New Molecular Marker Technologies for Pearl Millet Improvement

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At a time when most of the world still viewed molecular technology as a luxury, for use only with major staple crops, a DFID-JIC-ICRISAT project anticipated as early as 1991 the application of molecular diagnostics in the breeding of orphan crops for developing countries.

The first molecular marker-based genetic linkage map of pearl millet [*Pennisetum glaucum* (L.) R. Br.] was built with restriction fragment-length polymorphisms (RFLPs), the marker system of choice in the early 1990s (Liu et al. 1994). This map has served as the base for subsequent pearl millet marker-based studies at JIC (Busso et al. 1996, 2000; Devos and Gale 2000; Devos et al. 2000; Liu et al. 1996, 1997). The RFLP framework in the consensus map now available (Fig. 1, see pages 18–19 of this issue) is based on 173 (out of 500 available) mapped *Pst*I genomic clones from inbred line Tift 23DB, which has now become the base genotype for pearl millet molecular genetics. The clones are available as DNA or, in some cases, as DNA sequences, and have been distributed freely worldwide.

ICRISAT was able to build one of the very early molecular marker facilities in the CGIAR system in the early 1990s, and has used this facility for pearl millet diversity assessment (Bhattacharjee et al. 2002), mapping population skeleton map construction (Azhaguvel 2001; Kolesnikova-Allen 2001), and marker-assisted backcrossing (Sharma 2001). The markers and maps have also been used at CAZS and IGER in the UK, Université d'Orsay in Paris, and Tifton in the USA, to map and tag genes controlling important traits in the pearl millet crop. These include downy mildew resistance (Jones et al. 1995 and 2002; Azhaguvel 2001; Kolesnikova-Allen 2001), foliar disease resistance (Morgan et al. 1998), drought tolerance (Yadav et al. 2002), plant height (Azhaguvel 2001), flowering time, and the multiple phenotypic changes that occurred when pearl millet was domesticated – the so-called 'domestication syndrome' (Poncet et al. 2000, 2002).

Molecular marker technologies have moved on, particularly with the development of the polymerase chain reaction (PCR) that allows the rapid and inexpensive amplification of small quantities of DNA precisely targeted to known regions. The amplification commences from small lengths of DNA of known sequence known as primers. Depending on the primers, different segments of DNA are amplified in the PCR reaction. The program was quick to develop the first microsatellite markers (simple sequence repeats – SSRs) in pearl millet (Allouis et al. 2001; Qi et al. 2001). Some 100 markers, of which 60 are mapped (Fig. 1), are now available either as DNA primers for laboratories without the facility to make them themselves, or as DNA sequences of the flanking regions of the SSR. A silver-staining detection system has been developed that is more suited for SSR applications in developing countries because it does not require the use of radioactive labeling. We aim to continue development to about 200 SSRs but are already anticipating the next technological development, single nucleotide polymorphism (SNPs) for application in pearl millet (Fig. 2), which can also be handled by PCR.

The uptake of molecular marker technology at ICRISAT is central to the program, not only for applications in the breeding program, but also as a developing country-based test bed, and as an intermediate technology for further transfer to commercial and national laboratories in India and Africa. Recent work with the new SSR markers has determined that optimum working conditions – for example, amplification regimes and Mg^{+} levels – can vary markedly with locally supplied chemical resources.

The development of the pearl millet maps and markers has provided a nucleus around which other millet resources and technologies have been developed. Among these is the first pearl millet bacterial artificial chromosome (BAC) library (Allouis et al. 2001). This library is necessary for experiments that identify the precise location of particular pearl millet genes in order to be able to clone them.

The very first UK plant genome database is MilletGenes, which is based at JIC. MilletGenes was initiated with DFID funding and has now been incorporated into the BBSRC-funded UK CropNet programme. MilletGenes collates all genome related data – maps, markers, DNA sequences and images – on pearl millet, finger millet (*Eleusine coracana* Gaertn.), foxtail millet [*Setaria italica* (L.) P. Beauv.], and tef [*Eragrostis tef* (Zucc.) Trotter], a related crop of importance in Ethiopia. Among the new technologies is genetic transformation of pearl millet, achieved both in a small PSP-funded project at Bangalore in India, in an EU-INCO project at the University of Hamburg, Germany, and at Foodtek in Pretoria, South Africa.

