Development of Multi-Parent Advanced Generation Intercross (MAGIC) Mapping Population for QTL Analysis in Rice

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ABSTRACT

A major advantage for researchers in plant and animal genetics lies in the ability to create experimental populations. Such populations mix well characterized founder genomes in controlled pedigrees, and facilitate the investigation of both the genome itself and its relationship with traits and the environment. Traditional experimental populations combine the genomes of two parents with contrasting phenotypes to identify regions of the genome affecting the trait. However, each of these populations captures only a small snapshot of the factors affecting the trait due to the narrow genetic base. It is only possible to detect those genomic regions which differ between the two founders, and all alleles occur with high frequency in the population. Recently, a multi-parent advanced generation intercross (MAGIC) strategy has been proposed to interrogate multiple alleles and to provide increased recombination and mapping resolution. The main objective of developing MAGIC populations is to promote intercrossing and shuffling of the genome, thus increased precision and resolution with which QTLs can be detected due to the increased level of recombination. The Global MAGIC is an attempt to combine traits from several gene pools that have been adapted to different environments. In addition to being a source of potential novel variation, the Global MAGIC population provides useful materials for studying the relative contributions from different crop ecotypes and the level of recombination among exploited genomes constituting this population. An eight Parent-based rice MAGIC population was initiated to develop by employing 2-way, 4-way and 8-way cross along with 8-way intercross hybridization. Nonetheless, the 2-way (Single cross), 4-way (Double cross hybrid) cross has completed during the first year of research program (2018-2019) and subsequently, 8-way cross giving rise to 2 sets (*8-wcp set1) having genome ABCDEFGH and 8-wcp set-2 with genome EFGHABCD*) of 8-way cross F_1 genotypes has accomplished at Aman season of second year (2019-2020) of the project. The eight-way crosses of set 1 (*8-wcp set-1*) were further intercrossed (Boro 2019) with the reciprocal eight-way crosses set 2 (*8-wcp set -2*) for establishing altogether 85 eight-way intercross combinations (ABCDEFGH/EFGHABCD) and then, we render this 1-85 group as BUMpop (Bangabandhu University Mapping Population). Progeny from each of the eighty-five 8-way intercrosses were grown, keeping ~ 4 offspring per cross constituting the population size as $85 \times 4 = 340$ lines. The seeds of BUMpop were progressed to the F_6 generation via single seed descent (SSD) method in the field.

Key words: MAGIC, QTL, candidate gene, variety and rice

INTRODUCTION

Traditionally, breeders and geneticists use biparental populations for mapping and varietal development. However, biparental populations have limited allelic variation as they only segregate for QTL that differ between the two parents. An alternate approach is to create multi-parental populations derived from elite parents, in which each line represents a combination of alleles inherited from multiple parents. Ideally, a diverse set of lines is generated simultaneously that can be readily used by breeders and geneticists. To achieve this, the selected parents must be (a) diverse in their traits, (b) good combiners, and (c) have elite features, which will result in new and favorable allelic combinations. Further, these diverse multi-parent lines serve as a genetic population to map QTL and to understand genetic architecture. Recently, a multi-parent advanced generation intercross (MAGIC) strategy has been proposed to interrogate multiple alleles and to provide increased recombination and mapping resolution (Cavanagh et al. 2008). The advantages of using multi-parent populations are that: (1) more targeted traits from each of the parents can be analyzed based on the selection of parents used to make the multi-parent crosses; and (2) increased precision and resolution with which QTLs can be detected due to the increased level of recombination (Cavanagh et al. 2008). Multi-parent populations are now attractive for researchers due to the development of high-throughput SNP genotyping platforms and advances in statistical methods to analyze data from such populations. Advanced Inter-crossed Lines (AILs) are the fixed populations serving as permanent resources derived from MAGIC and are similar to recombinant inbred (RI) populations derived from bi-parental crosses. AILs are generated by randomly and sequentially inter-crossing a population initially originating from a cross between two inbred lines (Darvasi and Soller 1995); however, in our case multiple parents will be used. Advanced intercrosses (AIC), which will be derived from multiple parents, can be directly used as a commercial variety. In addition, due to increase recombination events in small chromosomal regions, it can be used for fine mapping (Huang and George 2012). Lines derived from early generations can be used for QTL detection and coarse mapping, while those derived from later generations may potentially yield markers very close to the QTL due to increased crossing-over events after every inter-mating cycle.

