POLLINATION AND BINDING SEEDS PROCESS ON DI- AND TETRAPLOID RED CLOVER (*TRIFOLIUM PRATENSE L.*) IN TRANSYLVANIA'S CONDITIONS

POLENIZAREA ŞI PROCESUL DE LEGARE AL SEMINŢELOR LA TRIFOIUL ROŞU DI- ŞI TETRAPLOID (*TRIFOLIUM PRATENSE L.*) ÎN CONDIŢIILE DIN TRANSILVANIA

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Abstract: *In this paper the di- and tetraploid red clover seedlings will be compared, under the aspects of pollination, fertility and binding seeds process. After a vast amount of research, the results obtained were that, often, the low seed yield in di- and tetraploid red clover is caused by of one of these factors: the improper pollination and fertility perturbance.The purpose of the experiments during the years 2004-2006, was to determine the pollination effects and to offer important clues about di- and tetraploid red clover seed production in Transylvania's condition. Red clover is a typical allogamous plant in which the auto incompatibility system determined by the S alleles is very strong, the pollination is compulsorily done by honey and bumble bees. The red clover flowers are very attractive to a large amount of insects, but because of the flower structure, only some sort of insects can do the pollination. The efficiency of the activity of a pollinator depends not only on the flower structure, but also on the meteorological conditions.*

Rezumat: *În această lucrare se vor compara semincerii de trifoi roşu di- şi tetraploid sub aspectul polenizării entomofile, fertilităţii şi procentului de legare al semințelor. În urma numeroaselor cercetări, s-a constatat că, în mod frecvent, producţia scăzută de sămânţă la trifoiul roşu poliploid se datorează polenizării necorespunzătoare şi perturbării fertilităţii. Obiectivul experienţelor, efectuate pe parcursul anilor 2004-2006, este acela de a determina efectele polenizării şi de a oferi unele indicii asupra producţiei de sămânţă la trifoiul roşu di- şi tetraploid, în condiţiile din Transilvania. Trifoiul roşu este o specie tipic alogamă, la care sistemul de autoincompatibilitate, determinat de alelele S, este foarte puternic, polenizarea de către albine şi bondari fiind obligatorie.Florile trifoiului roşu atrag un mare număr de insecte, dar din cauza structurii florale, numai anumite specii de insecte pot îndeplini funcţia de polenizatori. Eficienţa activităţii unui polenizator depinde nu numai de structura florală, ci şi de condiţiile climatice.*

Key words: *di- and tetraplid red clover, pollination, fertility, seed production.*
Cuvinte cheie: *trifoi roşu di- şi tetraploid, polenizare, fertilitate, producere de sămânţă*

INTRODUCTION

Starting with the 5th decade of the 20 century, an increasing interest has been shown, especially in the Scandinavian countries, for the red clover autotetraploid forms.

The numerous investigations have shown that by comparison with the diploid forms of red clover, the tetraploids are highly superior in what the following aspects are concerned: fodder (biomass) production, persistence and resistance (HERTZSCH, 1959, JULÈN, 1958, PAMFIL şi SAVATTI, 1984).

The advantages that red clover has to offer under the aspect of seed production and fodder qualities are visibly diminished by the low seed production, a limitative factor in culture expansion.

As seen by the different researchers (BRAGDØ-ASS, 1969, JULÈN, 1970, SAVATTI, 1973, BARBUR, 1974, DENIS, 1980, SAVATTI, 1998, MUNTEAN, 2002), the causes which appear

to contribute to red clover fertility diminutions, as well as to seed production can be synthesized as follows:

- *morphological and physiological causes* which affect the floral organs (length and diameter of corolla tube, nectar column high, quality and quantity of nectar, flavour and colour of flowers). The flower dimensions have an obvious negative influence over the pollinators flight intensity and under this aspect the tetraploid red clover is disadvantaged;

- *cytological and genetically causes* which generate the multivalent chromosomes appearance; gametes and aneuploid plants, ovary abortion, ovules and embryos, over going to an certain number of alleles/locus, caused by abnormal meiosis on the tetraploidy level;

- *diminutions of some productivity elements of seedlings*: the number of seedlings/plant, the number of main and secondary ramifications/seedling, the number of flower heads (buds)/plant and the flower number/plant;

- *causes independent of the genetic base:* the irritating effect of haploid pollen, the same as diploid and meteorological conditions influence.

