A Seminar Paper on

Marker Assisted Breeding for Improving Productivity of Rice

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Marker Assisted Breeding for Improving Productivity of Rice[1]

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ABSTRACT

Rice productivity is threatened by climate change, sudden outbreak of diseases and pests. By using conventional breeding, it is very difficult to develop a resistant host against stresses. Marker assisted breeding (MAB) is an effective and environmental friendly method increasing the efficiency of conventional breeding but can not substitute the conventional breeding. In this paper, effects of gene introgression and role of MAB for improving productivity are reviewed. It is prepared based on secondary sources. Tightly linked markers with target genes are used in marker assisted selection (MAS). *Xa4, Xa5, Xa13, Xa21* and *Xa27* for bacterial blight*; Pi2, Pi5, Pi9,* QTL on chromosome 1, 2, 11 and 12 for blast; *Gm1* and *Gm4* for gall midge; *saltol* for salinity; *qDTY1.1, qDTY2.1* and *qDTY3.1* for drought; *Sub1A* for submergence; *yld1.1 yld2.1* and *GW6* for yield enhancing genes from particular donor parent are introgressed into susceptible one and thus, it is possible to develop resistant or tolerant rice lines through MAB. Rice productivity is also increased due to gene introgression. The introgression lines, DHA-10, BC2F3 2-8-2-36, RPIC-16-65-125, BR9157-12-2-37-13-15, BR9157-12-2-37-13-71, 3-11-11-1, 3-11-11-2, RPIC-16-65-125, 3-11-11-1, 3-11-11-2 and 9311 BC3F1 give more yield than their respective recurrent parent. *GW6* gene introgression line, SSL-1 produce 19% more yield per plant than 9311. IR 96322-34-260-B-5-1-1 (1,411 kg ha^{-1}) in severe drought stress and IR 96321-1447-651-B-1-1-2 (3,298 kg ha^{-1}) in moderate stress condition provide more yield than recurrent parent, Swarna (676 kg ha^{-1} in severe drought and 2,021 kg ha^{-1} in moderate drought) due to drought tolerant gene introgression. Submergence introgression line, BR9157-12-2-37-13-17 produce 3.44 t/ha whereas the recipient parent, BRRI dhan33 produce 1.73 t/ha. So, the role of MAB in rice productivity is indescribable.

Key words: rice, stresses, marker assisted selection, markers, gene introgression and yield

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Chapter 1

INTRODUCTION

Rice is the main food belonging to the genus *Oryza* in most of the countries in the world. Its productivity improvement is one of the main pillars of food safety, particularly for Asia and Africa (Suela *et al*., 2019). It is a good source calorie (one fifth) and protein (15%) (Rashid *et al*., 2017). It is invented in India and then forward to other country. In rice breeding, there is a conflict between yield and other desirable agronomic characteristics such as biotic and abiotic stress related traits. Therefore, the main objectives of rice breeding is to reduce the losses of yield during unfavorable environment and increase the yield during favorable environment. Several breeding approaches for increasing the yield potential include conventional breeding ie. pure line selection, pedigree selection, bulked method, single seed decent method (SSD); ideotype breeding; hybrid breeding; transgenic rice breeding; molecular marker assisted breeding etc. (Khan *et al*., 2015). China, India, Bangladesh produced 146.73, 118.00, 35.85 million metric tons from an area of 29.69, 44.00, 11.83 million hectares and average productivity is 7.06, 4.02, 4.55 metric tons per hectare respectively (USDA, 2020).

Arable land is decreasing due to industrialization and extension of urban area but the population continues to grow. A lot of land for rice cultivation is being converted to non-rice purposes. The yield performance also become fixed about 4.5 t/ha (BRRI, 2018). Besides, rice breeding is threatened by manmade occurrences hence, breeders should be ready to fight to any kind of stresses that is the outcome of uncertainty. New variety development is now a challenging and resource intensive effort. The biotic stresses (fungal, bacterial, viral and insect pests) for tropical wetland rice and abiotic stresses (drought and flooding) for non-irrigated rice negatively affect production (Peng, 2003; Khush and Virk, 2005). According to Das and Rao (2015), biotic (bacterial blight, blast and gallmidge) and abiotic stresses (salinity and submergence) are responsible for acute yield losses in rice throughout the world. Bailey-Serres *et al.* (2012) remarked that 70 % failure during harvesting in 2011 was happened due to two abiotic stresses (drought and submergence). Severe yield losses happen due to cold, heat, salinity and drought throughout the world (Wang *et al*., 2003). Among the biotic stresses, bacterial blight, blast and sheath blight are the main diseases that cause a severe damage in rice productivity. Stem borer, plant hopper (especially brown plant hopper) and leaf folder are the major insect pests interfering the growth and productivity of rice (Jiang *et al*., 2012). *Xanthomonas oryzae pv. oryzae* (Xoo) is one of the major bacteria, responsible for bacterial blight of rice which causes severe yield losses. Hasan *et al.* (2015) reported that superior variety development that is resistant to biotic and abiotic stresses is the main goal of present day due to appearance of new diseases, pests, and uncertain climate change. Moreover, the report of United Nations High Commissioner for Refugees (UNHCR, 2020) shows that, about 9,15,000 Rohingya refugees from Myanmar are living at Cox's Bazar District in Bangladesh and they utilize the existing facilities and resources (FAO, 2020). Over the next 30 years, the rice production must be doubled to fulfill the demand of global population. Hence, rice breeders must think about time saving, cost effective breeding approach to improve rice productivity. If they use traditional breeding methods, it requires ten to twelve years for obtaining superior varieties (Collard and Mackill, 2008). In conventional breeding methods, it is very difficult to identify the undesirable genes that may be remained along with many generation. Several kinds of marker makes the conventional breeding more effective.

However, marker assisted breeding provides solution for all difficulties arising in rice breeding. It uses markers that are tightly linked with gene of interest and so, target genes are easily identified. Thus, generations of backcrossing are reduced (Hasan *et al.*, 2015). Kottearachchi (2013) observed that unwanted gene that is present in the same chromosomal region of desired gene can be removed easily by using DNA based markers. Cost of production is reduced by solving the problems of conventional breeding through marker assisted breeding in rice, thus it becomes more economic. Marker assisted breeding (MAB) is an efficient, economical and environmental friendly approach for pyramiding all stress resistant genes in a genotype and thus, host become resistance (Das and Rao, 2015). Chukwu *et al.* (2019) reported that breeders are able to select the specific region of chromosome containing target gene by exploiting the benefit of markers.

By considering the above situations, this review paper is made to satisfy the following objectives:

- To review the effects of gene introgression by using marker assisted selection
- To investigate the role of marker assisted breeding in rice productivity

Chapter 2

MATERIALS AND METHODS

The seminar paper is completely a review paper because it is prepared based on the information collecting from secondary sources. During preparation of the manuscript, I studied many corresponding papers, journals, reports, published books, scientific publications etc. I also used the library facilities of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). I went many websites for collecting latest information such as google, google scholar etc. I got information from Bangladesh Rice Research Institute (BRRI). I enriched my knowledge with the suggestions and information of our course instructors. My major Professor helped me in many ways for preparing the paper. After collecting enough information, it was sequentially accumulated together for better understanding.

