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Enhancing Abiotic Stress Tolerance to Develop Climate-Smart Rice Using Holistic Breeding Approach

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Abstract

Agricultural land and resources reduced annually because of climate change thus it is necessary to further increase the productivity of the major staple food rice to sustain food security worldwide. However, rice productivity enhancement is one of the key challenges in abiotic stress-prone environments. The integration of cutting-edge breeding approaches and research management methods in the current varietal improvement pipelines can make a step-change towards varietal improvement for the abiotic stress-prone environments. Proper implementation of breeder's equations in the crop improvement pipeline can deliver a higher rate of genetic gain. Single Seed Descent based Rapid Generation Advance (RGA) technique in field and greenhouse is the most promising innovations and low-cost, high-throughput marker-assisted selection approaches are applied for rapid and efficient selection for abiotic stress-tolerances. Also improving efficiency, intensity, and accuracy of selection and reducing breeding cycle time through holistic rice breeding that can play an important role in developing climate-smart abiotic stress-tolerant rice for target environments. This information can use as the future direction for rice breeders and other researchers.

Keywords: abiotic-stress tolerance, high-night temperature (HNT), holistic rice breeding, QTL, gene, product profile, rice

1. Introduction

Rice is the major staple food for more than half of the world population that supplies 30–50% daily calorie intake. Rice security is synonymous with food security in Bangladesh. If rice production hampers because of different abiotic stresses then food security also become vulnerable. Abiotic stresses such as salinity, drought, flood, high-night temperature (HNT), and heat/high-temperature increase enormous challenges that limit agricultural production and food security.

Coastal agriculture faces these challenges because salinity affects directly more than one million hectares of agricultural land in Bangladesh. Salinity causes due to chemical weathering of minerals that release salts in the soils as Na, Ca, Mg ions; coastal agricultural land is inundated by salt-water during flash flood/tidal upsurge; unplanned saltwater intrusion into the shrimp gher (shallow shrimp cultivation pond) in the southern coastal zone and lifting groundwater with EC >3.0 dS/m for irrigation.

Several studies revealed that the detrimental effects and suitable genetic [1, 2] and physiological mechanisms of salt-stress tolerance in various sensitive growth stages of seedling stages like early seedling stage during seedling establishment in the field after transplanting, and different susceptible stages of reproductive phase such as panicle initiation/emergence, booting, flowering/heading, spikelet fertility-sterility, and seed set, yield and other salt tolerance-related traits [3–10].

For maintaining food security and sustainability in rice production, both drought and heat/high-temperature tolerance is important in the respective stress-prone rice-growing areas for increasing rice production sustainably. However, steady growth in the rice sector is crucial during this pandemic situation to sustain self-sufficiency in different rice-growing countries in South Asia including Bangladesh.

Drought is also an important abiotic stress that threat for rainfed ecosystem. This stress adversely impacts on rice production. Drought tolerance is a complex polygenic trait with a complicated phenotype that affects various growth and developmental stages sensitive to drought-stress in rice. Different drought-responsive QTLs and genes regulate the degree of sensitivity or tolerance of rice through triggering signal transduction pathways to several drought conditions [11].

High-night temperature (HNT), different abiotic stress from heat stress, is emerging abiotic stress because of climate change. This stress (HNT) is drawing the attention of plant breeders and physiologists due to its detrimental effects on rice productivity. HNT varies 25-30°C that adversely affects yield and grain quality such as chalkiness in rice. This stress was reported in the Rajshahi region of Bangladesh (M. A. Rahman, unpublished data).

Fragile flood-prone environments belong to 18% of areas of Bangladesh that suffer from varying degrees of flooding causes due to flash flood submergence, monsoon flood, and irregular rainfall. The flood adversely influences the rice production of more than a million ha of land in Bangladesh. Submergence tolerant high-yielding rice varieties are grown by the farmers of the flash flood-prone areas. However, deepwater rice (DWR) is cultivated in areas where flood water-depth varied from 1.5 to 2.0 m and these areas are more vulnerable to rice production as there is no high-yielding DWR variety for this harsh ecosystem in Bangladesh. Thus, rice-growing areas under unfavorable environments need to enhance productivity by developing climate-smart rice to cope with the harmful effects of climate change.

In this review, we discuss the abiotic stresses and the development of climate resilient rice addressing adverse effect of climate change.

2. Abiotic stresses influencing rice production and food security

2.1 Heat tolerance

2.1.1 Strategies to enhance heat tolerance

Heat/high temperature tolerance is important in the heat-prone rice growing areas for increasing rice production sustainably. To address climate change,

screening and breeding for higher level of heat tolerance is needed. Strategies such as agronomic intervention through sowing time adjustment, chemicals/plant hormones application, genetic and genomic approaches [12], breeding for heat resistant variety development, marker-assisted introgression of *qEMF3* QTL [13] for developing and selecting cultivars with early morning flowering (EMF) before temperature rise are involved for improving heat tolerance.

QTLs associated with heat tolerance related traits using bulked segregant analysis in Rice to evaluate the genetic effect of QTLs controlling heat tolerance at flowering stage in rice. A population comprising 279 F₂ individuals developed from 996 (heat tolerant)/4628 (heat-sensitive cultivar), was investigated for their segregation pattern of the difference in seed set rate under normal/optimum temperature condition and stress/high temperature condition that exhibited normal distribution, suggesting the polygenic control on the heat tolerance [14].

Eleven QTLs identified for heat tolerance using RIL population derived from IAPAR-9 (sensitive)/Liaoyan241 (heat tolerant) at the heading and flowering stage in rice. Four major QTLs such as *qNS1*, *qNS4*, *qNS6*, and *qRRS1* found stable in both seasons/years in various environments [15].

Jagadish [16] dissected QTLs for relative spikelet fertility during anthesis in rice *qtl_1.1* (38.35 Mb) and *qtl_11.1*, 24.16 Mb, QTL contributor is Azucena) and one (*qtl_10.1*, 20.14 Mb from Bala). Total 24.1% phenotypic variation was explained by these three QTLs.

