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**Research Article**

# RESPONSE OF B CHROMOSOME TO MUTAGEN IN *PLANTAGO LANCEOLATA* L

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**ABSTRACT**

The effect of B chromosome on chiasma frequency and distribution in number of pollen mother cells (PMCs) was studied in *Plantago lanceolata*. The effect of chemical mutagens on chiasma frequency in carrier and non-carrier PMCs was also studied and compared with that of control. The treatment of Hydrazine-hydrate (HZ) along with Dimethyl-Sulfoxide (DMSO) as a penetrant carrier was given in two different ways. Firstly, both the chemicals were mixed and treated and secondly, sequential treatment of DMSO and HZ was given. Chiasma frequency in carrier and non-carrier plants was calculated in response to mutagenic treatment and compared with that of controls. B chromosomes were found to influence chaisma frequency during mutagenic treatments, however, the frequency of B chromosomes remained unchanged.



## INTRODUCTION

*Plantago lanceolata* is a carrier species of genus *Plantago*. It is commonly known as narrowed-leaved plantains and belongs to the family Plantaginaceae. B chromosomes have been known since 1927 when Longley[1] reported them in maize. Various detailed investigations were carried out on different aspects of B chromosomes in different plants such as *Trigonella, Impatiens balsamia,* Pearl millet etc. Rome[2] reported differential fertilization by carrier and non-carrier pollen grains in maize. John and Hewitt[3] found the evidence that chiasma formation and genetic recombination are influence by B chromosome, whereas Carter and Smith-White[4] did not find any effect of B chromosome on chiasma frequency. In 1984 three reports from different groups of authors came out about genetic recombination and influence of B chromosome on chiasma frequency. Sharma *et.al.*[5] reported about genetic diversity among Plantagos and karyotypes of *P.Lanceolata L.* with special emphasis on nuclear chromosome. The effect of B chromosome on chiasma frequency in Pearl millet was studied by Jaya and Pantulu [6]. Kumar and Raghuvanshi [7]also reported the influence of B chromosome on chiasma frequency in *Plantago coronopus* and their studies clearly revealed that the B chromosome had no significant effect on mean chiasma frequency and plant-to-plant variation, however, variation between and within PMCs affected in statistically significant manner.

Mutagens have become potential tools in crop improvement programmes. Along with the identification of different potential chemical mutagens, the scope for increasing the frequency and spectrum has broadened. The comparative low cost of chemical mutagens and ease of application has made an unappreciated interest in the artificial induction of mutations with these agents.

The present investigation is based on the mutagenic effect of Hydrazine-hydrate with Dimethyl-sulfoxide as penetrant carrier in *Plantago lanceolata* with response to B chromosome and chiasma frequency.

## MATERIALS AND METHODS

The seeds of *P.lanceolata* were obtained from I.A.R.I. New Delhi, India. The seed lots were pre-soaked in distilled water for 20 hours and then the treatment of HZ along with DMSO was given in two different ways:

1. First treatment of DMSO and HZ given to the pre-soaked seeds were in mixed states i.e. 0.1% DMSO solution prepared in 0.05% HZ was applied for 3 hours.
2. Second treatment DMSO and HZ was sequential which seeds were treated in 0.1% DMSO for 3 hours and then transferred in 0.05% HZ and kept them for again 3 hours.

After the treatment seeds were washed properly and were sown in replicates of different sets along with the control set.

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For morphological investigations various observations were made such as, rate of germination, growth, height etc. and for cytological investigations the young spikes were fixed in acetic-alcohol (1:3) fortified with iron for 24 hours. Acetocarmine stain was used for squash preparations. An ethanol-butanol schedule was used to make the preparations permanent.

## OBSERVATIONS AND RESULTS

*Plantago lanceolata* has 2n=12 chromosomes. The carrier plants had 2n=12+1 B in some of the pollen mother cells while non-carriers had 2n=12. Non-carrier PMCs showed 6 bivalents after pairing at metaphase I (Fig.2) while the carrier PMCs had an extra chromosome along with 6 bivalents (Fig.3).

**Fig 1** Morphological view of non-carrier and carrier plants of Plantago lancolata L.

**Fig 2** Non-carrier PMC with 6 bivalents at Diakinesis

**Fig 3** Carrier PMC with 6 bivalents at Diakinesis

**Fig 4** Non-carrier PMC with 6 bivalents at Anaphase I

In carrier PMCs of the carrier plants, B chromosome does not take part in pairing, so that there is uneven separation of chromosomes during anaphase I (Fig.5). B chromosome migrated towards one pole and incorporated in one of the daughter nuclei. The second division is quite regular and B chromosome appears to divide at anaphase II and is passed on to the gametes and further inherited to the next generations.

**Fig 5** Carrier PMC with 6 bivalents at Anaphase I with a B chromosome

**Fig 6** Abnormal PMC at Anaphase I showing laggard with a B chromosome

**Fig 7** Abnormal PMCs at Anaphase I showing bridge formation

15 plants were selected randomly for the study of B chromosome frequency and chiasma frequency from both the treated and controlled plants.

