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Development and evaluation of iso-cytoplasmic rice restorer lines for different agro-morphological traits

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Abstract

A set of 390 iso-cytoplasmic restorer lines were developed from 25 popular rice hybrids grown in India through pedigree breeding exercising selection for spikelet fertility, yield and extent of panicle exsertion were evaluated for various agro-morphological traits. Correlation analysis showed that yield per plant was significantly correlated with number of tillers (0.208), panicle length (0.127) and spikelet fertility (0.134). Principal component analysis indicated that the first four principal components had eigenvalue of > 1.0 and cumulatively explained 74.3% of total variance. Grain characters namely, test weight (0.451), kernel length before cooking (0.418), kernel breadth before cooking (0.136) and spikelet fertility (0.402) contributed positively towards principal component I. Yield per plant showed a positively skewed distribution with panicle length and a nearly linear relationship with spikelet fertility. A core set of 21 genotypes capturing the entire range of phenotype for different traits, was identified from 390 genotypes using advanced M (maximization) strategy and statistically validated. Besides this, promising iso-cytoplasmic restorer lines originating from different hybrids were identified for various agro-morphological traits. This is the first report on developing iso-cytoplasmic restorers from popular rice hybrids, which can be used in developing heterotic hybrids when crossed with diverse non-parental CMS lines.

Key words: Agro-morphological traits, evaluation, heterosis, hybrids, iso-cytoplasmic restorers, rice

Introduction

Rice (*Oryza sativa* L.) is the staple food crop of more than fifty percent of the world's population (Khush 2005). In Asia alone, it is consumed by more than three billion people accounts for 30-50% of agricultural production and 35-75% of the calories (Khush 2005; Hossain and Fischer 1995). Green revolution enabled a quantum jump in rice production in India during the mid-1960s. However, since then, the yield levels have plateaued (Mann 1999). With the area under rice cultivation in India remaining constant during the last few decades (42.2 mha in 1989-90 to 43.86 mha in 2014-15), improving yield in rice is a high priority in order to cater the increasing demand for food and achieve food security (Anonymous, 2015). Additionally, the yield improvement has to address the constraint imposed by changing climate namely, limited water availability, less labour and other resources.

Hybrid rice technology provides 15-20% higher grain yield than the best semi-dwarf varieties (Virmani et al. 2003), thereby bridge the yield gap and meet the challenge of increasing rice production while sustaining the natural resource base Pattnaik et al. (2016). Although, India adopted hybrid rice technology in early 90s, the present area under hybrid rice is only 2.7 mha, which is not sufficient to make an impact on boosting rice production.

Majority of the rice hybrids produced across the world and all the hybrids in India is based on cytoplasmic genetic male sterility (CGMS) system (Virmani et al. 1997). It is a three-line system that involves combination of three parental sources namely, a cytoplasmic male sterile (CMS) line (A Line), a maintainer line (B line) and a restorer line (R line) (Gopala Krishnan et al. 2012; 2013). Hybrid breeding based on CGMS system in developing hybrids in crops is possible, only when effective restorer lines are available (Nematzadeh et al. 2003).

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One of the limiting factors in hybrid rice breeding is the availability of desirable parental lines, as the perfect maintainers and restorers are lower in frequency among the elite breeding lines. Therefore, targeted breeding for improvement of parental lines is an integral in hybrid rice breeding so as to improve breeding efficiency as well as to develop heterotic hybrids (Kumar et al. 2017). To meet the expanding horizons of hybrid rice improvement, it is essential to assemble, evaluate, improve and conserve the parental lines (Sabar and Akthar 2002).

Development of iso-cytoplasmic restorer lines from elite rice hybrids through a process of generation advancement followed by selection is a novel approach for development of new restorers. The iso-cytoplasmic restorer lines possess the same male sterile cytoplasm, which could minimize the potential conflict due to interaction between the cytoplasmic and nuclear genes (Kumar et al. 2017). Therefore, the present study was carried out with the objective of developing iso-cytoplasmic restorer lines from a set of 25 different popular rice hybrids grown in India, and their evaluation thereof to identify promising iso-cytoplasmic rice restorers which can further be utilized in development of improved rice hybrids.

Materials and methods

Experimental materials

The present investigation was began at the experimental farm in *kharif* 2010 at the Division of Genetics, ICAR-IARI, New Delhi. In the first step, pure seeds of twenty-five elite rice hybrids were grown and F_2 seeds were harvested on single plant basis. These F_2s and subsequent generation were grown in the field and subjected to generation advancement coupled with selection for yield per plant, spikelet fertility and panicle exsertion. During *kharif* 2013, a set of 390 iso-cytoplasmic restorer lines (suffixed with PIRL to represent Pusa Iso-cytoplasmic Restorer Line) were generated from 25 hybrids, which were grown in augmented design with 4 blocks and 8 checks. The checks consist of 4 maintainer lines namely, IR 79156B, IR 58025B, RTN 12B, Pusa 6B and 4 restorer lines namely, RPHR 1005R, PRR 78, Pusa 1609 and DRR 714. The crop was grown following recommended agronomic practices and plant protection measures to ensure proper crop growth. Data were recorded for various agro-morphological traits on five randomly selected plants for all the restorer lines. Observations were recorded for tiller number per plant, plant height (cm), panicle length (cm), number of spikelets per panicle, spikelet fertility percentage, days to 50% flowering and grain yield per plant (g). Parameters regarding grain quality *viz*., kernel length before cooking (KLBC), kernel breadth before cooking (KBBC) and their ratios were also recorded using E-Vision, Annadarpan (CDAC, Kolkata).

Data analysis

The relative contribution of 25 hybrids towards iso-cytoplasmic restorer line development was estimated. For each of the groups, iso-cytoplasmic restorer lines were compared separately. SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) was used for comparison of means, correlation, regression, cluster analysis, and principal component analysis. Core set was formed using advanced M (maximization) strategy and validated using PowerCore software (Kim et al. 2007).

Results

A set of 390 iso-cytoplasmic restorer lines were developed from 25 popular rice hybrids released in India (Fig. 1) and evaluated for various agro-morphological characters. Comparison of different groups of iso-cytoplasmic restorer lines were performed based on the hybrids from which they were derived. The highest proportion of iso-cytoplasmic restorer lines among the public sector hybrids was from JRH 8 (22), followed by Indira Sona (19) and DRRH 2 (18) (Table 1). As far as the iso-cytoplasmic restorer lines derived from private sector hybrids are concerned, PA 6129 (45), Suruchi 5401 (29) and Indam 200-017 (23) were the highest contributors.

