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A proposed mechanism for loss of heterozygosity in rice hybrids

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Summary

A phenomenon, loss of heterozygosity (LOH), was discovered in hybrid plants involving a selected plant (named AMR) of the Chinese rice cultivar 'Zhongxin No. 1' as one parent. In these hybrids and some of their progenies, somatic variations were manifested by molecular genotypes and/or morphological phenotypes in vegetative parts of the same plant. Random amplified polymorphic DNA (RAPD) markers for the parents have been followed through the F_3 generation. RAPD markers were uniformly present or absent in all plants within some or all F_2 panicle rows derived from F_1 hybrids involving AMR. In contrast, RAPD markers segregated in the Mendelian manner for dominant markers in panicle rows derived from control hybrids. Certain F_2 panicle rows from F_1 hybrids involving the special rice became fixed for all assayed RAPD markers. Genotype fixation was confirmed by molecular assays and field observations of the F_3 progenies. We propose a new biological mechanism, called 'assortment mitosis,' as being responsible for the observed phenomenon. The use of this mechanism in plant hybrids allows the development of uniform progenies as early as the F_2 generation. Therefore, the time required to obtain fixed non-parental type progenies for subsequent performance trials can be drastically shortened. Utilizing this mechanism in plant breeding represents a new approach and requires the modification of conventional plant breeding procedures.

Abbreviations: LOH – loss of heterozygosity; RAPD – Random amplified polymorphic DNA

Introduction

Breeding new inbred cultivars of self-pollinated crops, such as rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), and wheat (*Triticum aestivum* L. and *T. durum* Desf.), usually requires 10 or more years due to the need for many generations of selfing following hybridization of two parental lines (Fehr, 1987). Anther culture (Nitsch & Nitsch, 1969; Sunderland, 1970) or chromosome elimination following wide hybridization (Kasha & Kao, 1970; Barclay, 1975) can be used to shorten the breeding cycle of some crops. However, these techniques require time- or labor-consuming procedures to obtain haploids and the subsequent use of colchicine treatment for chromosome doubling to obtain homozygous dihaploids. Apomixis, asexual reproduction through seeds, would completely eliminate the need to achieve homozygosity in hybrids for maintaining uniformity in a plant population (Hanna & Bashaw, 1987). However, apomixis is not presently available for use in grain crops.

Loss of heterozygosity (LOH) has been reported in and is frequently a common feature of human cancerous cells (Knudson, 1985; Vogelstein et al., 1989; Chigira et al., 1993). Due to the detrimental effect of cancers and the fact that human beings cannot have offsprings by selfing, the genetic consequences of this phenomenon in humans has not been further investigated. Studies on LOH have suggested that a chromosomal mechanism, such as mitotic crossover, rather than a mutational event may be responsible for cancers and other hereditary diseases in human (Faruqi et al., 1994). Although mitotic pairing has been observed in tissue-culture regenerated plants (Wen et al., 1989) and homozygous regenerants have been observed in tissue culture experiments, LOH in somatic

cells of seed-propagated plants was not reported until recently (Wang et al., 1999a).

A specific plant (named AMR by us) from the Chinese rice cultivar 'Zhongxin No.1' resulted in some uniform F_2 panicle rows when it was used as a parent in crosses with U.S. rice cultivars (Wang et al., 1999a). Apomixis was not responsible for the early fixation of these hybrids because those uniform rice lines were not identical to heterozygous hybrids of the parental plants. The high frequency of occurrence of these uniform F_2 panicle rows precludes the possibility that random Mendelian segregation during meiosis and random gamete combination can be responsible for the accelerated homozygosity at most or all loci. Furthermore, the affected loci covered more than six of 12 rice chromosomes (Wang et al., 1999b), making mutation an unlikely cause of LOH.

In this study, a mechanism is proposed and evidence is presented to account for the loss of heterozygosity and somatic variations *in vivo*. The use of this phenomenon will require modifications in plant breeding procedures to achieve accelerated genotype fixation of hybrid progenies.