Integration of the Pearl Millet Map in the Grass Consensus Map

Today we know quite a lot about the 2,400 million base-pair *Pennisetum glaucum* genome. The seven chromosomes that make up the haploid complement are well mapped and have an unusual profile in which recombination is exceptionally biased towards the chromosome ends. As with other 'diploids' we are detecting several ancient duplications in the genome, and some 28% of the RFLP probes map to more than one locus. Some of the linkage groups now include the chromosome ends (the telomeres), although alignment with the cytological map has still to be achieved.

These results show complex relationships, within which can be detected the now classical evolutionary translocations that define the Andropogonae group within the grasses. These alignments are quite adequate to allow the rice genomic sequence, which is now becoming available, to be applied directly to pearl millet improvement. A comparative analysis of the small foxtail millet genome (C=450 Mb), a member of the *Paniceae* tribe which also includes pearl millet, with rice (C=400 Mb) revealed a simple relationship between the chromosomes of the two species (Devos et al. 1998). The larger pearl millet genome, on the other hand, appears to have undergone many rearrangements relative to foxtail millet and rice (Fig. 3, see color plate on page 21 of this issue) with the maps of rice, although gene orders have remained conserved within each of the translocated segments (Devos et al. 2000). Most of these rearrangements are likely to be specific to pearl millet. However, at least two could be identified that are common to all *Panicoideae* species analysed to date. Nevertheless, since both foxtail and pearl millet belong to the same tribe, it is clear that some species undergo and fix rearrangements more readily than others, and that the number of gross structural rearrangements alone is not a measure for evolutionary divergence. The comparative data further demonstrated the presence of a major duplication between regions of pearl millet linkage groups 1 and 4. The same duplication is present in rice and foxtail millet, and must therefore predate the divergence of the *Panicoideae* and *Oryzoideae* subfamilies. The integrated maps can now be exploited for a range of applications, including gene prediction, fine-mapping, identification of candidate genes, and elucidation of metabolic pathways.

Applications

Knowledge of the relationship between the pearl millet genome and those of other grass species has many applications. Firstly, the number of markers available for genetic studies has greatly increased. The availability of comparative maps will allow the use of sequences from the target species as well as genes from other grasses as probes in mapping and tagging studies. Secondly, since conserved colinearity extends to genes controlling key traits, comparative genetic maps may be used to predict the presence of genes. Extrapolation and prediction from one species to another will benefit all crop plants, but especially those 'orphan' species for which only limited genetic information is available. Comparative genome analysis provides a link between genetics and taxonomy. The occurrence of genome rearrangements that are common between some species and differentiate others are good indicators of phylogeny. It may also pave the way to gene isolation in pearl millet. The high degree of colinearity that exists at the gene level between grass species irrespective of their total DNA content, has already promoted the use of small genome species such as rice and sorghum as intermediates for map-based cloning of genes in large genome species such as wheat and maize (Kilian et al. 1995; Foote et al. 1997; Chen et al. 1997).

Figure 1 (see pages 18–19 of this issue). Updated JIC consensus map for pearl millet showing distribution of RFLP, SSR and isozyme loci across seven linkage groups and a linkage fragment. Because this is a consensus map derived from several mapping populations, not all markers are mapped against one another and therefore some markers are positioned with less precision than others. Black bars to the right hand side and green bars to the left hand side of each linkage group indicate the limits of precision of placement of some markers. The chromosomes of pearl millet (*Pennisetum glaucum*, 2n = 2x = 14) are now well mapped with restriction fragment length polymorphism (RFLP in black), sequence tagged site (STS in red) and microsatellite (SSR in green) markers. The markers are used both by breeders for marker-aided selection of genes controlling agronomic traits, and also by researchers for discovering new agronomic genes and for map-based gene isolation.

