

# AUTOGAMY OF ALFALFA (*Medicago sativa* L.) AND IT'S USAGE IN BREEDING

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## ABSTRACT

The possibility of using autogamous forms of alfalfa in the breeding of varieties with stable seed productivity in the conditions of insufficient number of insect-pollinators has been proved. Aim of present study was to evaluate the breeding value of alfalfa inbred lines with different degree of autogamy for creation of synthetic varieties. Inbred lines of the seventh- tenth generations were obtained with a high level of autogamy (41-76%) and seed yield at the level of the variety – standard. The splitting on the trait takes place among the descendants of the inbred generations on the both of the autogamous and self-incompatible plants. The invariable high-autogamous lines have been not obtained for 34 years of research. The autogamy levels above 40.1%, had 55% of lines in the tenth generation. Inbred lines have been created with forage yields at the standard-grade level and high seed yields. Synthetic varieties with seed yields of 0.300- 0.321 t/ha and different number of components (5-7) were formed on the basis of lines with high combining ability effects. Autogamous forms which were created do not lose the ability to cross pollination.

**Keywords:** Alfalfa; autogamy; inbreeding; inbred lines; synthetic variety.

## INTRODUCTION

Alfalfa is a valuable forage crop, which plays a significant role in the obtaining of vegetable protein and in improving the quality of feed. So, high demand for seeds, which due to the low yield of existing varieties is only partially satisfied. Among the objective reasons for the low seed productivity of the varieties, the most significant is the reduction of the level of cross-pollination due to the insufficient provision of crops by insect pollinators [1]. The expansion of the area of cultivation of culture was not accompanied by the settlement and acclimatization of its effective pollinators - representatives of wild bees. Bees do

little to help it pollination, as the former do not open flowers and the latter, although adapted to the opening of flowers of representatives of the legume family, but visit alfalfa only in the absence of other honey plants [1,2].

Due to irrational use of chemical agents to control pathogens, significant climate change on the planet, the number of insect pollinators decreased to a catastrophic level (25-30%) in 70-80 years of XX century at the end of the century - up to 10-12% [1,2].

To raise the level of seed productivity of alfalfa, as well as a number of other crops, in the

conditions of insufficient number of effective pollinators, scientists from several countries have proposed the use of the phenomenon of autogamy. Mutant forms that occur in populations and are characterized by the presence in the genotypes of altered *Sf* alleles of the *S* incompatibility gene (with a single-locus gametophytic system of self-incompatibility control in entomophilous species, as well as in alfalfa sowing), capable of forming seeds during self-pollination. Such forms have been identified by researchers in several countries [3-5].

In order to describe the benefits of autogamy, alfalfa plants must combine in one genotype mutations in the structure of the lock mechanism of the flower, which cause its self-disclosure (autotripping), with mutations that reduce the manifestation of the effects of inbred depression on both the yield of fodder and seeds. It is also possible to use autogamous plants prone to pollination in a closed bud. The conversion of a typical entomophily plant from a cross- to a self-pollinated type is a very difficult task, whereas it's possible [6,7]. This is evidenced by the report that most annual species in the genus *Medicago* L. evolved from the allogamy to the autogamy reproduction method [8].

The aim of the study is to create autogamous forms of alfalfa with pods setting level of 40-70% after artificial inbreeding, a minimum effect of inbred depression (not higher than 10%) on the yield of fodder mass and seeds and the subsequent use of such forms to create synthetic varieties.

The research task included to identify autogamous forms with pods setting level of 40-70% using artificial forced self-pollination of alfalfa flowers, study the manifestation of autogamy in inbred generations, investigate the yield of fodder and the seeds of selected inbred lines, and substantiate the possibility of using inbred autogamic lines to create synthetic varieties.

The objects of study are 50 collections samples of alfalfa and 8 varieties of different ecological and geographical origin, inbred lines obtained from them. Final aim of research was to evaluate the breeding value of alfalfa inbred lines with different degree of autogamy for creation of synthetic varieties.

## MATERIALS AND METHODS

### Materials

50 collections samples of alfalfa from the collection of the Vavilov's All-Russian Institute of Plant Growing (St. Petersburg) of different ecological and geographical origin (France, Hungary, Canada, Sweden, Chile, Turkey, Denmark) and 8 varieties of Ukrainian breeding establishments of the National Academy of Agrarian Sciences (NAAS) of Ukraine, including 3 varieties of the Breeding and Genetic Institute (Odessa), 2 - Institute of Irrigation Agriculture (Kherson), 2 - Vavilov's Poltava Research Station (Poltava), 1 - Feed Institute (Vinnitsa) were used for initial research. Later were used inbred lines of the first - tenth generations, created on their basis. The first autogamous synthetic variety Yaroslavna, created by plant breeders of the Institute of Agriculture of the National Academy of Sciences of Ukraine, was used as a standard in breeding trials.

