INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 203-208, 2015

RESEARCH PAPER OPEN ACCESS

Study of accessory chromosomes (Bs) transmission in Iranian leek (*Allium ampeloprasum* ssp.)

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Key words: B Chromosomes, Transmission, Persian leek (*Allium ampeloprasum*), B chromosomes transmission rates.

http://dx.doi.org/10.12692/ijb/6.2.203-208 Article published on January 20, 2015

Abstract

Persian leek (*Allium ampeloprasum ssp. Persicum*) is deemed to be an Iranian native vegetable. In this research, B chromosomes transfer examined in progenies of a series of controlled crosses between female and male parent that were formerly isolated on the basis of the absence and presence of B chromosomes. The average of the expected and observed Mendelian transmission of B chromosomes was compared using the x^2 test. The results showed that the difference is not significant. However, the reduction in the number of B chromosomes were observed in most crosses and it was found that In two thirds of cases Transfer of B chromosomes was declined, Confirming that the estimated average rate of B chromosomes transfer were 0.42 between male and female lines Which is considerably lower than the expected Mendelian transmission rate(0.50). In general, the female gamete transfer rate (0.48) is higher than the male gamete (0.39), although this difference was not significant. The data presented here show that the transfer of B chromosomes in *Allium ampeloprasum ssp. Persicum* has not provided effective way to accumulate B chromosomes.

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Introduction

Persian leek (Allium ampeloprasum ssp. Persicum), as a Iranian native plant, is a monocotyledon, herbaceous, tetraploid (2n=4x=32) as well as self-compatible plant, but it is placed at hetrogam plants group due to it's protandry nature (Panahandeh and Agayev, 2000; Dashti *et al.*, 2004). Presence of B chromosomes in Persian leek has been demonstrated (Panahandeh and Agayev, 2000).

At present, B chromosome has known in more than one thousand plant species and their characteristics recorded (Jones and Rees, 1982; Jones, 1995; Puertas, 2002; Jones and Houben, 2003; Camacho, 2004; Burt and Trivers, 2006). According to studies, 23,652 species of gymnosperms (approximately 9 % of the 260 thousand species), 8% of the total monocots and 3% of Eudicots possess B chromosomes and among them, the maximum number of species having Subsidiary chromosomes are belonged to *graminaceae* and apiaceae. This is Perhaps because of the abundance of species in this two family. However, there are also a high proportion of species with B-chromosomes in some other families. Among all Plants containing B chromosomes, two orders of *Commelinales* and *Liliales* have attracted more attention (Levin *et al.*, 2005; Palestis *et al.*, 2004). The main characteristics for distinguishing them, is that they are not exist in some individuals of populations and as a result, they are additional. There is a considerable heterogeneity in B chromosomes transfer in terms of the number of B chromosomes, male or female parent type and crosses related to odd or even number of B chromosomes or crosses where the number of odd or even B chromosomes is desired. B chromosomes that they are deemed to be as additional elements in most plant species and animals, do not follow Mendelian inheritance principles And often Transmitted to the next generation beyond the expectation. Mechanisms such as derive, can take different forms in this case. But, this process is usually performed in male, female or both parents in such a way as preferred separation before, during and after meiosis (Jones and Rees, 1982).

Longley (1927) examined the number of B chromosome in reciprocal crosses of maize plants with zero and one B chromosome. Transfer of B chromosomes has also been studied in *Allium schoenoprasum L* (Bougourd and plowman, 1996). In this research we considered results obtained from a series of experimentally designed crosses to estimate the frequency of transmission of B chromosomes through the maternal and paternal parent in Persian leek *(Allium ampeloprasum ssp. Persicum)*.