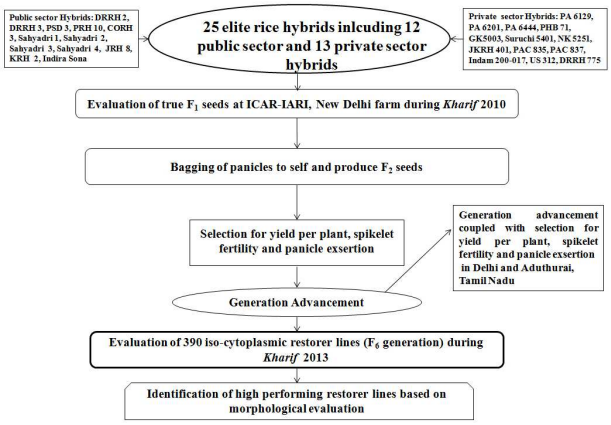

Fig. 1. A schematic diagram showing development of iso-cytoplasmic restorers from popular rice hybrids of India

Table 1. Number of selected iso-cytoplasmic restorer lines from each group of popular rice hybrids of India

Hybrids	No. of derived lines	Hybrids	No of derived lines
Public sector hybrids		**Private sector Hybrids**	
DRRH 2	18	PA 6129	45
DRRH 3	4	PA 6201	10
Pant Shankar Dhan 3	5	PA 6444	22
PRH 10	9	PHB 71	23
CORH 3	8	GK5003	22
SAHYADRI 1	11	SURUCHI 5401	29
SAHYADRI 2	14	NK 5251	14
SAHYADRI 3	7	JKRH 401	9
SAHYADRI 4	16	PAC 835	9
JRH 8	22	PAC 837	14
KRH 2	8	INDAM 200-017	27
INDIRA SONA	19	US 312	13
		DRRH 775	12
Total	141		249

Mean performance

The average performance of all the iso-cytoplasmic restorer lines derived from 25 hybrids was used to compare the superiority of a group. Iso-cytoplasmic restorer lines with better performance over other lines of same group were also given due importance. The mean performances of the iso-cytoplasmic restorer lines are presented in Supplementary Table S1.

For days to 50% flowering, the iso-cytoplasmic restorer lines derived from (Supplementary Table S1; http://isgpb.org) Pant Shankar Dhan 3 was earliest (84). For effective restoration, restorers having synchronized flowering duration as male sterile lines are required. Thus, lines with ~100 days to 50% flowering are most desirable. The iso-cytoplasmic restorer lines derived from PA 6444(102) followed by KRH 2 (102) and Sahaydri 1 (102) were found to be desirable in this regard. With respect to plant height, restorer lines with plant height more than male sterile lines are desirable. As the plant height of popular male sterile lines namely, IR 79156A, IR 58025A, RTN 12A, Pusa 6A and IR 68888A are less than 100 cm, restorer lines of plant height around 100-105 cm are highly desirable. Lines derived from PRH 10 (102.53), Indira Sona (103.52) and Sahyadri 1 (104.67) were found to possess the desired plant height. The iso-cytoplasmic restorers from US 312 (21.6; PIRL-183), PA 6444 (17.60; PIRL-377) and US 312 (13.8; PIRL-186) were found to produce highest number of productive tillers per plant. For panicle length, lines from NK 5251 (32.82 cm; PIRL-191) had shown highest panicle length followed by Indam 200-017 (31.92; PIRL-235), PAC 835 (31.70 cm; PIRL-529), PRH 10 (31.64 cm; PIRL-44) and DRRH 2 (31.32 cm; PIRL-22). *Per se* yield is the most important trait for comparing the performance of restorer lines as it reflects the performance of all component traits. Restorer lines derived from PAC 837 (13.76 g), JRH 8 (11.28 g) and PA 6129 (11.60 g) were found to possess higher overall mean yield. Several lines were selected with high performance from high performing groups likewise JRH 8 (21.5 g; PIRL-493), PA 6129 [(20.40 g; PIRL-311), (20.34 g; PIRL-310)] and PHB 71 (19.66 g; PIRL-380). As iso-

Table 2. A comparison of Pearson's correlation coefficient calculated for different morphological traits

	PH	NT	PL	YPP	TW	KLBC	KBBC	L/B	SF
DFF	0.449**	0.016	0.151**	0.040	–0.279**	–0.282**	–0.068	–0.129*	–0.385**
PH		0.019	0.375**	0.053	–0.233**	–0.202**	0.002	–0.142**	–0.313**
NT			–0.068	0.208**	–0.262**	–0.163**	–0.150**	0.024	0.036
PL				0.127*	–0.031	0.088	–0.314**	0.303**	–0.206**
YPP					0.153**	0.087	0.250**	–0.149**	0.134*
TW						0.488**	0.511**	–0.101	0.301**
KLBC							0.063	0.569**	0.185**
KBBC								–0.778**	0.096
L/B									0.065

Where, DFF = days to 50% flowering, PH = plant height, NT = number of tillers number per plant, PL = panicle length, YPP = yield per plant, TW = test weight, KLBC = kernel length before cooking, KBBC = kernel breadth before cooking, L/B= kernel length before cooking/ kernel breadth before cooking

cytoplasmic restorer lines are derived from rice hybrids by continued selfing and selection, they all possess the sterile cytoplasm from female line (CMS). Wild abortive cytoplasm (WA) has significant effect on the panicle exsertion; it is observed that lines carrying WA cytoplasm show incomplete panicle exsertion (Gangashetti et al. 2004). Thus, variation in range of panicle exsertion observed in each generation and through exercising appropriate selection pressure on the level of panicle exsertion in segregating generations, there is an opportunity for rectifying/ improving panicle exsertion in the hybrids as well as in restorer lines. Spikelet fertility is another important trait which would help in harvesting better yield. Restorer lines from PAC 837 (88.49%: PIRL-538), PRH 10 (85.18%; PIRL-43), Pant Shankar Dhan PC3 (83.33%; PIRL-32) and PA 6129 (80.95%; PIRL-287) have shown highest spikelet fertility as compared to lines of other groups.

Correlation among different traits

Correlation analysis is used to determine the predictive relationship that can be exploited in selection of desirable genotypes. Simultaneous selection for traits contributing to a desirable character can help in maximizing selection gains for that trait in the subsequent segregating population (Norain et al. 2014). Estimates of correlation coefficients of traits (Table 2) indicated that yield per plant have highest positive correlation with number of tillers (0.208), panicle length (0.127) and spikelet fertility (0.134), which is corroborated by earlier reports in rice (Sarkar 2006; Kole et al. 2008; Sharifi et al. 2013). Kernel length before cooking has negative association with days to 50% flowering, plant height and number of productive tillers per plant. Kernel breadth before cooking has also shown highest positive correlation with yield per plant (0.250). Therefore, selection of kernel breadth before cooking can help enhance the mean yield performance and *vice-versa*. Such associations such as yield with panicle length (Naik et al. 2005) and kernel breadth (Reddy et al. 1997) have been reported in rice. Significant negative correlation was observed for spikelet fertility with days to fifty percent flowering (–0.385), plant height (–0.313) and panicle length (–0.206). Test weight has also shown significant negative correlation with number of tillers (–0.262), plant height (–0.233) and significant positive correlation with kernel length before cooking (0.488), kernel breadth before cooking (0.511) and yield per plant (0.153). Therefore, grain yield can be improved through improvement of traits that show positive and significant association with grain yield.

