Hybridization Methods with Paddy

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EXTENSIVE use of paddy selections of hybrid origin appears to be confined to Japan and the U. S. A.; the percentages of selections of hybrid origin under issue in these two countries, according to the World Catalogue of Genetic Stocks, are 64 and 47, respectively. The percentage of hybrids listed from the rest of the rice-growing countries is under three. Systematic work on the hybridization of paddy in Ceylon is still in its infancy. The present paper records the results of preliminary studies in technique.

ANTHESIS

At Peradeniya, opening of the glumes commences after 10 a.m. and ceases by 2 p.m. Blooming reaches its peak during the interval 11.15 a.m. to 11.45 a.m. Most of the spikelets are observed to have opened by 12 noon. On rainy days with very cloudy weather blooming takes place even as late as 4.30 p.m. The glumes generally remain open for 39 to 62 minutes, though in some instances the period may exceed 90 minutes or more.

Ordinarily a panicle completes emergence from the flagleaf in about 3 days. The spikelets commence opening a day or two after emergence. If the panicle emerges before noon, blooming may commence on the same day. It was observed that the dehiscence of the anthers, even within the same variety, takes place either simultaneously with the opening of the glumes, or before, or soon after they emerge from the glumes. All the spikelets in a panicle complete blooming by the 8th day from the commencement, with the majority opening on the 3rd day. Thus panicles which were in the 3rd day of blooming are preferred for emasculation, particularly when earlier blooming was forced by isolating the panicle, as the largest number of flowers respond on that day.

EMASCULATION METHODS

In the method normally adopted at Peradeniya, all the spikelets which have bloomed previously are clipped off from the panicle 3 to 2 hours before the natural time of blooming. The spikelets are then emasculated immediately afterwards. At Peradeniya, the period 7.30 a.m. to 9.15 a.m. is found to be the most suitable and the most convenient time to emasculate. It is observed

that the anthers handled after 9.30 a.m. often dehisced. Emasculation is always commenced with the top spikelets, and progress downwards because of the basipetal nature of flowering. Moreover, the likelihood of pollen falling into the open spikelets below is minimised. Immediately after emasculation, each spikelet is examined with a lens in order to ensure the absence of pollen. All the unemasculated spikelets are then clipped off, and the panicle is covered with a paper bag. Instruments are sterilized with methylated spirits during the operation.

A variety of emasculation methods is adopted by rice-breeders. Torres (1923) describes a method in which the upper portion of the glumes are clipped off transversely the previous afternoon and emasculated; pollination is done the next morning. Sharngapani (1924) describes a method used in Bengal, in which the two glumes are gently pulled apart with the fingers and the stamens removed with a pair of fine forceps. This method unless very carefully followed leads to the damage to the lodicules. Ramiah (1927) reported a method which has been successfully adopted in Coimbatore and elsewhere, in which the panicle is enclosed in a brown paper envelope 1 to 1½ hours before the natural time of blooming. On the removal of the envelope 15 to 30 minutes after inversion, all the spikelets which are due to open on that day open in a flush, forcing the extrusion of undehisced anthers. The anthers are then removed without difficulty. The efficacy of this method was investigated in Peradeniya.

Paper covers of seven colours were tested. The covers measured 14 in. by 4½ in. Covers 1–5 were of tissue paper, and covers 6 and 7 were of thick, opaque paper. Panicles of *Panduruwi* which were selected for each treatment were examined the previous evening and the spikelets which had opened previously were removed. The bags were inverted over the panicles the next morning at the times specified in Table I—