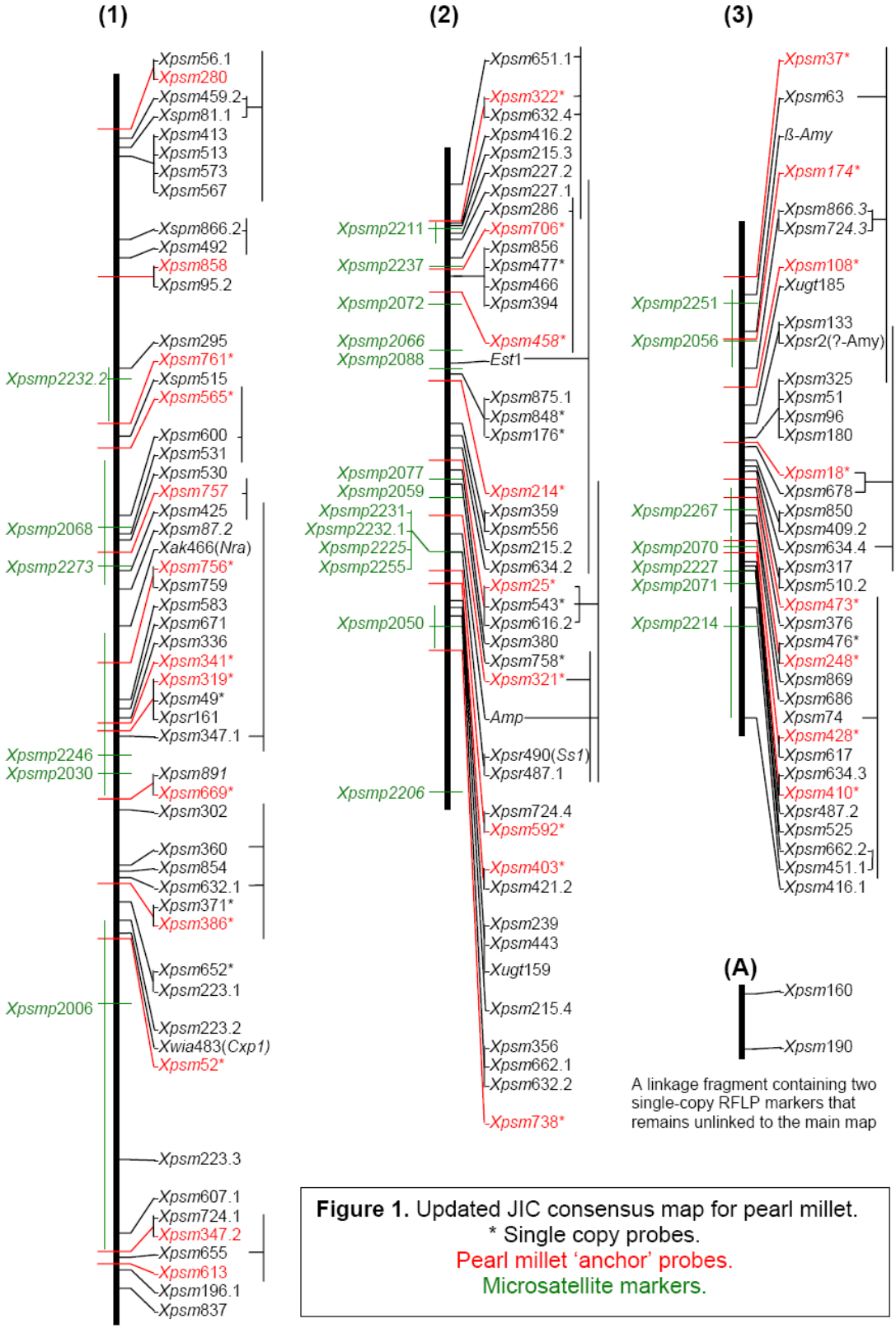