Materials and methods

A specific plant (AMR for assortment mitosis rice) identified from the Chinese rice cultivar 'Zhongxin No.1' was crossed with three commercial U.S. rice cultivars 'Kaybonnet', 'M202', and 'L202'. Zhongxin No.1 is a japonica rice derived from a cross involving the Chinese rice line 84-15, which was proposed to be apomictic (Chen, 1992) but was later proven to be normally sexual (Shi et al., 1996). Two other Chinese rice cultivars, 'Dragon Eyeball 100' and 'Bloody Sticky', were crossed with the U.S. cultivar 'Rosemont' as control hybrids. Dragon Eyeball 100 and Bloody Sticky are indica rice genotypes, whereas all U.S. rice cultivars are classified as javanica-type.

The F_2 of AMR and control hybrids were planted in panicle rows comprised of from 5 to 15 plants each. The random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990, 1991) was used to determine the genotype of all F_1 hybrids and F_2 progenies. F_2 panicle rows uniform for all assayed RAPD markers were selected for the verification of genotype fixation by fingerprinting 24 F_3 plants. DNA was also separately extracted from leaves and/or young panicles of different tillers from AMR hybrids and then assayed by the RAPD technique. RAPD assays and

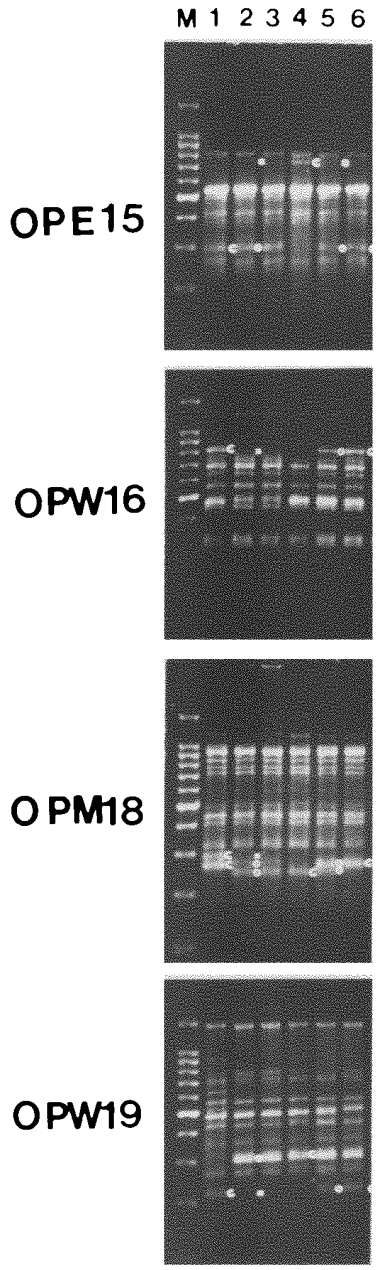

Figure 1. RAPD profiles of leaf DNA extracted from M-202 (lane 1), two F_1 plants of AMR × M-202 (lanes 2, 3), AMR (lane 4), one F_1 plant of AMR × L-202 (lane 5), and L-202 (lane 6) assayed with four Operon Technologies' random primers. Half-circles indicate the RAPD markers that are polymorphic between the two parents, square bars indicate the lost markers and full-circles indicate the markers expected in the hybrids. Molecular size markers (lane M) are 1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp from top to bottom with the 500 bp marker in a two-fold concentration.