In controls, out of 15 plants, 5 were found to be carriers. Among carrier plants B frequency ranged from 25.0%-32.0% (Table 1).The average of chiasma frequencies at metaphase I for non-carriers and carriers were 0.736 $\pm$ 0.007 and 0.742 $\pm$ 0.005, respectively. In carrier plants , the non-carrier PMCs had the average chiasma frequency value 0.782 $\pm$ 0.007 while in carrier PMCs it was 0.670 $\pm$ 0.007 (Table 2). There was no significant variation in mean chiasma frequencies between carrier and non-carrier plants. However, the plant-to-plant variation in carrier and non-carrier plants as well as non-carrier and carrier PMCs of carrier plants were found to be statistically significant (Table 3).

**Table 1**

| Doses | Total No. of Plants Studied | B-carrier plants | Non-carrier plants | Percentage of B-carrier plants | Frequency of B-carrier PMCs in carrier plants | | |
|---|---|---|---|---|---|---|---|
| | | | | | Min. % | Max. % | Average % |
| Control | 15 | 5 | 10 | 33.3 | 25.0 | 32.0 | 28.50 |
| Treatment I (0.1%DMSO+ 0.05% HZ) for 3 hours | 15 | 5 | 10 | 33.3 | 13.9 | 18.0 | 15.95 |
| Treatment II (0.1%DMSO for 3 hours + 0.05% HZ for 3 hours | 15 | 6 | 9 | 40.0 | 22.0 | 26.1 | 24.05 |

**Table 2** Chiasma Frequency/ Chromosomes in Carrier and Non-carrier Plants at Different Treatments.

| Source of Variability | Chiasma Frequency/Chromosome A.M. $\pm$ S.E. | | |
|---|---|---|---|
| | Control | Treatment I (0.1%DMSO+ 0.05% HZ) for 3 hours | Treatment II (0.1%DMSO for 3 hours + 0.05% HZ for 3 hours |
| **Non-carrier Plants** | 0.736$\pm$0.007 | 0.761$\pm$0.004 | 0.751$\pm$0.003 |
| **Carrier Plants** | 0.742$\pm$0.005 | 0.738$\pm$0.008 | 0.722$\pm$0.004 |
| i. Non-carrier PMCs | 0.782$\pm$0.007 | 0.756$\pm$0.008 | 0.748$\pm$0.009 |
| ii. Carrier PMCs | 0.670$\pm$0.007 | 0.672$\pm$0.007 | 0.678$\pm$0.007 |

During both the treatments, various meiotic abnormalities were found. Some of the PMCs showed two chromosomes in between the poles at anaphase I, out of which one was laggard and other was B chromosome (Fig.6).The bridges were also observed at anaphase I in treated plants (Fig.7).

First treatment i.e. combined treatment of DMSO and HZ did not show any significant effect in frequency of B carrier plants i.e. 5 out of 15 were carriers. The frequency of B carrier PMCs in carrier plants were ranged between13.9% to 18.0% (Table 1). During this treatment, the chiasma frequency values of non-carrier and carrier plants at Metaphase I was found to be 0.761 $\pm$ 0.004 and 0.738 $\pm$ 0.008, respectively. The non-carrier PMCs of non-carrier plants had lower chiasma frequency than the PMCs of non-carrier plants (Table 2). The chiasma frequency per chromosome values showed significant variations among non-carrier and carrier plants as well as in non-carrier and carrier PMCs of carrier plants. Plant-to-plant variations, however, were insignificant during this treatment, except in carrier plants where the plant-to-plant variations were found to be statistically significant (Table 3).

The sequential treatment of DMSO and HZ for 3 hours each respectively showed 6 carrier and 9 non-carrier plants. The B carrier PMCs in carrier plants ranged between 22.0% to 26.1% (Table 1) . In treated plants the non-carrier plants had 0.751 $\pm$ 0.0 03 average chiasma frequency per chromosome and carriers had 0.722 $\pm$ 0.004. The mean chiasma frequency per chromosome values in non-carrier and carrier PMCs of carrier plants were found to be 0.748 $\pm$ 0.009 and 0.678 $\pm$ 0.007, respectively (Table 2). Variation between the values of chiasma frequencies among carrier and non-carrier plants as well as carrier and non-carrier PMCs of carrier plants, was found to be

significant. The plant-to-plant variation, however, was almost insignificant except in carrier PMCs of carrier plants (Table 3).