Materials and methods

Plant material and Parental separation

Persian leek annual plants were planted in medium containing perlite with proper humidity and temperature of 25°C. Parental separation Based on the absence and presence of B chromosomes carried out using counting of chromosomes at mitotic metaphase stage in Persian leek root samples (fig 1). For this purpose, the roots were separated at active cell division stage and pretreatment of samples was performed with α-Bromonaphthalene and 8-Hydroxyquinoline at room temperature and subsequently Samples were fixed with Carnoy 's solution 1 (With a ratio of 3:1). To enter the stage of staining, fixed samples were hydrolyzed with HCL 1N for 6-10 minutes at water bath with 60°C and afterward, staining was performed using basic Fuchsin (Singh, 2002).

Crossing

After flowering, to perform crosses, young flower buds were isolated in Small bags of paper Calc and flowers were covered in all stages except pollination and emasculation. Before anthesis, 10 to 12 flowers were emasculated and other flowers were removed. Using hairbrush, crosses were performed through transferring of pollen from opened anthers on the emasculated flower receptive stigma. To increase the chances of fertilization, this was repeated in 2 or 3 days. Crosses were performed between B^+ and B^- plants.

Evaluation of B chromosome transition in Progeny

After ripening of crossed flowers, seeds were harvested and after germination of harvested seeds in germinator with 21°C, Sampling of roots was conducted. After the pre-treatment, fixation, hydrolyze and staining, Slides were prepared and using microscope, B chromosome transition was evaluated at mitotic metaphase stage.

Data analysis

Data analysis was performed Using the x^2 test (df=1) at the probability level of 5%.

Results and discussion

Deviations from Mendelian inheritance

According to table1, it is clear that B chromosome is not transmitted by Mendelian inheritance. In order to test this fact that, whether Deviation of Mendel's laws is random or not, the mean values of expected and observed B chromosomes transmission were compared Using the x^2 test. Results showed that there was no significant difference between observed mean number of B chromosomes transmission through paternal or maternal parents and the average value of the expected transfer.

Table 1. Transfer of B chromosomes in offspring and the results of X^2 test for controlled crosses.

♀×♂	Distribution of B chromosomes in offspring				Total offspring	Average number of B chromosomes in offspring		X2	P	Average transfer Speed for each B Chromosome
	0B	1B	2B	4B		Expected	Observed			
0B×1B	7	2	0	0	9	0.5	0.22	2.78	NS	0.22
0B×1B	6	1	2	0	9	0.5	0.55	0.56	NS	0.55
0B×1B	7	3	0	0	10	0.5	0.3	1.6	NS	0.3
0B×1B	7	4	0	0	11	0.5	0.36	0.82	NS	0.36
0B×1B	4	1	0	0	5	0.5	0.2	1.8	NS	0.2
0B×1B	12	5	0	0	17	0.5	0.29	2.88	NS	0.29
0B×1B	2	3	1	0	6	0.5	0.83	1.67	NS	0.83
1B×0B	5	4	0	0	9	0.5	0.44	0.11	NS	0.44
1B×0B	4	3	0	0	7	0.5	0.42	0.14	NS	0.42
1B×0B	6	6	1	0	13	0.5	0.61	0.38	NS	0.61
1B×0B	11	10	0	0	21	0.5	0.47	0.05	NS	0.47

