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Developing Mini Core of Rice Germplasm for Submergence Tolerance

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We report here development of a mini-core subset of submergence tolerant rice genotypes containing 21 accessions representing the genetic diversity derived from 5716 accessions collected from flood-prone rice growing areas. The mini-core was developed using PowerCore software on the basis of five quantitative and one qualitative trait. The resultant mini-core had 12.52% of mean difference (MD%), 68.13% of variance difference (VD%), 168.38% of variable rate (VR%), and 98.21% of coincidence rate (CR%) with the core collection, which brought about full coverage of 6 traits. The diversity of mini-core and entire set estimated by Nei index was 0.676 and 0.536 respectively. Cluster analysis was done using UPGMA method. These 21 accessions were classified into four clusters containing 12, 5, 3 and 1 accession in clusters 1, 2, 3 and 4, respectively. In nutshell, the mini-core developed in this study is a representative subset of the rice germplasm collected from different parts of rainfed lowlands of India.

Key Words: Mini-core subset, Power Core, Rice (*Oryza sativa* L.), SAS, Submergence tolerance

Introduction

Genetic resources enable plant breeders to create novel gene combinations and select crop varieties that are more suited to the needs of diverse agricultural systems. Geographically, rice is being grown in lands as far as 50 ^{0}N (Aiwei, China) to 30 ^{0}S (New South Wales, Australia). It is grown at altitudes ranging from below sea level (Kerala, India) to 2761 m above the sea level (Jumulla valley, Nepal), thus making its presence in all the environments and all continents of the world (Chang, 2000). The adaptation to extremely variable conditions in rice offers a hope to combat the current challenges imposed by variable abiotic stresses, as well as means to cope with the adverse effects of climate change, to secure food and livelihood. This is because genes associated with tolerance to various abiotic stresses are probably available within the cultivated gene pool, offering considerable opportunities for genetic improvement. The approach involving identification of tolerant germplasm and associated QTLs /genes has paid rich dividends in developing stress-tolerant high-yielding cultivars for commercial utilization (Xu *et al*., 2006; Sarkar *et al*., 2009; Singh *et al*., 2009; Septiningsih *et al*., 2009). Since the introduction over fifty years ago, the adoption of high-yielding semi-dwarf rice cultivars in unfavourable lowlands and coastal areas has not been successful. Apparently, adoption of these improved rice varieties in favourable irrigated areas could make significant impact on yield improvement, but with limited success in unfavourable ecosystems, as in coastal belt of water logging and submergence prone areas. Developing rice varieties with wider adaptation and broader tolerance to prevailing stresses would be more viable for these areas, where abiotic stresses are variable and complex, and growing conditions are too risky to persuade farmers for investment in inputs (Singh *et al*., 2010). Insertion of genes from alien species could also improve the tolerance to stresses, however, identification of specific genotypes within the cultivated gene pool and their utilization in breeding through conventional or biotechnological approaches still remain the most trusted and successful approaches to date.

A wealth of plant germplasm is accessible worldwide, with about 6 million accessions held in over 1400 gene banks. Yet, these collections are barely tapped (<1%) by breeders, owing to the scarcity of information on their characterization, other than the taxonomic status and geographical origin (Glaszmann *et al*., 2010). Together more than one lakh germplasm lines of rice are available in the National and International rice gene banks, of which more than 30,000 lines are available at the ICAR-National Rice Research Institute, Cuttack. The vast germplasm collections are accessible, but their use in crop improvement programme is limited, efficiently accessing genetic diversity is still a challenge. The complete and high-quality sequence of the rice

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genome has thrown light on population dynamics and the impact of selection during domestication. Germplasm characterization has now gained analytical power for resolving the genetic basis of trait variation, diversity patterns, and their adaptations in a definite or variable environment. Although many rice germplasm accessions have been collected and conserved in the Indian gene bank, the utilization, characterization and management of these resources have not kept pace due to the recurring problem of duplication among accessions, which results in a high rate of genetic redundancy. To overcome this problem, Frankel (1984) suggested sampling of the collection to a manageable sample or 'core collection'. A core collection contains a subset of accessions from large collections that captures most of available diversity of species (Brown, 1989a; 1989b). Core sets are derived from the wide spectrum of genetic diversity of the whole collection, most of this diversity is expected to be retained. Extensive evaluation of the core set leads us to guide more efficient utilization of the entire collection (Brown, 1989b). Core sets have been made for different crops including rice. However, to make a core set taking germplasm based on their sensitivity of a specific abiotic stress is hitherto scanty. Therefore, the objective of this study was to develop a core set of rice germplasm accessions sensitive to submergence using data on both qualitative and quantitative traits.