Chapter 3

REVIEW OF FINDINGS

3.1. Basis of Marker Assisted Breeding

According to, Chukwu *et al*. (2019), marker assisted breeding (MAB) is not a substitute of traditional breeding rather helps to increase the effectiveness of it. Molecular selection started its journey from 1990s for improving the productivity of rice (Jonas and Koning, 2013). All living beings suffer much due to the effects of changing climate. Severe yield losses are occurred due to the biotic and abiotic stresses (**Table 1**).

Table 1. Reports related to the consequences of biotic and abiotic stresses during rice cultivation

Stresses	Causes	Consequences	References
Bacterial Blight	*Xanthomonas oryzae pv.oryzae*	Partial grain filling leads severe yield loss	Pradhan *et al*. (2015)
Blast	*Magnaporthe grisea*	70–80% yield loss during severe condition	Babujee and Gnanamanickha (2000)
Gall Midge	*Orseolia oryzae*	0.8% yield losses of total production	Biradar *et al.* (2004)
Stem borer	*Scirpophaga incertulas* (yellow), *S. innotata* (white)	Deadheart and whitehead, reduced plant vigor	Nogoy *et al*. (2016)
Submergence	Rain-fed lowland conditions	Hamper growth and average productivity	Das and Rao (2015)
Salinity	Saline containing water (Sodium), in southern region	>50% yield losses	Molla *et al*. (2015)
Drought	Shortage of rainfall	Severe yield loss	Das *et al.* (2017)

Source: Modified from Das and Rao (2015); Das *et al*. (2017).

So, rice breeders must have to do research for solving all arising problems such as, climate change, arising new stresses (biotic or abiotic) and increasing population for food security (Nogoy *et al.*, 2016). Breeders are able to identify many stress resistant genes with the help of markers (**Table 2**).

Table 2. Recent discovery of breeders to overcome current stress related problems

Recent Discovery	References
42 bacterial blight resistance genes such as *Xa5, Xa13, Xa21, Xa17, Xa29(t), Xa42* etc.	Chukwu *et al*. (2019)
100 blast resistance genes but 14 genes widely used namely, *Pi1, Pi2, Pi9, Pi20 (t), Pi33, Pi39, Pi40 (t), Pi47, Pi48, Pi54rh, Pi56, Piz, Piz-t,* and *Pigm*	Hayashi *et al*. (2010); Huang *et al*. (2011); Das *et al*. (2012); Hua *et al*. (2015); Liu *et al*. (2013)
11 gall midge resistance genes	Dutta *et al*. (2014); Hasan *et al*. (2015)
Submergence1 (*Sub1*) in chromosome 9 for submergence tolerance	Septiningsih *et al*. (2013); Manivong *et al.* (2014)
Saltol for salinity tolerance	Das and Rao (2015)
Dreb1, qDTY1.1, qDTY2.1, qDTY3.1, QTLs for drought tolerance	Reddy *et al*. (2009); Lin *et al*. (2007); Das *et al*. (2017); Sandhu *et al.* (2019)

Source: Modified from Das and Rao (2015); Das *et al*. (2017); Chukwu *et al*. (2019); Sandhu *et al*. (2019).

Highly resistant variety development is a useful approach to overcome the upcoming stresses and changing climate. So, it is necessary to compile all the resistant genes in a genotype that perform well in all climatic conditions with its strong resistant capacity (Das and Rao, 2015). It is possible to develop a resistant variety quickly incorporating target genes in early generation with more precision across biotic and abiotic stresses through MAB (Das *et al*., 2017).

3.2. Types of Marker Used for Marker Assisted Breeding

According to, Chukwu *et al*. (2019), there are three types of markers used for identification of gene of interest such as morphological markers, biochemical markers and molecular markers. Two types molecular markers based on the detection methods are PCR and hybridization based markers (Yang *et al*., 2015). Molecular markers are also called DNA based markers. The number of this marker has no boundary. It is able to scan the whole genome of rice through this marker and fix the features on each chromosomes at high density (Akhtar *et al*., 2010).

Molecular markers are more advantageous than others. The genetic distance for rice marker should be less than 5 cM. Intragenic marker increase the reliability of rice marker for phenotypic prediction. It is mostly used marker in rice breeding. Das *et al.* (2017) reported that RG556, pTA 248, Xa13prom, RG64, P28; RM444, RM547; SUB1BC2, RM10745, Os01g0197700, RM212, RM319 etc. all are DNA based marker used for selecting linked target genes to increase the resistance power and productivity of rice against different kinds of stresses.

3.3. Conventional Breeding and Marker Assisted Breeding

Though marker can be used in any stage of breeding, it is more advantageous during early stage. Thus, breeders give more attention with high priority on lesser promising lines than conventional breeding as undesired lines are discarded in very early generation (**Fig.1**) (Akhtar *et al*., 2010). Ribaut and Betran (1999) concluded that MAB is an early generation selection that makes a way to evaluate lesser lines in later generations. For instance, homozygous lines are screened at later generation (F_5 or F_6) during conventional bulk and single seed descent methods. It is possible to screen homozygous line at early generation (F_2) by fixing particular alleles through markers.