It has been found that B-chromosomes does not follow the rules of Mendelian inheritance. In most species, the Characteristics of non- compliance with Mendelian inheritance often leads to accumulation of B chromosomes in the next generation. However, the data presented here indicate that, transmission of B chromosomes in *Allium ampeloprasum ssp. Persicum* has not provided Effective way to accumulate B chromosomes. The average number of B chromosomes transition observed in the offspring is not significantly higher than the expected Mendelian transmission of B chromosomes. A reduction in the number of B chromosomes were observed in most of crosses and it was found that In two thirds of cases Transfer of B chromosomes was declined Confirming that the estimated average speed of B chromosomes transfer were (0.42) between male and female lines Which is considerably lower than the expected Mendelian transmission rate(0.5). Therefore, based on evidence from controlled crosses, accumulation by increasing the rate of transmission cannot help to survival of B chromosomes in this population. In general, the female gamete transfer rate (0.48) is higher than the male gamete (0.39), although this difference was not significant. Bougourd and Plowman (1996) stated that, accumulation of B chromosomes does not occur in chives. In fact a reduction in the transmission of B chromosomes to progenies is seen in compared to their number in parents. In controlled crosses, the overall average speed of B chromosomes transition for each B chromosomes is 0.4 that it is lower than that transition rate in expected mendelian (0.5) and this reduction occurs in the entire range of B chromosomes. It is not clear that in which step B chromosomes are lost. Like B chromosomes in rye, all B chromosomes of Chives show stability during mitosis and they are found with a fix number in all root tips somatic cells (Stevens and Bougourd, 1994). It is clear that without losing of B chromosome the number of B chromosomes in pollen may Possibly increased than expected number of them and potentially lead to increment of B chromosome number in progenies (Bougourd and Plowman, 1996). It was found that during meiosis, a slight decrease in B chromosomes can be seen, at least in the male line (Bougourd and Parker, 1979b). Therefore we assumed that losing remarkable number of B chromosomes must have occurred before meiosis Such that during mitotic division of pollen that occurs through B chromosomes impact on the appearance of pollen. In some progenies of crosses in which one of parents contain one B chromosome, we observed progenies with more than one B chromosome (fig 2).

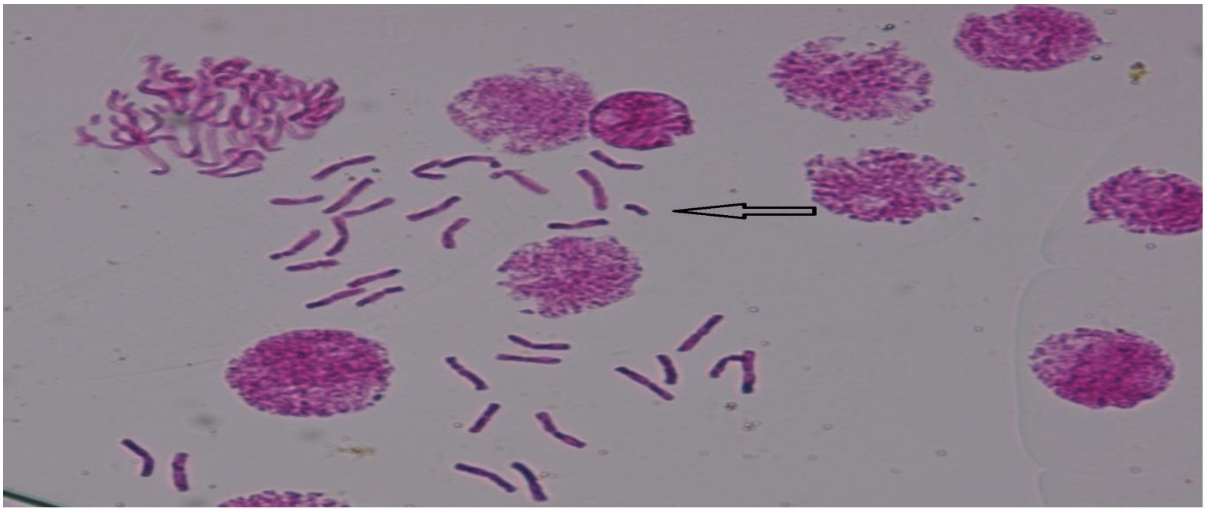

Fig. 1. Presence of B chromosomes at mitotic metaphase stage in Persian leek root samples.

This may be due to non disjunction of chromosomes in the male and female lines (Bougourd and Parker, 1979b). In the first mitotic division of male and female gametophytes, Non disjunction of B chromosome and Preferential distribution of cells, increased in gametes(Muntz, 1946; Hakans, 1948). This mode of transmission leading to accumulation of B chromosomes in the population. A small proportion of plants carrying the number of B chromosomes tend to accumulate B chromosomes (Puertas, 1985; Muntci, 1963). The formation of male gametes that contain B chromosomes, is predominantly depend on the reduction of univalent B chromosomes in meiosis and frequency of Direct non disjunction during pollen mitosis. Both parameters are directly estimated through microscopic examinations (Puertas *et al.,* 1986).

