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Bioprospecting for fertility restoration genes in wild relatives and land races of rice from different geographic regions

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Abstract

Hybrid rice development, a major advance in rice breeding, has demonstrated its immense potential in enhancing the rice productivity. In the three line system of hybrid rice, the restoration of fertility of the widely used wild-abortive type of cytoplasmic male sterility is controlled by *Rf3* and *Rf4*, two fertility restoration genes. Exploration for restorers in diverse genetic backgrounds is a critical step in hybrid rice breeding. As genetic resources constitute the major source of new genes/alleles, an attempt was made to assess the distribution of these two restorer genes in the native populations collected from the geographic regions known to be the primary and secondary centres of origin of rice. In addition, assays were performed on the populations of *Oryza rufipogon* and *Oryza nivara*, the wild progenitors of rice. The results suggest that the *Rf* genes are well distributed in the wild forms at a higher frequency, more in *O. rufipogon*, while in cultivated forms, their frequency is low, particularly for the *Rf3* gene. The genes and the alleles identified in the genetically divergent, low-performing land races and wild relatives can be successfully employed for the development of new hybrid rice cultivars.

Keywords: Assam, cytoplasmic male sterility, fertility restoration, hybrid rice, Jeypore, WA type

Introduction

Rice is the staple food of half of the world's population and people of several Asian countries fully depend on rice for their daily calorie intake. The introduction of high-yielding semi-dwarf varieties has heralded the green revolution. But, with yield levels stagnating, exploitation of hybrid vigour is considered to be a viable option to break the yield barrier in rice and the potential of hybrid rice was successfully demonstrated in China. But, the spread of hybrid rice in other Asian countries is low as *indica* hybrids could show only ∼15–20% yield advantage over inbreds, mainly due to the narrow genetic diversity present in *indica* source material (Hossain *et al.*, 2010) while hybrids from *indica/japonica* parents were reported to show ∼30–40% yield advantage over *indica/indica* hybrids (Yuan, 1994).

Cytoplasmic male sterility (CMS), a maternally inherited trait, which causes the production of non-functional pollen, is the key component of the three line hybrid rice programme (Kaul, 1988) and the wild-abortive (WA), is the most extensively used CMS type in hybrid rice (Yuan, 1977; Lin and Yuan, 1980). The WA type has been extensively investigated and investigations suggest that two nuclear genes, *Rf3* and *Rf4*, can restore fertility of the WA type (Zhang *et al.*, 1997, 2002; Tan and Trangoonrang, 1998; Kazama and Toriyama, 2014; Tang *et al.*, 2014).

Searching for restorer genes/novel alleles of the restorer genes in native landraces, the major source of genetic diversity (Brar and Khush, 2003; Sun *et al.*, 2003), is ideal as many desirable alleles may still be floating in the native land races which can be exploited (Tanksley and McCouch, 1997) further. As heterosis is known to be more in crosses

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from diverse materials, native land races from different geographic regions could be the ideal base materials. In addition, the wild forms, *Oryza rufipogon*, a perennial and *Oryza nivara*, an annual, the progenitors of cultivated rice (*Oryza sativa* L.) (Oka and Chang, 1962; Sampath, 1962; Sharma and Shastry, 1965; Wang *et al.*, 1992), are known to be gene reservoirs in rice. As gene flow is common between the cultivated and wild forms without any genetic barriers, a continuous array of intergrades were recorded and were considered to be the bridge between wild and cultivated forms (Oka and Chang, 1962). The variation reported in *indica* rice across a wide geographical area might be the result of free gene flow or can be due to the large number of seed dispersal routes on land to highly diverse geographic locations. Studies on the presence of the *Rf* genes in native populations at different geographic locations can provide us the evolutionary trends at different locations and also can help us to understand the origin and evolution of *Rf* genes. The present study reports the distribution of *Rf* genes in native land race accessions collected from two distinct geographic regions of India and collections of wild relatives of rice and molecular markers were employed for the study.

Materials and methods

The rice accessions used in the study are 184 collections from Assam, a geographic location in North Eastern India, reported to be associated with the primary centre of origin of rice (Richharia and Govindaswami, 1990) and 236 collections from Jeypore–Koraput tract of Eastern India, a region recognized as the secondary centre of origin of rice (Ramiah and Ghose, 1951; Ramiah and Rao, 1953). One hundred and sixty-three accessions of *O. rufipogon* and 157 accessions of *O. nivara*, collected for different geographic locations in India were also included in the study (online Supplementary Table 1).

Total genomic DNA was extracted from fresh young leaves employing the cetyl trimethyn ammonium bromide method (Doyle and Doyle, 1987) and PCR assays were carried out using the markers that are closely linked with *Rf3* (RM10305- Balaji *et al.*, 2012) and *Rf4* (RM6100- Singh *et al.*, 2005) genes. The primer sequences employed are: RM10305- F: (5′-CAGGAACCA ACCTTCTTCTTGACC-3′), R: (5′-GTCAGACTCCGATCTG GGATGG-3′) RM6100-F: (5′-TCCTCTACCAGTACCGCA CC-3′), R: (5′- GCTGGATCACAGAT CATTGC -3′).

