolution of the virulent populations of BPH poses a prime concern. The Srilankan rice cultivar Rathu Heenati was first reported as the potential donor providing resistance against four BPH biotypes [57]. Even after 30 years of deployment in the Philippines, Rathu Heenati still reported to provide resistance to BPH [58]. The cloning and functional characterization of BPH resistant genes provides unique opportunity to effectively use these genes in marker-assisted gene introgression program [53].

2.4 Gall midge

The Asian rice gall midge (*Orseolia oryzae*) is another serious insect pest of rice prevailing mainly in wet season in the South-East Asia, China, and India, while *Orseolia oryzivora*, a closely related species is prevalent in the Africa. The infestation of gall midge (GM) amounting an annual yield loss of \$550 million in different countries in Asia [59]. Till date, various genetic studies have identified 11 major resistance (R) genes providing resistance to 7 biotypes of the gall midge of rice which are prevailing mostly in South Asian countries [60–63]. Out of 11, eight of GM resistance genes (*Gm1*, *Gm2*, *Gm4*, *gm3*, *Gm6*, *Gm8*, *Gm7*, and *Gm11*) have been mapped successfully [64, 65]. Interestingly, none of the identified GM resistant genes confers resistance to all the biotypes of gall midge, while none of the GM biotype is virulent against all the identified resistance genes. Four gall midge resistance genes designated as *Gm1*, *gm3*(NB-ARC), *Gm2*(NB-ARC), and *Gm4* (NB-LRR) have been functionally validated and linked markers can be used for the marker-assisted introgression program [63, 66–68]. Marker-assisted introgression/pyramiding of gall midge resistant genes (*Gm1* + *Gm4*) and (*Gm4* + *Gm8*) in background of improved Samba Mahsuri and an elite rice hybrid DRRH3 respectively was attempted by Divya et al. [62] and Kumar et al. [69]. Further, marker assisted pyramiding of multi-genes conferring to bacterial blight, gall midge, blast along with Saltol QTLs for salinity tolerance was reported [18, 33].

3. Abiotic stresses

3.1 Drought

Among the abiotic stresses, drought is one of the most disruptive, and risky events of the ongoing climate change that affect millions of people every year across the world. Depending upon the intensity and pattern of rainfall, drought can occur from few days to few months or even to years [70]. The development of high yielding drought-tolerant rice varieties is the final goal of rice breeders to reduce the yield losses due to drought and to ensure the projected world food production. However, the development of drought-tolerant rice varieties is immensely tough due to the complex quantitative nature of trait [71, 72]. The selection of lines under differential level of drought and due to the occurrence of drought at different stages [73–75] is again not an easy task. In addition, the strong GxE interactions and low heritability of traits such as grain yield also add to the difficulty of the task [76]. Cost-effective modified breeding strategy involving combined phenotyping and genotyping selection approaches in the development and screening of large segregating populations covering high genetic variation have led to the successful identification of 12 major effect QTLs (*qDTY_{1.1}* on chromosome 1; *qDTY_{2.1}*, *qDTY_{2.2}* and *qDTY_{2.3}* on chromosome 2; *qDTY_{3.1}* and *qDTY_{3.2}* on chromosome 3, *qDTY_{4.1}* on

chromosome 4; $qDTY_{6.1}$ and $qDTY_{6.2}$ on chromosome 6, $qDTY_{9.1}$ on chromosome 9, $qDTY_{10.1}$ on chromosome 10, and $qDTY_{12.1}$ on chromosome 12) with consistent effects in background of widely cultivated, popular, high-yielding but drought-susceptible rice varieties, such as IR64, Swarna, Sabitri, MTU1010, Vandana and TDK1 [77–85].