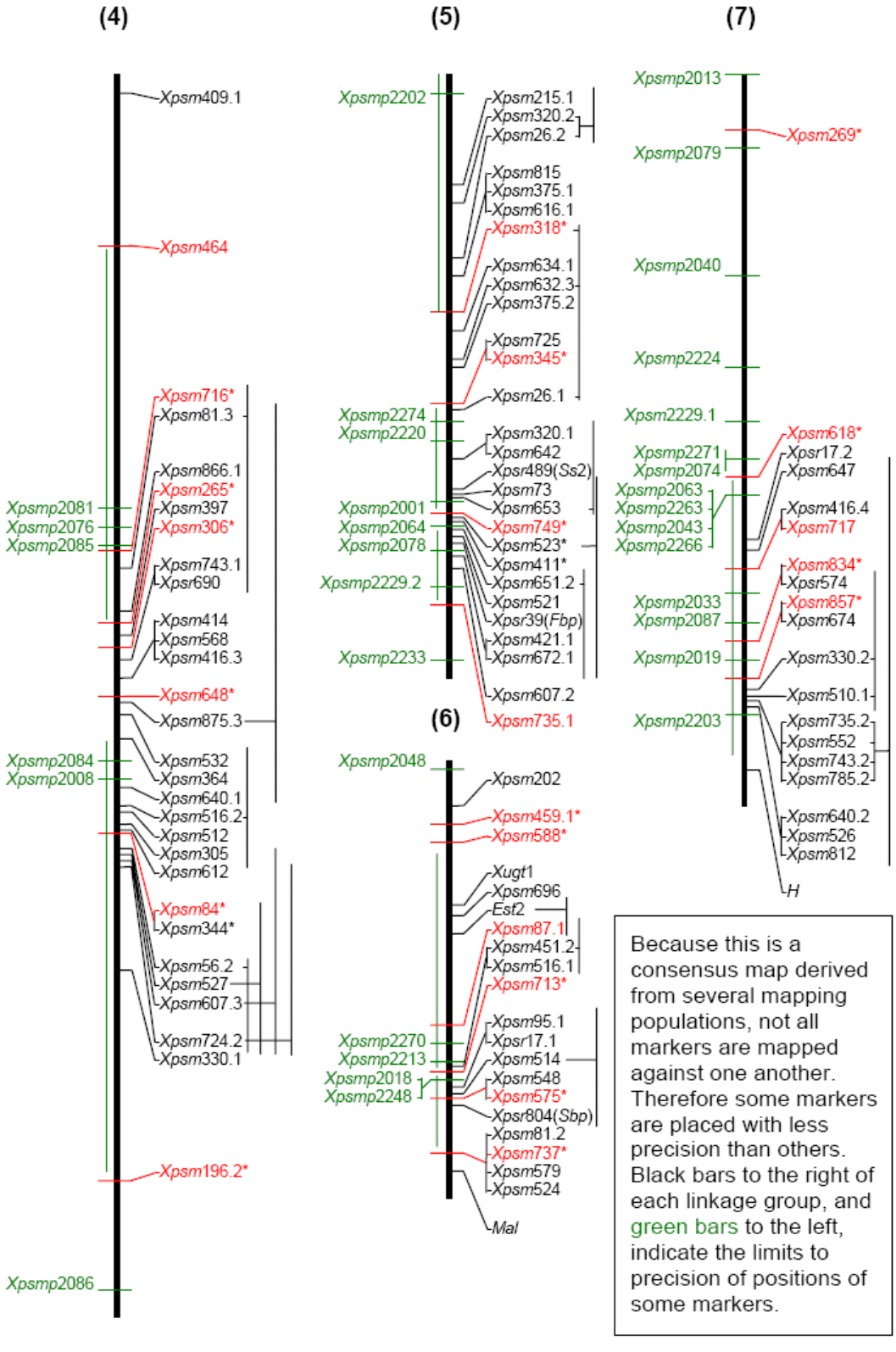