Rice is one of the world's leading staple foods. The rice production should be enhanced to meet the requirement of the increasing population. Genetic diversity between combining parents is an essential requirement for realization of heterosis in resultant hybrid. In order to increase the intra-variety genetic diversity based on existing germplasm resources, multi-parent advance generation inter-cross (MAGIC), a new breeding method using multiple parents' convergent cross, has been widely used to improve the genetic diversity of crops (Li, 2014; Cavanagh et al., 2008). Several major agronomic traits are controlled by multiple genetic loci, which make it difficult to study multiple complicated agronomic traits in bi-parental or natural populations (Wang et al., 2016). MAGIC populations, including multiple parents and alleles, have been used for linkage mapping and association analyses without the limitations associated with structured populations (Sallam and Martsch, 2015). There is currently ample evidence supporting the value of MAGIC populations for gene mapping and crop breeding (Li et al., 2013; Wei and Xu, 2016). Given the potential benefits of MAGIC populations, rice MAGIC populations using stress resistant rice ecotypes could be developed which might provide germplasm resources for future rice breeding.

Development and analysis of Multi-parent Advanced Generation Inter-Cross (MAGIC) populations have been conducted with several crop plants to harness the potential for dissecting the genetic structure of traits and improving breeding populations. We will attempt to develop a MAGIC population for rice from eight founder parents which are genetically diverse and carry many abiotic and biotic stress resistance, seed quality and agronomic traits relevant to rice improvement. Each of the eight founder parents were carefully selected as a donor for at least one major trait in an improved background. So that, populations will capture the majority of variation present in a given gene pool and consequently, developed MAGIC populations can be directly used as a commercial variety. The eight parents will be inter-cross using structured mating to

ensure the population would have balanced representation from each of the founder parents, followed by single-seed descent, resulting in recombinant inbred lines (RILs) each carrying a mosaic of genome blocks contributed from all founders.

The preliminary examination of MAGIC population will suggest the possibility of capturing various combinations of traits in the population, which is directly applicable in breeding programs. We can plan to disseminate, genetically characterize and evaluate the MAGIC lines in field trials through an evaluation network. This approach expands the trait data and will identify lines adapted to a range of production constraints (particularly stress tolerance). Thus, the goal will be to identify the best MAGIC lines with a combination of essential traits that are suitable for multiple environments.

Furthermore, exploiting the developed MAGIC population, QTL analysis followed by fine mapping of the AILs (Advanced Inter-crossed Lines) will be possible in order to develop trait-specific markers for application in breeding programs. All data (genotype and phenotype) may be stored in a database to enable breeders to improve their selections. Thus the MAGIC populations will provide the foundation for accelerating progress in breeding programs through identification of beneficial alleles at key genetic loci, as well as superior combinations of stress tolerance in the best MAGIC lines for the breeding programs.

METHODOLOGY

Four steps were followed for the development of MAGIC population; however, the first two steps (i.e. Founder selection and Mixing) have been partially completed at the first year of the project and chronologically, some partial task (i.e. mixing and advanced intercrossing) of the whole methods have concluded during second year as well as reporting time.

Founder selection

- The selection of the founders is one of the most important steps and depends heavily on the goals of the breede More diverse founder sets may provide biological insight into a wide variety of traits.
- Based on genetic or phenotypic diversity, either in a constrained set of material (e.g., elite cultivars, geographical adaptation) or material of more diverse origins (worldwide germplasm collections, distant relatives) is suggested as founder lines.
- Therefore, keeping thus view in consideration 8 founder lines viz. Chinnigura, Kataktara, Parija, Pokkali, BR-29 and Kalijira as well as two promising exotic collections from two different countries, Bashmati (India) and Double rice (China) assuming the genome A, B, C, D, E, F, G, H respectively, were selected from the large collected germplasm pool of the Department of Genetics and Plant breeding, BSMRAU

Mixing:

- Multiple parents were intercrossed to form a broad genetic base. Thus, the inbred founders was paired off and inter-mated which was termed as funnel
- An eight parent based rice MAGIC population was initiated to develop by employing 2-way, 4-way and 8-way intercross hybridization. Nonetheless, the 2-way (Multiple parents intercross, production of 4 F1s and harvesting seed separately), 4-way (Seedling raising of F1s, production of 2 sets of double cross hybrid (DCF1Hy) intercross program was completed during the year (2018-2019) and 8-way cross as well intercross hybridization has accomplished at the reporting year (2019-2020) of the project.
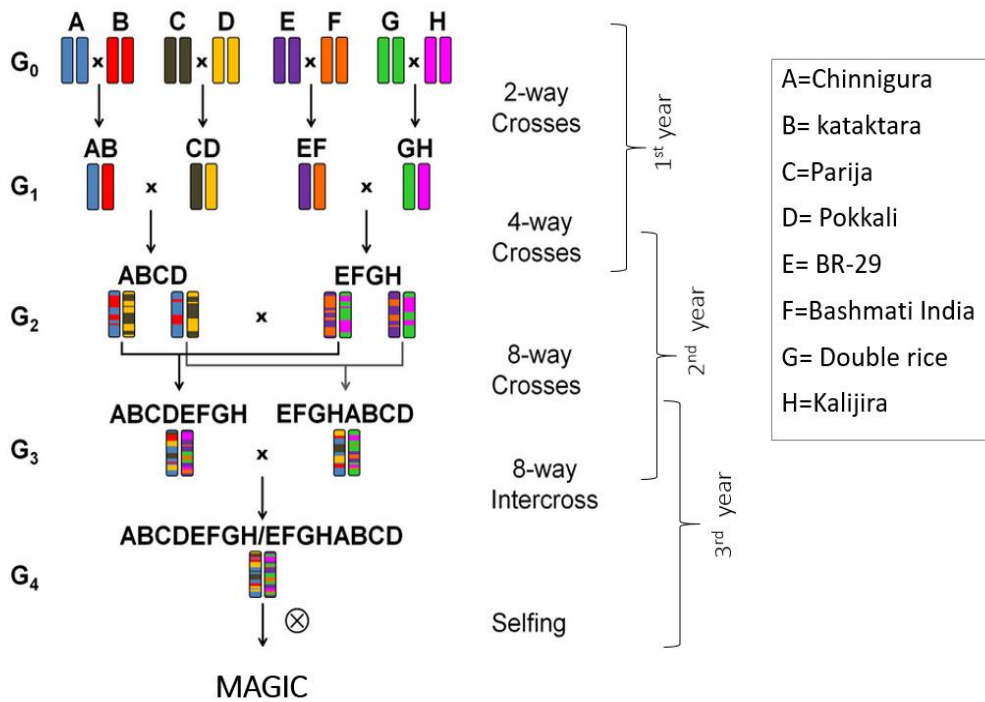
Figure 1: Breeding/ crossing scheme followed for development of rice MAGIC population

- In this stage we will get such lines whose genome is contributed by each of the founders.

Advanced Intercrossing:

- Mixing lines from different funnels will randomly and sequentially intercrossed as in the advanced intercross
- Main goal will be to increase the number of recombination
- At least six cycles of intercrosses will be required for constructing a good mapping population

Inbreeding

- Development of homozygous individuals.
- RILs produced through single seed descent (SSD)

RESULT AND DISCUSSION

Founder selection

Founder lines must be chosen based on genetic and/or phenotypic diversity of elite cultivars, geographical adaptation or material of more diverse origins (worldwide germplasm collections, distant relatives). Use of landraces as founders may introduce greater diversity. Thus, a MAGIC population is aimed to construct from 8 founder lines that had previously been characterized for a broad range of useful agronomic traits, including early maturity, eminent grain quality, broader adaptation, high yield potential, and drought tolerances as well as pest and disease resistance. Eight founder parents consisted of six popular rice varieties, Chinnigura, Kataktara, Parija, Pokkali, BR-29 and Kalijira as well as two promising exotic collections from two different countries; viz. Bashmati (India) and Double rice (China) will be employed in three rounds of intercrossing programme. The relevant details of the parents used in the study are presented in Table 1.