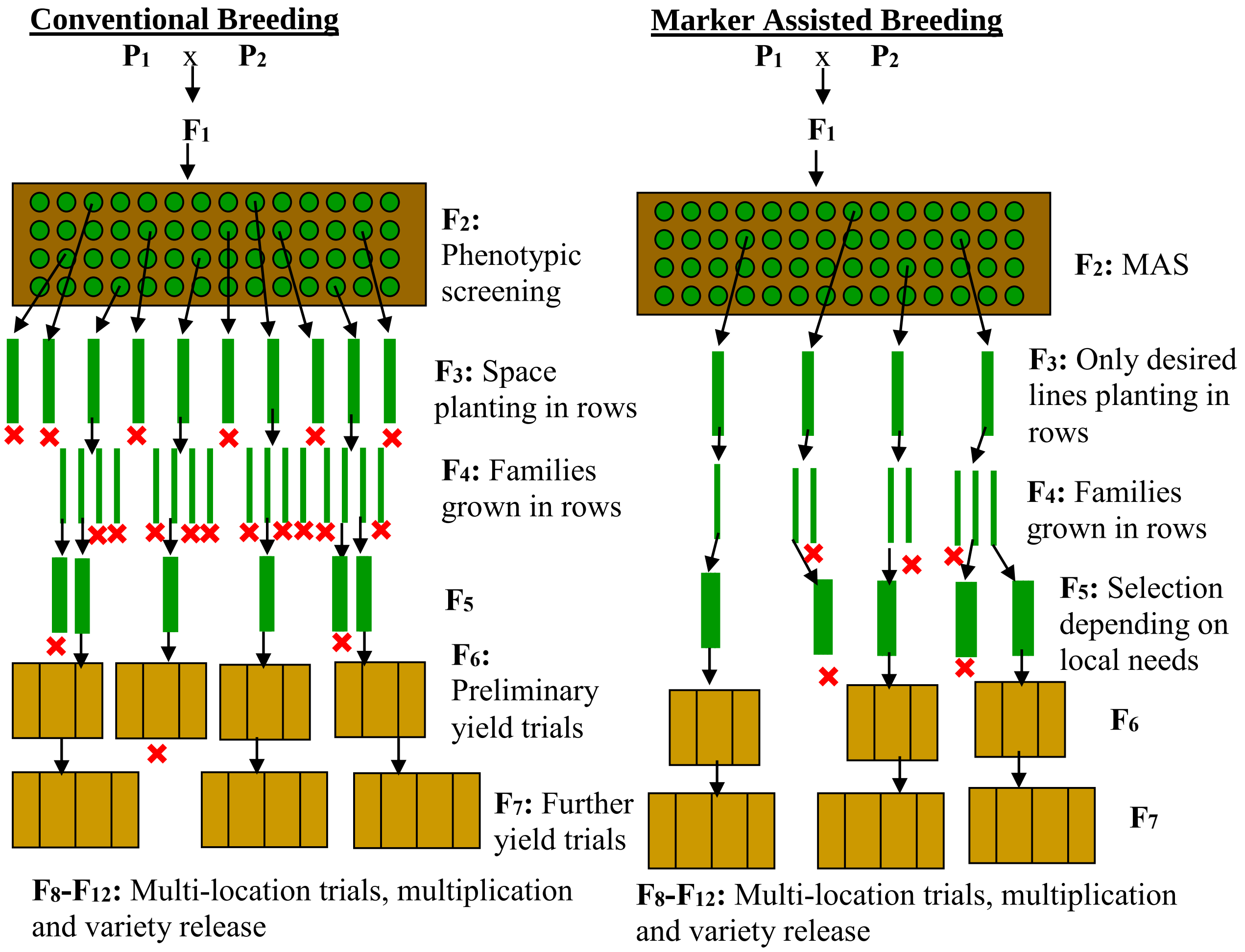

Fig. 1. Conventional Breeding verses marker assisted breeding (Akhtar *et al*., 2010).

3.4. Marker Assisted Gene Pyramiding

Transfer of more than one resistant or tolerant genes into a single genotype is called gene pyramiding (Collard and Mackill, 2008). It is helpful for preventing breakdown of resistance or tolerance against the stresses and possible due to the utilization of molecular markers though MAB (Das and Rao, 2015). According to Das *et al*. (2017), there are two steps for marker assisted gene pyramiding. One is accumulation step when all genes of interest (goi) are accumulated in one genotype. Another is fixation step when the accumulated goi are fixed into a homozygous state. Luo *et al*. (2016) introduced *Xa4* and *Xa21* genes into Mianhui 725 by using markers and thus, a line is developed such as, Wanhui 421 which attains 96.9% similarity of Mianhui 725 through marker assisted backcrossing. Wanhui 421(*Xa4*, *Xa21*) is crossed with IRBB27 (*Xa27*) and F_1 is achieved containing *Xa4, Xa21, Xa27* genes. At the same way, Wanhui 421(*Xa4, Xa21*) was crossed with 75-1-127(*Pi9*) and IR64 (*Sub1A*) and F_1 is achieved containing (*Xa4, Xa21, Pi9*) and (*Xa4, Xa21, Sub1A*) respectively. Backcrossing (5 times) is done to get more or less similar genetic background of recurrent parent (Wanhui 421). Subsequent crossing is done to obtain F_1 containing *Xa4, Xa21, Xa27, Pi9, Sub1A* genes. Selfing is done to get F_2 (*Xa4, Xa21, Xa27, Pi9, Sub1A*) (**Fig. 2**).

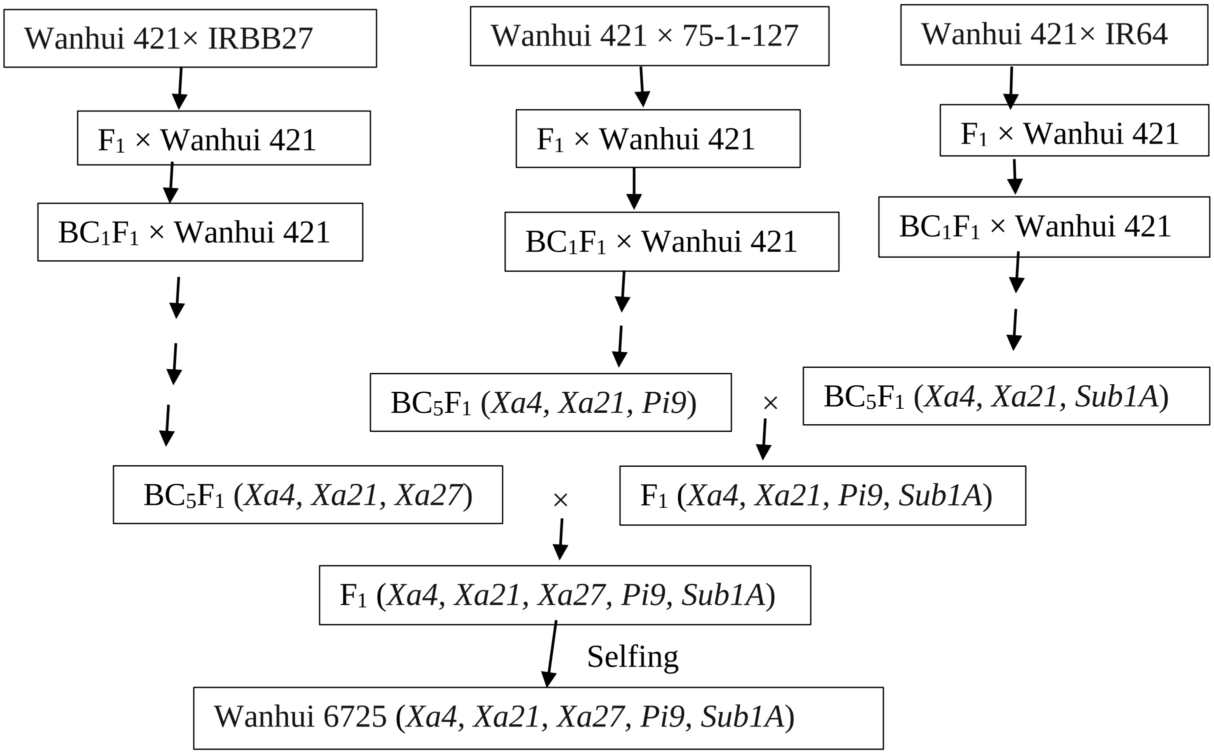

Fig. 2. Marker assisted backcrossing for accumulating different resistance genes in one genotype (Luo *et al*., 2016).

In all cases, desired individuals is selected based on marker information. For example, only 6 individuals are selected in heterozygous condition containing all desired genes from 92 individuals and 4 in homozygous condition are selected from 960 individuals. Among the selected 4, F2–281, was entitled as Wahhui 6725 possessing 95% similarity of Mianhui 725. NBS2-1, RM23887, RM224, 21, M124 markers are used for *Pi9, Sub1A, Xa4, Xa21, Xa27* genes respectively (Luo *et al*. 2016).

3.5. Marker Assisted Gene Introgression

3.5.1. Effects of Bacterial Blight (BB) Resistant Gene Introgression

According to Luo *et al*. (2016), the introgression line, Wanhui 6725 (*Xa4, Xa21, Xa27, Pi9, Sub1A*) and its hybrid, II-32A/WH6725 are resistant to 27 *X.oryzae pv. Oryzae* (*Xoo*) strains that are arising from 10 countries. The recurrent parent, Mianhui 725; its hybrid, II-32A/MH725 and CMS line II-32A are susceptible to 26; 23 and 24 *Xoo* strains respectively. Wanhui 421(*Xa4, Xa21*) and IRBB27 (*Xa27*) are resistant or moderately resistant to 22 and 24 *Xoo* strains respectively (**Table 4**). When lesion length (LL) less than or equal to 3.0 cm, it represents as resistant and 3.0 cm < LL ≤ 6.0 cm for moderately resistant; 6.0 cm < LL ≤ 9.0 cm for MS, moderately susceptible; LL > 9.0 cm for S, susceptible are used during disease scoring against BB.