Figure 1. Updated JIC consensus map for pearl millet.
* Single copy probes.
Pearl millet 'anchor' probes.
Microsatellite markers.



Because this is a consensus map derived from several mapping populations, not all markers are mapped against one another. Therefore some markers are placed with less precision than others. Black bars to the right of each linkage group, and green bars to the left, indicate the limits to precision of positions of some markers.

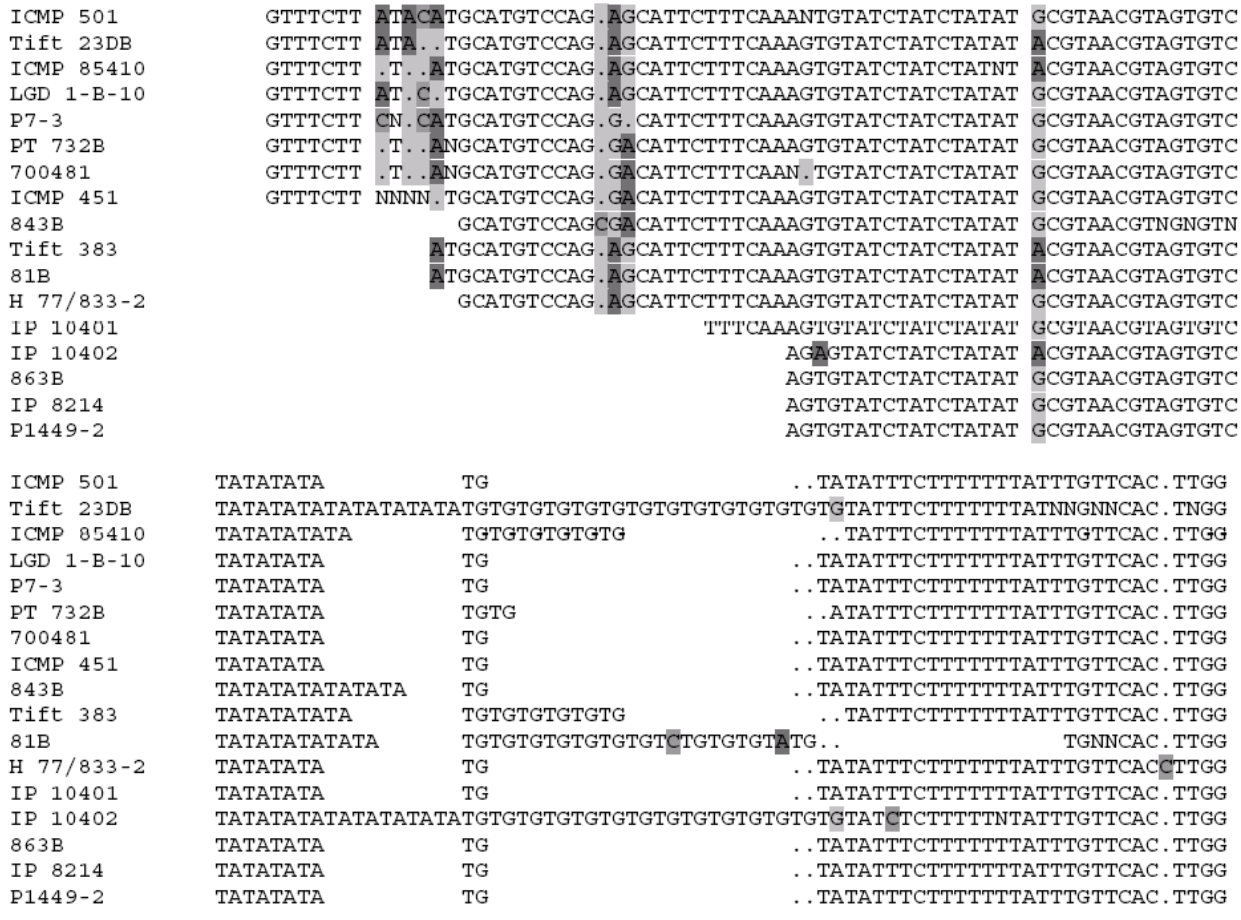


Figure 2. Single nucleotide polymorphism (SNPs) in pearl millet inbred lines. Molecular marker technology has moved on from the early days of restriction length polymorphisms (RFLPs), which are slow and expensive to apply, to simple sequence repeats (microsatellites or SSRs), which can be analyzed on automatic sequencing machines. We are now anticipating the next generation of markers, SNPs. In this DNA sequence analysis of 17 pearl millet inbred lines, variation in tandem repeat number at a microsatellite locus is shown (different numbers of TA and TG di-nucleotide repeats) along with SNPs and 'indels' (inserted or deleted base pairs shaded) in the flanking DNA sequence. SNPs are amenable to yet faster and more economic analysis than SSRs.

