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PRODUCTION OF SEEDS OF F_1 HYBRIDS OF TOBACCO (NICOTIANA TABACUM L.) RESISTANT TO TOBACCO DOWNY MILDEW (PERONOSPORA TABACINA ADAM)

Postepy Nauk Rolniczych
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For a number of years, many tobacco-producing countries have been pursuing studies on a large scale concerning the production value of F_1 variations of tobacco hybrids. Certain hybrids show clearly a heterozygous effect under certain conditions.

As a result of catastrophic spreading of tobacco downy mildew (Peronospora tabacina Adam) in Europe, many countries started to cultivate varieties of tobacco which resist the disease. However, it takes a number of years (6-8) to obtain results of the cultivation, and the problem is particularly difficult because it is possible to obtain varieties which resist the tobacco downy mildew only by crossbreeding.

These difficulties led tobacco growers to study the possibility of using for the cultivation of the first generation of (F_1) hybrids to local varieties together with varieties which resist the tobacco downy mildew (2, 3, 7, 8), such as Hicks Resistant and Hicks A_2 fixed. These varieties come from Australia. They were cultivated by

Dr. H. Lee from hybrids of the varieties Nicotiana tabacum L. and the wild species Nicotiana debneyi (which resist the mildew). Trial cultivations of the Hicks Resistant variety in certain European countries (and also in Poland) did not give positive results. The variety is not entirely stabilized yet under the conditions existing in Poland and is subject to dissociation. Furthermore, although it is resistant to tobacco downy mildew, it is greatly susceptible to virus necrosis of tobacco veins (potato virus Y) and black root (Thielaviopsis basicola Ferr.). As a result, the crops of this variety are smaller than those of the Polish cigarette varieties which resist the disease. Similar negative results were obtained in other European countries (8).

On the other hand, the preliminary results of the introduction of Hicks Resistant with local varieties in the cultivation of F_1 hybrids were satisfactory in many cases both in terms of the crops as well as in terms of their resistance to the mildew (2, 3, 7, 8). Test cultivations of F_1 hybrids of Hicks Resistant with certain varieties of the Paraguay type were introduced in France in 1961 on an area of 6,000 ha (8).

In Poland, tests of cultivations of F_1 hybrids of the same variety with Virginia Kaznowskiego and related varieties were also favorable. The cultivation of F_1 variety on an industrial scale will encounter considerable difficulties due to the cost of the production of seeds.

The most labor-consuming activity in the production of seeds of the F_1 hybrids is the cleaning of inflorescence, castration, and pollination of the blossoms. In addition, in these processes it is also necessary to cultivate the seedlings by eliminating new non-pollinated blossoms which continue to grow.

For this reason tests have been carried out for some time in various countries. The purpose of the test is to work out a practical method of crossbreeding. The tests, which were carried out by the All-Union Institute for Tobacco and Machorka [Machorka = inferior type of tobacco, cf. transl.] at Krasnodar (4) consisted of the planting of female and male varieties in alternate rows, castration of the female variety and its free pollination. However, the results obtained were unsatisfactory because of a low percentage of germinating cases, small number of seeds in the cases, and great losses of time used for the castration of blossoms.

In the experiment by Koelle (5) only 0.066% of seeds were obtained from castrated blossoms of the Forchheimer Ogrodowy variety growing next to the Havana IIc variety. In other words, castrated tobacco plants growing next to a fertile variety produced practically no

seed. Another method applied in the Soviet Union (4), which consisted of crossing without castration, also seems unsuitable when it comes to the production of F_1 resisting to mildew. Indeed, in F_1 a rather high percent of plants are obtained from self-pollination.

Another method which should be mentioned is the method described by Toskow and Spasow (6). The method consists in the placing on the stigma of the pistil a glass tube containing a pollen of the male variety. The tube isolated the stigma from anthers which were not removed. According to the authors named above, the method requires little work and the production of 1 kg of hybrid seeds (which were enough to plant 5 ha of land) cost 200 levas.