Principal component analysis

Principal component analysis (PCA) is a tool for describing the genetic variation present among genotypes and distinguishing selected genotypes based on similarities in one or more traits (Ariyo 1987). First four principal components with Eigen value of > 1.0 explained the cumulative variance of 74.3%. PCA of quantitative traits found that, the first principal component accounted for 24.6% of the total variability, wherein grain related characters namely, test weight (0.451), KLBC (0.418), KBBC (0.136) and spikelet fertility (0.402) were contributing positively (Table 3).

Table 3. Principal component analysis for various agro morphological traits

	PC1	PC2	PC3	PC4	PC5
DFF	–0.456	–0.050	0.197	–0.028	0.214
PH	–0.406	–0.076	0.412	–0.027	–0.007
NT	–0.121	0.024	–0.200	0.714	0.367
PL	–0.151	0.317	0.535	0.052	–0.455
YPP	0.056	–0.165	0.375	0.632	–0.089
TW	0.451	–0.164	0.396	–0.095	0.059
KLBC	0.418	0.268	0.325	–0.045	0.508
KBBC	0.166	–0.594	0.195	–0.082	0.192
L/B	0.136	0.639	0.039	0.045	0.143
SF (%)	0.402	–0.051	-0.149	0.259	–0.534
Loadings					
Eigenvalue	2.455	2.219	1.463	1.290	0.713
Difference	0.236	0.756	0.173	0.577	0.077
Proportion	0.246	0.222	0.146	0.129	0.071
Cumulative	0.246	0.467	0.614	0.743	0.814

Where, DFF= days to 50% flowering, PH= plant height, NT= number of tillers per plant, PL= panicle length, YPP= yield per plant, TW=test weight, KLBC= kernel length before cooking, KBBC= kernel breadth before cooking, L/B= kernel length before cooking/ kernel breadth before cooking, SF (%) = spikelet fertility percentage

Second and third principal component, explained 22.2% and 14.6% variation, respectively. Trait eigenvalue in every PC indicates its relative contribution of particular trait to the same. Traits like panicle length (0.317), L/B Vision ratio (0.639) have shown more contributions towards PCII. Plant height (0.412), panicle length (0.535) and yield per plant (0.375) for PCIII.

Regression analysis

Relationship between a dependent variable and one or more independent variables is essential to explain the conditional expectation of complex trait like yield. Crop yield prediction model is calculated with the use of linear regression technique, where the predictant is yield per plant and there are seven predictors namely days to fifty percent flowering, plant height, number of productive tillers, panicle length, test weight and spikelet fertility (Supplementary Table S2; http://isgpb.org). The regression coefficient of yield on number of effective tillers per plant (0.745) followed by panicle length (0.292) and test weight (0.199) were highly significant and positive with a probability value of < 0.01. Yield per plant was plotted against spikelet fertility (%) and number of tillers. Positively skewed distribution was observed between panicle length and yield per plant. A nearly linear relationship was observed between spikelet fertility and yield per plant (Fig. 2).

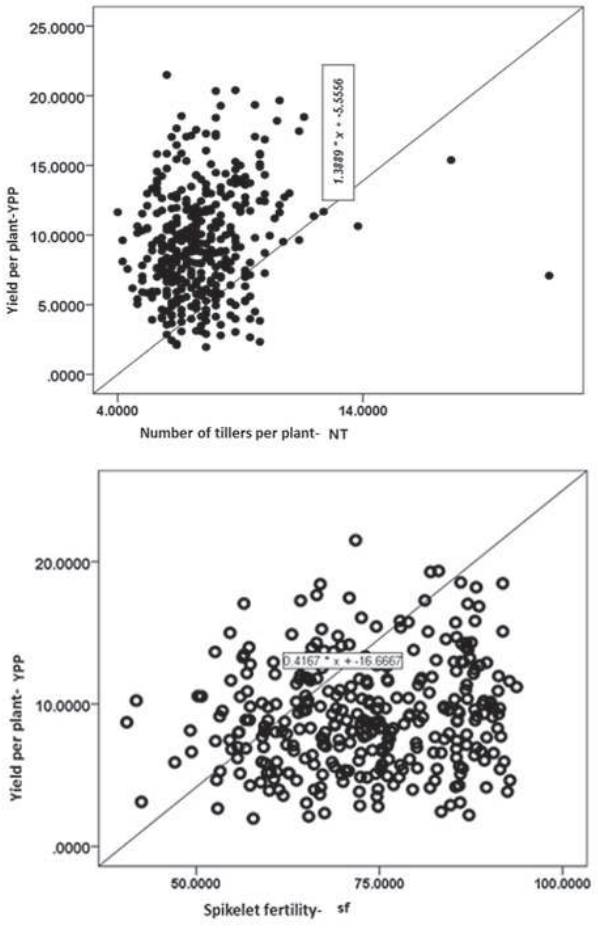

Fig. 2. Relationship of yield per plant with number of tillers per plant and spikelet fertility

Core set formation

A core set of 21 genotypes was identified from the entire set of 390 genotypes using advanced M (maximization) strategy implemented through a modified heuristic algorithm, which creates highly reproducible subsets representing all observations

Table 4. Average values for core collections using heuristic search

Predictors	Value
MD% (Mean difference percentage)	4.03
CR% (Coincidence Rate)	91.84
VD% (Variance Difference Percentage)	46.45
VR% (Variable Rate)	136.91

Where, Mean Difference (MD), Variance Difference (VD), Coincidence Rate (CR) and the Variable Rate (VR)

Table 5. Iso-cytoplasmic restorer lines and their parental hybrids used in constitution of coreset

Identified lines	Parental hybrids	Hybrids not used for constituting core set
PIRL-22	DRRH 2	DRRH 3
PIRL-37	PSD 3	Sahyadri 2
PIRL-46	PRH 10	Sahyadri 3
PIRL-80	Sahyadri 1	Sahyadri 4
PIRL-86	Sahyadri 1	GK 5003
PIRL-183	US 312	NK 5251
PIRL-236	INDAM 200-017	DRRH 775
PIRL-247	INDAM 200-017	PA 6201
PIRL-297	PA 6129	JKRH 401
PIRL-299	PA 6129	PAC 837
PIRL-356	PA 6444	
PIRL-377	PA 6444	
PIRL-381	PHB 71	
PIRL-390	PHB 71	
PIRL-429	Indira Sona	
PIRL-463	Suruchi 5401	
PIRL-478	Suruchi 5401	
PIRL-493	JRH 8	
PIRL-498	JRH 8	
PIRL-523	PAC 835	
PIRL-557	KRH 2	

Table 6. Performance of the coreset of iso-cytoplasmic restorer lines for agro-morphological traits