3.2 Salinity

Salinization of soil is another important crisis the world is facing nowadays. Salty soil which is widely distributed across the world is a major factor of rice yield reduction. The salt affected land in India accounts for 6.73 mha (million hectare) which is predicted to increase to 16.2 mha by 2050 [86, 87]. The complexity of salt tolerance mechanisms limits the development of high yielding salt tolerance rice varieties [88]. Salinity stress reported to affect rice grain yield from 20 to 100% depends on the severity of the stress and the duration of stress exposed to the rice crop [89]. Fortunately, the existing wide genetic variability in rice germplasm in response to soil salinity stress makes possible to develop salt tolerant rice varieties [90–92]. The identification and introgression of the trait (s)/genomic regions of interest are the well-known approaches for the development of salinity tolerant varieties [93, 94]. Marker assisted breeding approaches have been proven successful in developing new improved, high yielding salt tolerance rice varieties [95–100].

3.3 High and low temperature

Global changes in the climate conditions and increasing greenhouse gas emission led to a rise in earth's surface temperature in some past decades, and the temperature is predicted to rise by 2 to 4°C by 2050 [101]. The high temperature duration of 3–5 days, 5–7 days and above 8 days is generally considered as mild, moderate and severe heat injury, respectively [102] while low temperature ranged from 0 to 15°C and <0°C categorized as chilling and freezing stress, respectively. Over the past few decades, extensive efforts have been made in identification of genes/QTLs improving heat [103–105] and cold tolerance [106, 107] in rice, which are very complex trait.

4. Marker-assisted pyramiding of multiple QTLs/genes for abiotic/biotic stresses

The challenges from the climate change scenario require the development of climate-adapted rice varieties that combine the tolerance of various abiotic and biotic stresses to better sustain yield losses from unpredicted climate-related events. Recent developments in the identification of major QTLs/genes for drought, submergence, salinity, bacterial blight, brown plant hopper, gall midge, and blast and the successful introgression of identified QTLs to develop improved varieties tolerant of different individual stresses indicate that, with the advent of new marker technology, the development of varieties that combine tolerance of the various abiotic and biotic stresses prevalent in any region is feasible. Such varieties once developed can help farmers overcome yield losses and better farm income under the changed climatic conditions.

The identification of major effect QTLs for the grain yield under drought $qDTY_{12.1}$ [77], $qDTY_{3.1}$ [80], and $qDTY_{1.1}$ [78]; *Sub1*, the gene for submergence [108, 109]; and Saltol, the QTL for salinity [110, 111] using modern breeding tools has provided novel opportunities to the breeders to develop the rice varieties

Variety	QTLs/gene combinations	Targeted trait	Targeted country	Year of release
DRR dhan-42	$qDTY_{2.2} + qDTY_{4.1}$	Drought	India	2014
Yaenelo 4	$qDTY_{2.2} + qDTY_{4.1}$	Drought	Myanmar	2015
Yaenelo 5	$qDTY_{2.2} + qDTY_{4.1}$	Drought	Myanmar	2016
Yaenelo 7	$qDTY_{2.2} + qDTY_{4.1}$	Drought	Myanmar	2016
CR dhan-801	$qDTY_{1.1} + qDTY_{2.1} + qDTY_{3.1} + Sub\ 1$	Drought + flood	India	2017
Bahuguni dhan-2	$qDTY_{3.1} + Sub\ 1$	Drought + flood	Nepal	2017
Bahuguni dhan-1	$qDTY_{1.1} + qDTY_{3.1} + Sub\ 1$	Drought + flood	Nepal	2017

Table 2.
Marker-assisted derived variety released in various countries of South Asia for tolerance to drought and flood in rice.

tolerant of abiotic stresses. Marker-assisted backcrossing of *Sub1* to improve popular variety Swarna had been successfully demonstrated to provide a yield advantage under submerged conditions for up to 18 days [112]. Similarly, the development of drought-tolerant versions of IR64 and Vandna [84, 113] at IRRI that are shown to possess a 1.0 and 0.5 t/ha yield advantage under drought over IR64 and Vandna, respectively, and successful introgression of Saltol into BR28 [114] are some of the recent successes using marker-assisted introgression.