Preliminary tests involving the use of this method at Pulawy were not satisfactory - the placing of tubes on the stigmas of the pistil requires just as much time as the castration of the blossoms. In addition, the stigma and the style of the pistil break frequently under the weight of the tube.

As the present reports show, in most cases we use the so-called "classic" method for the production of hybrid seeds, which consist in the castration and pollination of blossoms. Data given in the literature with regard to labor productivity in the production of F_1 seeds by this method vary with different authors.

Jakowuk (4) reports that during one working day a skilled worker can castrate and pollinate 500 blossoms. Unfortunately, the author does not mention the number of hours of the working day, and he does not say whether the same worker also cleans the inflorescence at the same time. The given number of seeds indicates that the author assumes that all the blossoms have been fertilized, which in practice is unattainable.

In France (where seeds of F_1 hybrids of Hicks Resistant - 8.9) were produced on a large scale in 1961 and 1962), a skilled worker carried out 150 operations in one hour (8). The blossoms were castrated at the budding stage, when the petals of the crown began to turn pink. The castration consisted in the cutting by scissors of the crown at the base of the distension of the tube, and at the same time the tube was pulled upwards, so that the stamens grown onto the tube were cut off. The number of cases damaged in the process did not exceed 4%. The report also does not give any data concerning the time required for the cleaning of the inflorescence before castration and pollination.

Bajlow (1) made a penetrating comparison of various methods used to obtain hybrid seeds of tobacco. He examines three methods: 1) The usual method of castration of blossoms by using tweezers, without removing the blossom

crowns. After pollination the blossoms are isolated. 2) Gisquet's method - the castration consisted in the plucking of crowns with stamens. The inflorescence was isolated after pollination. 3) Pollination method without castration. When a pollen of the male variety was placed on the stigma, the stigma was isolated by pure vaseline applied directly from a tube. A similar method was applied in German experiments (5).

A comparison of labor productivity of one worker applying this method is given in the table on page 96.

The table shows that the "vaseline method" was the most productive one. However, even in this work the author does not mention whether he took in consideration in his computation of labor productivity the operation involving the cleaning of inflorescences from surplus blossoms and buds, or whether the time was used exclusively for castration and pollination. The author also discusses the number and quality of the seed capsules and states that in the years of 1957-1960 the largest percentage of fallen blossoms (6.4-13%) were observed in the application of the Gisquet method and the lowest number (0-3.7%) in the usual method. The only deviation from this norm was observed in 1959 (influence of atmospheric conditions). In the application of the "vaseline method" the percent of fallen blossoms was smaller (5.6-12%) than in the Gisque method. In the application of the "vaseline method" the smallest percentage of plants was obtained from self-pollination, as shown by an analysis of F_1. The seed capsules were well filled in this case and the seeds were of good quality.

Increase of Labor Productivity with the Application of the Vaseline Method in 1957 and 1958 (according to Bajlow) per 1 Worker in One Hour

Method	1957			
	Average Number of Blossoms			
	Castrated	Pollinated	Castrated and Pollinated	Percent as Compared to the Usual Method
1) Castration and isolation (usual method)	168	186	88.5	100
2) Castration without isolation (Gisquet method)	428	449	219	246
3) Without castration. Isolation by vaseline (vaseline method)	-	277	277	311

Table Continued

Method	1958 Average Number of Blossoms			
	Castrated	Pollinated	Castrated and Pollinated	Percent as Compared to the Usual Method
1) Castration and isolation (usual method)	209.3	209.3	104.6	100
2) Castration without isolation (Gisquet method)	437.3	434.3	217.9	207.3
3) Without castration. Isolation by vaseline (vaseline method)	-	427.0	427.0	408.2

In 1963, Poland started to produce hybrid seeds of tobacco on a production scale. The components of the crossing were the Pulawy variety of Virginia Kaznowskiego and related breeds with the Australian variety Hicks Resistant. The plantation of seeds in the production of hybrid seeds was located among other places at the IUNG Experimental Station Mokradki at Pulawy.