Table 4. Resistance score of 7 lines against 5 *Xanthomonas oryzae pv. Oryzae* strains originated from China and India

Rice Lines	Strain from China			Strain from India	
	HB17	HB21	JS49-6	A3842	A3857
Mianhui 725	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
II-32A/MH725	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
Wanhui 421	Moderately Resistant	Moderately Resistant	Resistant	Susceptible	Moderately Resistant
Wanhui 6725	Resistant	Resistant	Resistant	Resistant	Resistant
II-32A/WH6725	Resistant	Resistant	Resistant	Resistant	Resistant
II-32A	Susceptible	Susceptible	Susceptible	Susceptible	Susceptible
IRBB27	Resistant	Resistant	Resistant	Resistant	Resistant

Source: Luo *et al*. (2016).

3.5.2. Effects of Blast Resistant Gene Introgression

According to Yang *et al*. (2019), the recipient line, Feng39S is improved by the blast resistant gene (*Pi2*) from donor parent, Hua1201S through marker assisted backcrossing resulting in the production of 2 lines, DB16206–34, and DB16206–38 in BC_2F_5. In all steps, markers are used to select the desirable lines. 9311 is used as male parent for production of hybrid and the male parent is susceptible to blast. An evaluation trial of rice lines against blast is done in China (2018) and its results are demonstrated in **Fig. 3. A.** Hua1201S is resistant to leaf blast scoring 2. DB16206–34, DB16206–38 and their hybrids (DB16206–34/9311, DB16206–38/9311) are resistant to leaf blast showing disease score 1, 3, and 3 respectively. 9311, Feng39S and its hybrid (Feng39S/9311) are susceptible to leaf blast giving score 5, 8, and 4 respectively. These lines are also susceptible to neck blast showing more infection varying from 52% to 87%. Hua1201S, DB16206–34, DB16206–38 and their hybrids are resistant to neck blast demonstrating no or very little infection percentage. Murthy *et al*. (2017) reported that Samba Mahsuri (BPT5204) is a popular but blast susceptible variety improved by introgressing *Pi2* and *Pi5* genes from C101A51 and IRBL-5M through MAB. Both *Pi2* (DHA-1, DHA-2, DHA-10) and *Pi5* (DHB-19, DHB-25, DHB-27) introgressed lines show higher yield than recurrent parent, BPT5204 (**Fig. 3. B**). DHA-10 (5,680 kg ha–^{1}) is the highest yielder line among 7 lines and BPT5204 (4,973 kg ha–^{1}) is the lowest yielder.

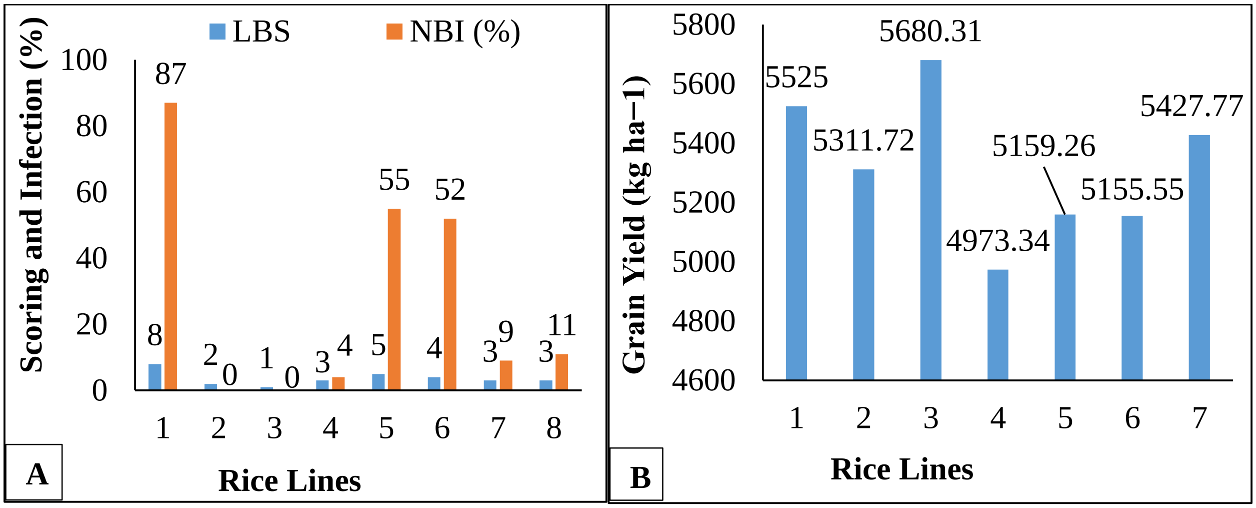

Fig. 3. Evaluation of rice lines against blast. **A** Blast scoring and Results of Infection (%) (resistant: 1–3, susceptible: 4-9); 1, Feng39S; 2, Hua1201S; 3, DB16206–34; 4, DB16206–38; 5, 9311; 6, Feng39S/9311; 7, DB16206–34/9311; 8, DB16206–38/9311; LBS, Leaf blast score; NBI (%), Neck blast infection (%) (Yang *et al*., 2019). **B** Performance for Yield; 1, DHA-1; 2, DHA-2; 3, DHA-10; 4, BPT5204; 5, DHB-19; 6, DHB-25; 7, DHB-27 (Murthy *et al*., 2017).

Nan *et al.*, (2019) reported an experiment at Khon Kaen University (KKU) and Khon Kaen Rice Research Center (KKRRC) in Thailand. Blast resistant QTL on chromosome 1 and 11 from Jao Hom Nin, 2 and 12 from P0489, bacterial blight resistant gene, *Xa5* from IR62266 are pyramided in RD6. The introgression lines are produced through marker assisted backcrossing and showed more grain yield than recurrent parent (**Fig. 4**). BC2F3 2-8-2-36 gives highest yield than other lines and recurrent parent at KKRRC in Thailand.

