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TECHNIC OF RICE HYBRIDIZATION IN CALIFORNIA[1]

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RICE HYBRIDIZATION IN OTHER COUNTRIES

The earliest record found by the writer regarding the artificial hybridization of rice dates back to 1901. In that year Hoshino (2),[3] of Japan, records having made two crosses of glutinous x common rice, one in the greenhouse of the College Botanical Garden at Sapporo, Japan, in May, 1901, and the other in a paddy field in August of the same year. He obtained 6 hybrid seeds in the greenhouse and 12 in the field. In making the crosses the glumes of the female parent (glutinous rice) were clipped off prior to emasculation and, therefore, the hybrid seeds were exposed to the air. It was not possible to tell by observation whether the hybrid seed possessed a glutinous or a common endosperm. Therefore, some of the hybrid seeds were cut and soaked in a solution of iodine. All the hybrid seeds turned violet in color as common rice does, whereas self-fertilized female glutinous seeds changed to a brown color. "It showed perfectly the phenomena of zenia in the rice endosperm." This was the first demonstration of zenia in the rice endosperm.

Koch (4) states that in 1907 Van der Stok started hybridization experiments with rice in Java. He crossed Karang Serang, an early-maturing variety of good quality, and Skrivimankotti, a high-yielding variety of poorer quality. Koch states that the best method of hybridization is as follows: Cut off the tops of the glumes with scissors a few hours before flowering (blooming), then remove the exposed anthers with a fine needle and pollinate a few hours after with pollen from the male parent. The panicles are then enclosed in a gauze envelope, which is protected at night and during rain by a little cover of dry leaves. By this method Koch, in one case, obtained 43.3% of hybrid seed. However, at times no hybrid seeds were obtained, due apparently to nonviable pollen.

In India, Hector at Dacca and Parnell at Coimbatore began hybridization work with rice about 1913.

As late as 1913 Farneti (1), of Italy, claimed that rice flowers never opened before, during, or after dehiscence of the anthers.

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[3]Reference by number is to "Literature Cited," p. 40.

Therefore, natural crossing was impossible. All the different rice varieties present must have originated as mutations. His attempts to cross rice resulted in failures. All the flowers artificially opened, whether or not the stamens were destroyed, remained unfertilized, whereas all those flowers not operated on set seed. However, Farneti reported that it was possible to cross rice provided sufficient patience and skill were used in removing the anthers by inserting a fine instrument through the minute openings at the points of the glumes. It was difficult, however, to avoid self-fertilization or injury resulting in sterility.

Sharngapani (6) gives the following description of the method used in Bengal for crossing rice:

"The two glumes are very gently pulled apart with the fingers—no forceps should be used—and the stamens removed with a pair of fine bent forceps. About two hours later, when the rice flowers begin to open, the emasculated flowers are pollinated, and the glumes are closed and tied up with a piece of fine silken thread. The tying helps to keep the glumes in their natural position. If the glumes are not tied up, they do not close properly, and the percentage of successful crosses diminishes greatly. The tying up also does the work of bagging, and no further bagging is necessary."

Torres (7) and Rodrigo (5), in the Philippine Islands, clipped off the glumes of the female parent in the emasculation process, which was done either in the morning before blooming began, or in the afternoon after blooming had ceased. The female was pollinated the following day if emasculated in the afternoon, or on the same day when emasculated in the morning. Rodrigo reports that the average number of pollen grains per stamen in three varieties ranged from 643 to 915. This means that each floret produces from 3,858 to 5,490 pollen grains, only 1 of which is necessary for fertilization.

RICE IMPROVEMENT WORK IN THE UNITED STATES

Rice is the most important cereal crop grown in many Asiatic countries. It is of only minor importance, however, in the United States where it has been grown since 1694. Louisiana, Arkansas, and Texas, in the order given, are the leading rice-producing states in the South, while on the Pacific Coast California grows considerable rice. In total production Louisiana ranks first and California second.

Rice improvement work in the United States consists largely of (a) the testing of varieties introduced from foreign countries, and (b) the testing of pure-line selections isolated from varieties introduced from foreign countries and from varieties grown commercially. The program to develop more desirable commercial rice varieties by hybridization was started in California in 1922 Some of the strains isolated from the first crosses made at that time are very promising.

FAULTY TECHNIC

E. L. Adams, in charge of the Biggs Rice Field Station from 1912 to 1918, attempted to hybridize rice, but no crosses were obtained.

The writer in 1920 and in 1921 tried to cross rice at the Biggs Rice Field Station, but without success. In each of these years spikelets which had emerged above the leaf sheath were carefully emasculated in the morning by gently pulling the glumes (lemma and palea) apart and removing the anthers with a pair of fine-pointed forceps. The emasculated panicles were then bagged. The following day about noon pollen was collected from panicles which were in bloom and the stigmas of the emasculated flowers were liberally dusted with this pollen. No hybrid seeds were obtained.

It seemed possible that the failure to obtain hybrid seed might be due to using bags of ordinary bond writing paper to inclose the panicles after emasculation and cross-pollination. Accordingly, in 1922, an experiment was conducted to determine the effect of using various kinds of cloth and paper bags on the setting of rice seed. This experiment indicated that the kind of bag has no appreciable effect on the number of seed set.

