JJBS

Volume 7, Number 1, March .2014
ISSN 1995-6673
Pages 71 - 74

Jordan Journal of Biological Sciences **Short Communication**

Secondary chromosomal association in kidney bean (*Phaseolus vulgaris* L.)

Girjesh Kumar and Nitu Chaudhary*

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

Received: October 12, 2013 *Revised: November 30, 2013* *Accepted: December 4, 2013*

Abstract

The present study documents the mutagenic efficacy of gamma ray and sodium azide on the chromosomal association pattern of bivalents and meiotic behavior in *Phaseolus vulgaris* L. The seeds were irradiated with different doses of gamma rays viz.10 krad, 20 krad, and 30 krad from a ^{60}Co source and thereafter, the seeds were treated with 0.3% of freshly prepared sodium azide solution for three hours, respectively. The results clearly show the formation of various types of secondary chromosomal association among bivalents. Secondary association is defined as the tendency of bivalents to lie in pairs having diffused connections. The phenomenon of secondary pairing manifested from metaphase I stage and persisted upto metaphase II stage. The bivalents lie side by side and end to end to form secondary pairing. A secondary association between bivalents is considered to be of great significance as it is being taken as an indicator of ploidy in plants. Apart from secondary chromosomal association, other meiotic abnormalities were also noticed. These include precocious movement of chromosomes at metaphase I/II (2.83%), stickiness at metaphase I/II (3.45%), stickiness at anaphase I/II (2.20%), bridge (1.25%), unorientation (0.94%), micronuclei (1.88%) etc. This phenomenon, along with other meiotic aberrations affects the pollen fertility considerably.

Keywords*: Phaseolus vulgaris* L., Secondary chromosomal association, Chiasma frequency, Meiotic aberrations, Pollen sterility.

1. Introduction

Paradoxically meiosis is an event of high evolutionary significance which aims to precisely half the chromosome complement and ensures the viability of gametes. All organisms, irrespective of their evolved complexity, meiotically reduce the chromosome number at the start of sexual reproduction, compensating for fertilization and maintaining the diploid chromosome set from generation to generation (Golubovskaya, 1979). All steps in the process of meiosis are so well orchestrated that even a miniscule change can lead to severe alterations in the phenotypic expression. So, the study of the meiotic process is essential for efficient planning of breeding programmes.

Secondary associations or secondary pairing is defined as the close proximity of bivalents or chromosomes in pairs having diffused connections during meiosis (Darlington 1965). Kuwada (1910) was credited for the discovery of this phenomenon in *Oryza sativa*. Later on, Ishikawa (1911) reported this phenomenon in *Dahlia variabilis* followed by Marchal (1912) in *Amblystegium*, Darlington (1928) in *Prunus* and Lawrence (1931) in the species of *Dahlia*. Since then, the phenomenon had been observed in many plant species. It is of great significance as it presents a clue to the analysis of polyploidy where numerical considerations are not available or fail to elucidate it (Matsura, 1935., Agarwal, 1983). Regarding the origin of secondary associations of bivalents, different views have been put forth by different authors in different plants (Darlington, 1928; Lawrence, 1931; Heilborn, 1936; Jacob, 1957; Gupta and Roy, 1973).

The kidney bean (*Phaseolus vulgaris* L.) is the world's most important grain legume for direct human consumption (Goncalves *et al*., 2008). It is a rich source of protein (21.25%), fat (1.7%) and carbohydrate (70%). Besides this, it also contains 0.16 mg iron, 1.76 mg calcium and 3.43 mg zinc per 100gm of edible part (Kaur and Mehta, 1994). Brazil is the world's greatest common bean producer, producing more than 2.2 million tons, which represents 17.3% of the world's production (Goncalves *et al*., 2008). The phenomenon of secondary association has earlier been reported in many plants such as *Cicer arietinum*, *Prunus*, *Taraxacum*, and *Ocimum*, etc. The present study is an illustration of the behavior of secondary association of bivalents in *Phaseolus vulgaris* L.

* Corresponding author. Tel.:e-mail: nituchaudhary23@gmail.com.

2. Materials and Methods

2.1. Procurement of Seeds

Seeds of inbred lines of *Phaseolus vulgaris* L. variety PDR-14 (Uday) were obtained from Indian Institute of Pulses Research (IIPR), Kanpur, India.