Development of 2-way (Single cross) and 4-way intercrosses (double cross) hybrids

For developing the MAGIC population, traits targeted from the selected parents were higher yield and attributing traits *i.e* wider adaptability, resistance to biotic and abiotic stress etc. One more objective of the present study was to bring diversity from China, and India in the genetic background of Bangladeshi rice cultivars. For the development of initial four

Details of the parents used in development of MAGIC population

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Kalijira	A popular traditional variety with fine grain and faboulous aroma called "prince of rice", very popular among food lovers which has unique flavor with more nutritional value, similar in appearance to basmati and jasmine rice but with a shorter grain length
Chinigura	Chinigura is a variety of aromatic rice mostly popular and cultivated in Bangladesh. This rice variety is totally white in color, thin and long in size with little bit round lower edge and pointy head.
Double rice	A Chinese land race having multiple grained rice, the unique rice variety (or species!) that has two grains in a single rice seed, quite similar to *'Biram Sundori'* of Bangladesh or *'Laila Majnu'* of Nepal having two (sometime three) grains in a rice seed
Kataktara	A moderately drought tolerant variety at seedling stage , local popular fine grain variety for regular consumtion, suitable for for broadcast/direct seeded/upland Aus rice ecosystem
Parija	Parija is a short durated aush variety, seed can be sowed using direct seeded rice method to harvest in 90 days or transplanted 18-day old seedlings to harvest in 75 days to achieve the maximum yield under changed climate without hampering Aman cultivation.
pokkalli	**Pokkali** is a unique saline tolerant rice variety that is cultivated using extensive aquaculture in an organic way in the water-logged coastal regions. Pokkali is famed for its peculiar taste and its high protein content, grains are extra large—has several medicinal properties. In the past, Pokkali provided the energy to fishermen to stay at sea all day long
Basmati	**Basmati** is a variety of long, slender-grained aromatic rice which is traditionally from the Indian subcontinent, has a typical *pandan*-like flavor caused by the aroma compound 2-acetyl-1-pyrroline, giving basmati its distinctive spicy fragrance and flavor.
BR-28	A popular Boro season variety of Bangladesh Rice Research Institute (BRRI), Plant height 90 cm, rice medium slender and white, moderately resistance to blast, high yielding, most popular term as "mega variety" of Bangladesh

Basmati India   Kalijira   Pokkali   Katak tara

Mukta gacha   Parija   Double rice   BR-29
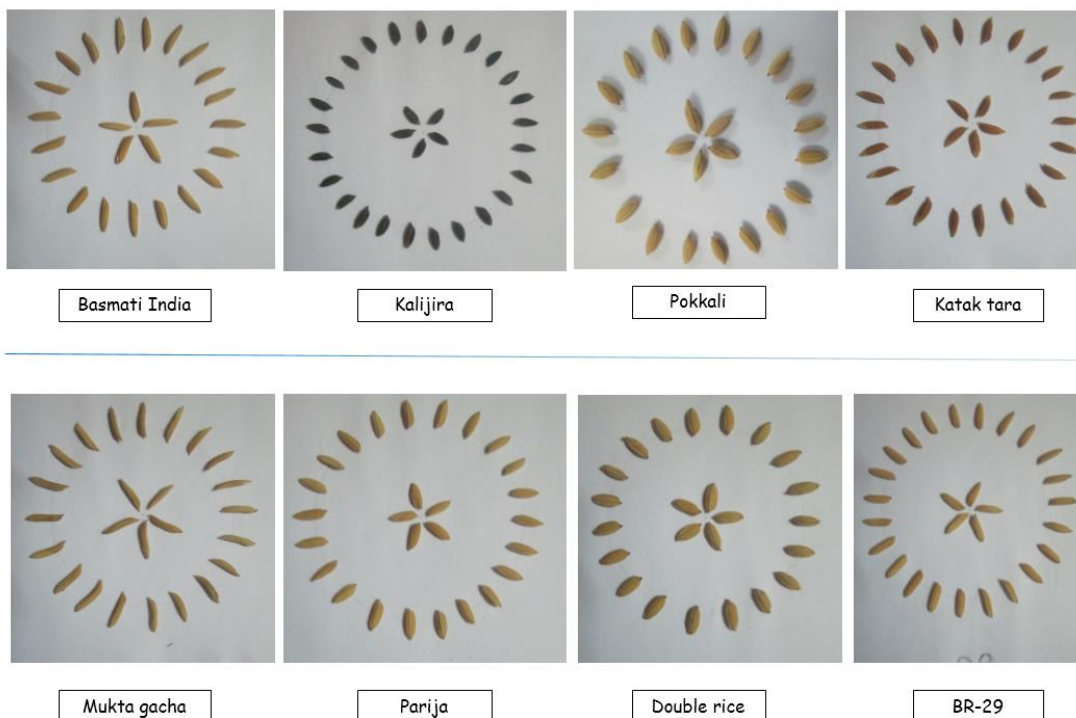
Figure 2: Grain morphology attributes the parents used in the development of MAGIC population