Table 1. Number of uniform and segregating RAPD markers in F_2 panicle rows of the crosses 'Dragon Eyeball 100' × 'Rosemont' (control 1), 'Bloody Sticky' × 'Rosemont' (control 2), 'AMR' × 'Kaybonnet', 'AMR' × 'M202' (two F_1 hybrids), and 'AMR' × 'L202'

F_1 hybrids & characterization	F_2 panicle rows									
	1	2	3	4	5	6	7	8	9	10
Dragon Eyeball 100 × Rosemont (No. RAPD markers assayed = 10)										
No. uniform markers	0	0	0	2	1	0	1	1	0	1
No. segregating markers	10	10	10	8	9	10	9	9	10	9
Bloody Sticky' × 'Rosemont (No. RAPD markers assayed = 12)										
No. uniform markers	0	2	3	0	2	3				
No. segregating markers	12	10	9	12	10	9				
AMR × Kaybonnet (No. RAPD markers assayed = 4)										
No. uniform markers	0	1	2	1	0	2				
No. segregating markers	4	3	2	3	4	2				
AMR × M202 #1 (No. RAPD markers assayed = 14)										
No. uniform markers	14	14	13	13	11	13	13			
No. segregating markers	0	0	1	1	3	1	1			
AMR × M202 #2 (No. RAPD markers assayed = 14)										
No. uniform markers	14	14	13	13	14	13	14	14		
No. segregating markers	0	0	1	1	0	1	0	0		
AMR × L202 (No. RAPD markers assayed = 14)										
No. uniform markers	3	2	11	11	12	12	12	11		
No. segregating markers	11	12	3	3	2	2	2	3		

Table 2. Morphological traits of parents and F_2/F_3 panicle rows of AMR × M202 and AMR × L202 hybrids

Lines	No. Plants	Plant height (cm)	Heading (Days)	Flag leaf width (cm)	Grain shape	Glume
AMR	17	112.4 ± 6.5	97	1.48 ± 0.15	short	pubescent
M202	17	109.0 ± 4.3	115	1.66 ± 0.10	long	glabrous
L202	18	101.5 ± 3.6	91	1.46 ± 0.08	long	glabrous
AMR × M202						
row 3080 F_2	17	99.7 ± 5.3	106	1.69 ± 0.07	short	pubescent
row 3089 F_2	18	104.7 ± 6.2	105	1.72 ± 0.08	short	pubescent
row 6509 F_3	18	83.6 ± 7.3	103	1.56 ± 0.12	short	pubescent
row 6511 F_3	17	87.2 ± 9.3	107	1.59 ± 0.17	short	pubescent
row 6516 F_3	18	87.4 ± 5.3	97	1.58 ± 0.09	short	pubescent
row 6518 F_3	18	90.6 ± 6.1	99	1.64 ± 0.10	short	pubescent
row 6560 F_3	18	87.2 ± 4.5	101	1.61 ± 0.07	short	pubescent
row 6581 F_3	11	83.7 ± 7.5	101	1.55 ± 0.13	short	pubescent
AMR × L202						
row 2183 F_2	17	124.7 ± 10.0	79	1.59 ± 0.18	long	segregating
row 2189 F_2	18	117.5 ± 6.6	76	2.51 ± 0.20	long	pubescent
row 2203 F_2	16	106.0 ± 5.7	77	2.59 ± 0.28	long	pubescent
row 2206 F_2	18	105.9 ± 5.3	77	2.58 ± 0.13	long	pubescent
row 2209 F_2	17	107.2 ± 4.8	74	2.44 ± 0.28	long	pubescent

Southern hybridization procedures described by Wang et al. (1999a) were used.

Results and discussion

The AMR plant identified from the Chinese rice cultivar 'Zhongxin No.1' when crossed with commercial U.S. rice cultivars resulted in some uniform F_2 panicle rows (Wang et al., 1999a; Table 1). Apomixis cannot account for the early fixation of these hybrids because the uniform rice lines are neither genetically nor morphologically identical to the heterozygous F_1 hybrids (Figure 1 & Table 2). AMR × M202 progenies, which had 13 or 14 uniform molecular markers (Table 1), had grain shape and glume pubescence of the maternal parent, but their heading dates were intermediate to the two parents and their plant heights were shorter than either parent (Table 2). On the other hand, all AMR × L202 progenies were uniformly long-grained and earlier in heading than both parents. Four panicle rows of AMR × L202 that had 11 or 12 uniform RAPD markers (Table 1) were uniform for wider leaf blade than either parent and were also uniform for glume pubescence. The panicle row 2183, which had 3 uniform and 11 segregating RAPD markers (Table 1), had narrow leaf blade as the two parents and was segregating for glume pubescence (Table 2). The high percentage of uniform F_2 panicle rows precludes the possibility that random Mendelian segregation during meiosis and random gamete combination are responsible. An alternative mechanism must be invoked to explain the accelerated rate at which homozygosity was reached at most or all loci.