2.2. Treatment Procedure

Dry and healthy seeds of *Phaseolus vulgaris* L. variety PDR-14 (Uday) were selected for gamma irradiation. The seeds were irradiated with three doses of gamma rays viz. 10 krad, 20 krad, and 30 krad, respectively from a ^{60}Co source at IARI, New Delhi. Thereafter the gamma irradiated seeds were treated with 0.3% of freshly prepared sodium azide (NaN_3) solution for about 3 hours with intermittent shaking, respectively. The seeds were then thoroughly washed with running tap water to remove the residual traces of sodium azide. Excess moisture was blotted off with the help of filter paper. One set of untreated seeds was kept in distilled water to act as control. The treated seeds were then sown in experimental pots in replicates along with the control to raise the M1 population.

2.3. Cytological Preparation

At the time of flowering, randomly selected floral buds of appropriate size were fixed in Carnoy's fixative (1:3, glacial acetic acid: absolute alcohol) for 24 hours at room temperature (25 ±2ºC). The fixed buds were later transferred to 70% alcohol (ethanol) and stored at 4 ºC for preservation. The slides were prepared using acetocarmine squash technique. Photomicrographs were taken from temporary slides. The cytological data were scored from the permanent slides which were made by passing the temporary waxed slide over a mixture of glacial acetic acid: butyl alcohol for a few minutes, followed by mounting on Canada balsam. Pollen sterility was calculated on the basis of stainability of pollen grains with 2 % acetocarmine (Shinde and More 2010).

3. Results

The course of meiosis was normal in control plants with 11 bivalents at diakinesis and metaphase I (Figure-1A) and normal segregation of 11:11 at anaphase I (Figure-1B).

The phenomenon of secondary association manifested from diplotene stages, whereas no glimpse of this phenomenon has been reported during pachytene stage. At diakinesis stage 1 or 2 tetravalent were found (Figure-1C). Secondary pairing by the bivalents has been observed in two patterns - end to end (Figure 1D-F) and side by side (Figure 1G and 1H). Multivalents were also observed (Figure-1I). At metaphase I/II stage, the bivalents and univalents showed different configurations and were found associated in the groups of II, III, IV, V, VI, VII and VIII. The type and frequency of pollen mother cells with secondary association has been listed in table-1. It is observed that the spectrum of pollen mother cells showing secondary association formation increased with an increase in the dose of mutagen.

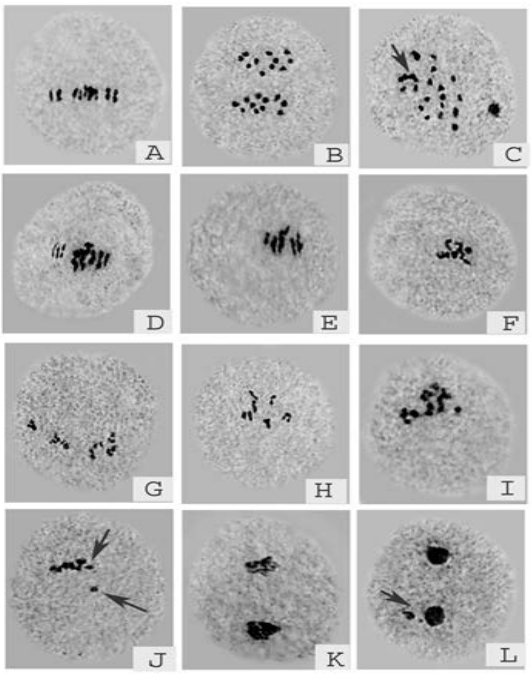

Figure 1. A. Normal metaphase (n=11); **B**. Normal anaphase (11:11); **C.** Tetravalent association of bivalents at diakinesis; **D-F-** Bivalents showing end-to-end secondary associations at metaphase; **G-H-** Bivalents showing side by side secondary associations at metaphase; **I**.A multivalent; **J**. Precocious movement of chromosomes at metaphase I; **K**. Stickiness at both pole during anaphase-I; **L**.Micronuclei at telophase I.