### Methods

*Self-pollination technique:* In order to determine the level of autogamy of the investigated samples, an artificial self-pollination is performed. In order to pollen tubes to germinating into pistil of the flower of the alfalfa, it is necessary to open a shuttle, which consists of two spliced petals and contains stamens and a pistil, that is to carry out "tripping", with this stamen-uterine column hitting for the surface of the vane. Opening of the flower is possible only once and supplementary pollination is impossible. The self-pollination of the flowers is carried out in the stage of the direct vane, the inflorescences are prepared by the following method: all the damaged, faded flowers, buds are removed (Fig. 1). For self-pollination, the legs of the metal tweezer seize the flower from the outside and slightly squeeze it, open it, "tripping" (Fig. 2).

The prepared inflorescences are placed under a butter-muslin isolator. The label indicates the type of pollination, date of holding, the number of pollinated flowers. Sterilization of the instruments is carried with 96° ethyl alcohol before of pollinated of each new inflorescence.



**Fig. 1. The flowers in the stage of direct vane**



**Fig. 2. Self-pollination flower**

After 40–45 days, the insulators collect, based on the number of beans that are involved in artificial pollination of 100 flowers, determine the level of autogamy. Plants with a low degree of autogamy were those who setting of 10 to 20 pods per 100 tripping flowers, with an average level - of 20.1 to 40 pods and a high - of more than 40.1 pods.

In the breeding nurseries, plants were placed a checkrow (70x70 cm). The plants individually were mowed, dried and threshed in stationary conditions after maturation the pods. The seeds were purified and weighed on the VLKT-500 scales.

Seed yields of 12 breeding specimens were studied in a nursery of a preliminary variety testing. The method of sowing is wide-row, row spacing 45 cm, seeding rate - 3 kg / ha, standard - Yaroslavna variety, repetition four times, the placement of plots is randomized, the area of the plot is 20 m<sup>2</sup>. Crop care, observation and accounting were performed in accordance with the methods of field research by B. A. Dospekhov [9].

## RESULTS AND DISCUSSION

The research of the possibility using autogamy of alfalfa in the breeding process for the creation of varieties with a sustainable yield of seeds in conditions of insufficient cross-pollination, was began in 1978 at the Institute of Agriculture in the

department of fodder crop genetics. Further research continued at the National University of Agriculture (now the National University of Life and Environmental Sciences of Ukraine).

The study of the original collection specimens made it possible to isolate from the 50,000 plants only 213 that were set the pods. These plants, as shown by studies, had mutational changes in the gene of incompatibility of the S - Sf allele, which led to the level of binding in tripping from 10 to 53 pods per 100 flowers. The study of the inheritance of autogamy by plants of ten inbred generations made it possible to conclude that among the descendants of all generations there is a split-up on both autogamous and self-incompatible plants (Table 1).

There have been no obtain constant high-autogamy lines for 34 years of research. Only in the ninth generation of inbreeding we receive line № 194, the autogamy level of all 25 plants which was high - from 52 to 73% (Table 1).

It should be emphasized that subsequent inbred generations have always received from medium- and high-autogamy plants. In the tenth generation, we were able to obtain more than 55% of lineages with autogamy levels above 40.1% among descendants. But these results were obtained in 2015, anomalous in temperature during alfalfa bloom. High air's temperatures may slightly increase the level of manifestation of the trait.

**Table 1. Distribution of inbred lines by the degree of autogamy**

Origin	Number of lines with autogamy level, pcs.				Total lines, pcs.
	0-10 %	10,1-20%	20,1-40 %	more 40,1%	
I <sub>1</sub> —I <sub>2</sub>	29	33	34	25	121
I <sub>5</sub> —I <sub>6</sub>	19	21	44	28	112
I <sub>8</sub> —I <sub>9</sub>	30	33	32	29	124
I <sub>10</sub>	2	6	9	22	39

For fodder culture, the important feature is the yield of fodder, so studied the manifestation of the feature at all stages of the breeding process. In studying the productivity of the feed mass of inbred lines, a large influence of inbred depression on this trait was established. In the first inbred generation, more than 70% of the 63 lines studied reduced the feed yield relative to the parent yield, with a maximum reduction of 32%. In the second generation, a similar trend persisted - 52% of the 58 lines studied reduced the forage yield, the maximum decrease was 41%. But among the plants of the first and second inbred generations it was possible to select plants whose productivity of forage was high. These data are in line with those of other authors who have shown that the maximum decrease in forage productivity occurs in plants of the first inbreeding generations, but the selection of high-yielding plants leads to the creation of lines with high forage productivity [10]. In our studies, the selection of plants that did not experience the effects of inbred depression on forage yield allowed the creation of lines of the fourth and subsequent generations with the manifestation of the trait on the level of the first Ukrainian variety with partial autogamy Yaroslavna - synthetic wich has five-components.