Marker assisted gene pyramiding is an effective breeding strategy to transfer more than one tolerance/resistance genes into a single rice line in order to achieve durable and broader resistance level which can prevent the breakdown of tolerance/resistance against specific races/pathogens [115]. Pyramiding of BLB resistant genes such as *xa5* + *xa13* + *Xa21* [30, 31], *Xa4* + *xa5* + *Xa21* [29] and *Xa4* + *xa5* + *xa13* + *Xa21* [70] had been reported to provide durable resistance in rice against bacterial blight disease. Pyramiding of blast resistance genes *Pi9* and *Pita* has proven effective to combat the blast incidence and increase the durability of blast resistance genes [116].

Rice lines pyramided with multiple disease resistance genes (*Xa4*, *Xa21*, *xa5*, *Bph18* and *Pi40*,) has conferred resistance against BLB, blast, and BPH disease [29]. Among abiotic stresses, recently, drought and flood tolerance were combined using marker assisted pyramiding of the drought QTLs ($qDTY_{1.1} + qDTY_{2.1} + qDTY_{3.1}$) and submergence gene (*Sub1*) together in a popular rice variety, Swarna [19]. The marker-assisted derived rice varieties have been released in different countries (Table 2).

5. QTLs/gene pyramiding through multiple parents crossing

To tackle the multiple problems of rice cultivation under ongoing climate change, a high yielding climate smart new rice lines with superior grain quality is the urgent need to intensify the sustainable rice production. Genomics-assisted breeding (GAB) was attempted to introgress and assemble multiple QTL/genes-*qDTY_{1.1}*, *qDTY_{2.1}*, *qDTY_{3.1}*, *qDTY_{12.1}*, *Sub1*, *Gm4*, *Pi9*, *Pita2*, *Bph3*, *Bph17*, *Xa4*, *xa5*, *xa13*, *Xa21* and *Xa23* into the background of a high yielding breeding line suited for lowland ecosystem of rice under Stress Tolerant Rice for Africa and South

Asia (STRASA) project at IRRI. Developed climate resilient rice lines carrying 6–10 QTLs/genes combinations for tolerance to multiple biotic (BLB-*Xa4*, *xa5*, *Xa21*, *xa13* and *Xa23*; Blast-*Pita2*, *Pi9*; BPH-*Bph3* & *Bph17* and gall midge-*Gm4*) and abiotic stresses (yield under drought stress- *qDTY_{1.1}*, *qDTY_{2.1}*, *qDTY_{3.1}*, *qDTY_{12.1}* and submergence- *Sub1*) as well as superior grain quality traits are free from undesirable linkage drags and can be released as varieties on different countries after evaluation in the national system or can be used as an elite parental lines for making diverse crosses targeted to achieve high genetic gain [117]. Recently, introgression lines with 7 to 10 QTLs/genes for multiple-biotic stresses (blast, BLB, BPH and GM) with drought QTLs in background of Swarna has been reported [14]. A MAGIC (multi-parent advanced generation intercross) population developmental strategy has also been proposed to examine the effect of multiple alleles to provide high grain yield, better grain quality, and tolerance to a wide range of multiple biotic and abiotic stresses [118].

An increase in rice productivity through introgression of multiple traits which can improve rice adaptability under dry direct seeded (DSR) and additionally carrying traits for abiotic/biotic stresses looks a promising breeding strategy to adapt with changing climate, limited water and labor resources and increase rice yield under mechanized DSR conditions. QTLs for traits that increase adaptability to direct seeded rice conditions such as root traits [nodal root number (*qNR_{4.1}*, *qNR_{5.1}*) and root hair density (*qRHD_{1.1}*, *qRHD_{5.1}*, *qRHD_{8.1}*)], early vegetative vigor (*qEVV_{9.1}*), early uniform emergence (*qEUE_{1.1}*, *qEUE_{11.1}*), grain yield under DSR conditions (*qGY_{1.1}*, *qGY_{8.1}*, *qGY_{10.1}*), and lodging resistance (*qLDG_{4.1}*) had been pyramided with abiotic stresses (drought QTLs-*qDTY_{1.1}*, *qDTY_{2.1}*, *qDTY_{3.1}* and *qDTY_{12.1}*) as well as biotic resistance (gall midge-*Gm4*, blast- *Pi9*, *Pita 2*, bacterial leaf blight- *Xa4*, *Xa21*, *xa5*, *xa13*, and brown plant hoppers-*Bph3* and *Bph17*) using MAS approach [119, 120].

