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ON APPLYING THE METHOD OF IMMUNOGENETIC ANALYSIS IN PLANT BREEDING

In recent years it has been accepted that, in plant breeding, genetics with its different branches, with molecular biology and biochemistry, play the role of generators of new ideas. Below we shall deal in greater detail with one branch of genetics — immunogenetics, which makes use of serological and hybridological methods of study.

Several investigators have tried to apply the serological method in plant breeding. It is well known that the most difficult problem in plant breeding is the selection of parental combinations and of the corresponding offspring. In plant breeding, individual selection is applied for practical purposes. Since segregating generations consist of a great number of different genotypes, a hybridological analysis is very labour- and time-consuming. It is for this reason that the application of the serological method in plant breeding is of great interest. Thus, Doubly and collaborators (1960) discovered that the titres of antisera of a host plant correlate with its resistance. So it appeared that flax is susceptible to the rust fungus if the titres of the antiserum of the rust fungus are with the flax antigen 1 : 160 or 1 : 320, and is resistant to the rust fungus if the titre amounts only to 1 : 20 or 1 : 40.

Edgecomb (1931) compared globulins in wheat cultivars. He succeeded in disclosing a parallelism between the serological relationship of cultivars and the number of chromosomes.

Nelson and Birkeland (1929) studied the similarity of globulins in 5 wheat cultivars by means of the precipitation reaction; the serological relationship was expressed in percentages. These investigators established the fact that the heterological reaction (e. g., the reaction of the antiserum of cultivar A to the protein of cultivar B) was always below 100 per cent. However, as we shall see later, this need not be the case.

Frey and associates obtained interesting results in the application of the serological method in plant breeding. Kleese and Frey (1964) claim that it is highly significant, in plant breeding, to predict the combinative value of the components of crossing. For this purpose a series of crossings are performed with cereals, and the variability of the offspring of each cross is determined over the period of one or more generations. The above-mentioned investigators tried to determine the suitability of the components of crossing by determining the concentration of their common antigens by means of the quantitative pre-

cipitation reaction. For that purpose they used dilutions of antigens and a constant amount of an antiserum. At the same time Kleese and Frey made use of the reciprocal precipitation method, performing the determination of antigens by means of the antiserum of a standard cultivar in all cultivars, and, parallelly, the determination of the common antigens by means of the antisera of all cultivars to the protein antigen of a standard cultivar (a cultivar selected as the principal cultivar for comparison). In this way they attempted to determine the serological relationship of the standard cultivar with other cultivar, using the formula: (the turbidity of the heterological reaction : turbidity of the homological reaction) × 100. However, the interpretation of the results of the precipitation reactions used does not show the serological relationship in the cultivars under comparison (cultivars A and B, cultivars A and C, cultivars A and D, etc.), but only the concentration of the common antigens in the couples of the cultivar under comparison. This is also indicated by the circumstance pointed out by the authors that in three cases the heterological reaction yielded a closer relationship than the homological one (e.g., the anti-A serum yielded a more intense reaction with cultivar B than with cultivar A).

Regardless of the above methodological drawback, Frey and his associate established the fact that the weaker the serological relationship between the parental combinations studied (A and B, A and C, A and D, etc.), the larger the variance of the grain yield of the given cross. (It would have been more correct to say: the lower the concentration of common antigens, the greater the variance of the grain yield of the given cross; Pavel).

The phenomenon that in the case of certain combinations under study (e.g., A and C, A and D) the value of the heterological reaction exceeds the value of the homological reaction (i.e. the genetic relationship is above 100 per cent) was explained by Smith and Frey (1970) by the circumstance that a definite cultivar contains a larger amount of the corresponding antigen(s). For instance, the reaction of the protein solution of cultivar A and of the antiserum of cultivar A is 100 cent; however, the reaction of the protein solution of cultivar B and of the antiserum of cultivar A is, say, 120 per cent. Consequently, cultivar B contains more corresponding proteins (resp. antigens) than cultivar A.

Smith and Frey try to remove this drawback by using the following formula in the processing of experimental data:

$$A/B\,\% = \sqrt{\frac{\text{anti A}\rightarrow\text{B}}{\text{anti A}\rightarrow\text{A}} \times \frac{\text{anti B}\rightarrow\text{A}}{\text{anti B}\rightarrow\text{B}}} \times 100,$$

where A/B represents the relationship between cultivars A and B, anti-A→B designates the reaction of A-antiserum with B, anti-A→A signifies the reaction of A-antiserum with A, etc.

This method, indeed, makes it possible to determine the serological relationship between two cultivars. Bearing still in mind the data presented by Kleese and Frey, it is possible to replace the idea of a serological relationship by the idea of a genetic relationship. It must be noted here, however, that in the method of the reciprocal precipitation method of Smith and Frey one must use the so-called standardized antisera (having the same capacity).