Core set	DFF (days)	PH (cm)	NT	PL (cm)	YPP (g)	TW (g)	KLBC (mm)	KBBC (mm)	L/B	SF (%)
PIRL-22	95.00	95.40	11.40	31.32	9.64	26.00	8.10	2.46	3.29	74.56
PIRL-37	78.00	90.00	10.00	23.60	7.26	24.00	7.94	2.05	3.88	80.02
PIRL-46	90.00	101.40	7.20	26.64	9.32	22.00	8.67	2.16	4.01	89.30
PIRL-80	100.00	107.00	9.60	25.88	19.34	30.00	7.32	3.24	2.26	83.08
PIRL-86	87.00	113.60	8.40	24.94	7.66	29.00	7.64	3.19	2.40	89.63
PIRL-183	92.00	102.00	21.60	26.76	7.08	14.00	6.91	2.35	2.93	78.51
PIRL-236	97.00	135.20	5.20	31.56	5.84	25.00	7.32	3.04	2.41	72.33
PIRL-247	90.00	118.40	7.20	26.30	3.12	17.00	6.49	2.39	2.72	42.50
PIRL-297	87.00	103.60	8.60	20.56	13.80	26.00	7.44	2.97	2.50	79.67
PIRL-299	86.00	99.40	5.60	22.22	9.62	26.00	7.05	3.22	2.19	76.24
PIRL-356	102.00	110.60	7.80	23.94	10.54	20.00	8.01	2.58	3.11	50.71
PIRL-377	95.00	102.00	17.60	22.56	15.38	13.00	6.79	2.51	2.71	77.89
PIRL-381	89.00	118.00	13.20	27.80	15.72	20.00	8.50	2.81	3.03	85.40
PIRL-390	98.00	130.40	7.80	28.66	11.74	16.00	7.66	2.69	2.84	64.08
PIRL-429	83.00	75.40	7.00	28.54	4.88	21.00	8.24	2.27	3.63	72.40
PIRL-463	105.00	108.40	6.00	24.94	8.04	16.00	6.96	2.19	3.18	60.84
PIRL-478	107.00	128.00	9.80	24.84	15.76	18.00	6.80	2.70	2.51	78.96
PIRL-493	87.00	122.20	6.00	29.72	21.50	27.00	8.49	2.92	2.91	71.75
PIRL-498	81.00	88.20	7.20	20.82	10.60	29.00	7.60	2.91	2.61	73.52
PIRL-523	114.00	122.20	8.00	26.42	10.52	17.00	6.80	2.58	2.63	56.00
PIRL-557	102.00	136.80	9.80	25.06	2.34	11.00	6.42	2.58	2.49	67.51

Where, DFF = days to 50% flowering, PH = plant height, NT = number of tillers number per plant, PL = panicle length, YPP = yield per plant, TW = test weight, KLBC = kernel length before cooking, KBBC = kernel breadth before cooking, SF (%) = spikelet fertility percentage

classes for various traits while ensuring there is least redundancy. The core set was further validated with statistical indicators namely, mean difference (MD%), variance difference (VD%), coincidence rate (CR%) and variable rate (VR%) for continuous variables. Mean difference percentage (MD%) exhibits the difference in averages of genotypes between the core set and the entire collection. In our study, MD value was 4.03, which indicated that the mean of the core set is similar to the mean of the entire set of iso-cytoplasmic restorers. Variance Difference Percentage (VD%) depicts the difference in distribution pattern. A large value of VD (46.51) is the indication of difference of variance between core set selected by 'PowerCore' from entire collection. Variable Rate (VR%) helps to compare the coefficient of variation values existing in the core collections with that of the entire collection, thereby determining how good it is being represented in the core sets (Kim et al. 2007). VR values (Table 4) show an average value of 136.91%. Coincidence Rate (CR%) shows whether the distribution ranges of each variable in the core set are well represented as compared to the entire collection and the core obtained using the HCC method is a representative of the whole collection. Results obtained show that the average CR value is 91.84% (Table 4). In general, a CR value of 80% indicated that the synthesized core set provides a fair representation of the whole accessions (Hu et al. 2000). The MD, VD and VR values obtained from the present analysis shows that the statistical consistency between the core and entire collections is good. The core set comprised of 21 restorer lines namely, PIRL-22, PIRL-37, PIRL-46, PIRL-80, PIRL-86, PIRL-183, PIRL-236, PIRL-247, PIRL-297, PIRL-299, PIRL-356, PIRL-377, PIRL-381, PIRL-390, PIRL-429, PIRL-463, PIRL-478, PIRL-493, PIRL-498, PIRL-523 and PIRL-557. The representation of lines based on the parental hybrids from which they have been

produced is presented in Table 5. Lines derived from 14 hybrids were used for constituting core set. Two lines were selected from seven hybrids *viz*., Sahyadri 1, INDAM 200-017, PA 6129, PA 6444, PHB 71, Suruchi 5401 and JRH 8 for making the coreset. Agronomic performances of iso-cytoplasmic restorer lines present in the corset were also compared (Table 6). Several promising restorer with higher yield performance were identified namely, PIRL-493 (21.50 g), PIRL-80 (19.34 g) and PIRL-381 (15.72 g). Percentage of genotypes in core set were also calculated and presented in (Supplementary Table S3; http://isgpb.org) showing that the distribution of variables was divided into class intervals and genotypes from each class interval were captured in constructing core set. Z test was also done to compare the difference between core set and entire set, and was found to be non-significant for all the traits (Supplementary Fig. 1; http://isgpb.org). Therefore, hieuristic core collection is an efficient tool for developing core sets, even when the entire collection represents unequal diversity and differentiation.

Discussion

In the assessment of iso-cytoplasmic restorers, superior lines throughout different groups *vis-a-vis* superior groups, based on their performances, were identified. The identified lines can be screened for the presence of fertility genes and their fertility restoration behavior by crossing with CMS lines (Singh et al. 2016; Kumar et al. 2017). Based on association among different traits observed in the present study, it can be realized that simultaneous selection for number of tillers, panicle length and spikelet fertility would enable improvement of yield per plant. In regression analysis also, it was observed that the contribution of number of effective tillers per plant followed by panicle length and test weight towards explanation of yield per plant was highest and significant. Hence, selection for these characters will help enhancing yield as they were mutually and directly associated with grain yield. Principal component analysis (PCA) employs orthogonal transformation to convert a set of variables into linearly uncorrelated variables called principal components such that the first principal component has the largest possible variance. PCA showed that the first four principal components are sufficient to capture the entire variability observed for different traits in the isocytoplasmic restorers evaluated in the present study. Traits showing higher variability can provide higher genetic gain in breeding programs (Gana et al. 2013; Varthini et al. 2014) and had been used (Chakravorty et al. 2013) in rice for subdividing observed variation and studying inter relationships among different traits. Our results show that heuristic core collection method was efficient in developing a core set of 21 isocytoplasmic restorers from a highly diverse set of 390 isocytoplasmic restorers derived from 25 diverse rice hybrids, ensuring 100% coverage of phenotypic traits without the comparison of relative characteristics within the genotypes.