MATERIAL AND METHOD

The biological material studied consisted of four species, two diploids (*Select and Roxana*) and two tetraploids (*Napoca-Tetra and Apollo-Tetra),* created at the University of Agricultural Science and Veterinary Medicine Cluj-Napoca, in the conditions from Cluj-Napoca and the Experimental Agro-Zootehnical Station Jucu and two species (*Roxana and Napoca-Tetra*), under the conditions from Viseu de Jos village, Maramures.

The biological material analyzed for seed number/flower head, binding seed number/flower head, was calculated by using the variability grad of characters under estimative values: $\overline{X}$ (mathematical average), S $\overline{X}$ (standard aberration of mathematical average) and s% (the variation coefficient) determined after SĂULESCU şi SĂULESCU (1967), CEAPOIU (1968), SAVATTI şi colab. (2004).

The interpretation of the variability coefficient (s%) was done in this way: under 10% - a low variation of characters, between 10-20%- middle variation and over 20% -a high variation (CEAPOIU, 1968; POTLOG şi VELICAN, 1971).

The signification difference between diploids and tetraploids was determined by using the "t" test (SĂULESCU and SĂULESCU, 1967).

Fertility is given by the seeds number on flower head, offering to us the most important indication over the red clover potential seed production. Still in the same content we can discuss about medium seed process binding or relative fertility that depends directly on the seeds number and flower number on flower head.

To determine the identity of the pollinators and their frequency, the numbering of the pollinators was carried out on a 100 mp surface, on red clover belonging to both ploidy levels, starting with 8 o'clock in the morning until 17.oo in the afternoon, during the years 2004-2006.

RESULTS AND DISCUSSION

Among the complex factors that determine the red clover fructification, the pollinating insects have a very important function (HAWKINS, 1962, 1971; PALMER, 1967, HOLM, 1972; CIURDĂRESCU and SAVATTI, 1971). Under the structural aspect of pollinator's community, it was observed during the years 2004-2006, in Cluj-Napoca's conditions, over all, that the number of pollinators was higher on second harvest for seed. By showing the day work of pollinators, the numbering variation was observed FOR each species, between 8 and 17 (table 1).

It has been ascertain that in general the individual pollinators number oscillates during

the day time, like this, around 8 the pollinators' number is small, increasing slowly to 12 a clock, when the first maximum it is registered, after this the pollinators' number is decreasing to 14.Around 16.00 the second maximum is registered, smaller in amplitude.

Table 1

The daytime numbering variation of pollinator insects

Species	HOUR				
	8-9	10-11	12-13	14-15	16-17
Apis mellifera	73	215	264	138	156
Bombus lapiderium	20	33	37	26	35

To rely upon this information we can subscribe to the opinion of HORBER (1965), ALLES (1970), RUSZKOWSKI and BILINSKI (1970), who assert that pollinators' activity decreasing between hours 14-16 can be because of the fact that between these hours high air temperatures are registered, this fact determines partial retracting of insects in their nests.

By analysing the frequency timetable of honey bees and bumble bees in the pollination process, the observation made over 8 hours, for the two pollinator species, it can be seen that in general, the number of honey bees which visit the red clover field is greater than the bumble bees number, without an absolute generalization (table 2).

In addition, a high frequency of pollinators can be noticed, on diploid forms, with small exceptions.

Table 2

Daily frequency of honey and bumble bees on red clover during the maximum flowering period (per ha)

Year	Variants	2n	4n	2n	4n
		Honey bees	Bumble bees	Honey bees	Bumble bees
2004	An II mowing I 204	850.3	705.6	662.6	482.8
2005	An II mowing II 2005	157.3	132.9	107.8	77.7
2006	An II mowing II 2006	569.2	736.4	618.8	613.6

Daily frequency has been induced by frequency timetable totals, and the total number of pollinators at the existent plants on 100 mp, has been calculated by gathering daily frequencies during maximum flowering period (when over 70% flower heads are in bloom). The obtained data were related to a hectare.