2.2 Drought stress tolerance

Improving grain yield is the key and universal objective of any crop breeding programs including rice. Identification of yield and yield-related traits and their introgression into adapted varieties is one of the best strategies to increase grain yield under drought. A number of yield QTLs identified in different chromosomes of rice under drought stress. QTL *qDTY12.1* was the first identified major grain yield QTL on chromosome 12 in rice under drought at the reproductive stage [17]. Another large-effect QTL for grain yield, *qDTY1.1*, was identified on chromosome 1 [18]. Moreover, other QTLs with major effect such as *qDTY2.2*, *qDTY3.1*, *qDTY3.2*, *qDTY4.1*, *qDTY6.1*, *qDTY9.1* controlling drought tolerance in rice were reported by several investigators [19–22]. The identified QTLs should be consistent in multiple genetic backgrounds and various target environments [23, 24]. Efficient QTL stacking of the major effect QTLs in the adapted varietal background is necessary to achieve higher grain yield under drought [25].

QTLs related to drought tolerance in rice have been shown in **Table 1**. However, only a few QTL studies on grain yield under drought stress have been reported. Most of the QTLs detected for regulating drought stress in rice analyzed for different important drought-related traits such as osmotic adjustment [48, 49], drought avoidance [32], root and shoot responses [50], photosynthesis and whole plant response [51] to drought tolerance.

2.3 Flood/submergence tolerance

Flood-prone ecosystems are fragile characterized by varying level of flooding, erratic precipitation that affect the rice production of more than one million ha in Bangladesh. Deepwater rice is cultivated more than 100000 ha in Bangladesh and the typical deepwater rice with nodal tillering, kneeing ability to keep the top three leaves in the air (above the water level) to capture and use solar energy for photosynthesis, internode elongation ability to prevent drowning with high yielding potential comparing with local varieties like Hijoldigha, Laxmidigha, Kartiksail,

Drought-tolerance traits/indices	Cross combination and mapping population	Molecular marker used	% Phenotypic variation	No. of identified QTLs for drought tolerance	References
Root penetration index, root and tiller number	CO39/Moroberekan (RILs)	RFLP	8.0–14.0	39	[26]
Drought traits related with osmotic adjustment and dehydration tolerance	CO39/Moroberekan (RILs)	RFLP	—	1	[27]
Root morphology and distribution	IR64/Azucena (Double-haploid)	RFLP	6.0–22.0	—	[28]
Root traits related with drought	IR58821–23-B-1–2-1/IR52561-UBN-1–1-2 (RIL)	AFLP & RFLP	6.0–27.0	—	[29]
Cellular membrane stability	CT9993–5-10–1-M/IR62266-42–6-2 (DH)	RFLP, AFLP & SSR	11.8–54.3	9	[30]
Drought resistance osmotic adjustment and root traits	CT9993/IR62266 (Doubled haploid lines)	RFLP, AFLP & SSR	8.0–38.0	5	[31]
Drought avoidance, leaf rolling and drying	Bala/Azucena (F5 population)	RFLP, AFLP & SSR	7.4–25.6	17	[32]
Root traits (Seminal and lateral root length; adventitious and lateral root number)	IR1552/Azucena (RILs)	SSR	—	23	[33]
Morphological and physiological traits	IR64/Azucena (Doubled haploid Lines)	RFLP	—	15	[34]
Root-penetration ability	Bala/Azucena (RILs)	RFLP & AFLP	—	18	[35]
Reproductive-stage drought tolerance	Vandana/Way Rarem (F ₃ -derived lines)	SSR	33.0	—	[17]
Grain yield under drought stress	CT9993/IR62266 (Doubled haploid lines)	AFLP		1	[36]
Seedling stage drought tolerance	Indica/Japonica (Azucena) (RIL)	RFLP, AFLP & SSR	10.0–27.0	7	[37]
Morphological and physiological traits related to drought avoidance	Azucena/Bala (RIL)	RFLP, AFLP & SSR	—	—	[38]
Grain yield under lowland drought stress	Apo/2*Swarna (RILs)	SSR	13.0–16.0	1	[39]
Grain yield performance under aerobic condition	Three populations, Apo/(2)*Swarna, Apo/(2)*IR72, and Vandana/(2)*IR72	SSR	39.0–66.0	1	[40]

Drought-tolerance traits/indices	Cross combination and mapping population	Molecular marker used	% Phenotypic variation	No. of identified QTLs for drought tolerance	References
Yield performance under drought stress	Two populations Basmati334/Swarna and N22/MTU1010 (F _{3:4} population)	SSR	—	—	[41]
Reproductive-stage drought stress	Aday Sel/IR77298-5-6-B-11 (backcross inbred lines (BILs))	SSR	19.0	9	[20]
Yield under lowland drought in different environments	R77298/Sabitri, (BC1 derived)	SSR	—	1	[21]
Drought stress at reproductive stage	Two populations Kali Aus/IR64, Kali Aus/MTU1010 (RILs)	SSR	6.0–9.0	2	[42]
Grain yield and yield characters during reproductive stage	IR64/Cabacu (RILs)	SNP	—	1	[43]
Grain yield under stress at reproductive stage	Swarna/WAB (Backcross inbred lines)	SSR	—	1	[44]
Reproductive stage drought tolerance	Teqing/Lemont (Introgression lines)	SNP	—	—	[45]
Reproductive stage drought tolerance	IR55419-04/2 [*] TDK1 (BC ₁ F _{3:4} population)	SSR	36.0	6	[46]
Ratio of deep rooting (RDR)	3 populations (RILs, mini-core collection and landraces)	SSR, SNP	—	6	[47]

Table 1.
Useful QTLs responsible for drought-stress tolerance in rice.