Uniform F_2 progenies are observed only when AMR is one of the parents and F_2 seeds are planted in panicle rows. Thus, we tested the hypothesis that different tillers (thus panicles) of each F_1 plant may have differing genotypes that are not of the F_1 genotype expected from the cross. The random amplified polymorphic DNA (RAPD) technique was used to test this hypothesis. A RAPD marker is generally dominant so that both maternal and paternal markers are expected to be present in F_1 hybrids and then segregate in a Mendelian manner in F_2 progenies. DNA was separately extracted from leaves of different tillers and then assayed by the RAPD technique using primers that produced polymorphic bands between the parents. Indeed RAPD results confirmed that, in two of three crosses involving AMR and U.S. rice lines, loss of heterozygosity occurred in F_1 hybrids so that RAPD markers from either male or female parent could be absent in all the F_2 plants in some or all panicle rows. In the cross between AMR and U.S. rice 'M202,' both of the two F_1 plants exhibited loss of heterozygosity in the sampled leaf tissues of the hybrid (Figure 1). The two F_1 hybrids have lost eight RAPD markers (two from AMR and six from M-202) but exhibited three markers each of AMR and M202 in the leaves of sampled tillers (Wang et al., 1999a). In contrast, all leaves sampled from a hybrid of AMR × L202 exhibited the RAPD bands from both parents. Nevertheless, loss of heterozygosity eventually occurred and was manifested in the F_2 progenies of AMR × L-202. Only in these two hybrids, did the loss of RAPD markers occur in all F_2 plants of some panicle rows resulting in different segregation patterns among different panicle rows. Loss of heterozygosity tagged by a RAPD marker was never observed in the control crosses.

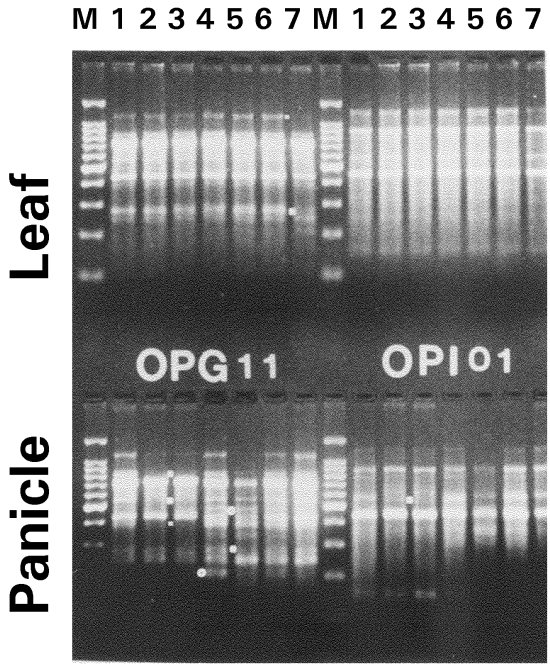

Figure 2. RAPD profiles for leaf (top) and corresponding panicle (bottom) DNA extracted from different tillers of a F_2 plant of the cross AMR × Kaybonnet having different panicle types and assayed with random primers OPG11 and OPI01. Square bars depict lost bands and circles indicate variant bands present. Note the presence of more variations among panicles, and between panicles and leaves, than among leaves.