Materials and Methods

Collection of Rice Germplasm

In almost all the countries of the South and Southeast Asia, rice productivity in rainfed lowlands is generally low compared to the favourable ecosystems (Sarkar *et al*., 2009). Farmers predominantly grow local tall, photoperiod sensitive rice genotypes in medium-deep and deepwater sub-ecosystems. High yielding semi-dwarf cultivars are rarely grown in these areas.

Multiple surveys and germplasm collection activities were undertaken at different years along the coastal and rainfed areas of the India that are prone to salt stress and flooding. The areas such as West Bengal, Bihar, Odisha and Assam are frequently encountered by excess water stress from the mighty rivers, the Ganges, Koshi and the Bramhaputra respectively. Thus in the present study, all these old collections conserved in the rice gene bank were used for screening experiment. Besides, some fixed breeding materials developed by International Rice Research Institute (IRRI), Philippines and the SAUs located in eastern India were also included.

Screening for Submergence

As we know that quality of floodwater influences the survival of plants, hence to screen the cultivars we focused on the susceptible cultivars and depending on the quality of floodwater, an assumption was made so that mortality of the susceptible check scores nearer to 100%. When the floodwater was clear, 8 days after complete submergence, we checked the susceptible cultivars. Extreme yellowing of leaves and softening of base was a harbinger of plant death and on that basis we took decision about the total days of submergence. Under clear water, in general, we gave submergence stress for 10-15 days depending upon the condition of susceptible check.

Plants were established using direct seeding in a field tank (40 m × 8 m × 0.8 m) in lines that were 20 cm apart and with 15 cm between hills. Chemical fertilizers were added as N:P:K at 0:30:30 kg/ha, respectively. Twenty-one-day old seedlings were completely submerged under 70-80 cm of water for 10-15 days. Plant height was taken before and after submergence to determine the elongation ability to identify which accession is suitable for flash flood versus stagnant water conditions. Finally, the number of survivors was counted after 10 days of de-submergence and percentage survival was determined as:

Survival (%) = (Number of hills after 10 days of de-submergence / number of hills before submergence) × 100 (Sarkar and Bhattacharjee, 2011).

Data Collection and Trait Evaluation

Data of six phenotypic traits were collected for all the 5716 rice genotypes. Out of these, five were quantitative and one was qualitative trait. The phenotypic traits include plant height before submergence, plant height after submergence, elongation, elongation %, survival % and submergence-type. The plant height before submergence and after submergence was recorded maintaining twenty-one-day old seedlings which were completely submerged under 70-80 cm of water for 10-15 days. Elongation was calculated as the difference between plant height after submergence and plant height before submergence. Submergence-type was divided into four categories: tolerant, medium tolerant, avoiding and susceptible. Survival due to complete submergence

depends on either quiescence or escape strategies (Sarkar and Bhattacharjee, 2011). Therefore, the plant which avoids the complete submergence for survival through greater elongation was termed as 'avoiding type' whereas the tolerant type restricts the elongation, remains inside the water and still survives under such situation. Survival percentage of 80 and above was considered as 'tolerant type' whereas above 50% to below 80% was considered as 'moderately tolerant'. Elongation and elongation % were calculated as follows:

Elongation = Plant height after submergence - Plant height before submergence

Elongation% = (Elongation / Plant height before submergence) × 100 (Sarkar and Bhattacharjee, 2011).