This is the first report on developing iso-cytoplasmic restorers from widely grown rice hybrids not only in rice but also in any crop. The core set of iso-cytoplasmic restorers can be used for further improvement of restorers while the promising iso-cytoplasmic restorer lines can help in developing heterotic hybrids, when crossed with diverse non-parental CMS lines.

Authors' contribution

Conceptualization of research (AKS, GKS); Designing of the experiments (AKS, GKS, AK); Contribution of experimental materials (AK, PK); Execution of field/ lab experiments and data collection (AK, GKS, PKB); Analysis of data and interpretation (AK, PKN, GKS, AKS); Preparation of manuscript (AK, GKS, AKS).

Declaration

The authors declare no conflict of interest.

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References

Anonymous. 2015. Agricultural Statistics at a Glance. Department of Agriculture, Cooperation and Farmers welfare, Ministry of Agriculture, and Farmers Welfare, Government of India.

Ariyo O. N. 1987. Mulitvariate analysis and choice of parents in Okra (*A. esculentus*). Ann. Appl. Biol., **116**: 335-341.

Chakravorty A., Ghosh P. D. and Sahu P. K. 2013. Multivariate analysis of phenotypic diversity of landraces of rice of West Bengal. American J. Exp. Agric., **3**(1): 110-123.

Gana A. S., Shaba S. Z. and Tsado E. K. 2013. Principal

component analysis of morphological traits in thirty-nine accessions of rice (*Oryza sativa*) grown in a rainfed lowland ecology of Nigeria. J. Plant Breed. Crop Sci., **5**: 120-126.

Gangashetti M. G., Jena K. K., Shenoy V. V. and Freeman W. H. 2004. Inheritance of elongated uppermost internode and identification of RAPD marker linked to *eui* gene in rice. Curr Sci., **87**(4): 469-475.

Gopala Krishnan S., Waters D. L. E., Katiyar S. K., Sadananda A. R., Satyadev V. and Henry R. J. 2012. Genome-wide DNA polymorphisms in elite *indica* rice inbreds discovered by whole-genome sequencing. Plant Biotechnol. J., **10**(6): 623-634.

Gopala Krishnan S., Singh A. K., Waters D. L. E. and Henry R. J. 2013. Molecular markers for harnessing heterosis. In: Henry R. J. (ed) Molecular markers in plants. Wiley, USA, pp 119-136.

Hossain M. and Fischer K. S. 1995. Rice research for food security and sustainable development in Asia: Achievements and future challenges. Geojournal, **35**(3): 286-298.

Hu J., Zhu J. and Xu H. M. 2000. Methods of constructing core collections by stepwise clustering with three sampling strategies based on the genotypic values of crops. Theor. Appl. Genet., **101**: 264-268.

Khush G. S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol., **59**: 1-6.

Kim K. W., Chung H. K., Cho G. T., Ma K. H., Chandrabalan D., Gwag J. G., Kim T. S., Cho E. G. and Park Y. J. 2007. PowerCore: a program applying the advanced M strategy with a heuristic search for establishing core sets. Bioinformatics, **23**(16): 2155-2162.

Kole P. C., Chakraborty N. R. and Bhat J. S. 2008. Analysis of variability, correlation and path coefficients in induced mutants of aromatic non basmati rice. Tropical Agric. Res. Ext., **113**: 60-64.

Kumar A., Bhowmick P. K., Singh V. J., Malik M., Gupta A. K., Seth R., Nagarajan M., Gopala Krishnan S. and Singh A. K. 2017. Marker assisted identification of restorer gene(s) in iso-cytoplasmic restorer lines of WA cytoplasm in rice and assessment of their fertility restoration potential across environments. Physiol. Mol. Biol. Plants, DOI 10.1007/s12298-017-0464-5.

Mann C. C. 1999. Crop scientists seek a new revolution. Science, **283**: 310-314.

Naik R. K., Reddy S. P., Ramana J. V. and Srinivasa Rao V. 2005. Correlation and path co-efficient analysis in rice (*Oryza sativa* L.), The Andhra Agric. J., **52**(1-2): 52-55.

Nematzadeh G. A. and Sattari M. 2003. A study of nucleus genome of some high yielding rice (*Oryza sativa* L.) varieties for application in hybrid rice technology. Iranian J. Agric. Sci., **34**: 213-219.

Norain M. N., Shamsiah A., Abdul R. H., Nor A. H., Haslinda A. M. and Wan A. W. A. 2014. Correlation analysis on agronomic characters in F_2 population derived from MR64 and Pongsu Seribu 2. J. Appl. Sci. Agric., **9**(18): 143-147.

Pattnaik S., Kumar V., Tiwari K. K., Prakash C., Singh A., Singh A. K., Behera N., Panigrahi J., Mithra S. V. A. and Mohapatra T. 2016. Physiological and molecular basis of water-deficit stress tolerance in F1 hybrids and their parental lines in rice. Indian J. Genet., **76**(2): 127-136. DOI: 10.5958/0975-6906.2016.00035.3.

Reddy J. N., De R. N. and Suriya Rao A. V. 1997. Correlation and path analysis in lowland rice under intermediate (0-50 cm) water depth. Oryza, **34**(3): 187-190.

Sabar M and Akthar M. 2002. Rice germplasm collection, conservation and utilization for hybrid rice. In Proc. of seminar on “Sustainable utilization of plant genetic resources for agricultural production” in Islamabad, Pakistan. Pp. 55-61.

Sarkar A. 2006. Genetic variability and genetic diversity in rice bean. Environ. and Ecol., **24**(1): 76-80.

Sharifi P., Dehghani H., Momeni A. and Moghadam M. 2013. Study the genetic relations of some of rice agronomic traits with grain yield by using multivariate statistical methods. Iranian J. Field Crop Sci., **44**(2): 273-82.

Singh A. K., Borah P., Ponnuswamy R., Sarma D., Roy A. and G. N. Hazarika. 2016. Identification of fertility restorers among Assam rice cultivars by phenotyping and molecular screening approaches. Indian J. Genet., **76**(1): 10-17. DOI: 10.5958/0975-6906.2016.00002.X

Varthini N. V., Robin S., Sudhakar D., Raveendran M., Rajeswari S. and Manonmani S. 2014. Evaluation of rice genetic diversity and variability in a population panel by principal component analysis. Indian J. Sci. Tech., **7**(10): 1555-1562.

Virmani S. S., Mao C. X. and Hardy B. 2003. Hybrid rice for food security, poverty alleviation, and environmental protection, Proceedings of the 4th International Symposium on Hybrid Rice, Hanoi, Vietnam, International Rice Research Institute: 407p.