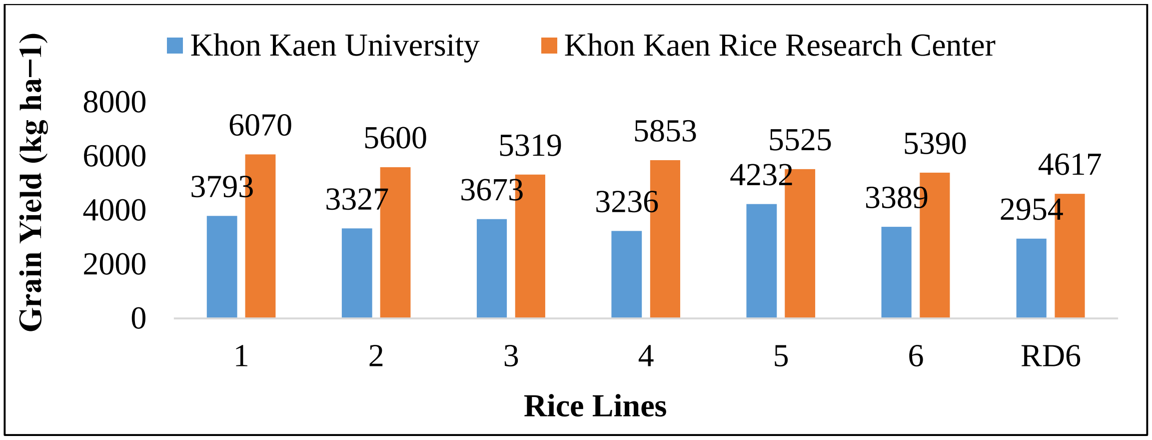

Fig. 4. Performance of 6 introgression lines including recurrent parent for yield in two locations. 1, BC2F3 2-8-2-36; 2, BC2F3 2-7-5-43; 3, BC2F3 2-8-2-19; 4, BC2F3 2-8-2-25; 5, BC2F3 9-1/15-1-28; 6, BC2F3 2-8-2-27 (Nan *et al*. 2019).

3.5.3. Effects of Gall midge Resistant Gene Introgression

Das and Rao, (2015) report that ILGP lines are developed by pyramiding many resistant genes such as *Gm1*, *Gm4* genes that are resistant to gall midge gained from Kavya Abhaya. These lines show more positive response ranging from 75 to 100%. Kavya (*Gm1*), Abhaya (*Gm4*) show more or 100% positive response to gall midge infestation. Whereas, the recurrent parent (ILP) is susceptible to gall midges and provide very little positive response (6.8%) against gall midge (**Fig. 5. A**). Kumar *et al.* (2017) improved a stable restorer line (RPHR-1005) of hybrid DRRH-3 by pyramiding bacterial blight and gall midge resistant gene. Improved Samba Mahsuri (ISM), SM1 and SM2 are produced from the cross of ISM (containing *Xa21*) with Abhaya (containing *Gm4*) and Aganni (containing *Gm8*), respectively. Bacterial blight and gall midge resistant gene are transferred to RPHR-1005 from the donors, SM1 (containing *Xa21*, *Gm4*) and SM2 (containing

Xa21, Gm8) through MAB. The introgression line, RPIC-16-65-125 shows better performance for yield and yield related traits than the recurrent and donor parent (**Fig. 5. B**).

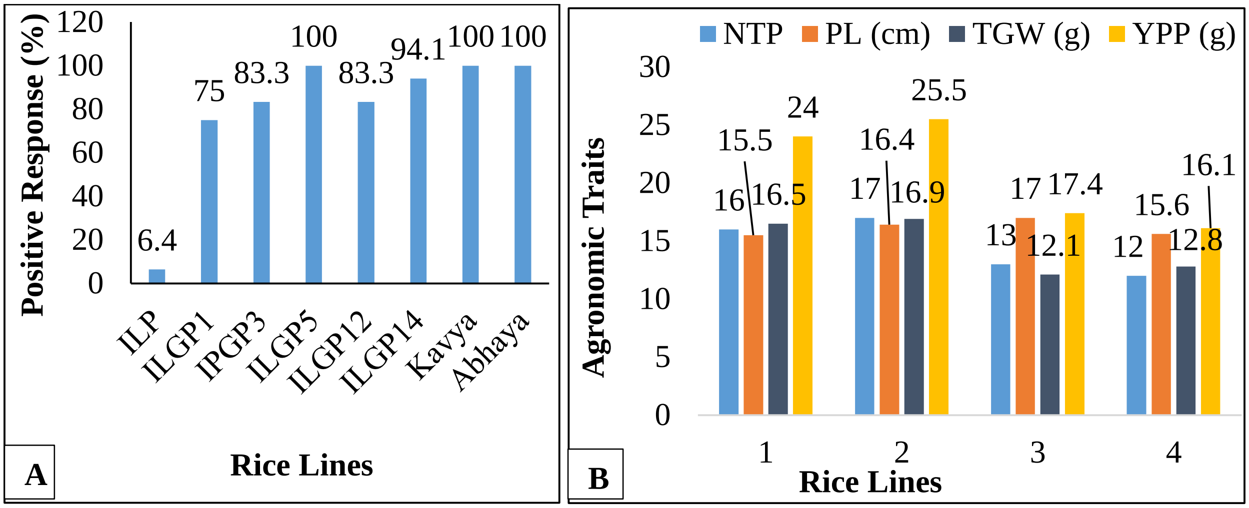

Fig. 5. Evaluation of rice lines against gall midge. **A** Positive Response (%); ILP, Improved Lalat Parent; ILGP, Improved Lalat Gene Pyramid) (Das and Rao, 2015). **B** Agronomic Performance; 1, RPHR-1005; 2, RPIC-16-65-125; 3, SM1 (ISM/Abhaya); 4, SM2 (ISM/Aganni); NTP, no. of productive tillers/plant; PL, panicle length (cm); TGW, 1000 grain weight (g); YPP, yield per plant (g) (Kumar *et al*., 2017).

3.5.4. Effects of Submergence Tolerant Gene Introgression

Luo *et al*. (2016) report that the introgression line, Wanhui 6725 (*Xa4, Xa21, Xa27, Pi9, Sub1A*) and its hybrid, II-32A/WH6725 are tolerant to two weeks submergence. The donor parent, IR64 (*Sub1A*) is also tolerant whereas the recipient parent, Mianhui 725; its hybrid, II-32A/MH725 and CMS line, II-32A are susceptible and can not recover rather, die or few survive with poor health (**Fig. 6. A**). Wanhui 6725, II-32A/WH6725, IR64 show 80% viability after recovery of submergence. According to Iftekharuddaula *et al.* (2016), submergence tolerant *SUB1* gene is introgressed into a short duration variety, BRRI dhan33 from BRRI dhan52 to improve the variety through MAB. Three introgression lines (BR9157-12-2-37-13-15, BR9157-12-2-37-13-17, BR9157-12-2-37-13-71) are selected that produce 1.24–1.71 t/ha higher yield and perform better for other agronomic traits than recurrent parent (**Fig .6. B**).

