Genetic Behavior of Distinct B Chromosome in Safflower

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Summary The present study documents the presence of 3 B chromosomes in safflower, which is one of the first such reports. During the present investigation, B chromosomes were found to induce a twofold effect on planting, firstly a reduction in chiasma frequency in euchromatic region, and secondly depression in the percentage of pollen fertility due to an increase in the number of unpaired chromosomes. Although the presence of B_s depressed the chiasma frequency of the regular complement, the frequency of A and B chromosomes does not appear to be related. No correlation between B chromosomes and phenotype has been reported. Thus, it can be predicted that the presence of additional DNA as B chromosomes can bring about some structural or numerical changes in the long run and may affect the genetic integrity of the plant and thus result in a changed genotype with negligible effect on the morphology and vigor of the plant.

Key words B chromosomes, Chiasma frequency, Pollen fertility, Safflower.

Since the first discovery of B chromosomes in maize (Longley 1927), these have been found in more than 1000 living species, (Beukeboom 1994). Cheng *et al.* (2000) and Kaniki (2009) enumerated the main characteristics of B chromosomes *i.e.*, they are present besides the normal diploid content of the plant, they are darkly stained, short in length, morphologically distinguishable from A_s, usually moving apart from A chromosomes and drifting in the nucleus as a univalent, and follow a non-Mendelian pattern of inheritance. Earlier works envisaged B chromosomes as dispensable supernumerary chromosome additions to the standard A chromosome complement of a species that may or may not be present in certain tissues, individuals, or populations. B chromosomes do not pair and recombine with any of the A chromosomes at meiosis, are often heterochromatic, and usually do not contain major genes except for ribosomal DNA (rDNA) sequences that have been mapped on many plant and animal B chromosomes (Jones and Rees 1982, Jones 1995). They could arrange themselves along with the A-chromosomes on the equatorial plane and move to the poles during anaphase. In some cases, they would lag in anaphase leading to their elimination from the cell (Masood and Fatemeh 2005).

B chromosomes may be transmitted through generations via non-disjunction of B chromosomes during pre- or postmeiotic mitosis and B genome carrier germ cells usually participate in gametogenesis and fertilization (Dhar *et al.* 2002). Nur (1962) has elaborated that B chromosomes are non-vital (occurring in only some individuals within a species) and typically have accumulation mechanisms that increase their transmission prior, during, or following gametogenesis. Chiasma formation and distribution, like other features of meiosis, are subject to stringent genetic control (Rees 1961, Baker *et al.* 1976) as several genes essential for encoding proteins required for pairing and synapsis are involved in catalyzing key steps in DNA breakage, repair, and recombination (Roeder 1997, Zickler and Kleckner 1999). Like A chromosomes, B chromosomes may significantly change the chiasma frequency thereby affecting the genetic constitution of the

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gametes as reported in *Festuca* (Jauhar and Crane 1990), *Aegilops* (Sheidai *et al.* 2002) and *Avena* (Sheidai *et al.* 2003).

There are a number of plants like *Plantago*, soybean, *Trigonella*, maize, rye, *Pennisetum*, *Crepis* *etc.* where B chromosomes have been reported, but reports of B chromosomes in safflower is rare. Thus, the present paper illustrates the cytological implications of extra B chromosomes in carrier and non-carrier plants in the natural population of safflower.

Materials and methods

From the natural population of safflower (*Carthamus tinctorius* L. var. A1) ($2n=24$), approximately 50 plants were randomly selected for screening regarding the presence of B chromosomes out of which 6 plants were reported to contain B chromosomes (B carriers). Young floral buds of the plants selected were fixed in 1:3 acetic alcohol solutions for 24 h, after which they were transferred to 70% alcohol and stored at 4°C. Slides were prepared using anther smear squash technique with 2% acetocarmine.

In order to calculate the chiasma frequency, about 100 pollen mother cells (PMCs) were studied from each plant at various stages from metaphase I to anaphase II.

To estimate pollen fertility, mature anthers were rubbed over glass slides and 1 drop of 2% acetocarmine was added. After 5 min of staining, the number of fertile (fully stained pollen grains) and sterile (without stain) pollen grains was counted under microscope.

Results

Out of 50 plants tested, only 6 plants (12%) were found to possess B chromosomes in the natural population of safflower. In plants No. 1 and 5, 2B chromosomes were reported, and in plant No. 2, 1B chromosomes was reported. In plant Nos. 4 and 3, however, 3B chromosomes were found. They were mostly round in shape, varying in number from 1–3, smaller than A-chromosomes and moving apart from them *i.e.* not pairing with them or among themselves as pictographed in the plates (Figs. A–L). In the control non-carrier plant, 12 bivalents were seen at diakinesis which arranged themselves at equatorial plate at metaphase I (Fig. A). At anaphase I, however, these bivalents separated and moved towards respective poles as univalents (Fig. B). After completion of meiosis, 4 daughter nuclei with a haploid set genome were found while those containing B chromosomes had the following configurations, $n=12+1B$ (Figs. C–I), $n=12+2B$ (Fig. J) where both B chromosomes were at one pole, or at different poles that neither pair with themselves nor with A chromosome, $n=12+3B$ (Figs. K–L) where two B chromosome were located closely and one moved apart. This might be due to the fact that their number varies between and within cultivars.