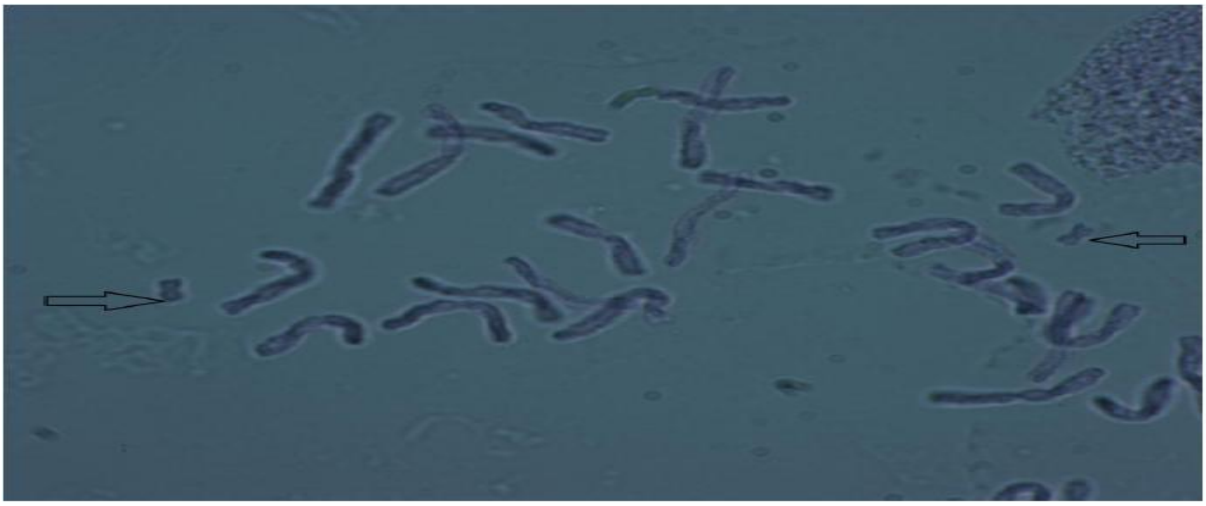

Fig. 2. Presence of two B chromosomes in some progenies.

Transmission of B chromosomes

According to (table 1), It is clear that in persian leek ((*Allium ampeloprasum ssp. Persicum)* B chromosomes transmitted to offspring with both male and femal parent. According to the results of the Transmission Rate, although a difference was observed but this difference was not significant. Longley (1927) examined the number of B chromosome in reciprocal crosses of maize plants with zero and one B chromosome. He intercrossed plants containing 1 and 2B chromosomes and results showed that B chromosome is freely transferred either with male gamete or female gamete. Additionally, recombinant gametes with a greater number of B chromosomes rarely occurred than expected. This is due to chromosome Non disjunction in anaphase step of meiosis and attributed to the subsequent performance of exceptional gametes. Reciprocal crosses of maize plants with zero and one B chromosome has been evaluated by Power and Dahl (1937). They observed that when a seed parent (female gamete) carrying B chromosome, this chromosome can be seen in half of the offspring. But when B-chromosomes are transferred by male parents (male gametes), it can be seen in less than one third of progenies. This result are interpreted to Means the selection process against to pollen carrying the extra chromosome, so that this selection is randomly occurred at first division of mitosis and meiosis and once again occurred regularly in second division without any cytokines. They concluded that the extra chromosomes is not entirely genetically inert and Affect the activity and function of the pollen.

The important result of this study revealed that, in *Allium ampeloprasum ssp. Persicum*, B chromosome has no effective cumulative mechanism. Such disaggregations of B chromosome include interesting benefits (Beukeboom, 1994). Because they are contrary to this opinion that, all B chromosomes are selfish genetic elements that despite the compatibility reduction of individuals carrying them, they make it possible to survive through meiosis drive (Jones, 1985).

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