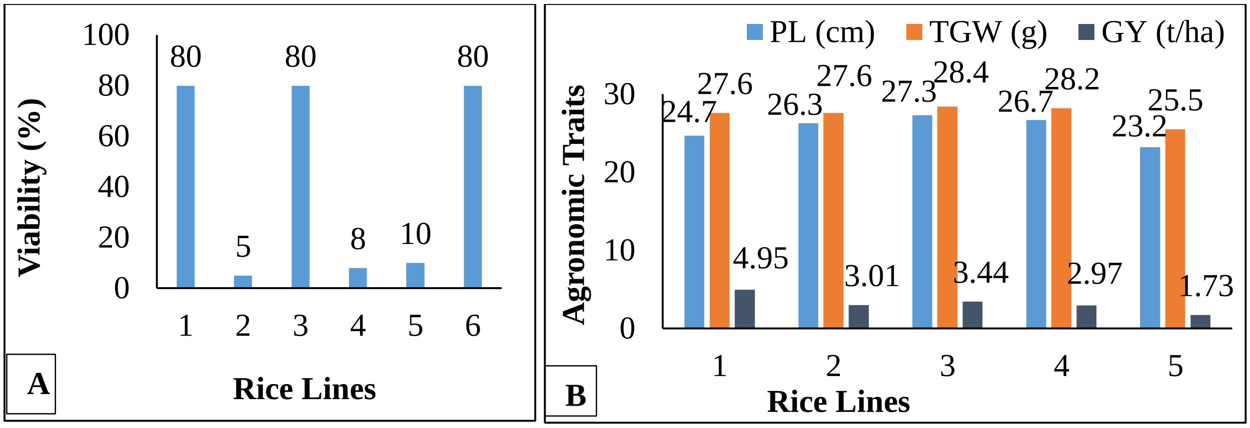

Fig. 6. Assessment of tolerance capacity of rice lines after recovery of two weeks submergence. **A** Viability % (1. IR64, 2. MH725, 3. WH6725, 4. II-32A, 5. II-32A/MH725, 6. II-32A/WH6725) (Luo *et al*., 2016). **B** Agronomic Performance in field trial; 1, BRRI dhan52 ; 2, BR9157-12-2-37-13-15; 3, BR9157-12-2-37-13-17; 4, BR9157-12-2-37-13-71; 5, BRRI dhan33; PL, panicle length (cm); TGW, 1000 grain weight (g); GY, grain yield (t/ha) (Iftekharuddaula *et al*., 2016).

3.5.5. Effects of Salinity Tolerant Gene Introgression

Thomson *et al.* (2010) report that SES score, shoot Na$^+$/K$^+$ ratio and root Na$^+$/K$^+$ ratio of the donor parent, FL478 (IR 66946-3R-178-1-1) is lower than others whereas more or less higher in recurrent parent (IR29) (**Fig. 7. A)**. The IR29 is sensitive and FL478 is tolerant and so, the performance of saltol lines is better than non-saltol or recurrent parent. For example, the saltol lines give 24-33% higher yield than the recurrent parent providing 20% yield under stress conditions compared to non-stress conditions. The reporter use SSRs markers for this study and test the performance of seedlings at 12 dS m^{-1} EC in a hydroponic system. According to Das and Rao (2015), saltol lines can survive under severe salinity stress representing in (**Fig. 7. B)**. ILP is used as recurrent parent containing bacterial blight resistant genes (*Xa4, Xa5, Xa13, and Xa21*) showing 5.8% survival ability. IR29, a susceptible one show very little survival ability to salinity stress. The donor parent, FL478 has 100% survival ability. ILGP, a saltol introgression line show 98% survival capacity to salinity stress.

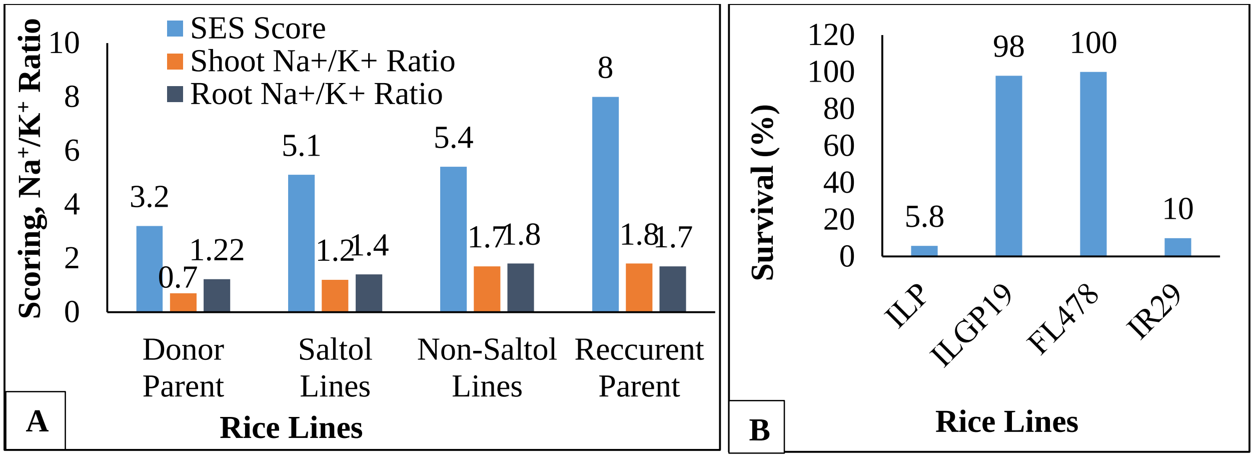

Fig. 7. Evaluation of rice lines for salinity tolerance. **A** Comparison for SES score, shoot Na^+/K^+ ratio and root Na^+/K^+ ratio (Thomson *et al.*, 2010). **B** Survival percentage against salinity stress (ILP, Improved Lalat Parent; ILGP, Improved Lalat Gene Pyramid) (Das and Rao, 2015).

3.5.6. Effects of Drought Tolerant Gene Introgression

Sandhu *et al.* (2019) conclude that IR 96321-1447-651-B-1-1-2 (*qDTY1.1*, *qDTY3.1*), IR 96321-558-563-B-2-1-1 (*qDTY3.1*), IR 96322-34-260-B-5-1-1 (*qDTY1.1*, *qDTY2.1*, *qDTY3.1*), IR 96322-34-223-B-1-1-1 (*qDTY1.1*, *qDTY2.1*, *qDTY3.1*) are developed by pyramiding drought tolerant genes into Swarna. These lines are isogenic to Swarna. Mean yield performance of introgression lines is mostly higher in both moderate and severe drought stress than the recurrent parent, Swarna (**Fig. 8**). (Sandhu *et al.*, 2019). IR 96322-34-260-B-5-1-1 represents highest yield (1,411 kg ha^{-1}) than others in severe drought stress and IR 96321-1447-651-B-1-1-2 (3,298 kg ha^{-1}) in moderate stress condition. The drought susceptible one, Swarna give lower mean yield than others in both two conditions.

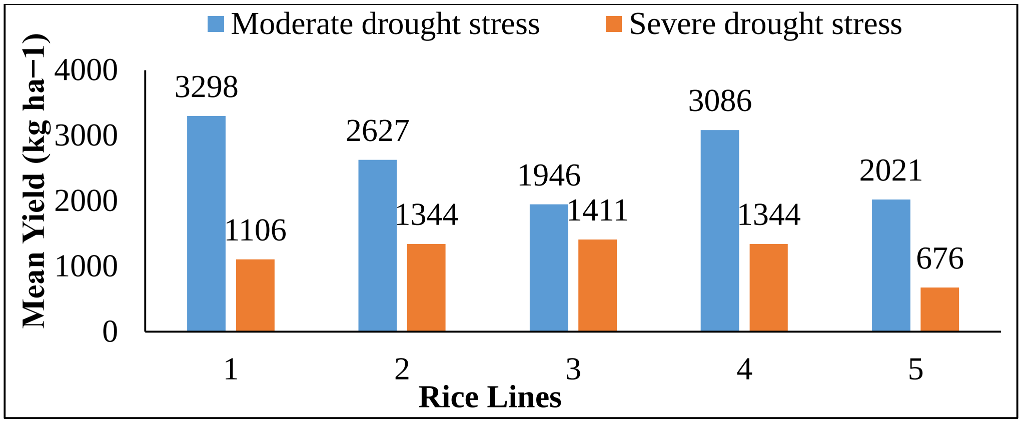

Fig. 8. Mean yield performance of five lines against moderate and severe drought stress in field trial. 1, IR 96321-1447-651-B-1-1-2, 2, IR 96321-558-563-B-2-1-1, 3, IR 96322-34-260-B-5-1-1, 4, IR 96322-34-223-B-1-1-1 and 5, Swarna (Sandhu *et al.*, 2019).