Somatic variations were also observed between leaf and panicle DNA of the same tiller, as well as among panicle DNAs (Figure 2), in an F_2 plant of the cross AMR × 'Kaybonnet' with panicles having

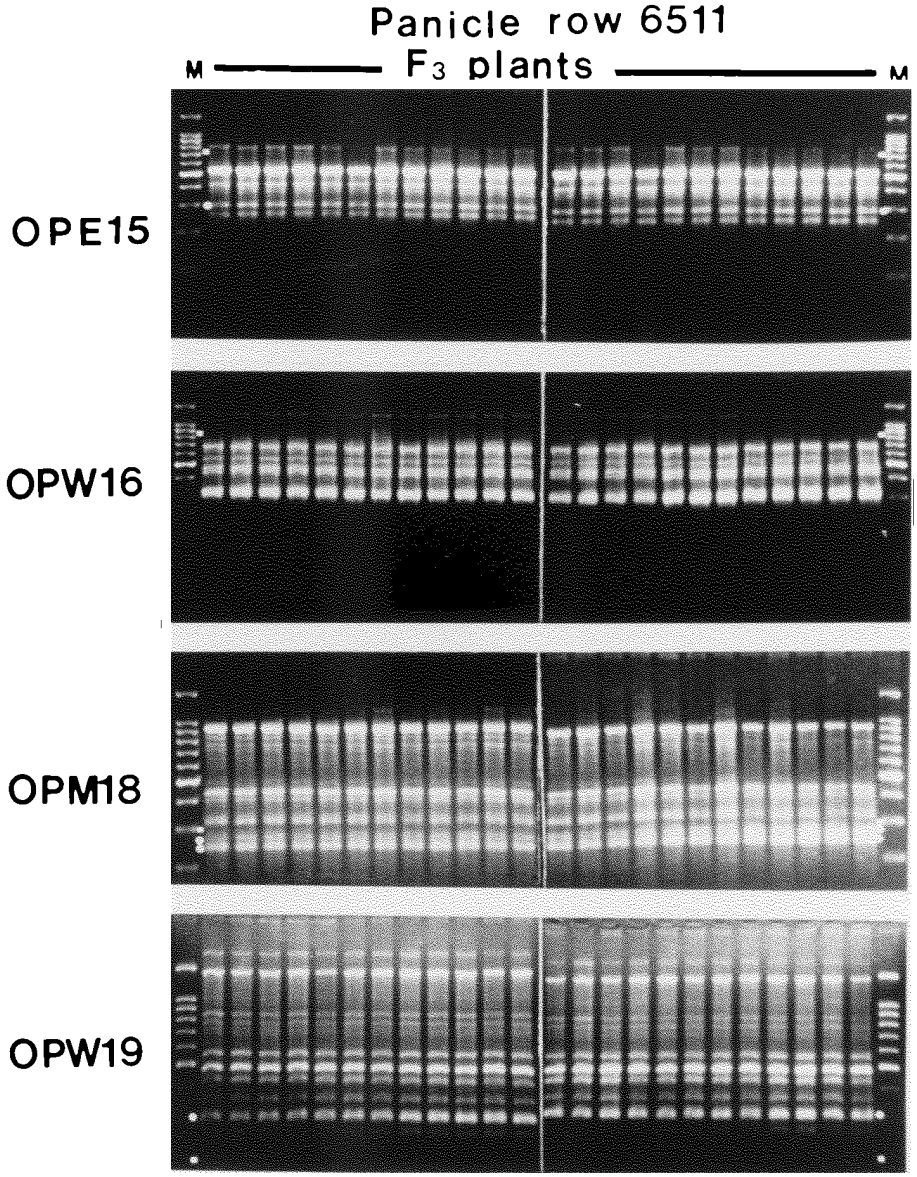

Figure 3. RAPD profiles for 24 individual plants in the uniform F_3 panicle row 6511 assayed with four primers. Square bars indicate the lost markers and full-circles indicate the markers expected in the hybrids. Note the uniformity for both the presence and absence of markers.