Table1. Secondary chromosomal associations among bivalents at metaphase-I/II in *Phaseolus vulgaris* L

Treatment doses	Total no. of PMCs observed	% of PMCs showing secondary association	% of PMCs showing secondary association among bivalents at M –I/II in different groups (I-VIII)								Other abnormalities	Pollen sterility (%)
			I	II	III	IV	V	VI	VII	VIII		
Control	365	-	-	-	-	-	-	-	-	-	-	2.87±0.13*
10kr+0.3%NaN_3	307	28.99	4.23	15.96	2.28	5.21	-	1.30	-	-	10.42	18.22±0.34*
20kr+0.3%NaN_3	339	40.70	4.12	19.17	3.83	6.78	2.35	2.06	0.88	1.47	13.27	30.07±0.22*
30kr+0.3%NaN_3	318	49.05	3.45	21.06	5.03	8.80	4.08	3.45	-	2.83	17.92	40.23±0.67*

Abbreviations : PMCs= Pollen mother cells, M-I/II=Metaphase I /II, NaN_3= Sodium Azide.
*Mean±SE

During the cytological observation, it was found that the tetravalent configuration was the common form of chromosomal association after bivalents which form the major share and were reported in 21.06 % PMCs. The percentage of PMCs showing tetravalent association varies from 5.21% at 10 krad+0.3% NaN_3 to 8.80% at 30 krad+0.3% NaN_3. Association of chromosomes in the configuration of VII was observed only at 20 krad+0.3% NaN_3 and was found to be 0.88%. Lowest number of univalents (3.45%) was found at 30 krad+0.3% NaN_3.Chromosomal associations in the group of III and VI were observed at all the doses. The treated sets also exhibited a wide array of other meiotic abnormalities apart from secondary chromosomal association. These include precocious movement of chromosomes at metaphase-2.83% at 30 krad+0.3% NaN_3 (Figure-1J), stickiness at anaphase-2.20% at 30 krad+0.3% NaN_3 (Figure-1K), micronuclei at telophase-1.88% at 30 krad+0.3% NaN_3 (Figure-1L), etc. A dose dependent increase in meiotic irregularities was observed with the mutagenic treated sets which ranged from 10.42% at10 krad+0.3% NaN_3 to 17.92% at 30 krad+ 0.3% NaN_3. The pollen sterility for control plants was found to be 2.87% which increases upto 40.23% at highest dose of treatment, i.e., 30 krad+0.3% NaN_3. The pollen sterility displayed an increasing trend along with the increasing doses of treatment.

4. Discussion

Although different views have been given regarding the formation of secondary associations, a satisfactory explanation has not yet been achieved. According to Thomas and Revell (1946), the fusion between heterochromatic regions of the involved bivalents is directly responsible for the formation of secondary associations. However, Lawrence (1931) and Malgwi *et al*. (1997) are of the opinion that homology existed between the paired bivalents, which resulted in a side by side association of bivalents in groups. There is a clear demarcation between multivalent formation and secondary pairing. Primary pairing at zygotene and chiasma formation among two or more homologous chromosomes resulted in multivalents whereas secondary association results from the loose association of bivalents without the existence of chiasmata (Katayama, 1965).

The occurrence of the phenomenon of secondary association has been considered to be a result of an artifact by many workers which may either be induced due to squash technique as obtained in *Luzula* by Brown (1950) and in *Carex* by Heilborn (1936) respectively or due to fixation as reported by Propach (1937). There has been a serious debate on the involvement of homologous chromosomes in the process of secondary pairing. Hirayoshi (1957) disagreed with the hypothesis which considers the involvement of homologous chromosome in secondary association and after examining careful results in *Oryzeae* and *Zizanieae* gave the conclusion that the secondary association may be a phenomenon operating under bio- and physico-chemical reactions and has no relation to the true homology of chromosomes.

Secondary pairing between bivalents has been considered as an indicator of polyploid nature of a species as reported in *Ocimum* (Mukherjee and Datta, 2005), and *Uraria picta* (Bhattacharya and Datta, 2010). According to Stebbins (1950), secondary association can be considered a phenomenon which depicts the polyploid nature of a species or genus, but elaborate phylogenetic predictions cannot be drawn from this as the secondary pairing between bivalents is considerably modified by other chromosomal changes. But amidst these explanations, Bhattacharya and Datta (2010) concluded that no inferences should be drawn regarding the polyploidy origin of species solely on the basis of secondary pairing. As an alternative, the cytological data must be co-related with locus specific molecular markers using FISH (Fluorescent in situ hybridization) to get a precise decision (Litcher, 1997). The phenomenon of secondary associations, along with other meiotic abnormalities, has some impact on pollen fertility as it was found to be significantly decreasing along with increasing concentrations of gamma ray +sodium azide.

Conclusively, it can be drawn from the above facts that the estimation of the strength of forces involved in the secondary association makes a foundation for assessing the impact of environmental factors on chromosomal association. The environmental factors modify the chiasma frequency by either altering chiasma formation or chromosome pairing. Since secondary pairing between bivalents is independent of chiasma formation, it provides accurate details about the effect of environmental factors on chromosome pairing.