Sampling Strategy and Data Analysis

Sampling the core collection was performed by the PowerCore software described by Kim *et al*. (2007). These variables were automatically classified into different categories or classes by the PowerCore programme based on Sturges' rule = 1 + Log2(*n*), where *n* is the number of observed accessions (Kim *et al*., 2007). Five genotypes namely FR 13A, Sabita, IR 42, Swarna and Swarna-Sub1 were preferentially included in the mini-core without validation using PowerCore because these are the check varieties.

The frequency distribution between entire set and core set for all the six traits were evaluated by the χ^2 test. The resulting mini-core was compared with the original core collection to assess its homogeneity. Nei genetic diversity index (Nei, 1972) was estimated for both the core and mini-core collections. Homogeneity was evaluated for the six phenotypic traits using the Newman-Keuls test for means, the Levene test (Levene, 1960) for variances, and the mean difference (MD%), variance difference (VD%), coincidence rate of range (CR%), and variable rate of coefficient of variance (VR%) according to Hu *et al*. (2000).

$$MD(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{|M_e - M_c|}{M_c} \times 100$$

where M_e is the mean of entire collection and M_c is the mean of core collection.

$$VD(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{|V_e - V_c|}{V_c} \times 100$$

where V_e is the variance of entire collection and V_c is the variance of core collection.

$$CR(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{R_c}{R_e} \times 100$$

where R_e is the range of entire collection and R_c is the range of core collection.

$$VR(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{CV_c}{CV_e} \times 100$$

where CV_e is the coefficient of variation of entire collection and CV_c is the coefficient of variation of core collection.

Coverage of all the phenotypic traits in the original core collection was estimated in the mini-core as proposed by Kim *et al*. (2007):

$$Coverage(\%) = \frac{1}{m}\sum_{j=1}^{m}\frac{D_c}{D_e} \times 100$$

where *D*c is the number of classes occupied in the minicore subset, *D*e is the number of classes occupied in the original core collection for each trait, and *m* is the number of traits, which is 6 in this case.

The cluster analysis was performed over the submergence traits using the un-weighted pair group method of arithmetic means (UPGMA) method.

Results

A total of 5716 accessions of rice germplasm were tested for submergence tolerance. Based on 6 traits, the heuristic search identifies 21 accessions as core set (Table 1).

Frequency Distribution of Submergence Traits

The frequency distribution for all the submergence traits between entire set and core set were tested using χ^2 tests. The χ^2 probability for distribution were non significant for PH(BS), PH(AS), elongation, elongation (%), and survival (%) but significant for submergence-type (Table 2) between entire set and core set. This indicates representative similarity between entire set and core set except for submergence type trait. The graphical distribution analyses of entire set for five quantitative traits were shown in Figure 1 (a-e).

Table 1. The minicore subset of 21 accessions along with their name and six traits developed using PowerCore software

Name	Source	PH (BS)	PH (AS)	Elongation	E (%)	Survival (%)	Submergence type
FR 13A	Odisha	49	65	16	32	93	Tolerant
Sabita	West Bengal	45	106.2	61.2	136	81	Avoiding
IR 42	IRRI, Philippines	38	72	34	89	0	Susceptible
SWARNA	Maruteru, Andhra Pradesh	33	68	35	106	20	Susceptible
SWARNA-SUB1	IRRI, Philippines	30	43	13	39	70	Medium Tolerant
AC-42238 (Kalam)	West Bengal	14	56	42	300	62	Medium Tolerant
AC-1786	Odisha	16	95	79	494	84	Avoiding
AC-42107 (Mugakatia); IC 575291	Odisha	25	84	59	236	16	Susceptible
Baikoili	Odisha	65	117	52	80	95	Avoiding
AC-975	Odisha	19	102	83	437	41	Susceptible
IR 84645-2-11-71-B	IRRI, Philippines	56	56	0	0	50	Susceptible
AC-42108 (Kankada Bichha); IC 575292	Odisha	19	87	68	358	34	Susceptible
Ranjei	Odisha	67	122	55	83	80	Avoiding
IRGC - 45059	IRRI, Philippines	47	75	28	60	100	Tolerant
RAU - 1415-12-7-6-4-3-8	Bihar	5	58	53	1060	7	Susceptible
IR64-SUB1	IRRI, Philippines	25	33	9	36	78	Medium Tolerant
AC-42268 (Bajal), IC 57544, JRS-215	West Bengal	34	126	92	271	65	Avoiding
AC-42103 (Gitanjali),IC 575287	West Bengal	37	109	72	195	118	Avoiding
AC-653	Odisha	12	82	70	583	53	Susceptible
AC-37983, Badshabhog	Chhattisgarh	32	0	0	0	25	Susceptible
AC-1303B	Odisha	76	101	25	33	65	Avoiding