Khoiyamtor, Lalmohan, and Shishumati. These local germplasm has the ideal ideotype for deepwater ecosystem but only limitation is low yielding ability. To address sustainable development goals (SDGs) and maintain food security, we need to increase the production in the abiotic stress prone environments such as salinity, flood/submergence, drought and heat-prone areas through horizontal expansion (expansion of arable land in abiotic stress prone areas which are not yet under cultivation) of abiotic stress tolerant rice varieties in these areas. However, Flood-prone ecosystems in Bangladesh are four types such as long time flooding zone (>35 days; 1.5–2.0 m water-depth), flash flood submergence zone (15–30 days; up to 1.5 m water depth), deepwater (> 2.0 m water-depth) zone and submergence during germination (10–12 cm depth) at relay Transplant Aman, direct seeded rice (DSR *Aus*) and broadcast Aman (B. Aman) Rice areas anaerobic germination In Asia, submergence affects rice yield adversely in 20 million ha, causing food insecurity. The *SUB1* gene governing submergence tolerance cloned and introgressed into a number of rice varieties in South Asia, South East Asia and Africa. Yield advantages of Sub1 varieties ranged from 1.0 to >3.0 t ha⁻¹ after submergence comparing with non-Sub1 varieties. These submergence tolerant varieties reached more than 3.8 million farmers within 3 years of release in Asia [52].

Biosynthesis of growth regulator (Gibberellin) and signal transduction is important pathways for internode elongation of the deepwater rice [53]. Two large-effect QTLs located on chromosomes 3 (*qGTIL3*, *qGLEI3*, *qGNEI3* located between 38 and 87 cM) and chromosome 9 (*qGTIL9*, *qGLEI9*, *qGNEI9* positioned between 16 and 88 cM) are controlling traits such as total length of internode (TIL), lowest elongated internode (LEI) and number of elongated internode number (NEI). Three factors involved to characterize deepwater rice internode elongation ability: (1) total length of elongated internode (TLEI); (2) elongated internodes number (EIN); and (3) minimum elongated internode (MEI) [54–57]. Among these, MEI is the main parameter for initiating the internode elongation of deepwater rice [54] because MEI is leaf stage- dependent and first starts of internode elongation at the sixth leaf stage in deepwater rice.

Catling (1992) [58] described the genetic basis of internode elongation during submergence of deepwater rice that is regulated by several minor and two major genes. Suge [59] identified one gene with neither allele is found dominant (incomplete dominance) that responsible for elongation ability. Internode elongation depends on the increasing activity of cell division and cell elongation in specific areas of the internode.

2.4 Salt tolerance

Salt-stress entails changes in different physiological and metabolic pathways, based on severity and duration of the stress, and eventually decreases rice productivity [10, 60–62].

Genetic characterization of salt tolerance related traits is important to estimate phenotypic co-efficient of variation (PCV), genotypic co-efficient of variation (GCV), broad-sense heritability and genetic Advance (GA).

If sufficient variation with high heritability and genetic advance exists in the germplasm for salt tolerance related traits; consequently there is possibility to improve the traits related with salt tolerance in rice by exploiting salt tolerant landraces/germplasm in the breeding programs.

Genetic component analysis (GCA) study showed that both additive and dominance gene effects controlled low Na-K ratio [63]. The combining ability analysis shows that both general combining ability (GCA) and specific combining ability (SCA) effects are also important for deciphering the genetics of salt tolerance. They also revealed that selection may be made in later generation under controlled conditions for minimizing environmental effects for low heritable traits like Na-K ratio.

The additive effects could enhance fixation of the desirable combination of genes and also additive gene action is correlated to narrow sense heritability [64]. Thus, selection for salinity tolerance may be made in the early generation. Understanding the gene action for salt tolerance in rice will be useful in the future breeding program [65].

2.4.1 Molecular basis of complex salt tolerance

The molecular dissection of salt tolerance has considerably enhanced using the molecular platforms for identifying quantitative trait loci (QTL) controlling related genetic variation in crops including rice [1, 2, 8, 60, 66–72].

Moreover, several QTLs related with physiological, agronomic traits conferring salinity tolerance at seedling and reproductive stage have been reported [1, 8, 71, 73, 74] including major QTLs for salinity tolerance such as *SKC1* [75] (a sodium transporter *OsHKT1*; 5 in the *SKC1* locus [76] and *Saltol* [71, 77] on chromosome 1

Rice growth stage	QTL/Gene name	Mapping population and parentage	Population Size	Salinity tolerant trait/ index	Flanking marker	Positive allele and Position (cM or Mb)	PVE (% R ²)	References
Seedling	<i>qSaltol</i>	RIL (IR29/Pokkali)	80	Na ⁺ uptake; Na ⁺ /K ⁺	RM140 - C1733S	Pokkali; 51.6–65.9 cM/13.87 Mb	39.2	[71, 77]
	<i>OsHKT1;5</i>	Advanced backcross	192	Shoot K ⁺ concentration	K159 - K061	Nona Bokra; 11.46 Mb	—	[76]
	<i>(qSKC-1)</i>	(Koshihikari*3/Nona Bokra)						
	<i>qST1</i>	RIL (Milyang 23/Gihobyeo)	164	Young seedling stage	Est12 -RZ569A	Gihobyeo; 40 cM	27.8	[78]
	<i>qST3</i>				RG179 - RZ596	Milyang 23; 138 cM	9.2	
	<i>qSES1</i>	F2 (NERICA-L-19/Hasawi)	153	SES	RM8094-RM582	NERICA-L-19	19.6	[79]
	<i>qSES6</i>				RM586-RM253	NERICA-L-19	39.7	
	<i>qSES10</i>				RM228-RM333	Hasawi	30.7	
	<i>qSES11</i>				RM536-RM287	Hasawi	37.2	
	<i>qSES2.1</i>	RIL (IR29/Hasawi)	142	SES	id2004774-id2007526	Hasawi; 64.8 cM	11.1	[80]
Reproductive	<i>qSES1.3</i>			SES	id1024972-id1023892	Hasawi; 170.0 cM	39.9	[1]
	<i>qSES3.1</i>	F2 (Capsule/BRRI dhan29)	94	SES	RM5626- R3M53	Capsule; 111.0 cM	23.0	[2]
	<i>qSES12.3</i>				RM252B RM27615- RM27877	Capsule; 31.0 cM	17.0	
	<i>qSL7</i>	RIL (93–11/PA64s)	132	Higher shoot length	SNP7–191–SNP7–226	PA64s; 86.31 cM	9.9	[81]
	<i>qNa1.7</i>	F2 (Cheriviruppu/Pusa Basmati 1)	218	Na (mmol g ⁻¹ dwt)	RM1349-RM7250	Cheriviruppu;; 31.06 Mb	13.5	[8]
	<i>qNaKR1.8</i>			Na/K Ratio	RM1349-RM7250	Cheriviruppu; 31.06 Mb	11.0	
	<i>qNFS2.1</i>	F2 (NSIC Rc222/BRRI dhan47)	92	Filled spikelet number	id2004774	BRRI dhan47; 40.1 cM	15.3	[73]
	<i>qPFS2.1</i>			Filled spikelet (%)	id2013434	BRRI dhan47; 122.1 cM	18.4	
	<i>qTS11.1</i>			Total spikelet (no)	id11000858	BRRI dhan47; 14.0 cM	15.8	