5.1 Steps in multiple-trait breeding

Three steps involved in multiple traits introgression scheme, (a) assemble first (b) line fixation and (c) line evaluation. In assemble first step, a complex crossing scheme utilized in transferring the desirable alleles/traits from all the targeted parents aimed to accumulate one copy of all targeted genes/QTLs in a single genotype. In line fixation step, gene based/SSRs/linked markers were utilized in each generation from F₂ to F₆ generation for tracking the presence of desirable alleles of targeted QTLs/in order to find homozygous plants carrying all the targeted QTLs/genes. Phenotyping of the homozygous lines for the targeted traits were performed in line evaluation step and proceed further for multilocation testing of promising lines in the targeted environments. The detailed description on traits, donors, QTLs/genes and markers associated that were used in genomic-assisted breeding program for the development of climate resilient lines at IRRI, Philippines (Table 3).

5.2 Challenges in multiple-trait breeding

The complex breeding program that targets combining tolerance of various abiotic and biotic stresses together in the various genetic backgrounds is unpredictable and more research is needed as such, little is known about the effect that each gene/QTL on the others. However, it is highly assumed that most of the QTLs/genes should work in an additive manner, as far as the targeted QTLs/genes are either located on different chromosomes or in different regions of the same chromosomes. Genomic interactions play a significant role in deciding the performance

Trait	Donor	QTLs/genes	Markers (SNPs/Indels/SSRs/gene based markers)	Reference
Biotic stress				
Blast	WHD-1S-75-1-127, Tadukan, IRBL9	<i>Pi9</i> , <i>Pita2</i>	<i>Pi9</i> : <i>Pi9</i> STS2, <i>Pi9</i> -659T, <i>Pi9</i> -1477G, MSU7_6_10381500 (M492 + M493), M891 (C), <i>Pi9</i> -659T, <i>Pi9</i> -1477G <i>Pita2</i> : MSU7_12_9177624 (M535 + M536), SnpOS00488(G), YL155/YL87, YL153/YL154	[40, 117, 121]
Bacterial leaf blight	IRBB60,	<i>Xa4</i> , <i>xa5</i> , <i>xa13</i> , <i>Xa21</i> , <i>Xa23</i>	<i>Xa4</i> : snpOS0054 (AG), RM224, MP1 + MP2 <i>xa5</i> : <i>xa5</i> S, <i>xa5</i> R, <i>xa5</i> DRR <i>xa13</i> : <i>xa13</i> -promoter (M478Lm + M479Lm + M480Lm), <i>xa13</i> F_130-147/ <i>xa13</i> R_1678-1662 <i>Xa21</i> : <i>Xa21</i> s_exon (M769 + M770), snpOS0061 (C), U1/I1, M1207 (T), pTA248	[117, 122]
Brown plant hopper	Rathu Heenati	<i>Bph3</i> , <i>Bph17</i>	RM589, RM586, RM190, RM8213, RM16556, RM586, RM589, RM190, RM7639, RM19311(linked markers)	[14, 117, 123]
Gall midge	Abhaya	<i>Gm4</i>	GM4_LRR-del_F, GM4_LRR-del_R	[49, 117, 124]
Abiotic stress				
Drought + submergence	IR96322-34-223-B	<i>qDTY_{1.1}</i> + <i>qDTY_{2.1}</i> + <i>qDTY_{3.1}</i> + <i>Sub1</i>	<i>qDTY_{1.1}</i> : RM431, RM11943, RM12233(linked markers), snpOS0071 (A), snpOS0074 (G) <i>qDTY_{2.1}</i> : RM324, RM3549, RM12868, RM12987, RM12995(linked markers), snpOS0078 (A), snpOS0079 (A) <i>qDTY_{3.1}</i> : RM520, RM16030, RM416(linked markers), snpOS0085 (G), snpOS0089 (C) <i>Sub1</i> : ART5, snpOS0040 (T)	[19, 117]
Drought	IR74371-46-1-1	<i>qDTY_{12.1}</i>	RM28099, RM28166, Indel 8, SnpOS00483(G), SnpOS00484(A)	[77, 117]
Cold	IR 83222-8-1-1-1-1-1-1, IR 66160-121-4-4-2, HGKN	<i>qCTS4a</i> , <i>qCTS11.1</i>	<i>qCTS4a</i> : RM349, RM17604, RM17623, RM3648, RM2799 <i>qCTS11</i> : RM26889, RM21, RM206	[125, 126]
Heat	N22/IR64	<i>qHTSF4.1</i> , <i>qHTSF4.2</i> ,	id4005120, id4011562	[104]
Salinity	Pokkali/IR29	<i>Saltol</i>	G11A, AP3206, RM3412, RM493	[110, 127]

