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Extent for use of indigenous specialty landraces, cultivars and basmati types in rice heterosis breeding

*Karam V. Mukerji, Dhirubhai Azad and Chandra Tata Johar

Department of Plant Molecular Biology, Faculty of Agricultural sciences, Savitribai Phule Pune University, Pune, India.

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Traditional landraces and basmati lines are useful pool of important traits and need to be analyzed for hybrid breeding utilization in rice. Furthermore, released cultivars have good agronomic characters which can be used directly in the hybrid breeding program. A study was conducted on 80 landraces, 5 basmati lines and 36 cultivars to characterize them, for fertility restoration traits and identification of prospective restorers and maintainers for Cytoplasmic Male Sterility (CMS) wild abortive line and IR58025A. The experiment was laid out in a randomized complete block design with two replications consisting of F₁ seeds along with their parents. Observations on spikelet fertility were carried out for the identification of restorers and maintainer lines. Out of the 121 genotypes analyzed, majority of the lines were either partial maintainers or partial restorers and 18 genotypes behaved as restorers while 16 as maintainers. These restorers can be used as male parents or to develop diverse restorers, while maintainers could be utilized for developing new CMS lines.

Key words: Hybrid rice, cytoplasmic male sterility, spikelet fertility, restorers, maintainers.

INTRODUCTION

Knowledge of genetic diversity in crop species is fundamental to its improvement. Genetic variability accumulated in the background of diverse plant types is immediately valuable for shaping new varieties and this forms the basic wealth on which plant breeders can operate for reconstructing the existing genotypes (Sujay, 2007). Among the different options available to increase yield, hybrid rice technology has been found to be the most feasible and pragmatic option (Yuan, 2009). However, yield potential of hybrids are stagnated in farmers' field due to lack of adequate research efforts in this direction (Byron, 2009). Furthermore, with the increase in hybrid rice yield and area, concern for various traits namely; cooking quality, disease and insect resistance, local adaptability and specialty uses has also increased.

In India, IR58025A, containing Wild Abortive (WA)

cytoplasm is the most widely used cytoplasmic male sterile line (CMS) in hybrid rice production. This cytoplasmic uniformity of a single source of CMS may lead to genetic vulnerability towards disease and insects as in the case of maize (Ullstrup, 1972), pearl millet (Kumar et al., 1983), and more recently in rice (Biswas, 1999; Sharma et al., 1999). Hence, there is an urgent need for diversifying our present rice CMS genotypes. Diversified CMS lines other than WA cytoplasm have been developed at China, Philippines, India and various other countries. More than 20 CMS sources have been developed (Fujii et al., 2010) from various accessions of cultivated rice and wild species.

Landraces provides a vast genetic variability for the present day rice improvement programme (Zhenshan et al., 1996). Though domesticated, un-adapted landraces are phenotypically less desirable such that plant breeders have recognized their worth for improvement of various traits namely; grain quality (Ghose et al., 1960), medicinal properties (Das and Oudhia, 2000; Shastri, 2004), and disease and pest resistance (Bhat and Gowda, 2004). Apart from these uses, some genotypes have special

*Corresponding author. E-mail: Karam.mukerji@unipune.ac.in

Table 1. Important traits observed in landraces and cultivars of rice.

Special features ¹	Traditional landrace/variety
Boiled rice	Dodda Bili bhatta, Dodda bhatta, Dodda
Chakkali (a snack) made from rice powder	mullare Shetgi, Sanna mullare
Drought tolerance	Azucena, Budda, Champakali, Dambarsali, Dodda bhatta, Gopal doddiga, Hakkalasali, Haldoddiga (Red), Moroberekan, Salumpikit Bangar gundu, Karigajavili, Malagkit Sung Song, Moroberekan Bangar Kaddi, Belgaum Basmati, Budda, Intan
Durable resistance to diseases and pests Early vigour	Aden Kelte, Alur sanna, Bangar Kaddi, Karigajavili, Padmarekha, Raj kamal, Putta bhatta Local, Rajkhaima
Good cooking quality	Badshahbhog, Huggi Bhatta, Kalanamak, Kumud, Gandha sali, Sindhagi local
Good cooking quality aromatic rice	Sanna mullare
Good quality flaking	Hannekattu, Ratanchuda, Ratanchudi
Good quality popping/puffing	Mysore sanna, Sanna bhatta, Vasane Sanna
Kesari bath (a traditional sweet) Medicinal properties	Bhatta Chittiga, Karibhatta, Karigajavili
Nutritive and satiety value	Gandha
Salinity tolerance	sali Pokkali
Submergence tolerance	Mattalaga, Neermulga,
Tolerance to low soil fertility	Neermullare Dambarsali
Traditional organic farming	Zigmaratgaya, Soratgaya, Padmarekha, Suggi
Very slender and medium grain type	Kaime Badshahbhog, Kalanamak

