RESEARCH NOTE

Monosomic analysis reveals duplicated chromosomal segments in maize genome

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Introduction

Maize (*Zea mays* L.) is a major cereal food crop in the world, and because of its economic importance it has long been a focus of genetic research. It has somatic chromosome number $2n = 20$ and regularly forms 10 bivalents during meiosis (Rhoades 1955). Recent advances in molecular cytogenetics have amply demonstrated the significance of aneuploids in evolution and crop improvement. Monosomics ($2n - 1$) are the most valuable among the various types of aneuploids used in cytogenetic and genetic studies, chromosome engineering and gene mapping (Weber 1991, 1994). Monosomy in polyploid species produces a relatively small phenotypic effect because a polyploid contains homoeologous chromosomes, thus a monosomic chromosome in a polyploid is not present in a truly hemizygous condition. Hence, nullisomic gametes ($n - 1$) function in allopolyploid forms and progeny of a monosomic plant includes parental monosomics (Sears 1954).

The individual chromosome of the genome in a monosomic has distinct effect on cellular processes and development. The loss of a chromosome in a diploid species has a far more drastic effect than in a polyploid species. Monosomy in a diploid plant causes genic imbalances consequently altering enzyme levels and modifies phenotype of monosomic plant (Birchler and Newton 1981). Monosomics in diploid crop plants were first discovered in the progeny of $3n \times 2n$ and $2n + 1 \times 2n$ crosses of maize (McClintock 1929; Einset 1943) and later artificially induced using X-irradiated pollen in maize (Baker and Morgan 1966) and tomato (Khush and Rick 1966).

Maize genome has long been known to possess duplicated genes (Rhoades 1951), and both isozyme (Wendell *et al*. 1986) and restriction fragment length polymorphism (RFLP) (Helentjaris *et al*. 1988) analyses have demonstrated that maize genome contains duplicated chromosomal segments with colinear gene arrangements. DNA sequence analysis has revealed the segmental allotetraploid origin of maize (Gaut and Dobley 1997). Weber (1973, 1983) utilized a genetic system (*r-X1* mutation on chromosome 10), which generates a high frequency of monosomics (10%–18%) to isolate a complete series of monosomics in maize. However, univalent chromosome behaviour in monosomics during meiosis remains poorly understood (Weber 1994). Here, we report the identification of monosomic plants for chromosomes 2, 4, 6, 8 and 10 of maize in the F_1 population of *R/r-X1* deficiency × Mangelsdorf's tester crosses. Cytogenetic analysis revealed that the monosomic chromosome remains unpaired at diakinesis and metaphase I in monosomics for chromosomes 4, 6 and 10. However, in monosomic 2 and monosomic 8, the monosomic chromosome frequently paired with other unidentified bivalents indicating the presence of duplicated chromosomal segments in maize genome.

Materials and methods

Development and identification of monosomics

Genetic stocks used for the development of monosomics were obtained from Maize Genetics Cooperation Stock Center, University of Illinois, Urbana, USA. Monosomics were developed in maize utilizing *r-X1* deficiency genetic stock and identified by the expression of recessive genetic markers and chromosome analyses (Weber 1994). The plants grown out of *r-X1* marker-bearing kernels (identified by mottled kernel colour phenotype) were crossed with pollen from Mangelsdorf's multiple chromosome tester possessing a recessive gene on each of its 10 chromosomes (see table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

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F1 plants grown out of colourless kernels (from *R/r-X1* deficiency × Mangelsdorf's tester crosses) were screened, and plants expressing *lg*, *j* or *g* recessive phenotypes were identified as presumptive monosomics for chromosomes 2, 8 or 10, respectively. These monosomics were confirmed by chromosome counts at diakinesis, metaphase I and anaphase I in pollen mother cells (PMCs). Monosomics for chromosomes 4 and 6 were identified by crossing semi-sterile plants of sub-normal stature (which also showed 9II + 1I chromosome associations at diakinesis) with monosomic tester. Monosomics 6 and 4 plants produced all kernels with white (*y*/*y*/*y*) endosperms and sugary (*su*/*su*/*su*) endosperms, respectively when test crossed while other monosomic types and diploids gave a 1:1 ratio of white (*y*/*y*/*y*) versus yellow (*Y*/*Y*/*y*) and sugary (*su*/*su*/*su*) versus non-sugary (*Su*/*Su*/*su*) endosperms, respectively.