**Table 3** Plant-to-plant Variation in Carrier and Non-carrier Plants at Each Dose.

| Doses | Sources of Variability | B=Total Chiasma Frequency/Total no. of Plants | X=Mean chiasma Frquency | S.D. (Standard Deviation) | S.E. (Standard Error) | Degree of Freedom (n-1) | t= (B-X)/S.E. |
|---|---|---|---|---|---|---|---|
| *CONTROL* | Non-carrier Plants | 0.736 | 0.73 | 0.0253 | 0.0080 | 9 | 0.750* |
| | Carrier Plants | 0.742 | 0.74 | 0.0153 | 0.0068 | 4 | 1.176* |
| | i. Non-carrier PMCs | 0.782 | 0.79 | 0.0196 | 0.0088 | 4 | 0.909* |
| | ii. Carrier PMCs | 0.670 | 0.68 | 0.0224 | 0.0071 | 4 | 0.408* |
| *DOSE I (DMSO+HZ) for 3hours* | Non-carrier Plants | 0.761 | 0.76 | 0.0153 | 0.0048 | 9 | 0.208 |
| | Carrier Plants | 0.738 | 0.73 | 0.0231 | 0.0103 | 4 | 0.777* |
| | i. Non-carrier PMCs | 0.756 | 0.76 | 0.0211 | 0.0094 | 4 | 0.426 |
| | ii. Carrier PMCs | 0.672 | 0.67 | 0.0180 | 0.0090 | 4 | 0.222 |
| *DOSE II DMSO for 3 hours + HZ for 3 hours* | Non-carrier Plants | 0.751 | 0.75 | 0.0117 | 0.0390 | 8 | 0.028 |
| | Carrier Plants | 0.721 | 0.73 | 0.0359 | 0.0146 | 5 | 0.568 |
| | i. Non-carrier PMCs | 0.748 | 0.75 | 0.0249 | 0.0102 | 5 | 0.167 |
| | ii. Carrier PMCs | 0.678 | 0.69 | 0.0235 | 0.0096 | 5 | 1.250* |

**Significant at P=0.50*

## DISCUSSION

The induction of biological damage by chemical mutagens depends upon various conditions of treatment prevailing prior to during or following the mutagenic treatment than Among these, pre-soaking of seed in water, presence and absence of oxygen during treatment are known to influence the biological activity of mutagens. [8,9,10]

DMSO has aroused considerable research interest in biology for its use as a penetrant carrier and potentiator of certain compounds.[11]Bhatia[12]reported significant increase in chlorophyll mutation frequency in *Arabadiopsis* by applying EMS in combination with DMSO to growing points. Enhanced mutagenic efficiency of EMS with DMSO has also been observed in rice by Reddi[13] and Anwar *etal.*[14]

The most important aspect of B chromosomes is their possible effect on endo and exo phenotypes of the plant. When present in large numbers, the B chromosome have been shown clearly to affect vigour and fertility.[15,16] In case of *Plantago lanceolata* the presence of b chromosomes did not show any clear cut demarcation between carrier and non-carriers phenotypically (Fig. 1 A & B).

During present investigations possible mutagenic response of B chromosomes in *P. Lanceolata* was studied from different angles against the mutagen Hydrazine hydrate (HZ) along with DMSO (a penetrant carrier). Studies reveal that the frequency of B chromosomes remain unaffected during treatments in carrier plants. The carrier PMCs of the carrier plants also displayed almost similar range in controls as well as in treated plants (Table 1). Certain meiotic abnormalities viz. laggards, bridges etc. (Fig. 6 & 7) were observed in treated carrier as well as non-carrier plants but the B chromosome remained unaffected.

In controls, the average chiasma frequency per chromosome, values of non-carrier plants were lower than that of carriers however, in non-carrier plants, non-carrier PMCs had higher chiasma frequency values than carrier PMCs.

During combine treatment (DMSO+HZ) for 3 hours it was found that non-carrier plants had higher average chiasma frequency per chromosome value than that of carrier plants, suggesting that B chromosomes had adverse effect on chiasma frequency in presence of the mutagens. Among the carrier plants, however, the carrier PMCs had higher chiasma frequency than the non-carrier PMCs in contrast to the control suggesting that among carrier plants B chromosomes positively influence the chiasma frequency in carrier PMCs in presence of mutagens. Whereas the sequential treatment also supported the above results i.e. mutagens adversely affected the chiasma frequency per chromosome in carrier PMCs of carrier plants.

Thus, studies on chiasma frequency per chromosome values clearly reveal that B chromosomes influence the chiasma frequency in carrier plants as well as carrier PMCs of carrier PMCs of carrier plants during mutagenic treatments with respect to controls. As regards the plant-to-plant variation in non-carrier plants as well as carrier and non-carrier PMCs of the carrier plants at each dose i.e. control, treatment I, treatment II, it remains insignificant at probability 0.05% and 0.01%. However, at P=0.5, all the controls i.e. non-carrier plants, carrier plants, non-carrier and carrier PMCs have significant plant-to-plant variation while during treatment I only carrier plants had significant variation and during treatment II only carrier PMCs of carrier plants had shown significant variation.

Thus plant-to-plant variation studies clearly show that during mutagenic treatments the variations have tendency to become statistically insignificant in non-carrier and carrier plants in comparison to controls where they are significant.

Conclusively, above studies clearly indicate that B chromosomes definitely influence the frequency of chiasma formation as well as plant-to-plant variation as a response to the mutagen HZ in combination with penetrant carrier DMSO.

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