¹Based on indigenous knowledge gathered from farmers (Adopted and updated from Bhat and Gowda, 2004; Sujoy 2007; Ahuja et al., 2008; Bhatupadya et al., 2008 and Hanamaratti et al., 2008).

properties namely; flaking, popping, puffing and use in unique food preparation (Grist, 1983). Such rice genotypes are generically known as specialty rice and include the group of basmati type, aromatic non-basmati types, non-aromatic-basmati types and non-aromatic special purpose rice types (Shenoy and Kalagudi, 2004). The specialty rice genotypes are more often native landraces than improved high yielding cultivars (Shenoy et al., 2001). Genetic diversity, hence, provides a scientific basis for efficient utilization of germplasm resources in crop improvement. Therefore, a proper assessment of their genetic worth in crop improvement programs aimed at utilizing these in rice heterosis breeding is advocated.

The objective of the present study was to determine the genetic diversity among the landraces, basmati lines and popular cultivars of rice, for their subsequent use in hybrid rice breeding by determining their restoration behaviour. The identification of maintainers and restorers among these lines would help to diversify the genetic base in hybrid rice breeding.

MATERIALS AND METHODS

Plant material

Totally, 121 genotypes comprising of 80 landraces, 5 basmati lines and 36 released cultivars were used in this study. IR58025A, a well known wild abortive (WA) cytoplasmic male sterile (CMS) line was used for evaluation of fertility restoration phenotype of 121 lines. The line has stable sterility, aromatic long slender grain and has

been used in development of several hybrids in India and elsewhere (Mao et al., 2009). Most of the landraces used in this study are adapted to upland rice ecosystem and are known to have various special features as detailed in Table 1. The lines were made available by the Barwale Foundation, Hyderabad, India; the Agricultural Research Station (Paddy), Sirsi, India; and the International Rice Research Institute (IRRI), Los Baños, Philippines.

Evaluation for fertility restoration

The experimental materials were raised at the Barwale Foundation Research Farm, Hyderabad, India (Coordinates: 17°24'22 "N and 78°12'40"E), during wet season from June to November, 2011. The genotypes were crossed with a WA-CMS line IR58025A to produce 10 to 15 F₁ seeds per cross, during the dry season from December 2010 to May 2011. The pollen parent and the respective F₁ were planted side by side. About 25 day old seedlings from each of the crosses were planted, adopting a spacing of 20 x 20 cm.

Recommended package of cultivation practices was followed and single seedling per hill was planted. All 121 F₁ seeds were evaluated for spikelet fertility. Spikelet fertility was computed as percentage seed set based on panicles of main and two side tillers of individual plants (bagged with parchment paper before flowering) of each F₁. The seed set in each panicle was counted and used for calculating the percentage of spikelet fertility. The spikelet fertility percentage of individual plants was taken as the average of spikelet fertility of three panicles each from three plants. Plants in each population were classified based on spikelet fertility percentage into four classes, namely, restorer (more than 70% fertility), partial restorer (31 to 70% fertility), partial maintainer (1 to 30% fertility) and maintainer (0% fertility) (Sheeba et al., 2009). Further, data were recorded for days to 50% flowering, plant height, productive tillers per plant, panicle length, single plant yield and 100 grain weight.

Table 2. Morphological data of the check KMR3 and the lines identified as restorers.