obvious variant morphology. The molecular data support the hypothesis that hybrids that involve AMR as a parent often contain chimeric tissues or organs. Chimerism in plants can be caused by mutation, transposable elements, chromosomal variations, and differential placement of parental genomes in wide hybrids (McClintock, 1950, 1951; Fedoroff, 1983; Schwarzacher et al., 1992; Li et al., 1998). None of these could lead to uniform progenies with stable 2n chromosomal ploidy and assorted or recombined parental traits. Mitotic recombination has been reported in many living organisms including higher plants, sometimes leading to very few cells with the homozygous condition for those loci on the pair of chromosomes involved in somatic crossover (Hunt, 1987; Newton, 1989; Cote & Gyftodimou, 1991). However, this mechanism cannot result in homozygosity in most or all chromosomes of an organism. All the seeds of parents and hybrids in our studies were germinated in petri dishes before transplanting into pots. Twin-seedling was never ob-

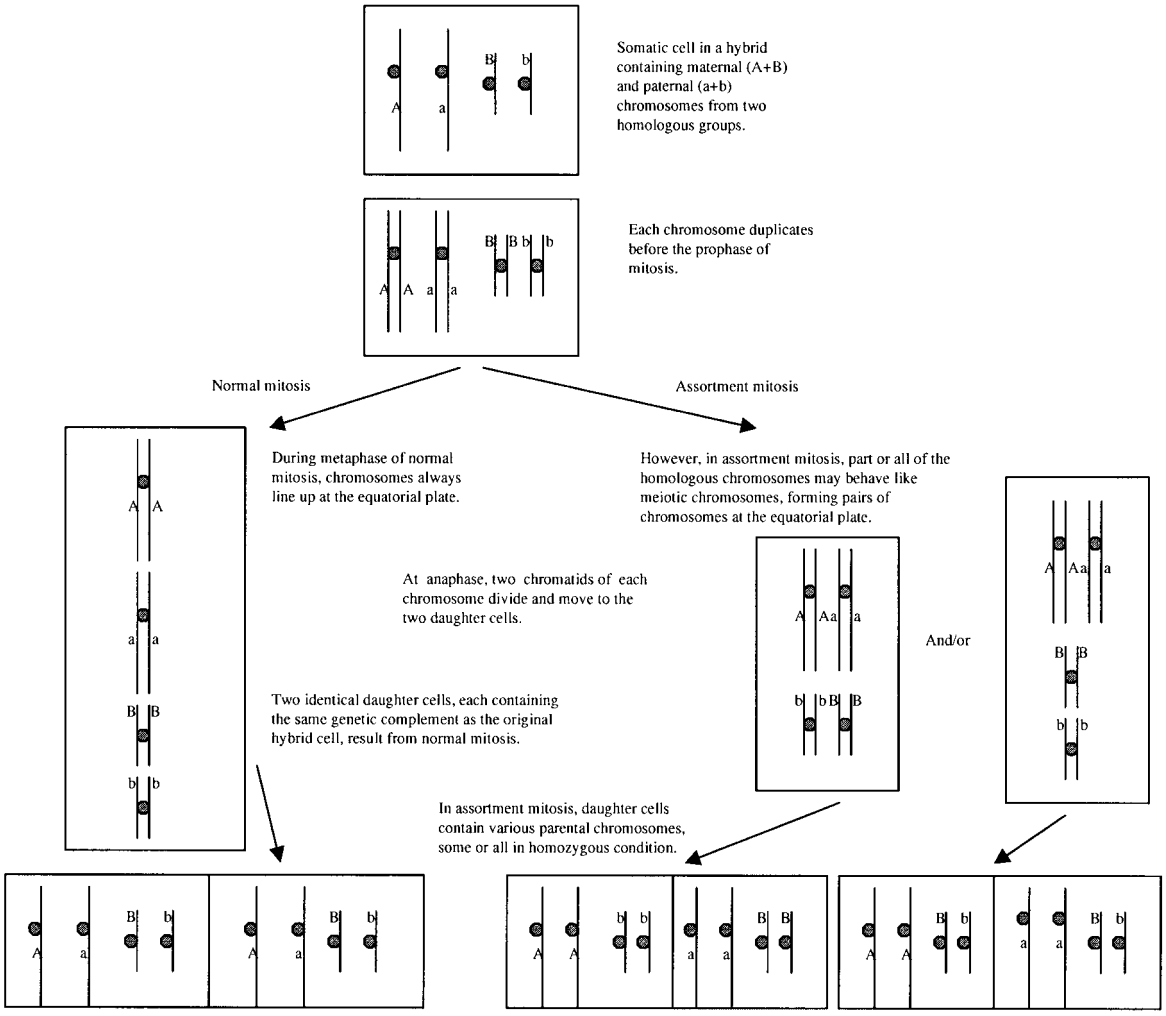

Figure 4. A diagram showing different chromosome compositions in mitotic daughter cells following 'assortment mitosis' in contrast to the heterozygous condition in two identical daughter cells derived from normal mitosis.

served, hence, it cannot explain the variations among tillers and the early fixation of genotypes in hybrids of AMR (Figure 3).

Therefore, we propose here a new biological mechanism to explain accelerated homozygosity in progenies of rice hybrids involving AMR as a parent. We call this phenomenon 'assortment mitosis' (Figure 4) because of the chromosomal assortment that occurs during this unusual mitosis. We hypothesize that some, if not all, homologous chromosomes in some somatic cells of AMR hybrids behave like meiotic homologues, i.e, they pair and then align along the equatorial plate at mitotic metaphase. The homologues, one each from the female and male parents, subsequently separate and randomly assort into daughter cells. Because there is no further division, the two chromatids of each chromosome separate to form two chromosomes, thus maintaining the 2n ploidy. Immediate homozygosity of all the loci on those homologues that have not undergone mitotic crossing-over is achieved by 'assortment mitosis.' Any single crossing-over would result in heterozygosity in the two daughter cells for the loci distal to the chisma. When the daughter cells of 'assortment mitosis' develop into germ-line cells and, eventually, into panicles or seeds, the anticipated result is uniformity or reduced segregation among plants in some panicle rows in subsequent generations. Occasionally, this somatic segregation can be manifested as chimera for a visible trait, such as panicle morphology.