According to Muthu *et al.* (2020), a popular rice variety, Improved White Ponni (IWP) is introgressed with three abiotic stress tolerant genes, drought (*qDTY1.1*, *qDTY2.1*), salinity (*Saltol*) and submergence (*Sub1*) through MAB from Apo, Pokkali FL478 and FR13A respectively. Backcrossed inbred lines (BILs) (3-11-11-1, 3-11-11-2) gather all four resistant genes showing better performance for yield and yield related traits than the recipient parent, IWP (**Fig. 9)**.

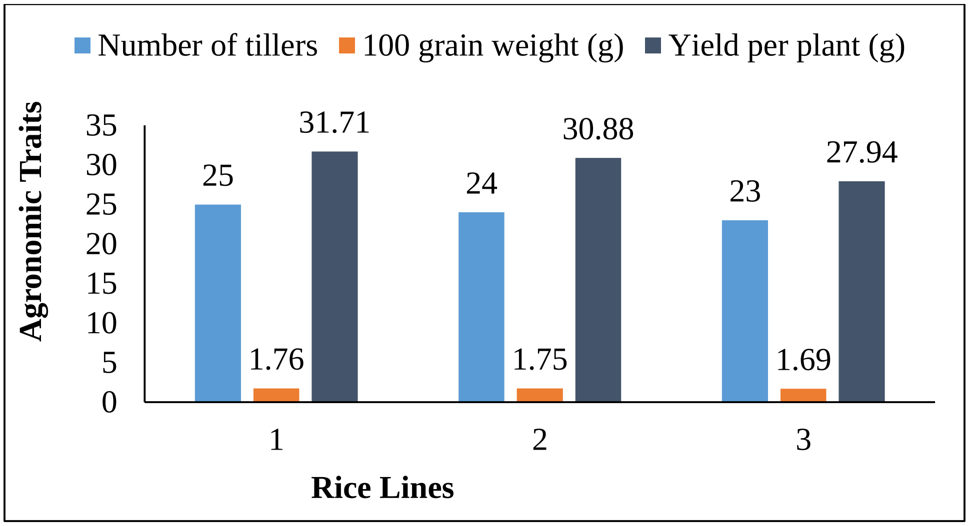

Fig. 9. Performance of rice lines for yield and yield related traits in field trial (1, BIL 3-11-11-2; 2, BIL 3-11-9-2; 3, Improved White Ponni (IWP) (Muthu *et al.*, 2020).

3.5.7. Effects of Yield Enhancing Gene Introgression

Liang *et al*. (2004) use *Oryza rufipogon* (IRGC 105491) as a donor parent of yield enhancing genes (*yld1.1* and *yld2.1*) and 9311 as a recipient and recurrent parent. 9311 is a popular parental line for hybrid seed production in China. The lines that carry the yield enhancing genes give better performance than the lines without the genes. Five BC3F1 lines are screened through MAS. The performance of introgression lines is better than the recurrent parent for yield and yield related traits. In case of grains per panicle, all represents more grains than 9311 (**Fig. 10. A**). Thousand grain weight is higher for all BC3F1 lines as compared to 9311 except BC3F1-3 and BC3F1-4. The derived lines show more yield per plant (>28 g/plant) than the recurrent parent (23g/plant) (**Fig. 10. B**).

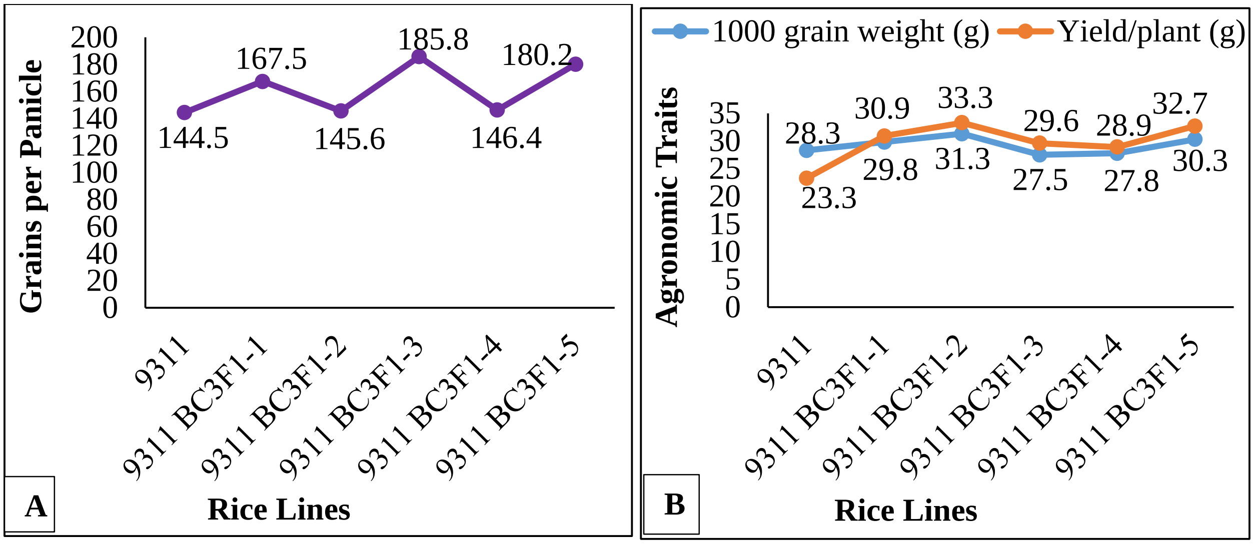

Fig. 10. Effects of yield enhancing genes for yield and yield related traits. **A** Grains per Panicle, **B** 1000 grain weight and yield per plant (Liang *et al*., 2004).

According to Li *et al*. (2014), *GW6* gene is transferred from Baodali to Zhonghua 11 (japonica type) and 9311 (indica type) through MAB. Three introgression lines for Zhonghua 11 and one for 9311 show better agronomic performance than recurrent parent (**Fig.11**). SSL-1 produce 19% more yield per plant than 9311.