Since there is a lack of studies concerning the appropriate methods of crossbreeding under conditions which prevail in Poland, Poland used a slight modification of the "classic method". The blossoms were castrated by tweezers, but the blossoms were not isolated after pollination by pollen of the Hicks variety Indeed, it was found that fertilization did not take place and the blossoms withered, probably due to the high temperature of the air under the insulators. The operations were carried out in the afternoon, when the number of flying insects was small and therefore there was little chance for outside pollination.

During the crossbreeding operations efforts were made to evaluate labor productivity of the workers engaged in the operations. The computation was made with regard to the parent variation of Virginia Kaznowskiego, which was planted over an area of 0.17 ha in spreads of 70 x 50 cm (to make the movements of the workers easier). The work started on 15 July 1963 and lasted until 2 September inclusive. During that period there were two holidays and three days of rain, so that the actual working time amounted to 12 days. An average of 12 persons worked

five hours a day during that period. The crossbreeding process consisted of three stages:

1. Preparation of infloresence for castration. This consisted of the removal of withered blossoms and undeveloped buds, leaving those blossoms which could be pollinated, i.e. those which have not developed yet (with pollen unopened), but have attained a clearly rosy color. The number of such blossoms, which were ready for one-time crossbreeding, varied from 4 to 19 (average 9.5).

2. The castration was performed by means of tweezers (cutting of crown and removal of anthers).

3. Pollination - pollinating stamens of blossoms of the Hicks Resistant variety were put intact with the stigma of the pistils, and in this way the pollen was put on.

The same person performed all these three operations, including the collecting and application of the Hicks Resistant pollen. According to our computations, one worker performed the following work within one hour: he castrated 124.4 blossoms or pollinated 213.3 blossoms, or castrated and pollinated 78.6 blossoms.

In comparison to the data given by Bajlow (1), labor productivity in Poland was slightly lower than in Bulgaria with regard to the "usual method". However, it should be noted that the preparation of inflorescence for castration is a labor-consuming job, that the ratio of time required for the cleaning of inflorescence and castration to the time used for pollination is 1.7 : 1 (1 hour 42 min. : 1 hour).

About four days after pollination it is possible to count the percentage of fertilized blossoms. The average proportion was 69.5% (the number of capsules germinating on a plant varied from 3 to 13, average 6.6).

On the whole, a total of 720 working hours were used to bring about crossbreeding over an area of 0.17 ha. In addition, the cleaning of inflorescence before the capsules became ripe and the collection of seed capsules and cleaning of seeds required a total of 188 hours. A total of 2.5 kg of seeds were obtained from the area mentioned above (for one-time crossbreeding). The average amount of seeds in one seed capsule amounted to 0.161 g.

Both the data obtained from literature as well as our own computations show that the production of F_1 seeds is extremely labor-consuming. We should also take in consideration the fact that even when the work is subjected to strict control, it is not possible to make sure that the crossbreeding will be done without any errors. The errors can occur at the time when inflorescence is prepared for castration, during the castration itself, or at the

time when new blossoms and buds are removed before the seed capsules become ripe. That may be the reason why there is a certain admixture of seeds from self-pollination.

For that reason the suggestion made by Bolsun (2) seems appropriate. Bolsun thinks that the problem must be solved by cultivating sterile male forms of the corresponding varieties. Male-sterile lines of certain varieties of tobacco are used in some countries (for example Austria, Fürstenfeld), and studies are under way to determine whether they can be used in the production of hybrid seeds. The use of such forms would reduce considerably the costs of labor involved in crossbreeding, because castration would become unnecessary and also it would not be necessary to clean the inflorscence before and after pollination.

Work designed to obtain male-sterile varieties is also carried out at the Institute in Pulawy (this applies among varieties to the variety Virginia Kaznowskiego). It must be assumed that these varieties can be used in the future in the production of hybrid seeds of tobacco.

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