In the same year (1922), the time of blooming of quite a large number of flowers in different rice varieties was observed (3), and it was found that more than three-fourths of the flowers under observation bloomed between 12 noon and 2 p. m. The results of this study indicated that previous failure might have been due to nonviable pollen and that the best time to collect pollen for crossing purposes was from 12 noon to 2 p. m.

Coincident with the above observations on the time of blooming further attempts were made to obtain certain rice crosses. A large number of spikelets were emasculated by gently pulling the glumes apart and removing the anthers with a pair of fine-pointed forceps. Two hybrid seeds were obtained. One of these hybrid seeds failed to germinate in 1923, but the other produced a hybrid plant.

The conclusion was reached that the pollen collected from spikelets which were in bloom probably was not viable. In 1923, therefore, pollen was collected from anthers carefully removed from florets which were just about ready to dehisce. At this stage of development the anthers are pushed up near the apex of the glumes and are easily seen by holding the florets up to the sunlight. This pollen was placed on the stigmas of certain emasculated florets, and pollen collected from spikelets which were in bloom was placed on the stigmas of other florets on the same panicle. In nearly all cases the pollen taken directly from the anthers before dehiscence, if sufficiently mature, resulted in fertilization, whereas the pollen collected from spikelets which were in bloom seldom produced hybrids.

It also was found, as previously observed by others, that the best practice in emasculating rice flowers is to clip off the ends of the glumes before removing the anthers, rather than gently to pull apart the glumes. The glumes (lemma and palea) are very delicate and easily injured at blooming time, and if handled are likely to dry up and result in sterility. By clipping off the lemma and palea at an angle of about 45 degrees with a small pair of scissors it has been found possible to remove the anthers very easily with little injury to the glumes or floret.

By clipping the glumes at an angle, removing the anthers with a pair of fine-pointed forceps, and using only pollen from well-developed anthers collected just before dehiscence, hybrid seed of all desired crosses have been obtained. Under field conditions, however, the percentage of seed set, even with this method, is comparatively low. On an occasional panicle 75% of the emasculated florets are fertilized, whereas on others many of the florets fail to develop. As an average, from 5 to 25% of successful fertilizations are obtained.

IMPROVED TECHNIC

The method now used in the hybridization of rice at the Biggs Rice Field Station is as follows: Usually in the morning before the rice begins to bloom, or less often in the afternoon after the daily blooming period has passed, all except 15 to 20 spikelets are removed from the female panicle. The glumes on the remaining spikelets are clipped off at an angle of about 45 degrees. This removes about half of the upper part of the lemma, but only the end and sometimes none of the palea. By clipping the glumes in this manner all six anthers are exposed. They can be easily removed with a pair of fine-pointed forceps, often in one operation.

It is best in removing the anthers to begin with the upper spikelet proceeding in order to the lower spikelets of the panicle. With this procedure there is less likelihood of pollen falling into an open flower below. After the anthers are removed the emasculated panicles are tagged and bagged in the manner usual for cereal plants. The same day or the following day, between 12 noon and 2 p. m., the male panicles are examined and a few panicles on which the anthers are pushed well up toward the apex of the glumes are collected. The glumes of the spikelets in which the anthers are well developed are gently pulled apart and one or more anthers are taken and placed in the emasculated female floret. It often is necessary to break the anthers open to insure that the stigmas are well covered with pollen. This can be determined with a hand lens. Often well-developed anthers will lose some pollen as soon as the glumes are pulled apart and the anthers are exposed to the air. Such anthers are ideal for use in cross-pollination and often several florets can be pollinated with a single anther in such condition. Several panicles are often required, however, to obtain sufficient pollen to pollinate 15 or 20 florets. This is owing to the fact that at any one time the anthers of only a small percentage of the spikelets are in the proper stage of development. If immature pollen is used, fertilization does not take place. After pollen has been placed on the stigmas of the emasculated florets, the panicles are again bagged and the bags are left on until the hybrid seed is mature.

It has been observed that the hybrid seed develops better within the bags than when the bags are removed after the seed starts to form. This probably is due to higher humidity within the bags, which appears to be an advantage in California, but may be a disadvantage in more humid countries.

The bags also protect the exposed seed from insects. The hybrid seeds when fully matured extend beyond the clipped glumes, and often they are considerably longer than normal seeds. The unprotected end of hybrid seeds appears to attract insects which soon destroy the entire seed if it is not protected.

Hybrid seeds often are shriveled and poorly developed and do not germinate as well as do the fully developed normally uncrossed seed However, well-developed hybrid seed germinates equally as well as uncrossed seed. Because of the poor development of some hybrid seed, very often only about 50% of hybrid seeds produce plants, even when they are started under very favorable conditions.

In Japan the improvement of rice by hybridization has been employed for many years. Japanese investigators have found that high temperatures and high humidity are favorable for fertilization in the rice plant. These factors can be controlled in a hothouse, whereas they can not be controlled under field conditions, and most of the Japanese rice crosses accordingly are made in greenhouses. The technic used by the Japanese is essentially the same as that described as now used at the Biggs Rice Field Station. Their percentage of successful crosses is doubtless higher, however, than that obtained at Biggs under field conditions.

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