Rice growth stage	QTL/Gene name	Mapping population and parentage	Population Size	Salinity tolerant trait/index	Flanking marker	Positive allele and Position (cM or Mb)	PVE (% R ²)	References
	<i>qYLD2.1</i>			Yield	id2004774	BRRI dhan47; 40.1 cM	14.6	
	<i>qDEG-S-2-2</i>	Backcross		Spikelet degeneration		6.8	34.44	[82]
	<i>qDEG-S-4-3</i>	Backcross		Spikelet degeneration		4.19	17.43	

SES: Overall phenotypic performance; SL: Shoot length; Na: Na⁺: concentration; K: K⁺: concentration; NaK-R: Na-K ratio, Sur: Survival; Chr: Chromosome number; PVE: Phenotypic variation explained.

Table 2.

Recently identified QTL for salt tolerance with controlling/responsible traits and phenotypic variation using different mapping populations at seedling and reproductive stages in rice.

(**Table 2**). Recently unraveled molecular basis of various rice landraces such as Pokkali [71, 77], Nona Bokra [76], Hasawi [1], Capsule [2], Changmaogu [74] and Horkuch [72] can withstand different levels of salt-stress at various sensitive growth stages of rice.

2.4.2 Enhancing genetic gain for salt-stress

Widely used tools for quantitative genetics such as genomic estimated breeding value (GEBV) and best linear unbiased predictions (BLUPs) are applied to evaluate the performance to construct high throughput new breeding populations for selecting the superior breeding lines when combined with genetic relatedness or kinship matrix/information (i.e. coefficient of coancestry) using pedigree information to calculate estimated breeding values (EBVs). This is a key parameter for selecting complex traits like salt tolerance and yield through estimating parents' genetic potential to produce better descendants on the basis of parent's own performance, pedigree information and progeny data. EBVs play important role to select parent for higher rate of genetic gain [83].

Theory of genetic gain in breeding and classical biometrical genetics indicates the improvement of performance compared to a standard or baseline. It is generally evaluated after advancing one generation to the subsequent generation and artificial selection is done [84]. However, genetic gain per year is also known as genetic trend that measured varietal performance over year by comparing benchmark or dominant varieties [85].

To maintain the food security under this Covid-19 situation horizontal expansion (increase of rice growing areas in high saline zone) of rice cultivation is needed areas in the coastal zone. Thus large areas will be brought under cultivation in high saline prone areas.

2.5 Modern breeding

Genomic assisted breeding (GAB) is regularly practiced for the genetic improvement of salt-tolerant rice applying various innovative tools for genomic breeding such as forward breeding, rapid breeding, and haplotype-based breeding [86]; 5G breeding methods such as genome sequence availability (genome assembly), characterization of germplasm at genomic and morpho-agronomic level, gene detection and understanding function, genomic breeding, and genome editing for enhancing superior performance of genotypes [87] could be used for enhancing efficiency and accuracy of breeding for complex traits related with abiotic stress tolerance. A precise SNP-assisted introgression of the *hst1*(*hitomebore salt-tolerant 1*) gene improved salt tolerance in the high-yielding rice variety was achieved through SNP based speed breeding [88].

Modern breeding emphasizes data-based parent selection. Local and exotic germplasms are subjected to genetic distance measurement, trait characterization through diagnostic trait markers, genomic selection, and breeding value estimation. Sometimes trait of interest (ToI) like salinity and submergence tolerance, aroma, disease resistance is found in local germplasm with low yield potential. Then that ToI is first transferred to an elite background possessing high yield potential for developing pre-breeding materials. After that, the elite line with the desired traits is used in breeding purposes to fulfill the product profile. **Figure 1** shows the schematic illustration for optimizing breeding scheme to achieve genetic gain.

