Asynaptic Variations in Induced Autotetraploid of Soybean

Girjesh Kumar and Priyanka Rai

Plant Genetics Laboratory, Department of Botany, University of Allahabad, Allahabad-211002, U.P., India

Received October 18, 2006; accepted November 18, 2006

Summary While working on colchiploidy in *Glycine max*, asynaptic variations was observed in the C₂ generation. The cytology and meiotic behaviour of this asynaptic tetraploid was studied. High and variable number of univalents were observed in this plant at diakinesis and metaphase I stage. In some of the PMCs 80 univalents could also be observed at diakinesis stage. The main and distinctive characteristic of the variant was the inability of the univalent chromosomes to congress at the equatorial plate, so that typical figures of metaphase I were never observed. The stages were hardly distinguishable and these were only identified on the basis of increase in chromosome number due to sister chromatid separation at anaphase II. Because of this abnormal meiotic behaviour aberrated tetrads were formed that gave rise to sterile pollen grains and pods were not formed. Some extrinsic or intrinsic agent might have acted on some genes responsible for synapsis and chiasma formation and resulted in complete disruption of chiasma assembly. Synaptic aberrations are potentially important source of information on the chiasma maintenance mechanism especially in a case when the system under study is an induced autotetraploid.

Key words *Glycine max*, Asynapsis, Autotetraploid, Meiotic behaviour.

Meiosis occupies a central role in the life cycle of all sexually reproducing eukaryotes, and it is through this process that eukaryotes achieve chromosome reduction, ordered chromosome segregation into haploid products and recombination of chromosomes. This involves a complex orchestration of events including chromosome pairing at zygotene, genetic exchange at pachytene, chiasmata formation at diplotene and chromosome segregation at anaphase I and II (Franklin *et al.* 1999). At least 1 chiasmata per bivalent is essential for orderly disjunction, otherwise, some homologues may migrate to same pole and form aneuploid gametes. The pairing and recombination of chromosome is under genetic control (Rees 1961).

A mutation in the genes controlling the meiotic recombination may lead to failure or early dissolution or reduction of chiasma formation. According to Gottschalk and Kleine (1976), chromosome pairing is under the control of 2 groups of genes designated *as* and *ds* which when present in recessive state, cause pairing to fail. The absence or failure of synapsis is termed as asynapsis while the immediate separation of the homologues following normal pachytene pairing is specified as desynapsis (Gottschalk and Kaul 1980 a, b). In autotetraploids, all genomes are identical and homologous chromosomes have equal opportunities to pair at meiosis. When pairing starts at different sites, multivalents are formed. However, the maintenance of the multivalence till metaphase I will depend on its frequency and chiasma localization.

While working on colchiploidy in *Glycine max*, an asynaptic variant was isolated in the C₂ generation of an induced autotetraploid. The present paper deals with the cytology and meiotic behaviour of this asynaptic tetraploid.

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* Corresponding author, e-mail: kumar_girjesh@yahoo.com, priyankarai30@yahoo.co.in

Materials and methods

Seeds of *Glycine max* were obtained from National Research Center for Soybean, Indore. Autotetraploidy was successfully induced using 'cotton swab' method with 0.4% solution of colchicine. The seeds from these induced autotetraploids were sown to raise C_2 generation. At the time of flowering, young floral buds were fixed in 1 : 3 acetic alcohol solution. After 24 h these buds were then transferred into 70% alcohol and stored at 4°C until use. Slides were prepared using another squash technique with 2% acetocarmine. Pollen fertility was determined using acetocarmine glycerine stainability test.

Observation

Meiosis in C_2 generation of the induced autotetraploids was observed. As compared to the C_1 generation, C_2 generation was found to stabilize itself in terms of chromosome behaviour as the bivalent frequency was found to be increased. The frequency of bivalents per cell was found to be 34.52 in C_2 generation as compared to 32.64 in C_1 generation of the induced autotetraploid ($4n=80$), while it was 20 in the normal diploid plants ($2n=40$). A few univalents, trivalents, tetravalents and multivalents were also observed in the autotetraploids (Table 1). All the meiotic stages were found to be quiet normal with chromosomes arranging themselves at equatorial plate in metaphase and segregated almost equally in anaphase stage (Figs. 1–3).