No.	Genotype	Category	DFF(d)	Ht (cm)	PN	PL (cm)	SPY(g)	100 gw(g)
1	KMR3	Check	95	120	7	23	19.5	2.6
2	Balesuli	landrace	86	139	6	24	12.0	2.6
3	Madras sanna	landrace	86	117	11	25	29.8	2.1
4	Dambarsali	landrace	82	101	4	23	12.6	2.8
5	Coimbatore	landrace	110	134	12	24	36.8	1.9
6	Salumpikit	landrace	74	112	11	25	11.4	1.8
7	Super basmati	Basmati	93	116	8	26	9.6	1.9
8	Amritsar basmati	Basmati	102	164	11	25	8.0	1.8
9	Sona mahsuri	Cultivar	107	105	9	22	38.0	1.5
10	IR 24	Cultivar	82	98	14	23	24.0	2.1
11	Mahsuri	Cultivar	104	131	8	25	39.1	1.6
12	IR 46	Cultivar	100	103	10	24	19.4	2.1
13	IR 56	Cultivar	84	89	15	25	32.2	1.9
14	IR 64	Cultivar	97	97	9	22	48.1	2.3
15	IR 74	Cultivar	107	75	13	21	22.6	1.8
16	IR 72	Cultivar	93	90	11	25	23.8	1.7
17	IR 26	Cultivar	81	111	10	24	19.1	2.1
18	Karuna (CO 33)	Cultivar	74	78	10	22	15.2	1.8
19	IR 66	Cultivar	97	104	10	28	35.3	2.2

DFF, days to 50% flowering; Ht, plant height; PN, number of productive tillers per plant; PL, panicle length; SPY, Single Plant Yield; 100gw, 100 grain weight.

RESULTS

The restoration behavior measured as percentage spikelet fertility in F₁ seeds showed variation among the genotypes tested (Supplementary Tables 1 and 2). The overall frequency of maintainers, partial maintainers, partial restorers and restorers were 13.2, 41.3, 30.6 and 14.9%, respectively. The highest numbers of maintainers (13) and restorers (11) were found from landraces and cultivars, respectively, while only two restorers and no maintainer were identified among basmati lines. Behavior of fertility restoration among different groups was as follows:

Group-wise analysis of genotypes with fertility restoration

Landraces

The majority of landraces used in the present study (Supplementary Table 1) were from India, except 'Chhomrong' which is from Nepal and 'Malagkit Sung Song' and 'Azucena' from the Philippines. Mean Spikelet fertility for 80 genotypes was found to be 29.36%. The frequency of maintainers, partial maintainers, partial restorers and restorers were 16.3, 45.0, 32.5 and 6.3%, respectively. Further, when fertility restoration behavior of the genotypes was assessed with respect to their special feature (Table 1), no distinct trend was observed in any group.

Basmati lines

A number of basmati rice varieties are under cultivation. In the present study, we used a total of 5 basmati lines. No maintainers and partial restorers were found among the lines, however three lines namely, Belgaum Basmati, Kasturi Basmati and Pusa Basmati 1 were found to be partial maintainers while Amritsar Basmati and Super Basmati were found to be restorers (Table 2).

Cultivars

The cultivars have a major advantage over landraces as they are high yielding and responsive to fertilizer. In the present study, cultivars from India, Nepal, Indonesia, Philippines, Africa, China, Taiwan and Japan were studied for their fertility restoration behaviour (Table 2). Spikelet fertility was taken for all F₁ seeds and the mean spikelet fertility value for 36 genotypes was found to be 43.43%. The frequency of maintainers was found to be 8.3% and that of partial maintainers, partial restorers and restorers was 30.6%, each. It was observed that 9 out of 10 lines from Philippines were restorers. ITA 112 and NERICA 3, both lines from Africa were identified as maintainers. Further, 'Chandan Nath-3' from China, Taichung 176 from Taiwan and Khumal-6, Khumal-9 from Nepal were identified as partial maintainers. Among the Indian cultivars 'Sona mahsuri', 'Mahsuri' and 'Karuna' Plant height was in the

Table 3. Morphological data of the check (IR58025B) and the lines identified as maintainers.

