PGR Newsletter
FAO - BIOVERSITY

Sections
- Latest (Fran�ais, Espa�ol)
- Editorial Office
- Back issues
- About the *PGR Newsletter*
- Instructions for authors
- Guidelines for referees

Search by language

- Articles in English
- Articles in French
- Articles in Spanish

All articles

search

- Contact us
- Bioversity International Home Page
- FAO Home Page

Sections > Short Communication

Published in **Issue No.142,** page 68 to 72

Characterization of red rice (*Oryza sativa*) varieties developed under on-farm dynamic management on Bohol, Philippines

A.R. Bertuso R. van Treuren F.A. van Eeuwijk L. Visser

- Figures
- Appendixes

Introduction

Farmer selection and breeding of local varieties are still practiced in many farming systems and are regarded as important components of on-farm dynamic conservation of plant genetic resources (Bretting and Duvick 1997). The farming system on Bohol, an island province of the Philippines, is comprised of mostly small- to medium-sized irrigated farms that combine both traditional and modern methods of cultivation under a wide range of ecological and soil conditions. Rice is the major food crop in Bohol. As a result of the Green Revolution, many traditional varieties were replaced with modern varieties during the 1970s. Nevertheless, local farmers are still involved in the improvement of available varieties and the development of new ones in order to meet the local agro-ecological conditions, their preferences and needs, and new market opportunities.

While most varieties exhibit a white pericarp, colour variation does occur in rice (Reddy et al. 1995). In the southern parts of Bohol, local farmers traditionally grow rice varieties with a red pericarp, which are preferred over white rice because of their supposed higher nutritional value and market potential. Local knowledge suggested that these farmers' varieties started as modern varieties with white pericarp in which colour variants emerged. However, how this colour variation was introduced into the modern varieties is currently unknown. Several processes may underlie the variation, including mutation within the modern varieties, seed contamination or gene introgression by pollen from other populations. According to the available information obtained from local farmers, grains with red pericarp, sometimes in combination with other off-type characteristics, were selected as starting material and used for seed multiplication in next generations. No conscious backcrossing to the modern varieties has been reported.

Different levels of genetic differentiation may be expected between the new varieties and their progenitors depending on the origin of the variation and the breeding strategy followed. For example, if colour variation has been introduced by mutation, the new varieties are expected to largely resemble their progenitors, even without any backcrossing. In the case where seed contaminants have been selected, high levels of differentiation between the new varieties and their progenitors may be expected. In the case of gene introgression by pollen, a smaller genetic distance can be anticipated. In previous studies, amplified fragment length polymorphism (AFLP) analysis has proved a valuable DNA fingerprinting technique in rice (Mackill et al. 1996; Zhu et al. 1998). This was used in the present study to compare four red rice varieties with their white putative progenitors. Moreover, the eight varieties were characterized by formal morphological analysis and by farmers' classification. The aim of this study was to obtain further insight into the most likely origin of the colour variation and to reveal the mechanisms that farmers have exploited to manage and develop genetic diversity.

Materials and methods

Study material

Eight rice (*Oryza sativa*) varieties were investigated. These were the four red rice varieties known by the local names, '36puwa', '66puwa', '77puwa' and 'RC18sel', and their respective putative progenitors, IR36, IR66, RC10 and RC18. The red varieties were collected in 1999 from three different communities on the island of Bohol (Philippines), whereas their putative progenitors were obtained from the Philippine Rice Research Institute (PhilRice).

AFLP fingerprinting

Seedlings were grown using seeds from each of the eight varieties. From nine randomly chosen seedlings leaf tissue was collected for molecular analysis. DNA extraction basically followed the methods described in Fulton et al. (1995) and AFLP procedures largely followed the protocol described by Vos et al. (1995). All plants were scored for the *Eco*RI primer E13 (E-AG) in combination with each of the *Mse*I primers M49 (M-CAG) and M51 (M-CCA). A total of 116 fragments were scored, of which 42 showed variation in band presence.

Morphological characterization

The eight varieties were grown in an experimental field in the province of Bohol. Each variety was represented by 960 plants. Ten randomly chosen plants per variety were scored for a number of quantitative and qualitative morphological characters based on the standard evaluation system for rice (IRRI 1996). The characters that varied among the eight varieties and that were used for subsequent analysis were leaf blade colour (light green, green), leaf length (cm), leaf width (cm), culm length (cm), culm number (number), culm strength (moderate, strong), scent (not, lightly), panicle length (cm), panicle type (intermediate, compact), panicle exertion (just, moderate, well), panicle threshability (loose, intermediate), lemma and palea colour (straw, brown furrows on straw), spikelet fertility (fertile, highly fertile), 100 grain weight (g), grain length (mm), leaf senescence (early and fast, intermediate) and maturity (number of days from seeding to grain ripening).