Virmani S. S., Virakamath B. C., Casal C. L., Toledo R. S., Lopez M. T. and Manalo J. O. 1997. Hybrid rice breeding manual. International Rice Research Institute, Philippines.

Supplementary Table S1. Comparison of *per se* performance of iso-cytoplasmic restorer lines derived from different hybrids

Genotypes	Days to fifty percent flowering					Plant height (cm)					No. of productive tillers/plant				
	Mean	Std Dev	Min	Max	CV	Mean	Std Dev	Min	Max	CV	Mean	Std Dev	Min	Max	CV
	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
CORH 3	92.13	2.47	89.00	96.00	2.69	107.23	6.38	97.00	115.40	5.95	7.13	0.43	6.40	7.60	5.99
DRRH 775	94.25	3.44	90.00	100.00	3.65	104.32	10.07	91.40	121.80	9.66	6.85	0.77	6.00	8.40	11.28
DRRH 2	91.50	4.53	80.00	97.00	4.95	96.53	5.92	77.20	104.20	6.13	7.63	1.60	6.20	11.40	20.99
DRRH 3	103.50	1.29	102.00	105.00	1.25	97.45	8.18	90.00	105.40	8.39	6.85	1.00	5.80	7.80	14.57
GK5003	94.77	6.52	82.00	105.00	6.88	106.36	4.78	95.60	115.80	4.49	7.38	1.02	5.60	9.80	13.81
INDAM 200-017	91.93	6.04	78.00	98.00	6.57	119.72	8.99	105.20	135.20	7.51	6.36	1.20	4.80	9.40	18.90
INDIRA SONA	89.37	9.51	78.00	104.00	10.64	103.52	10.63	75.40	120.20	10.27	7.28	1.25	5.00	9.40	17.11
JKRH 401	95.20	7.25	86.00	105.00	7.62	116.64	6.22	100.80	124.00	5.33	8.32	1.18	6.20	10.00	14.21
JRH 8	93.00	7.70	80.00	104.00	8.28	106.37	8.44	88.20	122.20	7.93	5.54	1.03	4.00	8.00	18.54
KRH 2	102.29	1.38	101.00	105.00	1.35	132.23	4.04	127.00	136.80	3.06	9.23	0.48	8.40	9.80	5.22
NK 5251	91.07	4.75	84.00	97.00	5.21	108.81	7.32	93.20	119.40	6.72	6.47	1.43	4.40	9.20	22.17
PA 6129	86.47	2.58	81.00	93.00	2.99	97.44	8.65	76.60	113.60	8.87	6.50	0.89	4.80	8.80	13.64
PA 6201	91.55	5.66	85.00	100.00	6.19	107.71	10.57	92.60	123.60	9.82	7.33	1.08	6.20	10.00	14.76
PA 6444	102.33	3.47	95.00	109.00	3.39	113.91	6.79	100.80	127.25	5.96	8.18	2.48	6.00	17.60	30.35
PAC 835	99.67	5.83	95.00	114.00	5.85	121.69	6.00	114.60	136.00	4.93	7.87	0.95	5.80	9.00	12.06
PAC 837	89.57	2.03	87.00	93.00	2.26	97.41	10.07	82.60	111.20	10.34	9.97	1.10	8.00	12.40	11.07
PHB 71	92.18	5.02	83.00	101.00	5.45	116.55	12.62	93.20	141.00	10.83	8.11	1.71	5.80	13.20	21.03
PRH 10	91.00	2.87	88.00	96.00	3.16	102.53	8.22	86.40	112.40	8.01	6.44	0.72	5.40	7.60	11.17
PSD 3	84.00	5.24	78.00	90.00	6.24	99.00	7.50	90.00	106.20	7.58	7.84	1.34	6.40	10.00	17.06
SAHYADRI 1	95.36	6.44	87.00	100.00	6.75	104.67	6.84	90.40	113.60	6.54	7.56	1.93	5.40	12.00	25.52
SAHYADRI 2	90.07	4.71	82.00	95.00	5.23	98.19	5.09	87.20	106.80	5.19	8.21	0.89	6.20	9.40	10.87
SAHYADRI 3	101.71	3.45	98.00	107.00	3.39	112.00	2.24	109.40	115.40	2.00	6.46	0.99	5.00	7.80	15.35
SAHYADRI 4	88.56	5.48	82.00	96.00	6.18	96.23	7.24	78.00	107.60	7.52	7.64	0.99	6.20	9.20	12.98
SURUCHI 5401	100.97	4.50	91.00	107.00	4.46	110.64	9.11	96.80	128.40	8.24	8.14	1.31	6.00	11.40	16.09
US 312	86.54	3.93	78.00	92.00	4.54	100.46	3.87	95.60	111.20	3.85	9.09	4.22	6.00	21.60	46.45
	Panicle length (cm)					Spikelet fertility (%)					Yield plant (g)				
CORH 3	28.34	1.51	26.00	30.40	5.34	85.11	4.46	75.33	91.17	5.23	7.45	3.71	2.20	12.96	49.79
DRRH 775	27.05	1.03	24.30	28.16	3.82	73.37	7.32	66.87	89.25	9.98	8.15	2.41	3.66	10.42	29.56
DRRH 2	26.75	2.03	23.42	31.32	7.58	83.72	5.00	74.56	91.41	5.98	10.69	2.73	6.24	18.20	25.56
DRRH 3	26.03	2.39	23.52	28.68	9.20	74.45	6.38	65.58	79.16	8.56	8.37	1.15	6.76	9.26	13.72
GK5003	25.31	1.17	23.02	27.30	4.63	75.70	15.41	50.24	92.75	20.35	9.77	3.14	3.84	17.10	32.10
INDAM 200-017	26.46	2.75	19.78	31.92	10.38	70.02	10.27	42.50	85.92	14.66	6.90	2.64	2.66	13.34	38.19
INDIRA SONA	27.45	1.51	25.06	30.34	5.50	64.82	14.12	47.08	90.41	21.79	7.36	2.93	3.04	13.48	39.88
JKRH 401	27.01	0.98	25.22	28.44	3.62	56.59	10.33	40.51	69.80	18.26	10.63	4.38	3.10	17.06	41.18
JRH 8	26.68	2.18	20.82	29.72	8.16	68.31	8.64	52.76	87.99	12.66	11.28	4.07	4.66	21.50	36.10
KRH 2	25.00	1.26	23.22	26.34	5.03	64.20	3.71	58.80	67.58	5.77	5.10	2.07	2.34	8.02	40.47
NK 5251	28.12	2.13	25.02	32.82	7.58	65.82	5.25	57.38	73.27	7.98	9.80	2.74	5.94	12.98	27.93
PA 6129	23.59	1.88	20.36	28.24	7.99	80.95	6.87	64.25	92.82	8.49	11.60	5.19	2.44	20.40	44.75
PA 6201	24.84	2.07	21.44	27.64	8.34	78.90	12.23	53.56	91.25	15.50	9.91	1.89	6.94	12.42	19.06
PA 6444	24.31	1.29	22.26	27.16	5.31	64.14	7.33	50.71	77.89	11.42	7.64	3.32	1.96	15.38	43.47
PAC 835	27.24	1.94	24.60	31.70	7.10	73.70	13.81	54.71	90.16	18.74	10.51	2.51	5.76	13.08	23.91
PAC 837	24.00	1.00	22.66	25.62	4.17	88.49	3.91	80.58	93.73	4.42	13.76	2.34	9.80	18.48	16.98
PHB 71	27.75	1.69	23.70	30.18	6.10	70.68	10.04	58.98	91.56	14.21	10.91	3.27	4.94	19.66	29.93
PRH-10	27.51	1.87	25.22	31.64	6.80	85.18	6.41	72.46	92.02	7.52	6.57	2.35	2.90	9.32	35.83
PSD-3	24.54	1.81	23.40	27.74	7.39	83.33	6.63	73.87	89.59	7.96	7.03	2.09	4.14	9.70	29.72
SAHYADRI 1	24.71	1.31	22.86	27.44	5.29	81.62	8.07	71.11	91.47	9.89	10.14	3.76	5.42	19.34	37.05
SAHYADRI 2	27.09	1.74	23.74	30.24	6.43	70.51	10.59	54.56	88.01	15.02	7.76	2.73	3.56	12.44	35.11
SAHYADRI 3	26.35	1.44	24.04	28.14	5.46	64.82	9.26	55.63	81.64	14.28	7.32	3.66	3.92	15.08	49.97
SAHYADRI 4	26.16	1.77	23.44	30.70	6.76	69.96	11.34	53.20	90.57	16.21	7.11	3.24	2.10	13.98	45.50
SURUCHI 5401	25.51	1.34	23.30	29.10	5.25	66.46	8.40	41.81	78.96	12.63	11.14	3.44	3.50	17.46	30.90
US 312	25.48	1.68	23.40	29.02	6.59	50.90	7.32	43.70	71.60	10.10	7.77	2.73	3.56	11.96	35.09