PH - Plant Height, BS - Before Submergence, AS - After Submergence, E - Elongation,
IRRI - International Rice Research Institute

Table 2. Comparison of frequency distribution for 6 traits between entire set and core set of rice

Traits	Df	$\chi 2$ value	Probability value
PH(BS)	1	0.6350	0.4255
PH(AS)	1	0.0636	0.8009
Elongation	1	0.1303	0.7181
Elongation (%)	1	0.9666	0.3255
Survival (%)	1	3.4994	0.0614
Submergence type	1	4.1188	0.0424

Df - Degrees of freedom

Comparison of Mean and Variance

Comparative values for the ranges, means, and variances of 6 phenotypic traits among the entire set and mini-core subset are presented in Table 3 and demonstrate that the mini-core subset covers the range of variation for each trait. The equality of mean and homogeneity of variance between entire set and mini-core was analyzed using SAS 9.2 software. The Newman-Keuls test results indicate the presence of homogeneity of means between the core collection and mini-core subset for 2 (33%) traits (PHAS and elongation) out of the 6 traits analyzed. Two (33%) traits (survival % and submergence-type) had homogeneous variances between the two collections as revealed by the Levene's test.

The MD%, the VD%, the CR%, and the VR% are designed to comparably evaluate the property of core collection with its initial collection. Over the entire 6 phenotypic traits, the MD% was 12.52%, far less than the significance level of 20% (Hu *et al*., 2000) between the entire set of 5716 and the mini-core set of 21 accessions selected by the PowerCore search. The VD% was 68.13%, far more than the significance level of 20% between the two collections, as four out of six traits are significant variance between entire set and the mini-core set (Table 3). The VR% compares the coefficient of variation values of the 6 phenotypic traits measured in the core collection with the mini-core subset in general and determines how well the variance is being represented in the mini-core. More than 100% of VR% is required for a core collection to be representative of its original collection (Hu *et al*., 2000). The mini-core had 168.38 VR% over its originating core, which is much more than the required VR%, indicating good