Farmers' classification

During the field experiment, all material was also evaluated for a number of traits chosen by a group of 42 randomly selected farmers from Campagao village (Bohol). The variable traits used for analysis were maturity (early, medium, late), number of tillers (number), yield (moderate, high), plant height (short, medium, tall), spikelet fertility (low, moderate, high), drought resistance (moderate, tolerant), panicle shattering (low, high), grain weight (moderate, high), panicle length (short, medium, long), cooking quality (no, yes), eating quality (no, yes) and volume expansion of cooked rice (low, moderate, high).

Data analysis

AFLP data

Genetic distances between varieties were calculated on the basis of those markers that were polymorphic across the total set of genotyped plants. As a precaution, markers that were present or absent only once across all observed plants were disregarded. AFLP patterns on varieties were characterized by a median profile across the plants within a variety and by a measure for intra-variety variation between the plants within a variety. The median profile per variety is obtained by ranking the observed band incidences per AFLP marker and taking the median value, i.e. the value halfway along the ranked band incidences. This procedure is best illustrated by an example. Suppose the observations for the plants 1–9 of a specific variety were 0 0 1 0 1 1 0 0 0, where 1 means the band was observed and 0 that the band was absent. Then these data are ranked from low to high, leading to the series 0 0 0 0 0 0 1 1 1. The median of this series, the fifth value in the ranked series, is 0, so that a 0 is noted for this marker in the median profile for this variety. Varieties were compared on the basis of these median profiles. Genetic similarity between two varieties was calculated as the number of similar markers divided by the total number of markers. This measure is known as the simple matching coefficient (Gordon 1981; section 2.3). Similarities were represented by means of a non-metric multidimensional scaling plot (Gordon 1981; section 5.3).

To express the amount of variation within varieties, the average standard deviation across the markers was used. This was calculated as follows. Let p be the observed band frequency within a variety for a particular marker, and n the number of plants for that variety. Then, calculate $p(1–p)/n$, take the average of this quantity across markers and, finally, take the square root of this average to obtain the desired measure for intra-variety variation.

Morphological and farmers' data

The variety by variable matrix of observations was transformed to a similarity matrix following a procedure outlined by Gower (see Gordon 1981; section 2.3) and implemented in GenStat (Payne 2002). Similarities were calculated for each variable separately and then the average of these similarities was calculated across variables (Gordon 1981; section 2.3). Similarities based on individual variables were Euclidean (1– $[(x_i – x_j)/\text{range}]$ 2, with x_i and x_j observations on varieties i and j) for ordinal and continuously measured traits, while simple matching (1 when $x_i=x_j$, 0 otherwise) was used for binary measured traits. Distances were visualized by a non-metric multidimensional scaling plot.

Results and discussion

For the red rice varieties '36puwa', '66puwa', '77puwa' and 'RC18sel', respectively, 24, 19, 14 and 12 polymorphic AFLP fragments were observed, with average standard deviations for intra-varietal variation ranging from 0.070 to 0.105. These levels of variation were considerably higher than those found for their putative progenitors IR36, IR66, RC10 and RC18 with, respectively, 2, 3, 1 and 6 polymorphic fragments and average standard deviations for intra-varietal variation ranging from 0.017 to 0.043. The six polymorphic markers for RC18 all resulted from a single individual plant that clearly can be considered to constitute an off-type.

The higher levels of intra-variety variation within the red rice varieties ruled out the possibility that they originated from the selection of mutations present within their putative progenitors, because in that case smaller differences in intra-variety variation would be expected between the members of red–white variety pairs. Considering the large differences in variation, it seemed more likely that gene introgression had occurred from other rice populations, thereby introducing novel variation within the modern varieties grown by the local farmers. Upon investigation of the rural areas on Bohol, it appeared that rice populations with red pericarp do occur around the farmers' fields within the study area.

For three AFLP loci, all four modern varieties were fixed for the same allele, whereas the red varieties were either fixed for the alternative allele or showed variation. This result may indicate a relationship between these AFLP fragments and pericarp colour, or point towards a common source of gene introgression into the modern varieties.

Differences in band frequencies between red varieties and their putative progenitors varied markedly among the red–white pairs. Fixations for different alleles were observed at eight loci for the pair RC18/RC18sel, at six loci for the pair RC10/77puwa, at two loci for the pair IR36/36puwa and at none of the loci for the pair IR66/66puwa. The lower levels of intra-varietal variation and higher numbers of different allele fixations with their putative progenitor observed for '77puwa' and 'RC18sel' suggested stronger selection for novel traits after gene introgression than in '36puwa' and '66puwa'.