Supplementary Table S1 contd.....

	Kernel length before cooking (mm)					Kernel breadth before cooking (mm)					L/B					Test weight (g)				
	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
CORH-3	7.10	0.39	6.64	7.71	5.52	2.62	0.17	2.31	2.83	6.41	2.72	0.14	2.50	2.96	5.32	23.25	2.19	20.00	25.00	9.41
DRRH 775	7.26	0.47	6.42	8.04	6.53	2.53	0.17	2.24	2.78	6.70	2.88	0.21	2.56	3.19	7.44	19.42	4.38	11.00	24.00	22.55
DRRH-2	8.26	0.36	7.46	8.97	4.40	2.39	0.21	2.17	2.94	8.66	3.48	0.36	2.67	4.13	10.23	24.89	2.95	18.00	31.00	11.85
DRRH-3	7.06	0.45	6.69	7.70	6.32	2.51	0.16	2.42	2.75	6.33	2.82	0.29	2.49	3.19	10.44	19.00	1.83	17.00	21.00	9.61
GK5003	7.32	0.22	6.88	7.64	2.98	2.58	0.13	2.39	2.85	5.02	2.84	0.14	2.63	3.11	5.08	19.59	2.72	15.00	24.00	13.88
INDAM 200-017	7.34	0.45	6.49	8.25	6.14	2.83	0.18	2.39	3.10	6.33	2.60	0.22	2.37	3.28	8.57	24.70	3.15	17.00	30.00	12.74
INDIRA SONA	8.00	0.29	7.44	8.39	3.62	2.47	0.20	2.16	2.92	7.93	3.26	0.27	2.60	3.77	8.29	22.32	3.28	17.00	29.00	14.72
JKRH 401	7.34	0.31	6.94	7.75	4.19	2.68	0.24	2.33	3.11	8.79	2.75	0.16	2.45	2.98	5.88	20.70	2.45	18.00	25.00	11.84
JRH 8	7.90	0.37	7.16	8.49	4.71	2.93	0.16	2.70	3.22	5.47	2.70	0.15	2.27	2.91	5.70	26.38	2.04	21.00	30.00	7.72
KRH 2	6.93	0.38	6.42	7.47	5.50	2.53	0.14	2.38	2.70	5.44	2.74	0.14	2.49	2.93	5.01	15.57	2.76	11.00	18.00	17.73
NK 5251	7.74	0.40	7.16	8.46	5.18	2.62	0.13	2.32	2.81	4.80	2.96	0.19	2.65	3.39	6.59	23.21	2.42	18.00	27.00	10.44
PA 6129	7.80	0.50	6.65	8.61	6.35	2.95	0.18	2.53	3.22	6.10	2.65	0.21	2.19	3.15	7.97	23.91	3.14	15.00	30.00	13.13
PA 6201	7.39	0.30	6.96	7.86	4.03	2.45	0.16	2.25	2.64	6.38	3.02	0.13	2.83	3.20	4.29	15.09	2.12	12.00	18.00	14.04
PA 6444	7.69	0.42	6.79	8.25	5.48	2.73	0.28	2.35	3.24	10.22	2.83	0.25	2.39	3.13	8.79	17.95	3.14	13.00	26.00	17.48
PAC 835	7.44	0.39	6.80	7.88	5.21	2.63	0.16	2.40	2.88	6.02	2.83	0.23	2.50	3.16	8.12	23.11	4.01	17.00	29.00	17.37
PAC 837	7.77	0.25	7.15	8.15	3.19	3.08	0.16	2.82	3.38	5.19	2.52	0.14	2.32	2.80	5.55	26.79	1.97	24.00	31.00	7.35
PHB 71	7.49	0.41	6.84	8.50	5.44	2.57	0.12	2.40	2.81	4.75	2.92	0.08	2.77	3.03	2.72	18.14	4.68	12.00	26.00	25.82
PRH-10	8.33	0.32	7.75	8.67	3.78	2.23	0.07	2.16	2.33	3.03	3.74	0.20	3.52	4.01	5.30	22.78	2.05	20.00	25.00	8.99
PSD-3	8.01	0.08	7.94	8.12	0.98	2.49	0.29	2.05	2.72	11.46	3.25	0.40	2.93	3.88	12.32	23.80	1.79	22.00	26.00	7.52
SAHYADRI 1	7.56	0.22	7.26	8.06	2.90	3.02	0.21	2.57	3.24	6.93	2.52	0.17	2.26	2.82	6.88	27.45	2.62	22.00	30.00	9.55
SAHYADRI 2	7.49	0.47	6.70	8.30	6.22	2.55	0.14	2.36	2.76	5.45	2.94	0.23	2.46	3.39	7.72	21.00	2.77	17.00	26.00	13.21
SAHYADRI 3	7.97	0.26	7.58	8.30	3.23	2.57	0.07	2.50	2.66	2.79	3.10	0.09	2.99	3.23	2.79	21.86	2.04	20.00	24.00	9.31
SAHYADRI 4	8.19	0.40	7.36	8.75	4.90	2.59	0.12	2.37	2.78	4.72	3.18	0.24	2.75	3.58	7.41	21.75	3.89	12.00	29.00	17.89
SURUCHI 5401	7.06	0.51	6.24	8.11	7.16	2.56	0.17	2.19	2.93	6.48	2.77	0.30	2.29	3.48	10.71	18.59	2.47	16.00	24.00	13.30
US 312	7.36	0.52	6.38	7.97	7.11	2.58	0.15	2.35	2.94	6.01	2.86	0.21	2.56	3.20	7.46	20.08	3.57	14.00	25.00	17.78