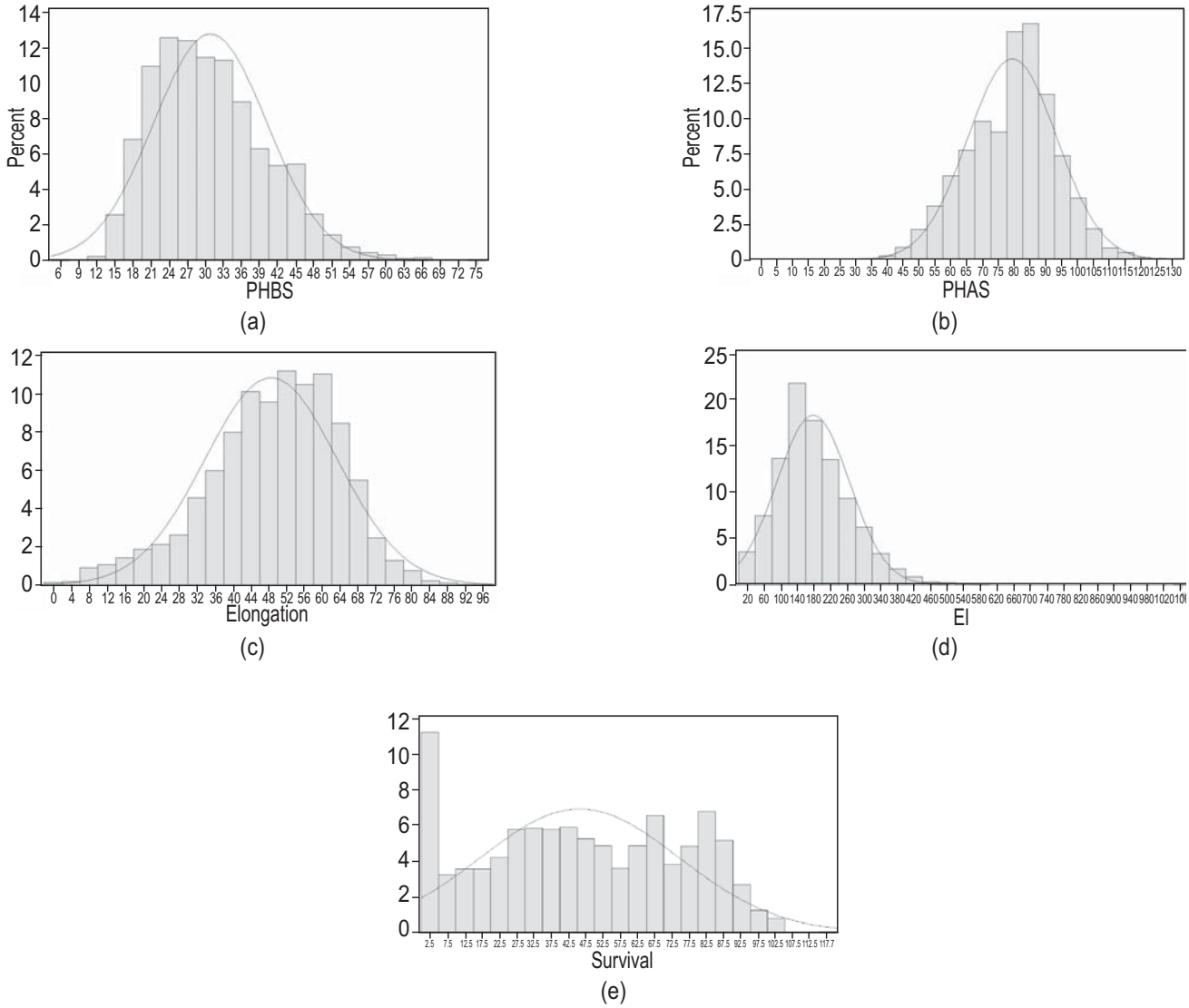

Fig. 1. Distribution analysis of five traits (a) Plant height before submergence (b) Plant height after submergence (c) Elongation (d) Elongation % and (e) Survival %

Table 3. Comparison of range, mean, and variance between the entire set of rice (*Oryza sativa*) collection and the mini-core for 6 phenotypic traits

Traits	Entire set			Core set			Tests	
	Range	Mean	Variance	Range	Mean	Variance	N-K	Lev
PH(BS)	5-75.68	30.82	88.45	5-76	35.38	372.44	**	*
PH(AS)	0-131	79.31	197.11	0-126	78.91	999.18	NS	*
Elongation	0-97	48.54	217.18	0-92	45.05	774.53	NS	*
Elongation (%)	0-1060	178.42	7587.29	0-1060	220.33	66165.97	**	*
Survival (%)	0-118	45.75	823.05	0-118	58.94	1074.29	**	NS
Submergence type	1-4	1.51	0.52	1-4	1.90	0.99	**	NS

PH - Plant Height, BS - Before Submergence, AS - After Submergence
*- significant at 1%, NS - Non significant, ** - significant at 5%