No.	Genotype	Category	DFF(d)	Ht (cm)	PN	PL (cm)	SPY(g)	100 gw(g)
1	IR58025B	Check	105	95	14	26	28.6	2.2
2	Biliakki	landrace	95	159	11	30.5	33.5	2.7
3	Bili Chitga	landrace	137	124	17.5	22.0	24.5	2.1
4	Bkb	landrace	124	187	9	25.5	17.5	2.4
5	Budda	landrace	102	105	11.0	21.0	16.2	3.0
6	Himali	landrace	88	94	10	22.25	37.8	3.4
7	Jadda bhatta	landrace	118	171	9	24.5	35.1	3.0
8	Karigajavili	landrace	111	143	8.5	30	26.7	1.9
9	Mysore sanna	landrace	111	129	15	23	15.5	2.1
10	Padmarekha	landrace	115	160	7.5	22.5	17.1	2.7
11	Raj kamal	landrace	116	100	17	25.5	19.4	2.2
12	Siddsal	landrace	118	153	8.5	24.5	14.6	2.7
13	Sindagi local	landrace	100	163	9.5	28	21.7	2.3
14	Malagkit Sung Song	landrace	102	126	10.5	21.0	26.4	3.1
15	ARC10550	Cultivar	95	147	7	27	31.5	2.8
16	ITA 112	Cultivar	85	85	11.5	20.0	16.9	3.5
17	NERICA 3	Cultivar	76	114	5	24	20.8	3.5

DFF, days to 50% flowering; Ht, plant height; PN, number of productive tillers per plant; PL, panicle length; SPY, Single Plant Yield; 100gw, 100 grain weight.

range of 75 (IR 74) to 164 cm (Amritsar basmati), with the mean of 109.7 cm. Most of the cultivars were found to be semi-tall in nature having plant height below 110 cm. Number of productive tillers per plant showed immense variation, minimum of 4 tillers were recorded for 'Dambarsali' and maximum 15 for RI 56, with a mean of 9.9. Panicle length was found to be in the range of 21 (IR 74) to 28 cm (IR 66), with a mean of 24 cm. Single plant yield showed vast variation ranging between 8.0 g (Amritsar basmati) and 48.1 g (IR 64), with a mean of 24.0 g. For 100 grain weight, 'Sona mahsuri' was recorded lowest at 1.5 g while 'Dambarsali' recorded highest at 2.8 g. The mean value for this trait was 2.0 g.

Agronomic data of maintainer

Agronomic traits among maintainers are represented in Table 3. DFF ranged between 76 (NERICA 3) to 137 (Bili Chitga) with a mean of 105.8. The DFF of cultivars and landraces was found to be below and above 100, respectively. Plant height is one of the critical factors in hybrid seed production and was in the range of 85 (ITA 112) to 187 cm (Bkb), with a mean of 132.6. Most of the landraces were found to be tall in nature having plant height above 120 cm. Number of productive tillers per plant were in the range of 5 (NERICA 3) to 17.5 (Bili Chitga), with a mean value of 10.7. Panicle length was found to be in the range of 20 (ITA 112) to 30.5 cm (Biliakki), with a mean of 24.5. Single plant yield showed vast variation that ranged between 14.6 (Siddsal) and 37.8 g (Himali), with a mean value of 23.8 g. For 100

grain weight, 'Karigajavili' recorded lowest at 1.9g while NERICA 3 and ITA 112 recorded highest (3.5 g) each. The mean value for this trait was 2.7 g.

DISCUSSION

For developing high yielding three-line rice hybrids, the basic step is to identify maintainers (having recessive fertility restorer gene/s) and restorers (having dominant fertility restorer gene/s) from a large germplasm collection (Siddiq, 1996). Extensive research work on identification of restorers and maintainers and the inheritance of fertility restoration has been done on the WA cytoplasmic source (Xie, 2009). Among the various sources of cytotesterility, CMS lines derived from the WA system have been found to be the most stable in terms of complete pollen sterility (Brar et al., 1998). Several studies by Singh and Singh (2000) and Malarvizhi et al. (2003) have reported higher frequency of restorers than maintainers for WA-CMS lines, while Kumari et al. (1997) and Salgotra et al. (2002) reported higher percentage of maintainers than restorers in their studies. In addition, most lines identified as restorers were *indica* and maintainer lines were landraces and *japonica*. The frequency of restorers for WA system is relatively high among improved *indica* cultivars than *japonica* rice (Zhuang et al., 1997).