We evaluated 1436 breeding lines for trait assay using 20-trait specific single nucleotide polymorphism (SNP) markers. These lines characterized for important traits such as disease (blast, bacterial leaf blight: BLB) and insect (brown plant

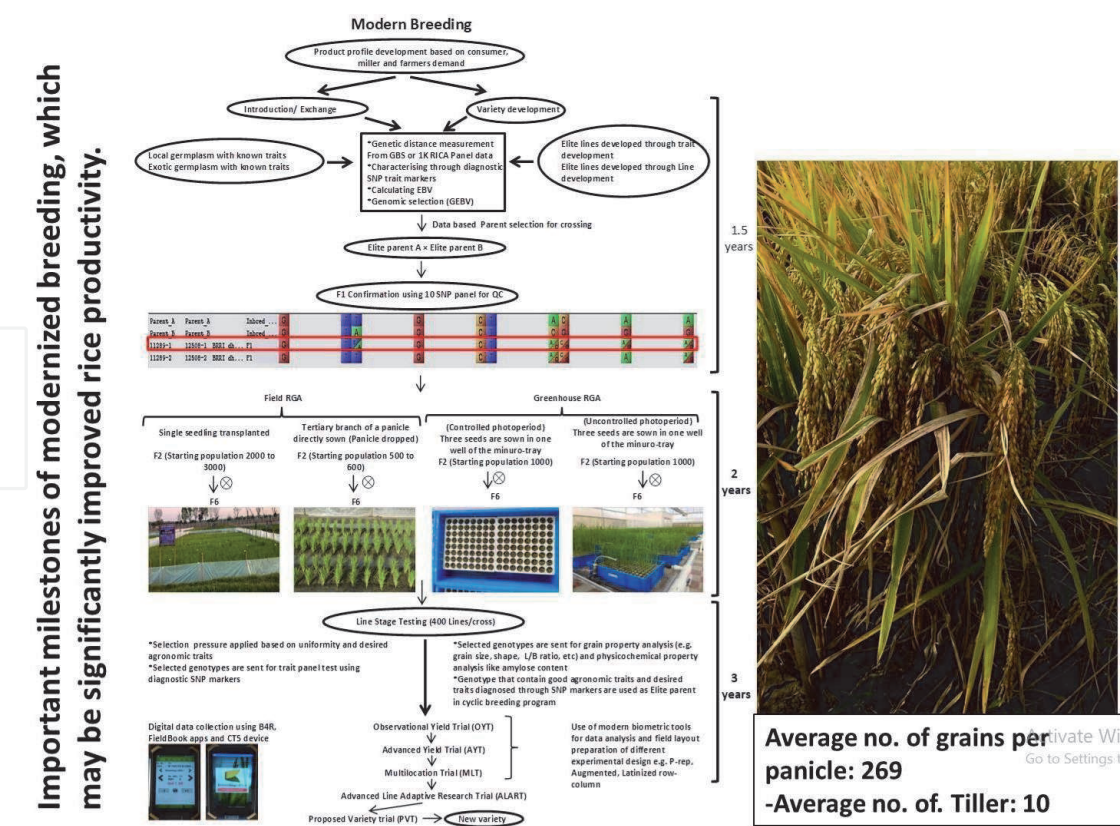


Figure 1.
Varietal development through breeding modernization for rapid varietal turnover and replacement for rice growers of target regions.

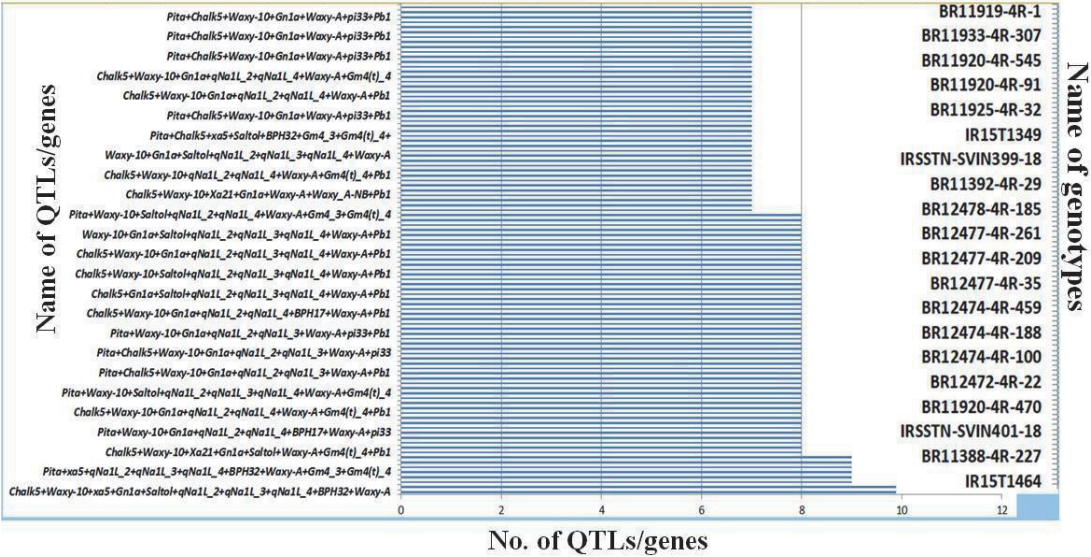


Figure 2.
Genetically important lines (GILs) in the STR Breeding program, T. Aman, 2020-21.

hopper: BPH; gall midge) resistance, grain quality (amylose content, chalkiness), grain number (*Gn1a*) and salinity tolerance at seedling stage (sodium exclusion, SES) traits. Out of 1436 breeding lines, 100 lines harbored the 7–10 QTLs/genes that regulating trait of interest (**Figure 2**) that designated as Genetically Important Lines (GILs). Each line assayed against QTLs and genes of interest to assess the presence or absence of useful traits. IRRI developed trait specific SNP markers used (<https://gsl.irri.org/>) and SNP genotyping assay was carried out by Intertek as an external service provider. The trait-based SNP markers associated with the traits for