Differences in selection patterns were also suggested by the results of the scaling plot of the AFLP data, placing '36puwa'and '66puwa' close to their putative progenitors IR36 and IR66, whereas '77puwa' and 'RC18sel' were found more remote from their putative progenitors RC10 and RC18 (**Figure 1A**). Results observed for the morphological data resembled those of the AFLP data, as the varieties occupied similar plot positions as for the AFLP data, with the exception of RC18 and '36puwa' (**Figure 1B**). With respect to the relative distances between the local varieties and their putative progenitors within the pairs of white–red varieties, the farmers' data largely resembled the AFLP data, although the differences within the pair RC18/RC18sel were not as large as observed for the AFLPs (**Figure 1C**).

The scaling plots of the three data sets all indicated a close relationship between IR66 and '66puwa'. This finding is in line with the information of farmers that selection in '66puwa' was performed only on red pericarp, while the other features of IR66 were consciously maintained (see also **Appendix 1**). In contrast to selection patterns employed to obtain '66puwa', selection resulting in the other three new varieties was more complex. Whereas AFLP and farmers' data showed convergence between IR36 and '36puwa', the two varieties were found more distinct based on the morphological data. Furthermore, RC18 was found closer to 'RC18sel' based on the morphological data and farmers' data than based on the AFLP data. According to the information obtained from the farmers, off-types from IR36 were not only selected based on red pericarp, but also on longer panicles and higher spikelet fertility. In addition to red pericarp, culm length was also selected in the development of 'RC18sel'. Selection of these additional characters, possibly in combination with correlated characters, may have contributed substantially to the observed differentiation, albeit that for each variety pair such differentiation was only evident in one of the characterization approaches. Successful phenotypic selection apparently has not yet resulted in strong genetic differentiation between '36puwa' and IR36.

No information could be obtained about additional selection criteria in RC10. Strong selection for off-type characteristics in RC10 may explain the large differentiation between RC10 and '77puwa' observed for all three data sets. Scent could have been an additional selected character, as selection for this character is known to occur on Bohol.

In the farmers' data analysis, the configuration of the white–red variety pairs with respect to each other differed markedly from those based on the AFLP and morphological data. This finding may be related to the way material was evaluated by the farmers, assuming a strong focus on within-pair comparisons, rather than on determining precise relationships between the pairs.

Despite the self-fertilizing nature, out-crossing rates may vary from 1% - 5% in cultivated rice (Singh 1983). Particularly for the pairs IR36/36puwa and IR66/66puwa, introduction of the novel variation by gene introgression seemed more likely because of fertilization by pollen from neighbouring stands than to seed contamination. In the latter case, it would have been quite unlikely to observe such close genetic resemblance between the white and red varieties within these pairs.

The combined results suggest that following gene introgression by pollen, selection schemes were employed by local farmers depending on their preferences, resulting in new phenotypes with new combinations of traits. The study illustrates the role that farmers play in the development of new varieties and in increasing the level of intra-varietal diversity of uniform modern varieties. Moreover, the study shows that modern varieties are used for further breeding and selection by farmers because they currently do not meet their requirements exactly.

Acknowledgements

The role of the farmers of Bohol by making their varieties available for analysis and by providing associated information is gratefully acknowledged. This research was funded by the Community Biodiversity Development and Conservation Program through the Centre for Genetic Resources, The Netherlands. This program is funded by the HIVOS/NOVIB Biodiversity Fund, the International Development Research Centre (IDRC), the Swedish International Development Cooperation Agency (SIDA) and the Development Fund of Norway. The authors would like to thank Teresita Borromeo of the University of the Philippines Los Banos for providing the seeds of the modern varieties and for her supervision during the experimental study in the Philippines.

References

Bretting PK, Duvick DN. 1997. Dynamic conservation of plant genetic resources. Advances in Agronomy 61:1–51.

Fulton TM, Chunwongse J, Tanksley SD. 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Molecular Biology Reporter 13:207–209.

Gordon AD. 1981. Classification. Chapman and Hall, London, UK.

IRRI. 1996. Standard Evaluation System for Rice. IRRI, the Philippines.

Mackill DJ, Zhang Z, Redoña ED, Colowit PM. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39:969–977.

Payne RW. 2002. The Guide to GenStat, release 6.1, part 2: Statistics. Lawes Agricultural Trust, Rothamsted Experimental Station, UK.

Reddy VS, Dash S, Reddy AR. 1995. Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarp. Theoretical and Applied Genetics 91:301–312.

Singh BD. 1983. Plant breeding: principles and methods. Alyani Publishers, India.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

Zhu J, Gale MD, Quarrie S, Jackson MT, Bryan GJ. 1998. AFLP markers for the study of rice biodiversity. Theoretical and Applied Genetics 96:602–611.

Contact PGRN-manuscripts@cgiar.org about this page