CRITICAL ASPECTS IN CONSERVATIVE BREEDING OF TETRAPLOID RED CLOVER *(Trifolium pratense* L.)

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Abstract. *The seed set, in two red clover cultivars (Select-1, diploid and Apollo-tetra, tetraploid) was analysed in an experiment in which the two cultivars were planted in merging plots. An obvious decrease of no. of seeds/inflorescence was noticed in the diploid cultivar, especially in plants situated near by the tetraploid cultivar. On the contrary, in tetraploid red clover plants there has been noted a significant increase of seed set, this increase being the greatest when the proximity to the diploid cultivars was very tight. It is concluded that in experimental fields with tetraploid clover, if a diploid cultivar is used as control, the tetraploid variants could be easily underestimated from seed production point of view due to the fact that the diploid control, with its "overproduction" of pollen, becomes the main pollinator of tetraploid variants. It is also recommended that, in seed production plots of red clover cultivars with different ploidy levels, a strip of 7-8 cm should be eliminated before harvest to avoid both mechanical and biological contamination.*

Key words*: red clover, diploid, tetraploid, conservative breeding.*

INTRODUCTION

The advantages the tetraploid red clover offers in terms of yield and forage qualities are significantly reduced because of the low seed production, limiting factor of its expansion in cultures.

In the context of the conservative breeding of the haploid pollen the disturbing effect was less studied.

The causes contributing to fertility diminution and implicitly to seed production of tetraploid red clover may be:

• Morphological and physiological causes affecting floral organs;

• Cytological and genetic causes, caused by abnormal meioses at tetraploid level;

• Reasons beyond the genetic basis (disturbing effect of haploid pollen and the influence of environmental conditions).

It is known that colchicine has no polyploidy effect on all ongoing mitoses during the treatment. Due to this fact plants not entirely are polyploid may occur, certain diploid sectors existing. These plants, called mixoploide, lead to major troubles in pollination, producing triploid embryos. Most of them abort implicitly producing a general fertility decline (JULÉN, 1954; SKIEBE, 1966; SAVATTI et al., 1986). Within diploid capitulas only diploid seed will form because the haploid pollen, in case it it mixed with the diploid pollen on diploid stigmata, has a considerably higher competitive power. The formed diploid seeds will contribute to the descendants' evolution through haploid pollen, to the strong perturbance of fertility in a tetraploid population.

The research of PANKIW (1972), SAVATTI et al. (1984, 1986, 1992), emphasizes that biological contamination of red clover seedlings depends more on the abundance of blossoming, on the field dimensions, than on the isolation distance of the di- and tetraploid red clover lots. Biological contamination reaches highest peaks on the parcels' edges, gradually decreasing towards their centre.

JULÉN (1956) considers that the distance between the seedling lots with a different degree of ploidy does not exert any influence on their seed production; MAIZONNIER (1969)

and SAVATTI (1992) instead, consider that the haploid pollen has a disturbing effect on the productive efficiency of the tetraploid red clover seedling lots.

Given the controversial issues, it was deemed appropriate to elucidate this problem.

MATERIAL AND METHODS

The studies were conducted on two varieties of red clover, Select 1 (diploid) and Apollo-tetra, created at UASVM Cluj-Napoca. Both varieties were sown on 60 cm close plots, each plot with an area of 1,800 square meters. The binding percentage was determined by comparing the seed number to the number of flower in each capitulum. As control plant the row of plants closest to the source of foreign pollen was used. The analysis was carried out for each variety, 1.800 capitula, thus, a total of 3.600. Correlations between the distance from the source of haploid pollen or diploid pollen, respectively and the degree of seed binding were established.

RESULTS AND DISCUSSION

Haploid pollen influence on tetraploid seed binding is obviously negative in their vicinity to the diploid culture. It results, from Table 1, that the Apollo-tetra variety displays the lowest plant seeds binding percentage in the first two rows neighboring the Select 1 diploid variety, at a 1.20 m distance. Beginning with the 1.80 m distance, the binding percentage increases significantly, because at a 7.20 m distance the increase of the percentage values is very distinct and very, very significantly distinct. The presented data clearly show that up to a 1.20 m distance the negative influence of the haploid pollen manifests itself, in an obvious way, on the binding percentage; between 1.20 m to 6.60 m this influence is reduced, while beginning with 7.20 m no disturbing effect of the haploid pollen on the tetraploid red clover plant binding percentage, to be observed, which leads us to believe that the flying of pollinators from one plot to another takes place on small portions, fact also reported in the literature (PANKIW 1952; SAVATTI et al., 1992).