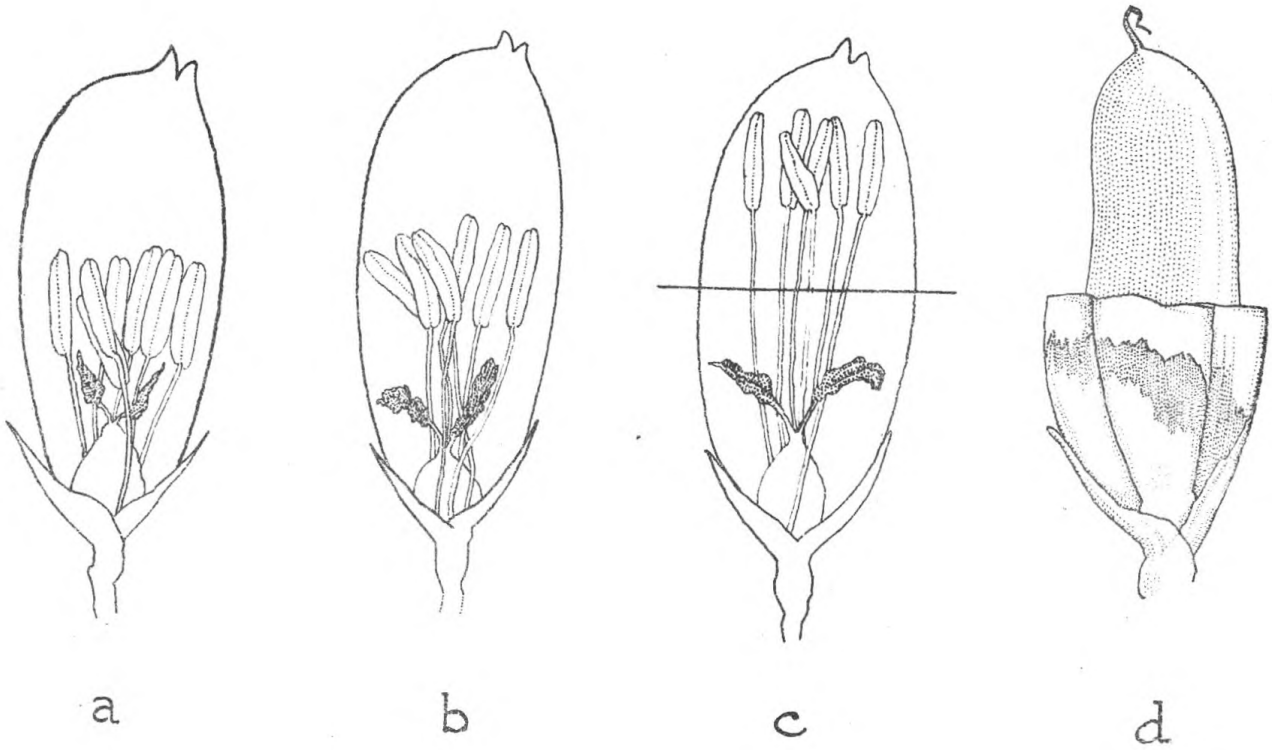
Table I

	Time of inverting bags	*1st Spikelet*		*3rd Spikelet*	
		Interval between treatment and blooming	*Temperature inside cover at time of blooming*	*Interval between treatment and blooming*	*Temperature inside cover at time of blooming*
	A.M.	*Min.*	*°F.*	*Min.*	*°F.*
1. White	7.41	177	85·1	183	86·0
2. Blue	7.43	114	85·1	159	86·5
3. Green	7.48	129	86·9	142	86·9
4. Yellow	7.47	136	87·8	174	87·4
5. Red	7.54	134	86·0	173	88·7
6. Brown	7.51	121	86·0	131	88·7
7. Black	7.45	104	86·9	126	87·8
8. Control	—	177	—	216	—

The results presented in the above Table clearly show that the black and brown covers were the most effective. The results obtained in this experiment has been confirmed in subsequent tests. It is evident that the

The stage at which the anthers are clipped off and the kind of seed which is formed, in the emasculation technique developed by the writer.

Fig. 1.



number of spikelets which open under the conditions prevailing at Peradeniya is, however, comparatively small, except when the covers were inverted after 10 a.m.; but at such a time there is considerable risk of the anthers dehiscing when handled. This method accordingly appears unsuitable for Peradeniya conditions.

The hot water immersion method of Jodon (1938) was tested in Peradeniya with satisfactory results. The results with this treatment were, however, not consistent. It was tested on many different varieties over a period of six months. The initial temperature of the warm water used ranged from 98·6 °F to 111·2 °F. and the period of immersion ranged from 3 to 14 minutes. The time of the commencement of immersion varied from 8·25 a.m. to 9·52 a.m., but was not an important factor. The most satisfactory results were obtained by the use of water at an initial temperature of 111·2 °F. for a period of 10 minutes. The atmospheric temperature which ranged between 77 °F. and 87·8 °F. during the treatments did not seem to have any effect on the response to this treatment. The varieties *Panduruwi* and *Taichu No. 65* responded well, while *Vellai illankalayan*, *Aikoku*, and *Norin 25* gave poor or no response. The other varieties gave inconsistent results. Fine-grained varieties seem to respond better than coarse-grained varieties. Differences in behaviour, however, cannot be solely explained on morphological grounds. Factors like panicle age seem to affect the response to this treatment. Panicles whose age from the time of emergence is under three days, give little or no response.