combinations of 2-way crosses, viz Chinnigura X Kataktara, Parija X Pokkali, BR-29 X Bashmati India, Double rice X Kalijira, seeds of eight founder parents were sown during *Aman* 2018 at different sowing dates to match the flowering time of the respective parental pairs (Table 2). During *Boro* 2018, F_1 seeds of *Chinnigura X Kataktara*, *Parija X Pokkali*, *BR-29 X Bashmati India*, *Double rice X Kalijira* were sown and after confirmation of hybridity, 4-way inter-crosses (double crosses) were attempted in four combinations and a total of 303, 4-way inter-cross hybrid seeds were harvested (Table 3) which was sown to perform 8-way cross during *Aman 2019*.

Table 2: Details of the 2-way intercrosses (Single cross hybrids) performed during the Aman and Boro seasons of 2018

2-way a intercrosses (Single crosses)	Crossing scheme during Aman 2018			Planting scheme during Boro 2018		
	Genome considered	*Floret subjected to crossing*	*No. of hybrid seed recovered*	*Date of sowing*	*Seed place for germination*	*No. of seedling recovered*
Chinnigura X kataktara	AB	140	25	06.01.19	15	11
(Parija X Pokkali)	CD	120	26	06.01.19	15	13
BR-29 X Bashmati India	EF	254	22	06.01.19	15	9
Double rice X Kalijira	GH	135	18	06.01.19	15	11

Table 3: Details of the 4-way intercrosses (Double cross hybrids) performed during the Boro season of 2018 and planting plan of Aman 2019

4-way intercrosses (DCHs)	Crossing scheme during Boro 2018			Planting scheme for Aman 2019		
	Floret subjected to crossing (Approx.)	*Genome combined*	*No. of double cross hybrid seed recovered*	*Seed place for germination*	*No. of seedling recovered*	*Date of transplanting*
(Chinnigura X kataktara) x (Parija X Pokkali)	>300	ABCD	146	150	137	4/8/2019
(Parija X Pokkali) x (Chinnigura X kataktara)	>250	CDAB	93			
(BR-29 X Bashmati India) x (Double rice X Kalijira)	>302	EFGH	45	60	53	
(Double rice X Kalijira) x (BR-29 X Bashmati India)	>290	GHEF	19			

Development of 8-way cross and 8-way intercrosses population

One initial MAGIC population will be developed by inter-crossing eight elite lines that include elite and modern varieties known to exhibit high yield potential, good grain quality, and tolerance to a range of biotic and abiotic stresses (Table 1). However, during the first year of the project, two-way crosses were exploited to derive 4-way crosses for which several such 4-way crosses/double crosses were made. The 4-way crosses/double crosses so developed were selected in such a manner that no parent was represented more than once (*e.g. ABCD/CDBA and EFGH/GHEF)* in the 4-way cross. The last stage involved intercrossing of these 4-way crosses to derive 8-way crosses.

Out of 190 individual available from two sets of four-way crosses (*Double cross hybrids build up during Boro 2018 Table 3*), several independent plants were selected and crossed (approximately 1000 floret in total), to obtain 8-way F_1 populations that involved reciprocal cross (*independent crosses assuming each double cross hybrid set plant once as father and once as mother*) combinations i.e.