The PCR mix consists of 1 unit of Taq DNA polymerase, 5 pmol of each primer, 10× PCR buffer with 20 mM $MgCl_2$, 2.5 mM dNTPs in a final volume of 10 µl. The PCR reactions were performed using the same profile for both the markers (initial denaturation at 94°C for 4 min, followed by 94°C for 30 s; 55°C for 45 s and 72°C for 1 min for 35 cycles with a final extension of 7 min at 72°C) on a thermal cycler (PTC-200 Thermo cycler; Bio-Rad, Germany). The amplified products were fractionated on 3% agarose gels at 75 V for 1.5 h and stained with ethidium bromide and the gel images were recorded with Multi Image system (Alpha Innotech, USA).

Results

The results suggest that in *O. rufipogon*, the wild progenitor, majority (76.69%) of the accessions possess at least one of the fertility restorer genes while the value in the accessions of *O. nivara* is 59.87% (Table 1, Fig. 1). In the accessions of Assam and Jeypore, the presence of these genes was around 30–35% of the accessions suggesting their presence is low in the cultivated rice.

Table 1. Distribution of fertility restoration genes in different populations

		O. rufipogon	*O. nivara*	Assam	Jeypore
1.	*Rf3* (homozygous) +	10 (6.13)	3 (1.91)	3 (1.63)	2 (0.84)
2.	*Rf3* (heterozygous) H	7 (4.29)	3 (1.91)	1 (0.54)	–
3.	*Rf4* (homozygous) +	45 (27.60)	23 (14.64)	50 (27.17)	67 (28.38)
4.	*Rf4* (heterozygous) H	16 (9.81)	3 (1.91)	1 (0.54)	17 (7.20)
5.	*Rf3* + *Rf4* (+ +)	18 (11.04)	11 (7.00)	1 (0.54)	–
6.	*Rf3* + *Rf4*(H) (+ H)	2 (1.22)	1 (0.63)	–	–
7.	*Rf3*(H) + *Rf4* (*H* +)	6 (3.68)	7 (4.45)	2 (1.09)	–
8.	*Rf3*(H) + *Rf4*(H) (H H)	2 (1.22)	2 (1.27)	–	–
9.	*Rf3* different allele (D3)	–	–	–	–
10.	*Rf4* different allele (D4)	19 (11.65)	10 (6.36)	–	2 (0.84)
11.	Accessions without any restorer gene	38 (23.31)	94 (59.87)	126 (68.47)	148 (62.71)
	Total number of accessions	163	157	184	236

Figures in parenthesis indicate the frequency (%) in the population.

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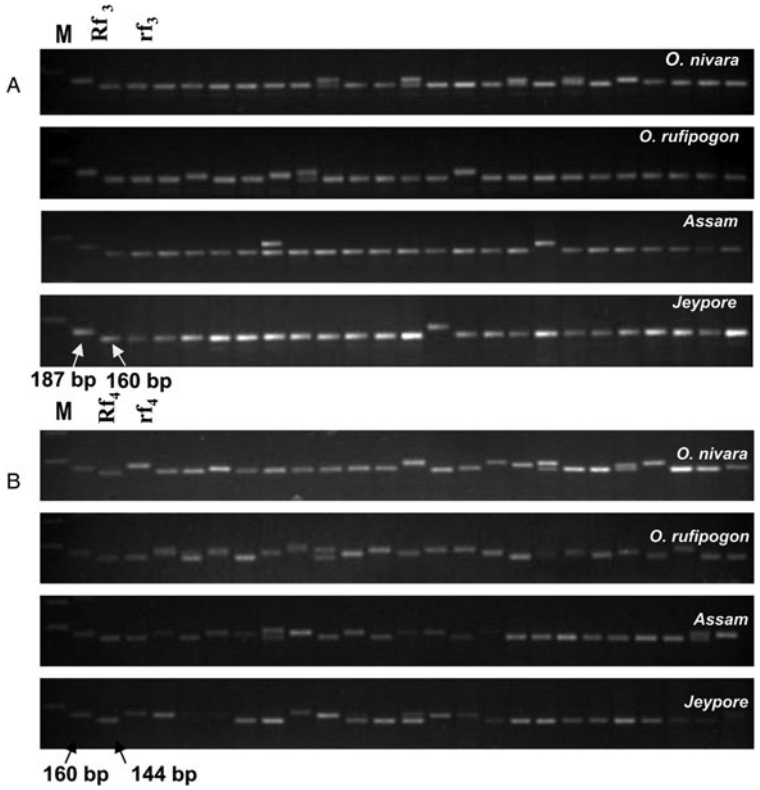

Fig. 1. PCR analysis for identification of fertility restoration genes in different populations. **A** – for *Rf3* gene. **B** – for *Rf4* gene. **M** – Mol. Wt. Marker; **Rf_3** – IR42266-29-3R; **rf_3** – CRMS 31B; **Rf_4** – IR42266-29-3R; **rf_4** – CRMS 31B.