Meiotic analysis

Anthers from putative monosomic plants were smeared in 2% acetocarmine for 2–3 min on dry slides. Chromosome numbers were determined from at least five well spread PMCs at different stages of meiosis. The behaviour of univalent chromosome was studied by recording the percentage of meiocytes possessing different chromosome associations at various stages of meiosis.

Results and discussion

Maize monosomic ($2n - 1 = 19$) plants were isolated using *r-X1* deficiency genetic stock carrying a deletion on long arm of chromosome 10 covering the *R* locus. This deletion causes nondisjunction of chromosomes at second embryo sac mitosis producing $n - 1$ and $n + 1$, egg cells (Simcox *et al*. 1987). *R/r-X1* plants with dominant marker genes on each of the 10 chromosomes on test crossing with Mangelsdorf's tester stock, yielded 54.3% coloured (*Y*/*y*, *R*/*r*) and 45.7% colourless (*Y/y, r-X1/r*) kernels. Colourless kernels carrying *r-X1* deficiency and mutant plant phenotypes mostly produced monosomes. The transmission of *r-X1* deficiency through female parent was 45.7%. The *r-X1* deficiency was in heterozygous condition and hence 50 per cent kernels are expected to carry the deficiency. This was indicated by the χ^2 test performed on the individual progeny. However, the pooled data showed a marginally significant deviation from 1:1 ratio ($\chi^2 = 6.35$; $P < 0.05$). Weber (1983) reported 40%–45% transmission of *r-X1* deficiency through eggs.

Identification of monosomics

Monosomics for chromosome 2 expressed liguleless leaf phenotype due to hemizygous condition of recessive gene *lg*. Two monosomic-4 plants were identified after test crossing with monosomic tester (see table 2 in electronic supplementary material). Monosomic-6 plants were identified by the cytological analysis (figure 1, *a*&*b*). Plants monosomic for chromosome 6 were also confirmed by the genetic test. On test crossing with *yy* tester, the monosomic 6 produced only the white kernels with *y/y/y* endosperm. However, some of the endosperms might possess *y/y* or *y/y/y/y* constitution due to non-disjunction of *y* carrying chromosome 6 during embryo sac development.

Monosomic-8 plants expressed japonica stripes on the leaves due to hemizygous condition of recessive allele *j* and also contained 9II +1I at diakinesis and metaphase I. However, few plants carrying deletion for *J* locus also expressed the japonica phenotype but these plants could be distinguished from monosomic-8 because these flowered earlier and were vigorous than the monosomics. Weber (1991) also reported the deletion for *J* locus and observed that the deficiency carrying plants are larger and flower earlier than monosomics. Lin (1987) gave the cytological evidence for the deletion of *J* locus produced by the *r-X1* deficiency and reported partial monosomic 8 plants. In the present study, the deletion for *J* locus was detected by the presence of a heteromorphic bivalent or two univalents in addition to nine bivalents at diakinesis and metaphase I in the large plants expressing japonica stripes. Monosomic-10 expressed golden plant phenotype and also had 9II + 1I at diakinesis and metaphase I. Two plants also expressed golden plant phenotype due to deletion of *G* locus during embryo sac mitosis in the *R/r-X1* plant. These plants were vigorous and showed 9II + 2I or 9II + 1 heteromorphic bivalent.

Meiotic chromosome behaviour in monosomics

Monosomics provide the ideal opportunity to analyse univalent chromosome behaviour because each meiotic cell contains a univalent chromosome. The behaviour of univalent chromosome at pachytene revealed that in monosomics 4, 6 and 10, the monosomic chromosome remains as a univalent. In the monosomic-6, illegitimate (fold-back) pairing was observed in univalent chromosome 6 near the end of long arm. Weber (1994) reported that this behaviour of univalent 6 may be because it is attached to the nucleolus and hence less free to move during synapsis. However, McClintock (1933) stated that illegitimate pairing is the characteristic behaviour of unpaired chromosome segments.