ABCDEFGH and EFGHABCD (Table 4), respectively symbolized this two sets as *8- wcp set1* and *8-wcp set 2* (Table 4). The eight-way crosses of set 1 (*8-wcp set1*) were further hybridized/intercross with the reciprocal eight-way crosses set 2 (*8-wcp set 2*) for establishing altogether 85 eight-way intercross (Table 5) combinations (ABCDEFGH/EFGHABCD) and then, we render this 1-85 group as BUMpop (Bangabandhu University Mapping Population). We hope to address whether this extra round of inter-crossing increased the levels of recombination enough to enable direct fine-mapping of QTLs using the MAGIC population. The term 'MAGIC group 1–85, as will be used hereinafter, refers to these 85 subpopulations.

Genetic analysis of 6 x 6 diallel population for yield contributing traits in rice

Yield is a polygenic character resulting from the interaction of yield contributing characters influenced by environmental fluctuations. Selection is the important aspect in a crop improvement program, but it is difficult to make improvement through direct selection on the basis of phenotypic performances only. Therefore, parents should be selected or chosen based on their genetic value for any plant breeding program.

Diallel analysis provides an effective means of obtaining rapid information about the genetic features of the homozygous lines. Selection of parental lines in terms of their ability to combine in hybrid combinations and subsequently use them for developing pure lines or hybrid varieties depend on their nature of combining ability. The study of combining ability also offers scope in partitioning the genetic component of characters into additive and non-additive components. The parents with high GCA could be used for developing inbred variety while crosses showing high SCA could be used for developing hybrid variety.

Keeping such views in mind a 6 x 6 diallel population was extracted and genetic analysis was performed by an MS student of the Department of Genetics and Plant Breeding. General Combining Ability (GCA) of the parents and Specific Combining Ability (SCA) of the F_1's were estimated. Moreover, the heterosis of developed cross combinations was assessed inorder to select the promising F_1's. And finally, few prospective general and specific combiners was selected and planted in Aman 2020 season for using in relevant crop improvement program. Having recognition of such lines, the breeders can develop high yielding varieties of rice. These improved varieties of rice can be produced on commercial scale to increase yield in addition with supplying quality seed to farmers at low cost saving foreign exchange. All the data sheet of performed analysis has been attached in the supplementary table (Supplementary Table 1-7) for better exploration of the generated diallel population.

Table 4: Details of the 8-way cross performed during the Aman 2019 and planting plan for Boro season of 2019

8-way cross hybridization	Population Symbol	Base Population		Crossing sheme during Aman 2019			Planting Plan for Boro 2019		
		4-way crosses (Double cross hybrids)	Assumed Genome	No.of floret subjected to crossing (Approx.)	Genome combined	No. of 8-way cross seed recovered	Seeds placed for germination	No. of seedling recovered	Date of Trasplanting
[(Chinnigura × Kataktara) × (Parija × Pokkali)] X [(BR-29 × Bashmati India) X (Double rice × Kalijira)]	8-wcp set 1	(Chinnigura × Kataktara) X (Parija × Pokkali)	ABCD	>550	ABCDEFGH	432	300	227	14.01.2020
		(BR-29 × Bashmati India) X (Double rice × Kalijira)	EFGH						
[(BR-29 × Bashmati India) X (Double rice × Kalijira)] X [(Chinnigura × Kataktara) × (Parija × Pokkali)]	8-wcp set 2	(BR-29 × Bashmati India) X (Double rice × Kalijira)	EFGH	>400	EFGHABCD	178	178	145	
		(Chinnigura × Kataktara) X (Parija × Pokkali)	ABCD						

Table 5: Details of the 8-way intercrosses performed during the Boro season of 2019 and planting plan for Aman 2020

8-way intercross hybridization	Base Population		Crossing sheme during Boro 2019			Planting Plan for Aman 2019		
	8-way cross population	Assumed Genome	No.of plants subjected to crossing	Genome	No. of 8-way intercross seed recovered	Seeds placed for germination	Assume MAGIC population structure	Expected date of Trasplanting
8-wcp set 1 X 8-wcp set 2	8-wcp set 1	ABCDEFGH	> 54	**ABCDEFGH** / EFGHABCD	> 25 seeds/plant	10 seeds per plant	4 random seedling/ plant, thus, altogether 85 x 4= 340 individuals	2nd week of August, 2020
	8-wcp set 2	EFGHABCD						
8-wcp set 2 X 8-wcp set 1	8-wcp set 1	EFGHABCD	> 41	EFGHABCD / **ABCDEFGH**	> 25 seeds/plant			
	8-wcp set 2	ABCDEFGH						