Of the two genes assayed, the occurrence of *Rf4* was significantly higher than *Rf3* in all the populations and the presence of *Rf3* is very low in both Assam (3.8%) and Jeypore (0.85%) collections. The frequencies of *Rf4* (single gene) were 57.55, 49.06, 87.93 and 97.67(%), while the values for *Rf3* were 16.04, 11.32, 6.9 and 2.33 in *O. rufipogon, O. nivara*, Assam and Jeypore collections, respectively. While in most of the accessions, the genes are in homozygous state accessions with these two genes in a heterozygous state was also higher in the wild forms (17.19%) compared with cultivated forms (4.52%).

In the cultivated forms, the presence of both the genes in the same accession was very low. The frequency was 1.09% in Assam collections while none of the accessions collected from Jeypore contain both the genes in the same accession. In the wild relatives, the frequency of accessions having both the genes was 17.18 and 13.38(%) respectively for *O. rufipogon* and *O. nivara*.

The presence of a different kind of allele (the alleles having a different size than the expected sizes with the markers used) was observed only for the *Rf4* gene and in cultivated forms, such alleles are also very low frequency with only two alleles of a different size being recorded in the Jeypore collections. In the wild relatives, the frequency of a different allele was 11.65 and 6.36% of the accessions of *O. rufipogon* and *O. nivara*, respectively.

Discussion

The results suggest that the *Rf* genes were well distributed in the wild relatives while their distribution was low in the cultivated species thus supporting the view of Wang *et al.* (1992), who suggested that greater part of genetic variation in the genus *Oryza* remained intact in the wild relatives. Of the two wild relatives, the high frequency observed for both *Rf* genes in *O. rufipogon* suggest that the genetic wealth is intact in this wild progenitor, where out pollination rates are higher. The presence of new alleles in *O. rufipogon* suggest that this wild progenitor is a reservoir of allelic diversity that remained intact even in the absence of any selection pressure. It is also interesting to observe that the genes in heterozygous state are present in higher frequency in the wild forms (*O. rufipogon* – 22.08%; *O. nivara* – 12.1%) while in cultivated forms, the frequency of heterozygote's was very low, an observation that is in general agreement with previous studies on out-crossing rates (Morishima *et al.*, 1984; Barbier, 1989) of the species and might be related to their survival in the ever-changing environments. The low level of heterozygosity in the cultivated forms might be the result of self-fertilization and though selection was not practiced in the land races, the loss of these genes can be attributed to the self-pollination mechanisms evolved in the cultivated rice and non-

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requirement of an pollination mechanism in a self-pollinated crop.

One of the interesting observations of the study was the wide dissimilarity for the presence of two genes at the two centres of diversity. In both, though *Rf4* is more predominant, its proportion in the population varied. In Assam collections, the proportion of was around 85% while it was 97% in Jeypore collections. While the occurrence of both genes in the same accession was not observed in the Jeypore accessions, their combined presence was recorded in very few accessions of Assam, known to be the primary centre of origin of rice.

For the WA system, the effect of *Rf4* appeared to be slightly larger than that of *Rf3* (Jian and Zhang, 2012; Jian *et al.*, 2013) while the strong dominant gene *Rf3* alone could restore the fertility of WA type (Hossain *et al.*, 2010). From the reports, it can be assumed that different alleles of these genes show varying degrees of restoration depending upon the genotypes employed. Jian *et al.* (2013) had demonstrated varying levels of fertility restoration of WA type in rice by different *Rf* alleles (for *Rf3* and *Rf4*) from different genotypes using SSSLs (single segment substitution lines) suggesting the utility of the alleles from different genetic backgrounds in the improvement of hybrid rice. In this context, the new alleles observed for *Rf4* might be invaluable and though no new alleles were found for *Rf3*, the utility of the alleles available in the population can be examined further for their utility.

The modern varieties are likely to share a higher proportion of alleles with landraces than with wild relatives as most of the elite cultivars are the result of either through selection or hybridization of landraces. Use of new alleles from the wild germplasm may show better response than from the crosses involving landraces as the wild species, with an out pollination mechanism, could possess many favourable characters which can be exploited fully through further studies. As crossing barriers do not exist between *O. rufipogon, O. nivara* and *O. sativa*, the utilization of the *Rf* genes from the wild relatives is feasible and improvement of cultivated rice through introgression of valuable genes from wild germplasm was well documented. In addition, if the genes are introgressed from genetically divergent, low-performing wild or weedy donors, the alleles of interest are likely to be associated with positive transgressive variation in elite genetic backgrounds and can help in development of superior hybrid rice cultivars. Some of these land races that possess *japonica* traits can help in development of hybrids with better performance. The new alleles found in these populations will be great interest for hybrid rice breeding programmes in generation of new parental lines and hybrids.

The utilization of such a diverse gene pool that can provide a wide array of genotypes with *Rf* genes can save the breeders a lot of time as breeding for restorers is one of major activities of the hybrid rice programme. A systematic assessment of the expression levels of the *Rf* alleles in the promising accessions is an essential first step for identification of potential donors. It can be followed by sequencing and analysis to detect the functional aspects of the variation, if any, which can provide additional inputs for the development of superior hybrids. These accessions can also be the ideal experimental material for studies to understand the adaptation mechanisms to different stresses.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262117000090.

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