Seed productivity of the best autogamous lines from the first to the eighth generations was studied. In the first two generations of inbreeding, only 16 lines out of 121 tested were statistically indistinguishable from plants of the standard, not exceeding it, which observed and by other scientists [11,12]. It is well known that inbreeding depression has a negative effect on seed yield. It is necessary to select plants with the highest manifestation of the trait, which leads to positive consequences for overcome it. Such consistent selections have allowed the creation of lines with a minimum effect of inbred depression on an important utility character for alfalfa - seed yield. In the eighth generation, we received lines whose

seed yield exceeded the yield of plants of the Yaroslavna standard, for 3 lines it was statistically significant (Table 2).

10 lines were crossbreeding according complete diallelic scheme in 2012 in order to study the combining ability of inbred lines. Analysis of the obtained hybrids on the basis of trait "seed mass per plant" showed that only three lines had positive values of the effects of general combining ability - SF 98/35 (17.35), SF 98/16 (23.69) and SF 98/46 (0.74).

High the effects of the specific general combining ability of the trait "seed mass per plant" had hybrid combinations of lines: SF 98/35 / SF 98/16 (5,96), SF 98/46 / SF 98/25 (7.16).

High indices of the effects of specific combining ability (SCA) on the basis of trait "seed mass per plant had hybrid combinations of lines": SF 98/35 × SF 98/16 (5.96), SF 98/46 × SF 98/25 (7.16). Somewhat lower, but also high rates of SCA effects were determined in the combinations: SF 98/194 × SF 98/35, SF 98/35 × SF 98/38, SF 98/194 × SF 98/38, SF 98/16 × SF 98 / 13, SF 98/194 × SF 98/46. It should be noted that the combination of SF 98/16 × SF 98/35, had the highest performance in both directions of crossing, so it is advisable to use it to create alfalfa hybrids.

We have created 29 inbred lines of alfalfa of the seventh - tenth generations during our research. Synthetics with various number of components were formed from some of them. For this purpose, in isolated conditions, an equal number (20 pieces) of plants of each line were planted out on a plot in a fixed manner for maximum pollination by insect pollinators. Seeds (Sin 0) were collected from the entire plot and used for reproduction. In the preliminary variety testing were sown seeds of generation Sin 1, the results of the study are shown in Table 3.

**Table 2. Seed yields of autogamous lines  $I_8$  in the previous sort test (average 2010-2011)**

Title	Weight of seeds		% to standard
	average, kg/20 m <sup>2</sup>	t/ha	
Yaroslavna - standard	0,384	0,192	100
SF 98/25	0,373	0,186	97
SF 98/46	0,427*	0,213	111
SF 98/16	0,486*	0,243	127
SF 98/35	0,438*	0,219	114
SF 98/13	0,358	0,179	93
SF 98/38	0,409	0,204	106
SF 98/194	0,294	0,147	77
LSD <sub>05</sub>	0,021		

**Table 3. Seed yield of new synthetics (average, 2015-2016)**

Title	Components, pcs.	Weight of seeds		% to standard
		average, kg / 20 m <sup>2</sup>	t/ha	
Yaroslavna - standard	5	0,568	0,284	100,0
Syn- 4	5	0,512	0,265	93,3
Syn - 8	5	0,600	0,300	105,6
Syn - 9	5	0,630*	0,315	110,9
Syn - 7	6	0,632*	0,316	111,3
Syn - 5	7	0,643*	0,321	113,0
Syn - 6	7	0,602	0,301	105,9
LSD <sub>05</sub>		0,057		

The obtained results (Table 3) indicate the possibility of using autogamous inbred lines to create synthetic varieties with high seed productivity. 5 new synthetics surpassed the seed yield of the Yaroslavna standard by 5.6% - 13.0%, the seed yield was quite high - from 0.300 t / ha to 0.321 t / ha. It should be emphasized that the autogamous samples obtained do not lose their ability to cross-pollination. This is proved by our research on autogamous plants with genetically controlled recessive trait (white flowers), which were placed in an array of plants with blue flowers. All seeds from plants with white flowers gave offspring with blue flowers. The autogamous forms of alfalfa obtained by us have an easy opening of flowers under the influence of abiotic factors, which allows them to form an increased seed yield in adverse weather conditions.

## CONCLUSION

On the basis of many years of research the possibility of using autogamous forms of alfalfa in the selection of varieties with stable seed productivity in the conditions of insufficient number of insect pollinators has been proved. Inbred lines of the seventh- tenth generations were obtained with a high level of autogamy and seed

yield at the level of the variety - standard. Synthetic's varieties with different number of the components and high seed yield were obtained.

- 1 The splitting on the trait take place among the descendants of the inbred generations on the both of the autogamous and self-incompatible plants. The invariable high-autogamous lines have been not obtained for 34 years of research.
2. The autogamy levels above 40.1% had more than 55% of lines in the tenth generation.
3. Inbred lines with forage yields at the standard-variety level and high seed yields have been created.
4. Synthetic's varieties with seed yields of 0.300- 0.321 t / ha were formed on the basis of lines with high combining ability effects, and
5. Autogamous plants, which were created, do not lose the ability to cross pollination.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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