Meiotic analysis of a C_2 generation autotetraploid with stunted growth and very much-reduced flowering displayed highly increased number of univalents at diakinesis and metaphase I stage. In the normal C_2 autotetraploid, bivalents alongwith few univalents and multivalents were observed in most of the cases (Table 1). In few of the PMCs (Pollen Mother Cells) even 40 bivalents have also been observed (Fig. 1). Approximately 34.52 bivalents, 0.88 univalents, 0.36 trivalents, 1.66 tetravalents and 0.43 higher multivalents per PMC were observed in C_2 autotetraploids while fewer bivalents (21.79) and high and variable number of univalents (24.12 per PMC) were observed in the asynaptic plant (Table 1, Figs. 4–7) at diakinesis and metaphase I stage. In some of the PMCs 80 univalents can also be observed at diakinesis stage. The main and distinctive characteristic of the asynaptic plant was the inability of the univalent chromosomes to congregate at the equatorial plate, so that typical figures of metaphase I were never observed (Fig. 5). Instead of typical metaphase I and anaphase I, the PMCs exhibited widely scattered univalents in the cytoplasm (Figs. 5, 6). Typical telophase I was also not observed. Because of this atypical division in meiosis I, spindle assembly could not be formed in the second divisional stages and the chromosomes were seen lying scattered in the PMC (Fig. 7). The stages were hardly distinguishable and these were only identified on the basis of increase in chromosome number due to sister chromatid separation at anaphase II. Chromosome segregation was also found to be asynchronous hence variable number of chromosome complement could be observed at later stages of division. Later the 160 chromatids, depending on their proximity, were organized into different groups and formed varied number of different sized micronuclei. Abnormal chromosomal segregation resulted in aberrated tetrads that gave rise to sterile pollen grains (Fig. 9) while in the normal autotetraploids a high percentage of fertile pollen grains (Fig. 8) has been recorded. Pollen fertility in this asynaptic plant was registered to be only 17.9% in contrast to other C_2 generation plants in which it was observed to be 74.6% and the pod formation was not observed.

Discussion

The inability of univalents to congregate at the equatorial plate and widely scattered chromosomes in cytoplasm at metaphase and anaphase stages suggests lack of pairing at early prophase

Table 1. Chromosomal associations and pollen fertility in diploid, autotetraploid and asynaptic autotetraploid plants

Plant	No. of PMCs analyzed	Mean value/cell					Pollen fertility %
		Uni	Bi	Tri	Tetra	Penta	
Diploid	48	—	20	—	—	—	96.7
Autotetraploid							
C ₁	25	1.48	32.64	0.48	2.08	0.60	63.5
C ₂	69	0.88	34.52	0.36	1.66	0.43	74.6
Asynaptic autotetraploid	74	24.12	21.79	0.90	1.41	0.71	17.9

Figs. 1–3. Meiotic stages in autotetraploid plant ($4n=80$). 1, Diakinesis; 2, metaphase I; 3, anaphase I.

Figs. 4–7. Meiotic configurations in asynaptic autotetraploid plant. 4, $2IV+3III+10II+43I$; 5, $1III+7II+63I$; 6, $1II+76I+4$ separated sister chromatids; 7, $40I+80$ separated sister chromatids.

Figs. 8–9. Fertile and sterile pollen grains in asynaptic autotetraploid plant. 8, Fertile pollen grains; 9, sterile pollen grains.

stages indicating that this plant belongs to the category of asynaptic type. Also according to Peirson *et al.* (1997), in asynaptics, univalents never align at the equator at metaphase I, while in desynaptics, the bivalents as well as univalents align themselves at metaphase plate. This also supports our view that the present plant is of asynaptic type.