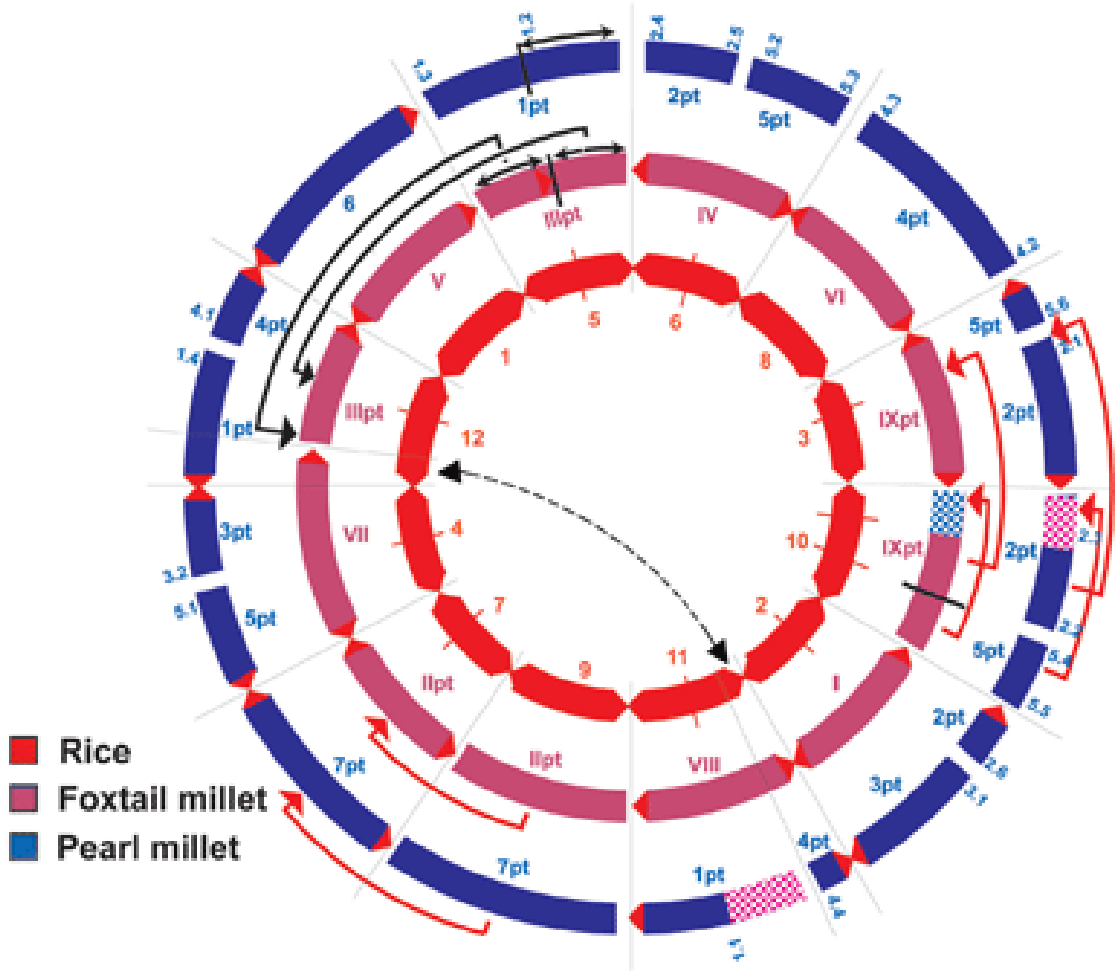


Figure 3. Relationships among the genomes of rice, foxtail millet and pearl millet based on comparative RFLP mapping studies (Devos et al. 1998, 2000). Rice chromosomes (in red) are numbered from 1 to 12 with arabic numerals. Foxtail millet linkage groups (in pink) are numbered with roman numerals and pearl millet linkage groups (in blue) are numbered with arabic numerals. Hatched areas indicate regions with little available comparative data. Red triangles indicate telomeres, double-headed arrows show inversions, and single-headed arrows denote evolutionary translocations. In pearl millet, due to the large number of rearrangements relative to rice, the majority of the arrows are omitted. The dotted arrow indicates the rice 11S/12S duplication.

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