Diakinesis analysis in the monosomics 4, 6 and 10 revealed that the monosomic chromosome remains as a univalent and forms 9II + 1I chromosome associations quite frequently. However, in few cells 8II + 3I associations were also observed suggesting precocious separation of a bivalent (table 1). In the monosomics 2 and 8, the monosomic chromosome paired with other unidentified bivalent, thereby, forming a trivalent configuration quite frequently (figure 1, *g*&*h*; table 1). However, no trivalent was observed at metaphase I in these monosomics. Moreover, in these monosomics, relatively higher per cent of PMCs had a univalent located at the metaphase-I plate (table 1). This indicates that the non-homologous (homoeologous) pairing was not strong enough to carry up to metaphase I. It was supported by the fact that the monosomic chromosome paired

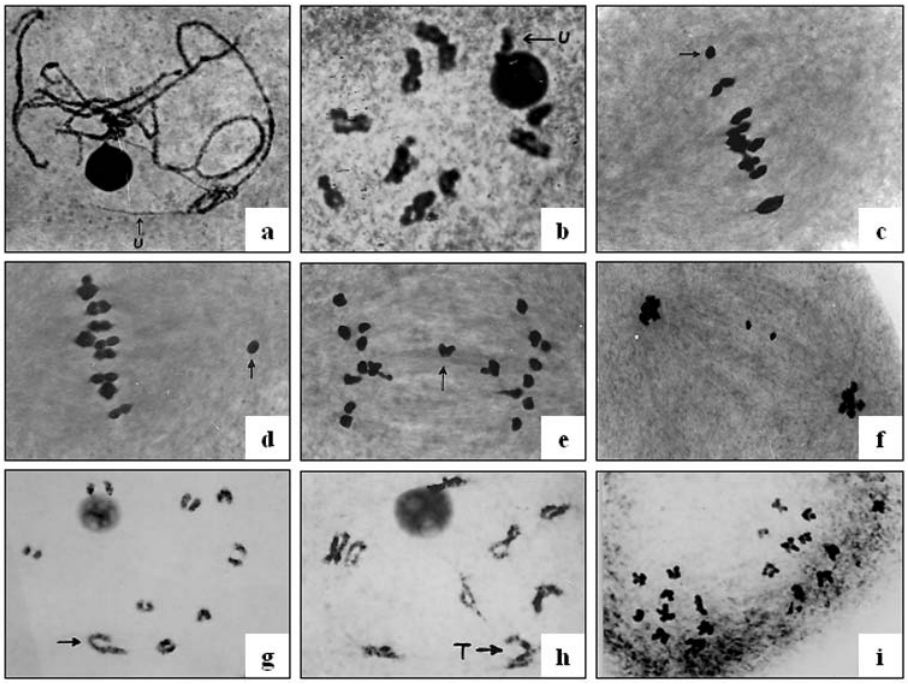

Figure 1. Behaviour of univalent chromosome during meiosis in maize monosomic-6 plant (*a–f*). (*a*) Univalent chromosome attached to nucleolus during pachytene. (*b*) Univalent chromosome 6 attached to nucleolus during diplotene. (*c*) Metaphase I showing 9II+1I with univalent chromosome arranged on metaphase-I plate. (*d*) Metaphase I showing 9II+1I with univalent chromosome lies off metaphase-I plate. (*e*) Anaphase-I showing 9 – 9 chromosome disjunction with univalent chromosome lagging at metaphase plate. (*f*) Late anaphase-I showing equational division of univalent chromosome and two lagging monads. (*g*) Diakinesis in monosomic 2 showing 8II+1III with precocious separation of most of bivalents. The trivalent formed by homoeologous pairing of univalent chromosome 2 with some unidentified bivalent. (*h*) Diplotene in monosomic 8 showing 8II+1III, the trivalent formed by homoeologous pairing of univalent chromosome 8 with some unidentified bivalent. (*i*) Prophase-II in monosomic-6 plant showing cell pair with 9 – 10 chromosomes, univalent had passed undivided to one pole during anaphase-I.

Table 1. Univalent chromosome behaviour during diakinesis and metaphase I in the monosomics analysed.