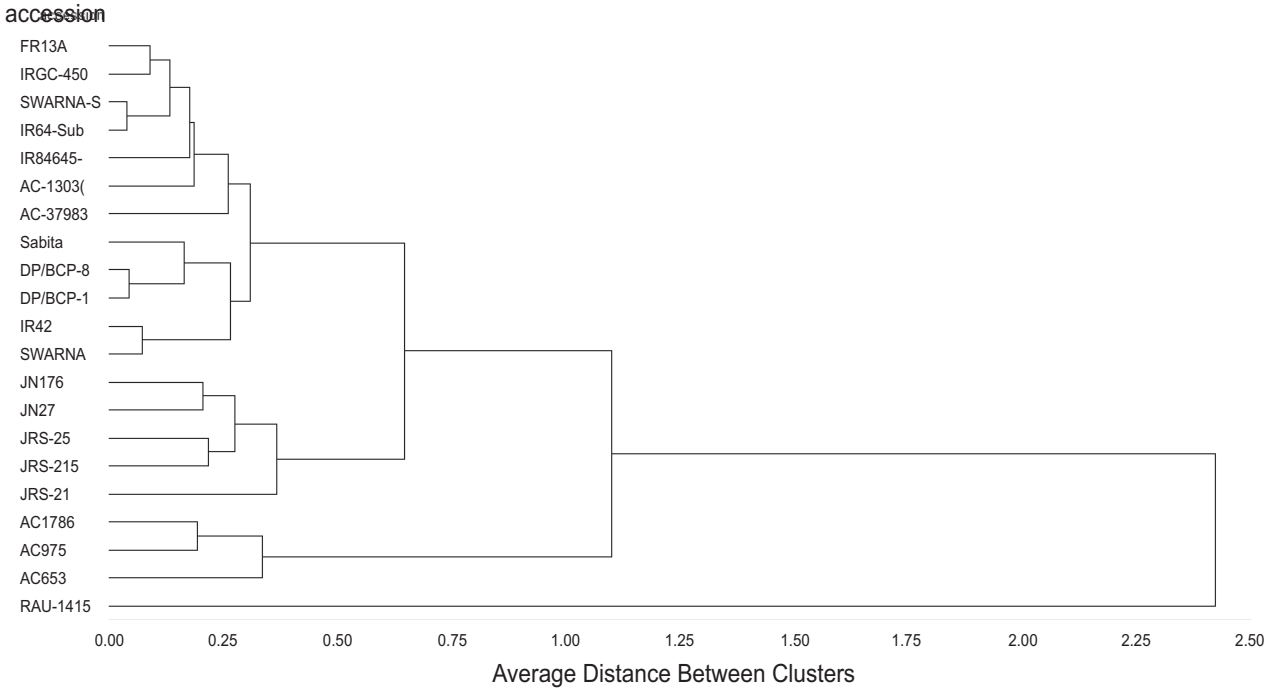

Fig. 2. Dendrogram of 21 selected accessions using UPGMA method

representation. The coincidence rate indicates whether the distribution ranges of each trait in the mini-core subset are well represented when compared to the core collection. The resulting CR% over the 6 traits was 98.21%, indicating homogeneous distribution of the phenotypic traits because it was larger than 80% (Kim *et al*., 2007). The calculated coverage value for the resulting mini-core was 100%, suggesting there is full coverage of all the diversity present in each class of phenotypic traits in the entire set of rice collection.

Cluster Analysis

A cluster analysis was performed using UPGMA method resulted into four clusters. Twelve accessions (FR 13A, IRGC-45059, Swarna-Sub 1, IR64-Sub1, IR 84645-2-11-71-B, AC-1303B, AC-37983, Sabita, Baikoili, Ranjei, IR 42 and Swarna) were grouped together in Cluster 1. Cluster 2 contains five accessions (Kalam, Kankada Bichha, Mugakatia, Bajal and Gitanjali). Three accessions (AC-1786, AC-975 and AC-653) grouped to form cluster 3 and cluster 4 contains only single accession RAU - 1415-12-7-6-4-3-8 (Figure 2).