Trait category	Trait	Trait specific markers (favorable allele)	Chromosome	QTL/Trait contributor
Blast	<i>Pb1</i>	snpOS00478 (T)	11	Pokkali 26869
Blast	<i>Pi9</i>	snpOS00451(C)	6	Pi9 (DQ285630)
Blast	<i>Pita</i>	snpOS00006 (C)	12	—
Blast	<i>qPi33</i>	snpOS00468 (T)	8	IR64
BLB	<i>xa13</i>	snpOS00493 (C)	8	IRBB60, some aus
BLB	<i>Xa21</i>	snpOS00061(C)	11	IRBB60
BLB	<i>xa5</i>	snpOS00054	5	IRRI 154, FR13A
Insect (BPH)	<i>BPH17</i>	snpOS00430 (G)	4	Rathu Heenati
Insect (BPH)	<i>BPH32</i>	snpOS00442 (G)	6	Honderawala, Rc222
Insect (Gall midge)	<i>Gm4(t)</i>	snpOS00466 (A)	8	Abhaya
Insect (Gall midge)	<i>Gm4(t)</i>	snpOS00467 (A)	8	Abhaya
Amylose	<i>Waxy</i>	snpOS00445 (C)	6	Wx(a) - except Basmati
Amylose	<i>Waxy</i>	snpOS00446 (T)	6	Wx(a)-Rc222, Exon10
Chalkiness	<i>Chalk5</i>	snpOS00024 (G)	5	Minghui63
Grain number	<i>Gn1a</i>	snpOS00396 (T)	1	Swarna (A8/AP9) allele
Salinity-seedling	<i>qNa1L</i>	snpOS00405 (A)	1	FL478
Salinity-seedling	<i>qNa1L</i>	snpOS00409 (C)	1	FL478, Capsule
Salinity-seedling	<i>qNa1L</i>	snpOS00410 (A)	1	FL478, Capsule
Salinity-seedling	<i>qNa1L</i>	snpOS00411 (T)	1	FL478, Capsule
Salinity-seedling	<i>Saltol</i>	snpOS00397 (T)	1	FL478, IR 107321-1-141-3-120

Table 3.
Useful traits, trait-based SNP markers and their contributors of favorable allele.

instance, snpOS00478 (*Pb1*), snpOS00451 (*Pi9*), and snpOS0054 (*xa5*), snpOS0061 (*Xa21*) etc. were applied for genotyping (**Table 3**).

These ten SNP markers produced 40% polymorphism across the *indica* germ-plasm derived pairwise combinations where in 95% of crosses made at least 1 polymorphic SNP marker was found within the IRRI rice breeding pool or *indica* subspecies (gsl@irri.org[<https://sites.google.com/a/irri.org/snp-genotyping-mmml/genotyping/quality-control-panel/indica-rice-qc-10-snp-panel>]).

Single seed descent method with the facility of rapid generation advance technique is expected to have better efficiency in the increment of genetic gain compared to pedigree and other methods of breeding [2]. From each cross, 200–400 fixed lines evaluated in line stage testing (LST) trial and selection performed using high selection pressure. Then the selected lines are evaluated in yield trials and include in new variety release system (**Figure 1**).

Modern biometric tools are used in data analysis and field layout preparation of several experiment design e.g. P-rep, Augmented RCB, Alpha-Lattice, Latinized row-column, RCBD. Observation trials are mainly conducted using P-rep, Augmented RCB, Alpha-Lattice design of experiment based on the entry number and land availability. Selected genotypes are subjected to grain property analysis (e.g. grain size, shape, L/B ratio, etc) and physicochemical property analysis (e.g. amylose content). Selected genotypes that hold good agronomic traits, grain quality

characters and desired traits diagnosed through SNP markers are used as Elite parent in cyclic breeding program.

During this period phenotypic data collection is aided with data collection machine (Grain counter, Nondestructive moisture meter, destructive moisture meter, Phenoapp, CT5) and different software’s like B4R (Breeding 4 Rice), FieldBook etc.

We need to explore a large number of cross combination derived fixed lines to experience a remarkable genetic gain with the shortest possible time. Line fixation can be accomplished within three years using the RGA [89, 90] facility which allows a breeder to contribute more in varietal development.

Salinity (T. Aman) Breeding Product Profiles (20% Breeding Program Focus)						
Country/Region: Salt affected coastal region and/ Whole Bangladesh		%Resource allocation for trait improvement	Market Leading Variety #1: BRRI dhan41 (Medium slender, high head rice yield, medium growth duration)			
Market Slot: T. Aman Rice (Medium slender to long slender grain)			Market Important Variety #2: BR23 (Medium bold, high yield potential, photo period sensitive)			
Trait Family	Key Economic Traits		Trait value	Standard Variety Assessment	Trait Benchmarking Details	Trait availability in the Breeding Program
Yield (Paddy)	Yield (>10% higher)		1-Must Have Trait	BRRI dhan41	> BRRI dhan41	Program is actively working with trait
Maturity	Intermediate /long range	10	1-Must Have Trait	BRRI dhan23	<= BRRI dhan23	Program is actively working with trait
Abiotic Stress Tolerance	Salinity tolerance	20	1-Must Have Trait	BRRI dhan67	> = BRRI dhan67	Program is actively working with trait
Biotic Resistance (Fungal)	Blast	20	1-Must Have Trait	BRRI dhan67	Standard Evaluation Scale = < 3	Trait Limited or NOT available
Biotic Resistance (Bacterial)	BLB	10	3-Trait Values	BRRI dhan67	Standard Evaluation Scale = < 3	Program has trait available
Biotic Resistance (Insect)	BPH	10	3-Trait Values	BRRI dhan67	= Rathuheenati (<i>bph32, bph17</i>)	Trait Limited or NOT available
Consumer Traits	Amylose content	15	1-Must Have Trait	BRRI dhan67	=> 24%	Program is actively working with trait
	Zinc content	5	3= Value added	BRRI dhan72	BRRI dhan72; => 24 mg/kg Zn	Program is actively working with trait
Yield (Economic)- Head Rice	Head rice recovery	10	1-Must Have Trait	BRRI dhan67	> 50%	Program is actively working with trait
Total		100				
Legend: 1= must have traits, 2= nice to have, 3= value added or game changer						

Table 4. Product profile with market demand-led traits for developing salinity tolerant rice (STR) variety for target region under STR breeding program at BRRI.

However, promising breeding lines selected in OYT will be recycled to initiate next cycle of breeding for population improvement. By applying this transformed/modernized breeding approach abiotic stress tolerant varieties with enhanced grain yield and quality traits will be developed.