Table 1

Influence of haploid red clover pollen on seed set in tetraploid cultivars, depending on distance from pollen source

Distance to the 2n pollen source	Tetraploids		± d	LSD
	$\bar{X}$	% seed set		
0,60 m	17,70	100,00	-	-
1,20 m	19,26	108,45	1,56	5% = 5,5
1,80 m	23,69	133,39	5,99*	5% = 5,3
2,40 m	23,50	132,32	5,80*	5% = 5,4
3,00 m	23,38	131,64	5,68*	5% = 5,2
3,60 m	23,39	130,57	5,69*	5% = 5,2
4,20 m	23,49	133,39	5,79*	5% = 5,3
4,80 m	23,80	134,00	6,10*	5% = 5,6
5,40 m	23,16	130,40	5,46*	5% = 5,4
6,00 m	22,26	125,34	4,56	5% = 5,0
6,60 m	24,20	136,26	6,50*	5% = 5,7
7,20 m	26,00	146,40	8,30**	1% = 7,6
7,80 m	28,30	159,34	10,60***	0,1% = 8,6
8,40 m	27,30	133,91	9,60***	0,1% = 8,4
9,00 m	27,30	133,91	9,60***	0,1% = 8,4
9,60 m	31,50	177,36	13,88***	0,1% = 9,7
10,2 m	31,80	179,11	14,10***	0,1% = 9,6
10,8 m	27,75	156,25	10,05***	0,1% = 9,2

Analyzing the behavior of the diploid form in terms of the binding percentage, we notice a fact scarcely mentioned in the literature, namely that the diploid pollen derived from tetraploid forms, has a disturbing influence on the diploid red clover binding percentage (Table 2).

The lowest percentage of bound seeds was highlighted in the plants in the vicinity of the tetraploid form, 0.60 m, respectively. Up to a 5.40 m distance from the pollen source a slight increase in the bound seeds percentage is noticed, basically, at this distance, no negative influence of the diploid pollen longer being observed.

By comparing the seed binding percentage on a 10 meter plot segment from the pollen source, it is noticed that it increases by 56% in the case of tetraploids and only by 23% in the case of the diploids.

Table 2

Comparative results on seed set in red clover cultivars with different ploidy levels depending on the distance between cultivars

Distance between pollen sources(m)																	
0,60	1,20	1,80	2,40	3,00	3,60	4,20	4,80	5,40	6,00	6,60	7,20	7,80	8,40	9,00	9,60	10,20	10,8
Diploid – $\bar{X}$ seed set																	
43,8	50,1	48,6	48,4	54,2	47,3	47,7	50,6	51,0	53,0	54,8	54,6	55,3	56,0	51,9	55,1	55,7	53,98
Tetraploid – $\bar{X}$ seed set																	
17,7	19,2	23,6	23,5	23,3	23,3	23,4	23,8	23,1	22,2	24,2	26,0	28,3	27,3	27,3	31,5	31,8	27,75
Tetraploid isolated from diploid – $\bar{X}$ seed set																	
									29,5								

The question is what does the disparity in the behavior of plants with different ploidy level represent?

According to the research conducted by JULÉN (1950), SAVATTI et al. (1986), the haploid pollen tubes compared to the diploid ones grow faster on tetraploid plant stigma, the fertilization being carried out more quickly. Consequently, the fertilization is mostly done with haploid pollen, a large number of triploid embryos resulting. To explain the phenomenon mentioned, Watkins (quoted by SKIEBE, 1966), believes that during the crossing of plants with different ploidy levels the triploids are easier obtained if the maternal genitor has a higher number of chromosomes.

According to WEXELSEN and VESTAD (1954), JULÉN (1954), SCHWEIGER (1958) the triploid embryos abort in an early stage of their development. Unlike the mentioned authors, MAIZONNIER (1969), by crossbreeding the tetraploid red clover with the diploid obtained a 3 ‰ triploid seed frequency, and only 1 ‰ in the case of mutual crossbreeding. The frequency of triploid plants, although not completely sterile, in a tetraploid population may occur, reducing thus the seed production.

We believe that the perturbing influence of the haploid and diploid pollen, especially in the confluence areas of the clover fields with different ploidy level may be due to a certain "specialization" of the Hymenoptera (*Apis* and *Bombus*) pollinators in pollination process of the two clover forms with different ploidy level. Due to the greater length of the 4n corolla forms, it is probably that the honeybees with a shorter trunk, renounce to "venturing" within the tetraploid clover field.

The nearby presence of red clover cultivars with different ploidy level, both in the plant breeding fields as well as in the in seedling lots, has certain disadvantages in terms of seed production.

In the tetraploid plant breeding fields, where as control plant using a diploid variety is used, this having high haploid pollen, the tetraploids will achieve a lower seed production and therefore the tetraploid biological material will be appreciated under its real value.

In what concerns the red clover seed lots with different ploidy level, sowed without isolation, before harvesting the seeds it is recommended to remove a 7-8 m wide strip, which can prevent biological and mechanical contamination of the harvested seeds .

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