Discussion

Phenotypic Property of the Mini-core Set

We developed a mini-core subset containing 21 entries of its parental population using a strategy based on heuristic searches. The MD% between the mini-core and core collections for 6 phenotypic traits (12.53%) was higher than the core collections developed by Kim *et al*. (2007), but it is within significance level of 20%. The VD% of the mini-core (68.13%) was higher than that of the reported studies for each of 10 test runs in rice (Kim *et al*., 2007) and 9 test runs in cotton (*Gossypium hirsutum* L.) (Hu *et al*., 2000). The VR% of the mini-core (187.38%) was larger than the test runs in Kim *et al*. (2007) and Hu *et al*. (2000), and the CR% of traits in the mini-core (99.21%) was larger than nine out of 10 test runs in Kim *et al*. (2007) and six of nine test runs in Hu et al. (2000). Core collections with low MD% and VD% and large VR% and CR% are considered to provide a good representation of the genetic diversity of the initial collection (Hu *et al*., 2000; Kim *et al*., 2007). Furthermore, the presented mini-core displayed 100% coverage for 6 phenotypic traits, demonstrating that the PowerCore software is effective in maintaining the diversity present in each class of the traits in the entire set of rice collection. In contrast, Brown (1989a; b) used several statistical models to suggest that at least 70% of the variation in an entire collection could be represented in a core composed of at least 10% of accessions. Therefore, this mini-core should be considered as a sound representation of rice genetic diversity found in the entire set of rice collection.

The diversity estimated by the Nei index (Nei, 1972) of the mini-core subset (0.676) was greater than the original core collection (0.536), as would be expected when genetically similar accessions were removed from the original core. The diversity existing in the mini-core is larger than that in rice landrace populations in Yunnan, China (Zeng *et al*., 2007), Indonesian rice populations (Thomson *et al*., 2007).

Core collections are subsets of the main collections, comprising selective accessions that represent most of the genetic variability contained in the entire collection. The main collection can be stratified into groups (subsets) sharing common characteristics according to taxonomy, geographic or ecological origin, and descriptors followed by sampling within these groups. A core subset provides in a way an entry point to the entire collection for accelerating the utilization of germplasm and should not be looked upon as a substitute of the latter. By maximizing the diversity studied in a reduced number of individuals through the use of core collections, the probability of identifying variants of interest for association studies involving complex traits is increased. Furthermore, the knowledge gained through the core collections allows the choice of optimal crosses for generating quantitative trait loci (QTL) mapping populations.

Cluster Analysis

The mini-core of 21 accession includes 9 susceptible, 7 avoiding, 3 medium tolerant and 2 tolerant accessions. Out of these, cluster 1 contains 2 tolerant and medium tolerant each; 4 avoiding and susceptible each. Cluster 2 includes 2 avoiding and susceptible each and 1 medium tolerant accession. Cluster 3 contains 2 susceptible and 1 avoiding accessions; and cluster 1 includes 1 susceptible accession. Thus, cluster 1 contains 100% of the tolerant, 57% avoiding, 45% susceptible and 67% medium tolerant accessions. Cluster 2 includes 29% avoiding, 22% susceptible and 33% medium tolerant accessions. 14% avoiding and 22% susceptible accessions involved in Cluster 3; and cluster 4 contains 11% accessions of the mini-core set.

The mini-core contains accessions from IRRI, Philippines and five states Odisha, West Bengal, Bihar, Andhra Pradesh, Chhattisgarh of India. These contribute 23%, 43%, 19%, 5%, 5% and 5% from IRRI, Odisha, West Bengal, Bihar, Andhra Pradesh and Chhattisgarh respectively. Cluster 1 includes 80%, 26%, 25% 100% and 100% accessions form IRRI, Odisha, West Bengal, Andhra Pradesh and Chhattisgarh respectively. Cluster 2 contains 22% Odisha and 75% West Bengal accessions; cluster 3 includes 20% IRRI and 22% Odisha accessions while cluster 4 contains 100% accession from Bihar.

Conclusion

The rice germplasm collection of **N**RRI consists of more than 5716 accessions, which were collected from different regions of the country and during different time periods. This study was aimed to establish a smaller set based on collections made during different time period. Enough phenotypic data is not available on all the 5716 accessions for use to construct core/mini core at one go. Hence, the only strategy is to develop a small set/mini core set for phenotypic evaluation which will be the representation of the entire 5716 lines. Five genotypes FR 13A, Sabita, IR 42, Swarna and Swarna-Sub1 were the check varieties so preferentially included in the mini-core. In conclusion, the mini-core of 21 accessions presented in this study is a good representative subset of the submergence tolerance rice core collection of 5716 accessions.

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