2.6 Product profile (PP) for target region

Product profile (PP) is a realistic roadmap for varietal development which addresses requirements of plant breeder, farmers, millers/market and consumers demand. Example of a product profile for salinity tolerant variety development for Southern Coastal Zone in T. Aman season is shown in **Table 4**. Variety development based on formal PP will be able to replace market leading variety with the new one; thus farmer could get early turnover from the new variety. However, the market leading variety may be or may not be a mega variety in target region. The way of developing PP is:

- Designating the target region that use common PP or trait requirements
- Identifying the market leading variety and second important variety
- Placing key traits into appropriate trait family
- Estimating the amount of emphasis or breeding efforts need to give in the breeding program for selecting trait.

3. Varietal development

3.1 Germplasm collection and characterization for salt-stress tolerance, and utilization

A total of 107 landraces collected from southern coastal zone of Bangladesh.

The genetic base of salt-tolerant donors needs to broaden for developing climate-smart rice [91] varieties for salt-affected regions with higher level of adaptation. All germplasm [92] were used for diversity analysis using a genome-wide set of 376 single nucleotide polymorphism (SNP) markers to identify and characterize novel sources of salt tolerance.

Three major clusters -the indica, aus and aromatic subgroups were identified. The largest group was indica, with the salt-tolerant Pokkali accessions in one sub-cluster, while Bangladeshi landraces, including Akundi, Ashfal, Capsule, Chikirampatnai and Kutipatnai, were in a different sub-cluster. The salt-tolerant landrace Hasawi and Kalarata clustered into a distinct aus group near to indica. Allelic diversity study at the major QTL *Saltol* shows different alleles at the *Saltol* locus for Akundi, Ashfal, Capsule, Chikirampatnai and Kutipatnai.

Sixty-nine landraces were further screened for physiological traits associated with salt stress at the seedling stage. Seven landraces such as Akundi, Ashfal, Capsule, Chikirampatnai, Jatai Balam, Kalarata and Kutipatnai uptake less Na and comparatively more K and maintain lower Na/K ratio in leaves. They efficiently restrict sodium transport root to the shoot.

Newly identified salt-tolerant landraces are genetically and physiologically different from known donors (Pokkali and Nona Bokra). These landraces can be used to develop salt-tolerant varieties with higher tolerance [10].

These landraces may be harbored novel sources of QTL/alleles for salt tolerance that will be useful in molecular breeding.

3.2 Participatory varietal selection (PVS)

Participatory varietal selection (PVS) demonstrates the varietal/line selection on the basis of farmers’ need/demand-based choice of varieties that differs from plant breeders’ selection process. Plant Breeders evaluation of varietal performance – mostly following statistical designed and quantitative data based methods which is usually different from the farmers [93–95]. However, variety selection criteria may vary according to gender, environmental condition, market demand and economic/social status [95–97]. Until now, breeding objectives in different countries have not been properly focused on the opinions of farmers, their preferences and needs for the adverse growing conditions of their regions [94, 98, 99]. To unravel this situation, participatory varietal selection is the important way of decentralized-bottom-up breeding or farmer breeding approach that integrates farmers and their complex criteria for variety selection into varietal development programs [99–103].

BRRI dhan47 (IR 63307-4B-4-3) was first selected through PVS and released as salt tolerant variety for *Boro* season in Bangladesh.

Different high-yielding rice varieties for salt (15 from BRRI and two from BINA), drought, submergence tolerance, upland rice varieties and other developed and released for Transplanted Aman (T. Aman-RLR) and Irrigated Ecosystem (Boro-dry season) (**Table 5**).

Variety name	Cross	Salient features with growing season	Year of release
Salt-tolerant varieties			
BR23 (BR716–7–2-1-1)	DA29/BR4	Moderately salt tolerant photosensitive <i>T. Aman</i> rice variety for Rainfed Lowland ecosystem	1988
BRRI dhan40 (BR5331–93–2-8-3)	IR4595-4-1-15/BR10	Moderately salt tolerant <i>T. Aman</i> rice variety for Rainfed Lowland ecosystem	2003
BRRI dhan41 (BR5828–11–1-4)	BR23/BR1185-2B-16-1	Moderately salt tolerant <i>T. Aman</i> rice variety for Rainfed Lowland ecosystem	2003
BRRI dhan47 (IR 63307-4B-4-3)	IR51511-B-B-34-B/TCCP266–2-49-B-B-3	Salt tolerant <i>Boro</i> rice variety for Irrigated ecosystem	2007
BRRI dhan53 (BR5778–156–1-3-HR14)	BR10/BR23//BR847–76–1-1	Salt tolerant <i>T. Aman (RLR)</i> rice variety	2010
BRRI dhan54 (BR5999-82–3-2-HR1)	BR1185-2B-16-1/BR548–128–1-3	Salt tolerant <i>T. Aman (RLR)</i> rice variety	2010
BRRI dhan55 (IR 73678–6-9-B: AS996)	IR64/ <i>Oryza rufipogon</i>	Moderately salt, cold and drought tolerant rice variety	2011
**BRRI dhan28- <i>Saltol</i> (IR 89573–84)	BRRI dhan28 ⁺ 3/FL478	Salt tolerant <i>Boro</i> rice line for Irrigated ecosystem	MABC product
**BR11- <i>Saltol</i> (IR 89574–7)	BR11 ⁺ 3/FL478	Salt tolerant <i>T. Aman (RLR)</i> rice line	MABC product
BRRI dhan61 (BR7105-4R-2)	IR64419-3B-4-3/BRRI dhan29	Salt tolerant <i>Boro</i> rice variety/Irrigated rice	2013