A major influence over pollinators has the climate condition, specially precipitations and temperature. PONOMAREV (after SERGHIEV, 1963) doing a study regarding the meteorological conditions over red clover and over reciprocal relations between bumble bees and honey bees, ascertains that the daily and seasonal course of pollination is characterized by a certain regularity and dependence on temperature.

The ecological importance of cold and bad weather, of long standing reverberates in pollinators' flight diminishing, causing, indirectly, intensity decrease of the red clover pollination.

To illustrate this supposition, under the experimental conditions from 2004-2006, in Cluj-Napoca, we will show the climatic factors and their correlation with the red clover pollination process (table 3).

From the shown data, it can be seen that the climatic conditions of 2004 are approaching the required parameters for accomplishing a proper red clover seed production.

If we make a comparison between two climatic years, one favourable (2004) and one unfavourable (2005), we will notice the fact that the overall climatic parameters, denote total values or average values which are approximately equal, excepting bigger quantity precipitation in 2005 with 28,9 mm, which justifies the classification of favourable and unfavourable years.

Table 3

Meteorological data for the experimental years 2004-2006 in Cluj-Napoca

2004					
	June	July	August	September	
Precipitation (mm)	67.6	123.2	97.2	79.0	X=91.8 mm
Temperature mean˚C	18.7	20.4	19.6	14.1	X=18.2˚
2005					
	June	July	August	September	
Precipitation (mm)	98.6	132.8	171.6	76.6	X=119.9mm
Temperature mean˚C	17.5	20.0	19.3	16.0	X=18.2˚
2006					
	June	July	August	September	
Precipitation (mm)	165.6	29.4	129.8	6.8	X=82.9 mm
Temperature mean˚C	17.7	20.1	11.5	14.4	X=15.9˚

Most of the researchers who were engaged with tetraploid red clover breeding (VALLE, 1959, JULÉN, 1970, ESKILSSON and BINGEFORS, 1972, SAVATTI, 1973, 1998, 2002) appreciated that tetraploid forms realize after more generations of selection just 70-75% from the fertility of their initial forms.

Even though literature data are often contradictory, a conclusion can be formulated that fertility of the tetraploids is lower than that of the diploids and improving this character constitutes a priority objective in plant breeding.

In the case of the tetraploid material, analysed during 2004-2006, the fertility presented a low level by comparison with diploids (table 4).

Table 4

The seeds number on flower head on di- and tetraploid red clover variety (Cluj-Napoca, 2004-2006)

Year and determination wing	Ploidy level	$\overline{X}$	$\pm s\,\overline{X}$	%	s%	d	t	Signification
2004 (II) mowing II	2n	64.0	4.50	100.0	22.3	-	-	
	4n	48.5	0.88	75.7	18.8	-15.5	-3.38	000
2005 (II) mowing II	2n	49.4	2.08	100.0	27.3	-	-	
	4n	36.4	1.52	73.7	27.5	-13.0	-5.01	000
2006 (II) mowing II	2n	39.5	3.11	100.0	37.0	-	-	
	4n	15.8	0.64	40.1	41.0	-23.7	-7.62	000

The numerical oscillation of the number of seeds/flower head, on both red clover forms with different level of ploidy, from one year to another, makes us believe that climatic factors put their mark on this character. Clearly favourable differences appear in the diploid form, while the tetraploid genotype realizes just 40-75% in comparison with the diploids' number of seeds/flower head, confirming in this way the experimental data presented in the reference material.

It has to be pointed out that, for both ploidy levels, considerable average values of the seeds number variability are registered, with high possibilities of selection in this sense, a fact emphasized by UZIK (1971) and SAVATTI et al. (1987).