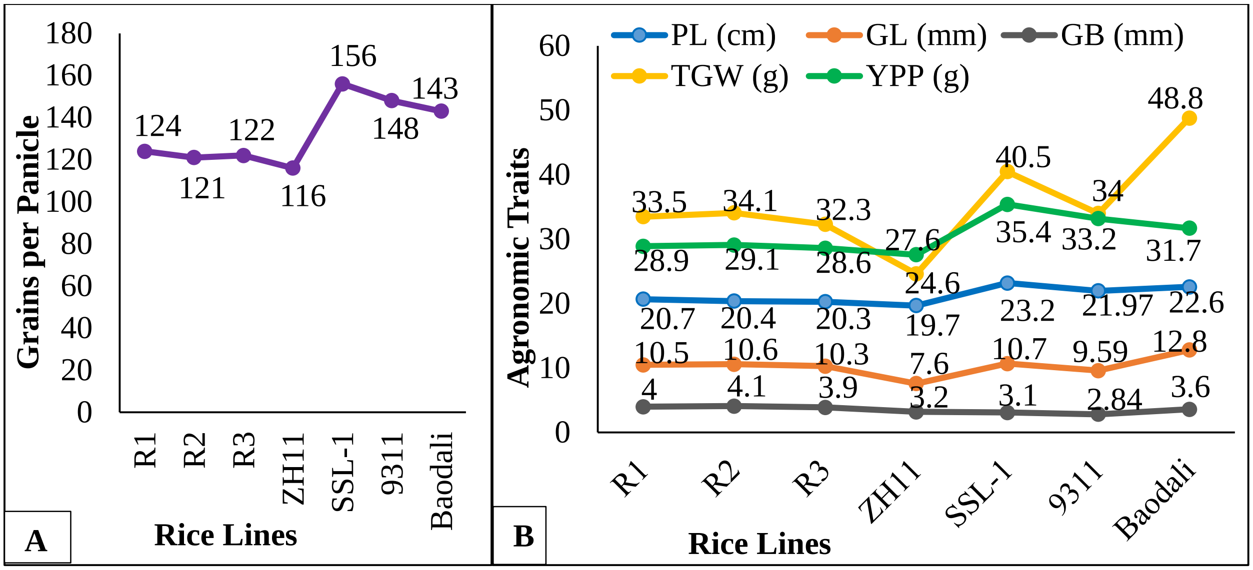

Fig. 11. Performance of *GW6* gene containing lines comparing with donor and recipient parent for yield and yield related traits. **A** Grains per Panicle, **B** Agronomic Traits. ZH11, Zhonghua 11; near isogenic line R1, R2, R3 for ZH11; SSL-1 for 9311; PL, panicle length (cm); GL, grain length (mm); GB, grain breadth (mm); TGW, 1000 grain weight (g); YPP, yield per plant (g) (Yuan *et al*., 2014).

3.6. Factors Affecting Marker Assisted Breeding

According to Akhtar *et al*. (2010), there are five factors to be considered during MAB ie. reliability, technical procedure for marker assay, level of polymorphism, cost, quantity and quality of DNA required. Markers should be reliable, polymorphic and cost-effective. Genetic distance should be less than 5 cM. The effectiveness of MAB depends on the distance between molecular markers (Nogoy *et al*., 2016). It should be closely linked to gene of interest. Intragenic markers can increase the reliability for predicting a phenotype. Sometimes, MAB requires large number of DNA with better quality and it is difficult to obtain. Technical procedure should be simple and quick.

3.7. Recent Progresses of Marker Assisted Breeding in Rice

Breeders have developed rice lines that are capable to survive against biotic and abiotic or any kind of climatic uncertainty through marker assisted breeding with the help of linked markers (Das *et al*., 2017). Many genotypes of rice and stress related traits are improved through marker assisted pyramiding (**Table 4**). At this time, many rice genotypes are available that are resistant to bacterial blight, blast, gal midge, drought, salinity, submergence etc.

Table 4. List of genotypes and traits improved by marker assisted selection

Improved Genotypes	Traits	Resistant Genes	References
Pusa basmati I	Bacterial blight	*Xa13 + Xa21*	Kottapalli *et al*. (2010)
Samba Mahsuri	Bacterial blight	*Xa5 + Xa13 + Xa21*	Kottapalli *et al*. (2010)
Wanhui 6725	Bacterial blight	*Xa4+Xa21+Xa27*	Luo *et al*. (2016)
Swarna sub1	Submergence	Sub1	Nandi *et al*. (1997)
IR64 sub1	Submergence	Sub1	Reddy *et al*. (2009)
Pusa RH10	Rice blast	*Piz5 + Pi54*	Singh *et al*. (2013)
Feng39S	Rice blast	*Pi2*	Yang *et al*. (2019)
Lalat	Gal midge	*Gm1+Gm4*	Das and Rao, (2015)
Swarna	Drought	*qDTY1.1+qDTY2.1+qDTY3.1*	Sandhu *et al.* (2019)
Tapaswini	Bacterial blight	*Xa4 + Xa5 + Xa13 + Xa21*	Dokku *et al.* (2013)
Mangeumbye	Bacterial blight	*Xa4 + Xa5 + Xa21*	Suh *et al*. (2013)
IR29	Salinity	*Saltol*	Thomson *et al.* (2010)
Dongan	Anaerobic germination	*AG1 + AG2*	Kim *et al.* (2019)

Source: Modified from Thomson *et al.* (2010); Das and Rao (2015); Luo *et al*. (2016); Das *et al*. (2017); Sandhu *et al.* (2019); Yang *et al*. (2019).

Chapter 4

CONCLUSION

In this review, the effects of gene introgression are described nicely. Wanhui 6725 and its hybrid, II-32A/WH6725 become resistant to 27 *Xoo* whereas recurrent parent, Mianhui 725; its hybrid, II-32A/MH725 and CMS line II-32A are susceptible. DB16206–34, DB16206–38 and its hybrid, DB16206–34/9311, DB16206–38/9311 become resistant against blast whereas recurrent parent, Feng39S; its hybrid Feng39S/9311 and male parent for hybrid, 9311 are very much susceptible to blast showing more infection varying from 52% to 87%. ILGP 1, ILGP3, ILGP5, ILGP 12 and ILGP14 can survive and show more positive response while recurrent parent, ILP can not survive or very little positive response to gall midge infection. WH6725 and its hybrid, II-32A/WH6725 can survive after two weeks submergence whereas the recipient parent, Mianhui 725; its hybrid, II-32A/MH725 and CMS line II-32A were susceptible and could not recover rather, die or few survive with poor health. ILGP can survive in salinity stress condition where ILP can not survive. IR 96321-1447-651-B-1-1-2, IR 96321-558-563-B-2-1-1, IR 96322-34-260-B-5-1-1, IR 96322-34-223-B-1-1-1 achieve tolerant capacity against moderate and severe drought stress whereas Swarna is susceptible to drought. All are possible by the use of marker linked to target genes.

The role of marker assisted breeding in rice productivity is indescribable. Marker assisted breeding increases the efficiency of conventional methods by using molecular markers. Blast introgression line, DHA-10 (5,680 kg ha−1) is the highest yielder line than recurrent parent, BPT5204 (4,973 kg ha−1). Blast and bacterial blight resistant lines, BC2F3 2-8-2-36 gives yield 6,070 kg ha–^{1} that is more than the recurrent parent, RD6 (4,617 kg ha–^{1}). Triple tolerant lines (drought, salinity and submergence) produce more (>31 g/plant) than recipient parent, IWP (28 g/plant). Submergence introgression line, BR9157-12-2-37-13-17 produce 3.44 t/ha whereas the recipient parent, BRRI dhan33 produce 1.73 t/ha. IR 96322-34-260-B-5-1-1 (1,411 kg ha^{-1}) in severe drought stress and IR 96321-1447-651-B-1-1-2 (3,298 kg ha^{-1}) in moderate stress condition provide more yield than recurrent parent, Swarna (676 kg ha^{-1} and 2,021 kg ha^{-1}). *GW6* gene introgression line, SSL-1 produce 19% more yield per plant than 9311. The derived introgression lines for yield enhancing genes show more yield per plant (>28 g/plant) than the recurrent parent, 9311 (23g/plant).

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