The morphological phenotypes of cultivars identified as maintainers and restorers is undoubtedly superior (Tables 2 and 3) than that of landraces. However, as seen in the past, introduction of cultivars leads to abandonment of traditional varieties, resulting in decreased

overall genetic diversity. Preferred by local consumers owing to their specialty features, landraces form the abundant wealth of secondary gene pool of rice. They are also known to have better adaptability to local environments (Bhat and Gowda, 2004). In the present study, among the landraces, the number of maintainers (13) was more than that of restorers (5). However, when compared to landraces, among cultivars, the number of restorers (11) was much higher than that of maintainers (3). This clearly indicates that landraces are closer to maintainers with respect to the fertility restoration genes. Furthermore, the relatively high frequency of restorers among cultivars indicates that with respect to fertility restoration, genes cultivars are closer to restorer gene pool. Since heterosis is often realized in terms of genetic distance, the distinct gene pools of maintainers and restorers can hasten hybrid rice breeding.

Among the landraces, "Dambarsali" and 'Salumpikit' which were identified as restorers are known to have drought tolerance, whereas 'Balesuli', 'Madras sanna' and 'Coimbatore' are adaptable to rain-fed ecosystem in India. We propose diversification of restorer line development by crossing the restorers from landraces with the restorers from cultivars to develop site specific restorers for hybrids. Furthermore, landraces could have different restorer genes, which should be elucidated by genetic and molecular marker studies. Interestingly, a number of landraces were identified as maintainers in our study, among which 'Budda' is known to have drought tolerance and early vigour which is essential for weed competitiveness. 'Karigajavili' is a genotype with multiple traits such as durable resistance to disease and pests, good cooking quality and medicinal properties. 'Padmarekha' and 'Raj kamal' have good cooking quality. 'Mysore sanna' is widely used in the preparation of 'Kesaribath' a traditional sweet of South India, popular in ceremonies, especially, marriages. 'Malagkit Sung Song' has durable resistance to diseases and pests. Further, 'Biliakki', 'Bili Chitga', 'Bkb', 'Himali', 'Jadda bhatta', 'Siddesal' and 'Sindhagi local' are adapted to upland rice ecosystem in India. We propose diversification of maintainer line breeding by utilizing the landraces efficiently, in order to incorporate specialty traits in hybrids.

Basmati lines demand a premium in the market and hence, utilization of 'Super basmati' and 'Amritsar basmati', identified as restorers in our study could hasten basmati hybrid breeding program. Among the maintainers identified from cultivar group, ITA 112 and NERICA 3 are very popular in Africa, whereas, ARC10550 is a released cultivar for North-eastern states of India. For restorers, nine lines from the Philippines were found to be restorers. These lines were developed by the International Rice Research Institute (IRRI), Philippines and are popular in various countries in Asia. Among Indian cultivars, 'Karuna' released in Tamil Nadu, 'Mahsuri' and 'Sona mahsuri' released in Andhra Pradesh were found to be restorers. Apart from high spikelet fertility of the F_1 , there

are some other desirable traits of a good restorer line. If a restorer qualifies some or all of these traits, then, it can be used in the hybrid breeding program. The traits include, tall stature (10 to 15 cm taller than A line), non-synchronous tillering and long panicles, high pollen production ability and high amount of residual pollen, resistance to insect pests and diseases, and sturdy culm (Xie, 2009). The morphological data of 18 restorers as compared with a well known restorer line KMR3 is detailed in Table 2. 'Madras sanna', 'Super basmati' and a popular cultivar 'Sona mahsuri' were found to have desirable flowering duration, height, and panicle length that can be used for developing medium duration rice hybrids. Furthermore, similar studies conducted at India and elsewhere have also identified cultivars from IRRI as potential restorers (Shi-hua et al., 2009; Mao et al., 2009; Lafarge et al., 2009). These lines have been actively utilized in hybrid rice breeding program.

It was observed that the frequency of maintainers among the elite breeding lines is rather low. Furthermore, even if some of the lines are identified as maintainers, they are susceptible to various biotic and abiotic stresses. Hence, there is a need to combine desirable traits through recombination breeding. Sometimes, even partial maintainers with many desirable traits can be used in the breeding program. Some of the desirable traits of the maintainer lines are relatively dwarf/semi-dwarf stature, good and synchronous tillering, high stigma exertion, high outcrossing potential, sturdy culm, resistance to insect pests and diseases, complete and stable maintenance of sterility (Xie, 2009). Out of the total 16 putative maintainers, 3 were cultivars and 13 were landraces. The morphological data of these lines, as compared with a well known maintainer line IR58025B is detailed in Table 3. From this study, two lines 'Himali' and 'ITA 112' were found to have desirable flowering duration, height and high grain weight that can be exploited for the development of new CMS lines.

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