The time and location that 'assortment mitosis' occurs may be a function of the genotype of the other parent. In the hybrid AMR × M202, 'assortment mitosis' must have occurred so early that most of the tillers originated from a daughter cell that no longer contained all chromosomes from both parents (Figure 1). On the other hand, 'assortment mitosis' must have occurred late in the ontogeny of hybrid AMR × L202. As a result, no leave tissues exhibited unique RAPD profiles that deviated from the expected F_1 profiles (Figure 1). The frequency of uniform panicle rows apparently depends on the time and location that 'assortment mitosis' occurs. Panicle rows grown from seeds of AMR × M202 had the highest frequency of uniform panicle rows (Wang et al., 1999a).

'Assortment mitosis' that leads to loss of heterozygosity can result in immediate homozygosity for a majority, if not all, of the loci in the rice genome. If these loci affect important and visible agronomic traits, the hybrid progenies can be phenotypically uniform and still maintain heterozygosity for less obvious traits. Therefore, this phenomenon can be used to shorten the breeding time required to develop new rice cultivars. Similarly, loss of heterozygosity may be used for breeding other crops in which homozygous inbred lines are needed if and when gene(s) for loss of heterozygosity can be found in or transferred to other crops. To detect and utilize LOH in crop improvement, panicles (or spikes) on F_1 hybrids and their offsprings must be harvested and planted separately, i.e. the F_2 seeds cannot be harvested in bulk. Extra effort in harvesting and planting the F_2 seeds may save many years of breeding by finding uniform inbreds in the F_2 or F_3 generation.

Whether the proposed mechanism can also account for the non-segregating F_2 population derived from another rice hybrid (Wu et al., 1999) needs to be investigated. If assortment mitosis is responsible for early genotype fixation in that cross, the gene(s) controlling this phenomenon must be present in one (more likely the SAR-3) of the parental lines of that cross. Additionally, it is interesting that the mechanism we proposed here is identical to the one suggested by Sybenga (2000) in his comment on our paper (Wang et al., 1999a).

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