Advancing the BUMpop up to F_4 generation through SSD method

Progeny from each of the eighty-five 8-way intercrosses were grown; ~ 4 offspring per cross as well as were advance by selfing, thereby the population size targeted was 85 × 4 = 340 lines (F_2-like). The F_2 seeds of BUMpop were progressed to the F_4 generation via single seed descent (SSD) method. The selfing procedure started in July 2020, and has completed in May 2020. It will take more two growing season to self the plants to F6:6 recombinant inbred lines (RILs).
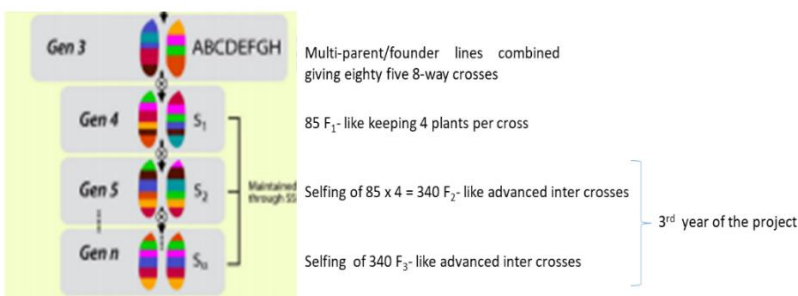

Figure 3: Activities performed at the Aman and Rabi 2021 season of the project

Selection of superior genotypes

Based on the initial performance (e.g. Plant height, panicle length, grain morphology, maturity status, aroma etc.), 23 super promising lines have already been selected as candidate varieties treating that population as SACVpop-1. All of the individual will be brought under preliminary yield trial in the coming Aman-2021 season. A good number of genotypes were possible to pick up as candidate variety.

Combining ability and heterosis study for yield and it`s contributing traits in rice

An experiment of 7 x 7 half diallel mating design was conducted to study the combining ability along with heterosis of 21 F1 hybrids generated through hybridization involving 7 diverse *Indica* rice parents (Supplementary Table 1-6). Additionally, the molecular genetic diversity was surveyed among the seven parental lines with seven RAPD markers (Supplementary Table 7 and Supplementary Figure 1 and 2). The relationship of genetic diversity with hybrid yield of aforesaid population was also assessed to formulate a strategy for marker-based prediction of heterosis. Overall, the hybrids performed better than the parental lines and check for the examined traits and exhibited clear hybrid vigor. Analysis of combining ability revealed significant GCA and SCA variances for all the studied characters. Combining ability analysis revealed that cross combination Double Rice x Pokkali was the best specific combiner for the traits pollen fertility percentage (%), panicle length (cm), filled grain/panicle and grain yield/ hill (g) while Muktagaccha

x BRRI dhan 29 and Basmati x Double Rice were the best specific combiners for grain length (mm) and thousand grain weight (g) respectively. In case of heterosis, Double Rice x Pokkali exhibited maximum heterobeltiosis for panicle length (33.33%) and filled grain/panicle (144.01%). Furthermore, Pokkali x Chinigura was recorded for producing the highest heterobeltiosis for number of tiller/hill (173.17%) as well as grain yield/hill (71.05%). Heat-map relaying on molecular genetic diversity exposed the highest genetic distance between Chinigura and Pokkali followed by Muktagaccha and Pokkali. Unweighted neighbor-joining tree was also clustered the parental lines into three clusters indicating the presence of considerable genetic diversity among the seven parental genotypes. Positive and significant correlation was found between molecular GD with specific combining ability and heterosis for all of the traits under study indicating that the prediction of hybrid performance using RAPD markers with present set of individuals is very high.

CONCLUSION:

The developed population will provide solutions to a range of production constraints particularly stress tolerance, development of variety with several agronomically beneficial traits as well as variety which can adapt to several diverse regions and suitable for diverse climatic conditions. Diverse founder lines had been intercrossed and accordingly a MAGIC population (BUMpop) of F_6 generation has been constructed. Furthermore, twenty-three super promising lines had also been isolated and placed for testing trial to sort out as candidate variety(s).

ACKNOWLEDGEMENT

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