	Diakinesis				Metaphase I			
	Chromosome associations (%)			Total PMCs	Univalent lies on MI plate(%)		Univalent lies off MI	Total PMCs
Monosomic type	9II + 1I	8II+ 1III	8II+3I	studied	Middle	End	plate(%)	studied
Monosomic 2	81.1	17.8	1.1	276	15.1	28.3	56.6	198
Monosomic 4	99.2	0.0	0.8	260	12.9	24.2	62.9	186
Monosomic 6	98.3	0.0	1.7	289	5.5	22.9	71.6	236
Monosomic 8	50.6	47.6	1.8	431	26.1	28.1	45.8	345
Monosomic 10	98.7	0.0	1.3	395	6.3	20.5	73.2	287

with the homoeologous bivalent at one end only. Hence, the trivalent associations observed at diakinesis might have resolved into a bivalent and a univalent at metaphase I partly due to chiasma terminalization and pulling force operating during the co-orientation movement of paired chromosomes. This assumption was also supported by the increased frequency of univalents arranged on metaphase-I plates in monosomics 2 and 8. These observations suggest that pairing did not involve longer segments of chromosomes and indicate the presence of small duplicated chromosomal segments in the genome of maize.

Weber (1983) observed 0.7% of the PMCs with a trivalent in monosomics of maize and suggested that the trivalent configuration is also generated when a univalent is adjacent or superimposed upon a bivalent and many of the trivalents might be artifacts. But he did not mention in

Table 2. Univalent chromosome behaviour during anaphase I and prophase II in the monosomics analysed.

	Late anaphase I				Prophase II				
	Chromosome disjunction pattern (%)			Total PMCs studied	Cell pairs with chromosomes (%)				Total PMCs studied
Monosomic type	10 – 9	9 – 9 + 1L	9 – 9 +2M		10 – 9	9 + M 9 + M	9 + M9	9 – 9	
Monosomic 2	40.0	46.9	13.1	130	52.2	24.8	5.3	17.7	113
Monosomic 4	62.4	27.2	10.4	125	65.1	22.1	0.0	12.8	86
Monosomic 6	60.5	30.0	9.5	210	61.9	20.0	3.8	14.3	105
Monosomic 8	52.7	36.7	10.6	150	54.4	22.4	2.4	20.8	125
Monosomic 10	56.2	40.4	3.4	146	61.0	18.2	0.0	20.8	77
Average	54.4	36.2	9.4		58.9	21.5	2.3	17.3	

L, laggard; M, monad.

which monosomics trivalents were observed. Helentjaris *et al*. (1988) using RFLP analysis in maize monosomics, discovered and mapped duplicate loci in the maize genome. They reported that 13 pairs of duplicate loci had one copy each in chromosomes 2 and 7, while 10 other pairs of duplicate loci had one copy in chromosome 3 and the other in chromosome 8. Thus, much of the maize genome is represented as low copy of duplicate-nucleotide sequences.

The observations on univalent chromosome behaviour at metaphase I revealed that in majority of cells, the univalent located off the equatorial plate and when the univalent was on the plate it was located at the end of the plate as if it was pushed towards one side (table 1). At anaphase I, the univalent passed undivided to one of the pole in more than 50% PMCs and this behaviour of univalent chromosome does not differ significantly in all the monosomics except in monosomic 2 where it is passed to one pole in 40% cells (table 2). In the 36.2% cells, the univalent was lagging at the equatorial plate or in the cytoplasm (figure 1, *c–e*). The lagging was highest in monosomic 2, may be due to higher frequency of univalent located at metaphase-I plate. So during anaphase I it may lag and show splitted halves. In 9.4% PMCs, the equational division of univalent chromosome was observed. The equational division of univalent was highest in monosomic-2 and lowest in the monosomic 10 (table 2). This may be because of relative length of the univalent chromosome. Weber (1983) observed that 52.9% cells contained no lagging chromosomes and 33.4% cells had one laggard at telophase-I in monosomics. However, 12.5% cells showed the equational division of the univalent chromosome.

The irregular behaviour of the univalent chromosome during anaphase-I was also reflected in the chromosome composition of prophase-II cell nuclei. In 58.9% cell pairs, the univalent was included in one of the cell (figure 1,*i*) while in 21.5% cell pairs the univalent was divided equationally and present as a monad. However, 2.3% cell pairs showed nine univalents plus one monad in one cell and only nine univalents in the other cell indicating loss of one monad. In 17.3% cells only nine univalents were present in each cell indicating the loss of monosomic chromosome (univalent) at anaphase I and the loss was comparatively more in monosomics 8, 2 and 10.

The cytogenetic studies on monosomics are useful for both the strategic and applied research in crop plants. Monosomic maize plants were generated using the *r-X1* deficiency system, and the monosomy was confirmed both genetically and cytologically. These chromosomally engineered stocks can successfully be exploited for genetic manipulation of crop plants.

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