Supplimentary Table S2. A regression study of various predictors on yield

Parameter Estimates				
Variable	Parameter estimate	Standard error	t value	Pr > |t|
Intercept	-18.826	4.679	-4.020	<.0001
DFF	0.054	0.033	1.650	0.100
PH	0.019	0.020	0.980	0.329
NT	0.745	0.129	5.780	<.0001
PL	0.292	0.095	3.070	0.002
TW	0.199	0.051	3.910	0.000
SF (%)	0.048	0.019	2.480	0.014

Where, DFF= days to 50% flowering, PH= plant height, NT= number of tillers number per plant, PL= panicle length, TW=test weight, SF (%) = spikelet fertility percentage

Supplementary Table S3. A comparison of percentage of genotypes used for constituting core set under different class-intervals

Classes	Percentage of genotypes in core set								
	DFF	PH	NT	PL	YPP	TW	KLBC	KBBC	SF
1	11.76	16.67	4.88	40.00	10.00	11.11	22.22	30.00	33.33
2	2.33	7.69	3.19	6.25	3.57	22.22	13.64	4.00	20.00
3	6.06	2.86	4.20	4.35	4.48	6.90	7.14	4.41	2.22
4	5.97	3.53	12.12	6.15	5.06	5.56	3.13	4.17	4.00
5	4.26	4.88	20.00	3.23	4.76	5.26	3.80	2.35	1.54
6	3.13	3.95	50.00	3.85	2.04	2.53	3.33	7.69	4.41
7	5.17	4.08	NA	5.36	7.41	1.92	3.57	5.00	6.67
8	5.26	7.69	100.00	5.56	11.11	5.48	8.70	8.70	3.57
9	12.50	16.67	NA	12.50	14.29	4.35	33.33	25.00	5.26
10	100.00	33.33	100.00	20.00	25.00	12.50			

Where, DFF= days to 50% flowering, PH= plant height, NT= number of tillers number per plant, PL= panicle length, YPP= yield per plant, TW=test weight, KLBC= kernel length before cooking, KBBC= kernel breadth before cooking, SF (%) = spikelet fertility percentage

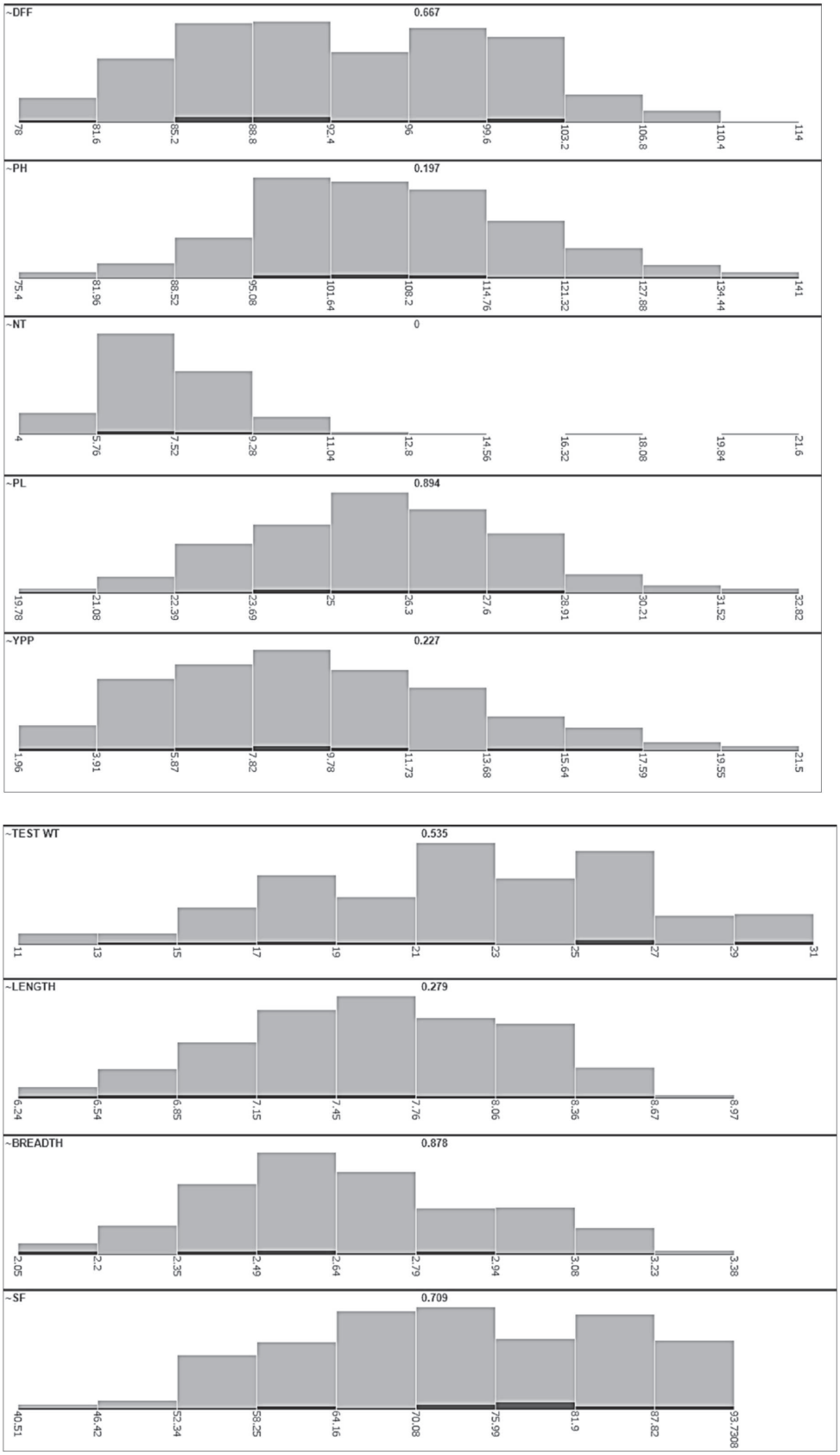

Fig. 3. Frequency distribution of coreset (dark) and whole set for each variable