Table 3.

List of traits, donors, QTLs/genes, and markers associated used in crossing program for the development of climate resilient lines at IRRI, Philippines.

of introgression lines pyramided with various drought grain yield QTLs in rice [128–131]. In some cases, epistatic interactions between different loci can enhance or reduce the effect of some of the genes/QTLs. Under such a situation, as an alternative strategy of identifying and advancing lines with different combinations of genes/QTLs- six, seven, or eight for different stresses and showing higher grain yield under nonstress conditions will also be selected and the best combinations that will show tolerance of a maximum number of abiotic and biotic stresses and the highest yield advantage will be advanced for testing. Plants carrying maximum number QTLs/gene but having negative interaction with grain yield and showing inferior plant type can be rejected for further advancement.

Maintenance of larger population size could also be a feasible strategy which can allow to select rare recombinants having maximum number of targeted QTLs/genes and also free from undesirable linkages. In previous studies, drought tolerant rice lines were developed through successful breakage of the linkages between loci for tolerance to drought and undesirable traits by fine mapping and maintain huge population size [132].

6. Role of genomic selection in multiple-trait breeding

Genomic selection (GS) in crop plants facilitates the rapid selection of superior accessions/genotypes and accelerate the breeding cycle targeted for higher genetic gain. It aims to use the genome-wide markers to predict the effects of all associated loci. The developed best prediction model is applied to the tested breeding material which has been characterized only genotypically but not phenotypically. The breeding estimated value called as GEBV (genomic estimated breeding value). The parental lines with higher GEBV can be selected as the candidate lines for future breeding programs. Most of the previous studied in cereal crops has shown great potential for GS to enhance the selection for grain yield and yield related traits [133, 134]. Multi trait genomic selection can be also implemented on phenotypic data of multiple traits *viz.* grain quality traits, grain yield and yield components, and reaction to the biotic and abiotic stresses, however it is important that a favorable genetic correlation exists between traits to implement genomic prediction model effectively [135, 136].

7. Conclusions

To solve the global issue of food security in the era of changing climate, novel approaches involving successful stacking of multiple genes/QTLs in a single rice line utilizing strategic phenotypic-genotypic selection could provide opportunity targeting genetic gain in rice. New advances in hybridization strategies, genomics, marker development, and sequencing permitted the opportunity to create multi-gene carrying high-yielding rice varieties to combat multiple stresses. The development of rice varieties carrying multiple QTLs/genes in homozygous conditions can address the production constraints faced due to both biotic and abiotic stresses simultaneously. These stress-tolerant rice varieties with desired grain quality can greatly help farmers in improving productivity under multiple stress conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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