Morphologically, the carrier and non-carrier plants were indistinguishable and no difference in the life pattern of the plants was noticed.

B chromosomes had a marked effect in reducing chiasma frequency in pollen mother cells. The range of chiasma frequency per chromosome of non B-carrier plants was between 0.78–0.85 (0.82 ± 0.049). The B carrier plants, however, possess 2 types of PMCs, one with the configurations $n=12+1B$, $n=12+2B$ and $n=12+3B$ and the other with $n=12$. The B carrier plants recorded a decrease in chiasma frequency in the range 0.70–0.75 (0.72 ± 0.035) While it was recorded to be between 0.75–0.84 (0.79 ± 0.038) in non-carrier PMCs of carrier plants. Thus it is clear that the presence of B chromosome significantly influenced the chiasma frequency.

As regards the pollen fertility percentage, it was recorded to be 98.12 ± 0.08 in non-carrier plants. However, in non-carrier PMCs of carrier plants it was recorded to be 97.35 ± 0.75 and 90.50 ± 0.62 in carrier PMCs. Thus B carrying plants are less fertile than the 0B type, whereas the Bs have no significant effects on plant vigor.

Fig. 1. Meiotic cells with no B chromosomes (A and B) and with B chromosomes (C–L). A: normal metaphase I, B: normal anaphase I. C and D: One B chromosome at metaphase I/II, E–H: One B chromosome at anaphase I, I: B chromosome involved in bridge formation. J: 2 B chromosomes at anaphase II. K and L: Three B chromosomes at metaphase and anaphase. Arrows show B chromosomes. Bar=10 μ m.

Discussion

Although there are reports mentioning the presence of the highest number of the B chromosomes in members of families Poaceae and Asteraceae (Jones *et al.* 2008), tracing B chromosomes, especially in safflower, is very difficult due to the problem of chromosome stickiness, poor staining ability and lack of research work on the cytology of safflower. Keeping in view its ethnopharmacological importance as a future protein factory and its property of reducing the cholesterol levels in blood, its cytogenetical aspects have been greatly ignored.

Our results shows that in safflower, B chromosomes have a definite influence on mean chiasma frequency as well as on fertility, as also supported by studies on rye (Müntzing 1949, Jones and Rees 1967) *Plantago coronopous* (Paliwal and Hyde 1959) and in *Impatiens* (Raghuvanshi and Mahajan 1982, Kumar and Dixit 2002).

According to Khush (1973), the genetic imbalance caused by the presence of B chromosomes, makes the pollen very sensitive to aneuploidy. As a rule, unbalanced male gametes ($1n+1$) cannot compete with euploid ones ($1n$) and extra chromosomes are transmitted at a reduced rate through males. They do not possess any visible phenotypic effect on the plant carrying them because they contain no major genes with phenotypic effects on organism development. As previously reported, B chromosomes were all detected in natural varieties and existing populations, so the researchers could not study from where B chromosomes had actually come. These kinds of obstacles have limited the formulation of hypotheses regarding the origin of B chromosomes (Cheng *et al.* 2000). The presence of B chromosomes in the future can create new and autonomous elements as components of the genome (Jones *et al.* 2008). The potential application of B chromosomes is for the modification and mapping of the A genome, and to investigate the numerical or structural heterogeneity of the chromosomes. In the long run, unlike all the normal chromosomes, the B chromosome, due to its distinctive origin, could be useful in studying aneuploid/allopolyploid variation in plants or, due to its misdividing activity, could result in fully/semi mortile grains. As regards the function of B chromosome, there is enormous evidence for their role in the modification of A-chromosome chiasma frequency and distribution as well as in causing sterility. Although reports show that the B chromosomes were mostly inert in nature having no effect of their own, when their number exceeds they can clearly effect the vigor and fertility of the plant (Camancho *et al.* 2000, Green 2004), except in the case of *Plantago coronopous* where a single B chromosome induced total male sterility (Paliwal and Hyde 1959), and in *Haplopappus gracilis* where the color of achene was changed by the presence of a single B chromosome (Jackson and Newmark 1960). Our results, showing a decrease in pollen fertility with an increase in the number of Bs as regard to plants carrying $0B_s$ were in concordance with studies conducted by González-Sánchez *et al.* (2004) in rye.

From our study it can be successfully deduced that B chromosome make useful experimental tools to study genome organization, their use in modulating and demodulating the frequency and distribution of recombination, to induce numerical heterogeneity in plants, in diploidizing allopolyploids, to study centromeres and to be development as plant artificial chromosomes; given that they can be structurally modified and their inheritance stabilized (Jones *et al.* 2008).

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