Variety name	Cross	Salient features with growing season	Year of release
BRRI dhan67 (BR7100-2R-6-6)	IR61247-3B-8-2-1/BRRI dhan36	Salt tolerant <i>Boro</i> /Irrigated rice variety	2014
BRRI dhan73 (IR78767-B-SATB1-28-3-24)	BRRI dhan40/NSIC Rc106 (IR61920-3B-22-1-1)	Salt tolerant <i>T. Aman</i> (RLR) rice variety	2015
BRRI dhan78 (IR77092-B-2R-B-10)	IR84645/IR84649	Dual tolerant (Salinity+ <i>SUB1T. Aman</i> rice variety	2016
BRRI dhan97 (IR83484-3-B-7-1-1-1)	IRRI 113/BRRI dhan40	Salt tolerant irrigated (Boro) Rice	2020
BRRI dhan99 HHZ5-DT20-DT2-DT1 (GSR IR1-5-D20-D2-D1)	Huang-Hua-Zhan/OM1723	Salt tolerant irrigated (Boro) Rice	2020
Binadhan-8 (IR66946-3R-149-1-1)	IR29/Pokkali	Salt tolerant irrigated (Boro) Rice	2010
Binadhan-10 (IR64197-3B-14-2)	IR42598-B-B-B-B-12/Nona Bokra	Salt tolerant irrigated (Boro) Rice	2012
Drought tolerant varieties			
BRRI dhan42 (BR6058-6-3-3)	BR14/IR25588-7-3-1	Moderately drought-tolerant <i>Upland</i> (DSR) rice variety	2004
BRRI dhan43 (BR5543-5-1-2-4)	BR24/BR21	Moderately drought-tolerant <i>Upland</i> (DSR) rice variety	2004
BRRI dhan56 (IR74371-70-1-1-B)	Way Rarem/2*IR5519-4	Drought-tolerant <i>T. Aman</i> (RLR) rice variety	2011
BRRI dhan57 (BR7873-5NIL)-51-HR6	BR11/5*CR146-7027-224	Drought-tolerant <i>T. Aman</i> (RLR) rice variety	2011
BRRI dhan66 (IR82635-B-B-75-2)	IR78875-176-B-2/IR78875-207-B-3	Drought-tolerant <i>T. Aman</i> (RLR) rice variety	2014
BRRI dhan71 (IR82589-B-B-84-3)	IR55423-01 (NSIC Rc9)/IRRI148	Drought-tolerant <i>T. Aman</i> (RLR) rice variety	2015
Submergence Tolerant varieties			
BRRI dhan51 (IR81213-246-237)	Swarna/IR49830-7-1-2-3	Flood-tolerant <i>T. Aman</i> (RLR) rice variety	2010
BRRI dhan52 (IR85260-66-654-Gaz2)	BR11*3/IR40931-33-1-3-2	Flood-tolerant <i>T. Aman</i> (RLR) rice variety	2010
BRRI dhan79 (BR9159-8-5-40-14-57)	BRRI dhan49*6/BRRI dhan52	Flood-tolerant <i>T. Aman</i> (RLR) rice variety	2017
Deepwater Rice variety			
BRRI dhan91 (BR10230-15-27-7B)	Tilokkachari/BRRI dhan41	Suitable for shallow flooded ecosystem	2019

*A large range of salt tolerant improve genotypes was grown in mother and baby trials of participatory varietal selection (PVS) in coastal areas of Bangladesh. BRRI dhan47 (IR 63307-4B-4-3) was finally selected and released as salt tolerant variety for Boro season in Bangladesh.

**First introgression of Saltol locus into the mega varieties and developed two salt tolerant rice genotypes, IR89573-84 (BRRI dhan28-Saltol) and IR89574-7 (BR11-Saltol) through marker-assisted breeding at IRRI that are under advance stage of testing for release in Bangladesh and Myanmar.

Table 5.
List of abiotic stress tolerant varieties released in Bangladesh for Upland rice (*Aus*), *T. Aman* (RLR- wet) and Boro (dry) season.

3.3 Marker-assisted selection

FL378 (IR66496-3R-78-1-1), a salinity tolerant recombinant inbred line derived from cross between IR29 and Pokkali was used as donor for *Saltol*. FL378 had the Pokkali introgression on the chromosome 1 from RM1287 (10.9 Mb) to RM493 (12.2 Mb) for about 1.3 Mb and its average tolerance score was around 4.7 [104–106]. The recurrent varieties were BR11, BRRI dhan28 and BRRI dhan29, three mega varieties of Bangladesh are widely grown in wet and dry season.

BRRI dhan28-*Saltol* seeds were developed at IRRI and FL478 as donor parents. The introgression lines of BR11-*Saltol* and BRRI dhan28-*Saltol* were evaluated in salt affected coastal district of Satkhira during dry season.

Moreover, *Saltol* QTL was introgressed into the genetic background of BRRI dhan49 [107] and Pusa44 and Sarjoo52 [92] through marker-assisted backcrossing. FL478 was used as a donor for *Saltol* QTL. A number of *Saltol* introgression lines (NILs: BRRI dhan49-*Saltol* lines) were developed [107]. Krishnamurthy [92] reported that the NILs PU99, PU176, PU200, PU215, PU229, PU240, PU241, PU244, PU252, PU263 of Pusa44 and SAR17, SAR23, SAR35, SAR39, SAR77, SAR87, SAR123, SAR136 NILs of Sarjoo52 exhibited salt tolerance with low salt injury score (SIS) of 3 or 5.

4. Conclusions

Effect of abiotic stresses increases due to worldwide climate change. The holistic breeding approach combines different cutting-edge/modern breeding strategies (data based parents selection for crossing, rapid breeding, genomics-assisted breeding and haplotype-based breeding) including efficient gene stacking facilitate the development of climate-resilient rice varieties. Genotypes could cope with the climatic threats, increase the varietal turnover of farmers, and contribute to meet challenges of abiotic stress-prone ecosystems through enhancing productivity and sustaining food security. Also, rice cultivation areas will be expanded under the high abiotic stress-prone areas where salt-stress is a key problem for rice production during both dry and wet seasons in different rice-growing countries including Bangladesh. Moreover, the stress related to HNT needs to be emphasized because this stress may also become the challenge for food security where the rice is a staple food.

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Conflict of interest

No conflict of interest.

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