Acknowledgements

The authors are extremely thankful to the Indian Institute of Pulses Research (IIPR), Kanpur, India, for providing the inbred seeds of *Phaseolus vulgaris* L and Nuclear Research Laboratory (IARI) for providing the gamma irradiation facility. Sincere thanks are due to all the members of the Plant Genetics Laboratory for their encouragement and support.

References

Agarwal PK. 1983. Secondary association of bivalents in *Cissus discolor* blume. *Cytologia*, **48**: 577–580.

Bhattacharya A. and Datta AK. 2010. Secondary chromosome associations in *Uraria picta* (Jacq.) DC. (Family: Leguminosae). *Cytologia*, **75**: 37–40.

Brown WS.1950. Spurious secondary association and asymmetric spindles in *Luzula*. *Cytologia*, **15**: 259–268.

Darlington CD. 1928. Studies in *Prunus*. I and II. *J. Genet.*, **19**: 213–256.

Darlington CD. 1965. **Cytology**, London: Churchill.

Golubovskaya IN.1979. Genetic control of meiosis.*Int. Rev. Cytol*., **58**: 247–290.

Goncalves VMC, Silverio L, Elias HT, Filho PSV, Kvitschal MV, Retuci VS, Silva C 2008. Combining ability and heterosis in common bean (*Phaseolus vulgaris* L.) cultivars. *Pesq. Agrpec. Bras. Brasilia*, **43(9)**: 1143-1150.

Gupta PP. and Roy SK. 1973. Primary and secondary chromosome association in *Euryale ferox* Salisb. *Cytologia*, **38**: 645–649.

Heilborn O. 1936. The mechanism of so called secondary association between chromosomes. *Hereditas* , **22**: 168-18.

Hirayoshi I. 1957. The chromosomal relationships in Oryzeae and Zizanieae. *Cytologia Suppl. Vol. Proc. Int. Genet. Symposia* 1956: 293-297.

Ishikawa M.1911. Cytologische studien von dahlien. *Bot. Mag.*, **25**: 1–8.

Jacob KM. 1957. Secondary association in the castor oil plant. *Cytologia* **22**: 380-392.

Katayama T. 1965. On so-called secondary association in rice plants. I Cytological observations, *Japan. J. Genet*.,**40.(1):**25-32.

Kaur J and Mehta U. 1994. Nutritional evaluation of new varieties of kidney beans (*Phaseolus vulgaris*). *Legume Res*. **17**: 17-21.

Kuwada Y.1910. A cytological study of *Oryza sativa* L.*Bot. Mag*., **24**: 267–280.

Lawrence WJC. 1931. The secondary association of chromosomes. *Cytologia* , **2**: 352-384.

Lichter P.1997. Multilocular FISHing : What's the catch? *Trend Genet.*,**13**: 475–478.

Malgwi MM. Oyewole SO. and Khan AU. 1997. Chromosomes and secondary associations in tetraploid *Cleome polyanthera* L. *Nucleus*, **40**: 20–25.

Marchal E.1912. Recherches cytologiques sur le genre *Amblystegium. Bull Soc R Bot Belg.*. **51**: 189–200.

Matsura H.1935. On the secondary association of meiotic chromosomes in *Tricyrtis latifolia* and *Dicentra spectabilis* Miq. *J Fac Sci Hokkaido Imp University*, Series V, **3 (5):** 251-60.

Mukherjee M. and Datta AK. 2005. Secondary chromosome associations in *Ocimum basilicum* L. and *Ocimum tenuiflorum* L. *Cytologia*, **70**: 149–152.

Propach H.1937. Cytogenetische untersuchungen in der gattung Solanum, Sect. Tuberarium. I. die sekundarpaarung. *Zeitschrift fur Inductive Abstummungs-und Vererbungslehre* **72**: 555-563.

Shinde MS. and More AD. 2010. Study of Pollen Sterility in Clusterbean ((L.) Taub.) Through Mutagenesis *Cyamopsis Tetragonoloba*. *Asian J Exp Biol Sci*., **1**: 31-34.

Stebbins GL. 1950. **Variation and Evolution in Plants**. Columbia Univ. Press, New York.

Thomas PT. and Revell SH. 1946. Secondary association and heterochromatic attraction. I. *Cicer Arietinum Ann Bot*., **10**: 159-164.