Though asynapsis of spontaneous origin has been reported in diploid soybean (Bione *et al.* 2002a) but asynapsis of spontaneous origin in an induced autotetraploid of *Glycine* has not been reported yet.

It is well known that early meiotic events are most susceptible to change by intrinsic or extrinsic agents. These might have acted on some genes responsible for synapsis and chiasma formation and resulted in complete disruption of chiasma assembly. Simchen and Stamborg (1969) concluded from their study that a mutation in highly conserved *rec* gene system might lead to the failure of chiasma formation and recombination. Ji *et al.* (1999) proposed that recombination modifies mutation in *rec* gene might reduce recombination to a point where no pairing occurs.

It might be an attempt to attain meiotic stability in the course of evolution. It is believed that in autotetraploids, there is gradual regularization of meiotic behaviour in succeeding generations by diploidization with increase in bivalent frequency at the cost of reduction in quadrivalents, trivalents and univalents as observed in *Vigna mungo* (Singh and Singh 1986). It has been known that a considerable decrease in the multivalent frequency is due to the effect of selfing-enhanced diploidization.

In the C_1 generation the frequency of multivalents was higher and in order to attain stability, the cellular machinery might have acted in a way to reduce pairing through some intrinsic agent to minimize the number of multivalents. This ultimately caused the reduction in pairing between the bivalents also and led to a case in which a complete lack of pairing was observed. Absence of pairing between homologous chromosomes in diploids is a common phenomenon reported in many plant species like *Zinnia* (Singh and Gupta 1981), *Chilli* (Rao and Kumar 1983), *Barley* (Kumar and Singh 2002), *Rice* (Reddi and Rao 2000), *Chickpea* (Kumar and Sharma 2001), *Anogeissus* (Rao and Kumar 2003) and *Soybean* (Palmer and Horner 2000, Bione *et al.* 2002a, b) but absence of pairing in an autotetraploid is quite interesting, since the homologues have more than 1 pairing partners and chiasma was not observed which suggests that this mutation might have affected specific genes for chromosome pairing.

Khawaja *et al.* (1998) observed few complete asynaptics in aneuploids of tetraploid *Lathyrus odoratus*. Lacadena (1965) also observed similar incidental asynapsis in nullisomic *Triticum triaristata* with $2n=6x-2=40$. Desynapsis after colchicine treatment of *L. odoratus* and *L. pratensis* has been described by Khawaja and Ellis (1987).

Behaviour of univalents is due to the fact that they have only one centromere, so that they are unable to maintain the normal bipolar orientation along the metaphase I spindle, therefore, univalents reach more independently to the poles (Peirson *et al.* 1997). The formation of a chiasma in normal cells provides a connection between homologues and restrains a poleward movement and causes tension at the kinetochores. In the absence of a chiasma, the resulting univalent kinetochores do not feel any tension and produce a signal that causes the cell to delay anaphase (Dawe 1998) and due to this the univalents were observed scattered in the cytoplasm and rest of the stages were not observed, since chromosomal movement was greatly affected. Such abnormalities often lead to the formation of polyads by rounding off of univalents into micronuclei and then into non-functional spores which might be the reason for the absence of pod formation.

The present investigation can provide useful information on chromosomal behaviour of soybean, as our knowledge on cytogenetics of this important biological system is very limited because of small size of chromosomes and absence of adequate cytological techniques. Since this plant was also morphologically affected with stunted growth and reduced flowering which shows that this mutation also affected the genes for plant height and flowering pattern were also adversely affected.

Moreover, synaptics are potentially important sources of information on the chiasma maintenance mechanism especially in a case when the system under study is an induced autotetraploid.

Acknowledgement

Authors are thankful to National Research Center for Soybean, Indore for providing seeds. Sincere thanks are due to all the members of Plant Genetics Laboratory for valuable help.

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