Proceeding from the reports of Frey and his associates it seems to be very attractive not to confine oneself to the application of the serological method in determining the genetic relationship between the

components to be crossed, but also to use the immunogenetic method for a simplified grouping of the offspring of a segrating generation (F_2 and F_3). In other words, the method of an immunogenetic analysis can be used in the selection of parental combinations as well as in the serological classification and selection of the offspring of crosses. In doing this, one has to use the method of Smith and Frey.

The need for the latter (e. g. serological) differentiation becomes apparent from the following discussion. Let us assume that we have a self-pollinating crop. At the same time we proceed from the fact that the two cultivars to be crossed differ in 10 genes. Then in the following generations $2^{10}=1024$ homozygotic and $3^{10}=59\,049$ all combinations will be formed (the ratio of homozygotes to all combinations is about 1 : 60)*. In practice, however, they cannot be distinguished.

Here one has to use a simplified method, namely the immunogenetic method, the main points of which are as follows.

1. Components of crossing (parental combinations) are selected on the basis of the genetic relationship, if possible, by choosing contrasting cultivars that differ in the grain yield or in some other agronomic characteristic. In this case one has to use the reciprocal precipitation reaction.

2. The grains of the F_3 generation are divided into three sets on the basis of a serological analysis: those similar in antigenicity to parent A, those similar in antigenicity to parent B, and the intermediate ones — I.

3. The grain yield off sets A, B and I is determined in the F_4 generation and, in case of need, a serological analysis of set I is randomly performed.

Schematically the immunogenetic method proposed here consists of the following stages:

1. Crossing (A×B) — 1 F_0 plant (5 F_1 grains).

2. Five F_1 plants (100 F_2 grains).

3. A hundred F_2 plants (2,000 F_3 grains). The serological analysis: one half of the grains of panicles, i. e. all together 1,000 grains.

4. Three sets (A, B and I) are sown separately — 1,000 F_3 plants (20,000 F_4 grains). A random serologic analysis of set I is performed.

5. Analysis of three separate sets of the grain yield (about 20,000 F_4 plants).

The method proposed essentially simplifies the breeding of the grain yield or any other nonvisible agronomic characteristic. Likewise, the time interval foreseen for studies is considerably shortened. In case a greenhouse is used, the whole study cycle lasts for three or four years.

* In 1000 plants there are 17 homozygotes and 893 heterozygotes.

REFERENCES

Doubly J. A., Flor H. H., Clagett C. O., 1960. Relation of antigens of *Melampsora lini* and *Linum usitatissimum* to resistance and susceptibility. Science **131** (3394) : 229.

Edgecomb A. E., 1931. Immunological relationship of wheats resistant and susceptible to *Puccinia rubigo-vera triticina*. Bot. Gaz. **91** (1) : 1—21.

Kleese R. A., Frey K. J., 1964. Serological predictions of genetic relationships among oat varieties (*Avena sativa* L.) and corn inbreds (*Zea mays* L.). Crop Science **4** : 379—383.

Nelson C. I., Birkeland J. M., 1929. A serologial ranking of some wheat hybrids as an aid in selecting for certain genetic characters. J. Agric. Res. 38 (3) : 169—181.

Smith R. L., Frey K. J., 1970. Use of quantitative serology in predicting the genotypic relationship of oat cultivars. Euphytica 19 (4) : 447—458.

Hsieh S.-C., Frey K. J., 1972. Serological predictions of genotypic relationships among rice *Oryza sativa* cultivars. Egyptian J. Genet. Cytol. 1 (2) : 288—299.

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IMMUNOGENEETILISE ANALÜÜSI RAKENDAMISEST SORDIARETUSES

Resümee

Tehakse ettepanek mitte piirduda seroloogilise meetodi rakendamisega ainult ristatavate vanematevormide geneetilise (seroloogilise) suguluse määramisel, vaid kasutada ühiste antigeenide määramist poolkvantitatiivse pretsipitatsioonireaktsiooni abil ka lahknevate põlvkondade seroloogilisel rühmitamisel. Ühtlasi on sobiv määrata valitud järglaste (seroloogiliste rühmade) saagikus. Teiste sõnadega, immunogeneetilist analüüsi tuleb kasutada nii vanematepaaride valikul kui ka järglaste seroloogilisel klassifitseerimisel ja valikul.

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ПРИМЕНЕНИЕ ИММУНОГЕНЕТИЧЕСКОГО АНАЛИЗА В СЕЛЕКЦИИ РАСТЕНИЙ

Резюме

При определении генетического (серологического) родства между скрещиваемыми компонентами предлагается не ограничиваться серологическим методом, а использовать также иммуногенетический анализ, состоящий в определении количества общих антигенов (полуколичественной реакцией преципитации) у расщепляющих потомков, объединяя их в серологические группы. Параллельно следует определить и урожайность у отобранных серологических групп. Другими словами, иммуногенетический анализ применяем как при подборе родительских форм, так и при серологической классификации и отборе потомков.

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