The average percent of seed formation, or relative fertility, depends directly on the seed number and flower number on flower head. In general, the variation curve of this character is nearly similar with that of absolute fertility, even though it appears as normal, like in the case of the tetraploids to be registered a slight deficit of binding percent.

With all of these contradictory opinions, most of the researchers consider that tetraploid red clover fertility deficit can be reduced by a rigorous selection during many generations, until 15% in comparison with diploid forms (AKERBERG et al., 1968, SCHWEIGER et al., 1972, SAVATTI and PAMFIL, 1984, SAVATTI at al., 1987).

In the studies made by us, high meaningful differences can be seen in year II of

vegetation (2004-2006), under diploids variety level. The tetraploid average represent just 62,7% of the diploid forms; values (table 5).

Table 5

The average percent of seeds binding on red clover di- and tetraploid types (Cluj-Napoca, 2004-2006)

Year and determination mowing	Ploidy level	$\overline{X}$	$\pm s\,\overline{X}$	%	s%	d	t	Signification
2004 (II) mowing II	2n	63.7	3.80	100.0	18.4	-	-	
	4n	45.4	0.67	71.3	44.1	-18.3	-5.35	000
2005 (II) mowing II	2n	50.1	1.95	100.0	17.6	-	-	
	4n	35.3	1.45	70.4	25.6	-14.8	-3.16	00
2006 (II) mowing II	2n	39.0	2.86	100.0	30.0	-	-	
	4n	15.0	1.16	38.5	0.2	-24.0	-7.96	000

It has to be mentioned the fact that in year II of vegetation both ploidy levels present variation coefficient with high or very high values, making it possible to improve this character by selection.

The research made during 2004-2006, has taken us to the surprising result that percentual differences of seeds number and binding percent, fertility, are much more different from a city to another, but also from a year to another. To elucidate this aspect we will present the seeds number and seedlings binding percent from an expanded ecological area, from the centre to the north of Transylvania (table 6).

Table 6

Seeds number in di- and tetraploid red clover seedlings field, in different locations (2004-2005)

year II, mowing I	Location	Ploidy level	Seeds number/flower head	
			2004	2005
2004-2005	Cluj-Napoca, Hoia	2n	56-70	61
	Jucu	2n	61	56
	Cojocna	2n	64	65
	Vişeu de Jos	2n	38-54	43
	Individual lot (Dej)	2n	38-54	49
	Individual lot (Gherla)	2n	46-49	48
	Individual lot (Gheorghe Coşbuc)	2n	39	45
	Cluj-Napoca, Hoia	4n	35	32
	Jucu	4n	30	36
	Cojocna	4n	32	30
	Vişeu de Jos	4n	29	26

For red clover seed production practice, from numbers presented above, the next surprising conclusion can be drawn: the seminal structure on both genotype types oscillated within obvious limits. The cause for this discrepancy can be put in account of different epochs of ingathering of first mowing for fodder, flowering and seeds binding having place in different climatically conditions, from where different aspects can result regarding red clover seedling yield fertility.

The same aspect is available for flowering periods of seedling yield, which can expand over 4-6 weeks. In general a decrease of the fecundated flower number can be noticed when the first mowing delays to the end of the flowering period (SAVATTI, 1998)

CONCLUSION

From the studies made it can be seen that some characters such as: seed number on flower head, flower number on flower head, pollinators, but also climatically condition have an important role in red clover fruition.

By analyzing the timetable of honeybees and bumblebees in the pollination process, it can be seen that in general the number of honeybees that visit red clover fields is higher than the number of bumblebees.

To analyse the pollination numerical variation for daytime flight, we observed every species, between 8 and 17, concluding that in general the pollinators' number oscillated during the daytime. This is mainly because of the temperature, which around noon is higher, causing in this way a low intensity of pollinators or even their going back in the nests; this observation is also available for precipitations.

Regarding fertility, the conclusion that can be formulated is that on tetraploid level it is reduced, in comparison with the diploids and the improving of this character is a priority objective in plant breeding.

The research made during 2004-2006, took us to the surprising results that percent differences of seed numbers and seed binding process, fertility, are much different from one city to another and from one year to another.

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