The writer usually adopts Jodon's method as a preliminary. The panicle which is to be emasculated is usually first treated with warm water at an initial temperature of 111·2 °F for 10 minutes. If the spikelets reacted and the stamens came out, there was no further difficulty. Otherwise the opening of the glumes was assisted with a needle. Even if the stamens remained inside after the forcing open of the glumes, it was not difficult to remove them, as the filaments would have elongated as a result of the warm water treatment. This preliminary treatment was also advantageously adopted in the new technique of emasculation developed by the writer and described below.

In the forenoon before a spikelet is due to open the anthers are pushed up by the rapidly elongating filaments, as shown in Fig. 1 *a* and *b*. Shortly after the anthers reach the apex as in Fig. 1 *c*, the glumes open. In this technique the upper portion of the glumes, together with all the anthers inside it are clipped off in one operation with a curved pair of scissors, when the anthers reach the stage shown in Fig. 1 *c*. It is quite simple to perform this operation, as the position of the organs inside the spikelet is clearly seen by holding it in transmitted light. This method is much easier to adopt in varieties in which the stigmas are pigmented as their exact position is more clearly seen through the glumes. Examination of the stigmas, after emasculation shows that there is no pollen on them, in spite of the fact that this operation is done close upon the natural time of blooming. A much large number of spikelets can be emasculated per day by this technique than is possible with any of the other methods described above. By immersing the panicle in

warm water 3 to 2 hours before the natural time of blooming, it is possible to make the spikelets reach the stage shown in Fig. 1 *c* within 10 minutes. This eliminates chances of the anthers dehiscing and also makes it possible to handle more spikelets in a day. In this method 1·2 per cent. selfing has been recorded. The cup-like nature of the emasculated spikelet facilitates the examination of the stigmas, and eases pollination. It is found necessary to keep the panicle bagged till the hybrid seeds mature, particularly during wet weather. The seeds which form assume the shape shown in Fig. 1 *d*.

POLLINATION METHODS

Many different methods are practised by rice-breeders for obtaining and applying pollen. Most of these were tested by the writer and the under-mentioned methods were found satisfactory.

A well developed, unopened spikelet, in which the anthers are just touching the roof of the glumes is removed. The glumes of the selected spikelet are gently pulled apart with the fingers and the stamens in which the anthers had not dehisced, are carefully extracted by means of their filaments. These stamens are placed on the palm of the hand or in a watch-glass and examined with a lens for the right stage of ripeness. In an anther which is about to dehisce, the two lobes assume a characteristic consistency, and release pollen grains when lightly pierced with a needle. An anther in such a stage of ripeness when taken on a mounted needle and gently rubbed on the inside of the glumes of the " female " spikelet, dehisces releasing a cloud of pollen. When partially burst anthers are used, the stigmas are lightly touched with it.

A method which the writer finds very effective is to place 4 to 5 ripe anthers in the spikelet of the ovule parent by about 11 a.m. The empty anther sacs are removed by 3 p.m., using a chip-blower or mounted-needle. This pratice, in addition to giving good results, greatly reduces the time that is taken up in pollination.

It is advantageous to delay the pollination till the 3rd day of emasculation, in order to detect any spikelets that may have been accidentally selfed. Selfed ovaries swell by the 3rd day. On the other hand, results obtained in Peradeniya indicate that there is an appreciable loss in receptivity by the 3rd day.

In pollinating spikelets in which the glumes had been forced open either by the method described by Sharngapani or Jodon, the writer has adopted a modification in order to prevent any damage to the lodicules. By inserting between the lemma and the palea, a pin possessing the shape of an inverted V, the palea is obliquely pushed backwards and is held in position by the left arm of the inverted pin, as shown in Fig. 2. This method of obliquely turning the palea to a side, instead of pulling both the glumes apart, prevents any damage to the lodicules. It also has the advantage of keeping the spikelet open till the entire operation of pollination is complete.

The use of a V-shaped pin to keep the spikelet open.

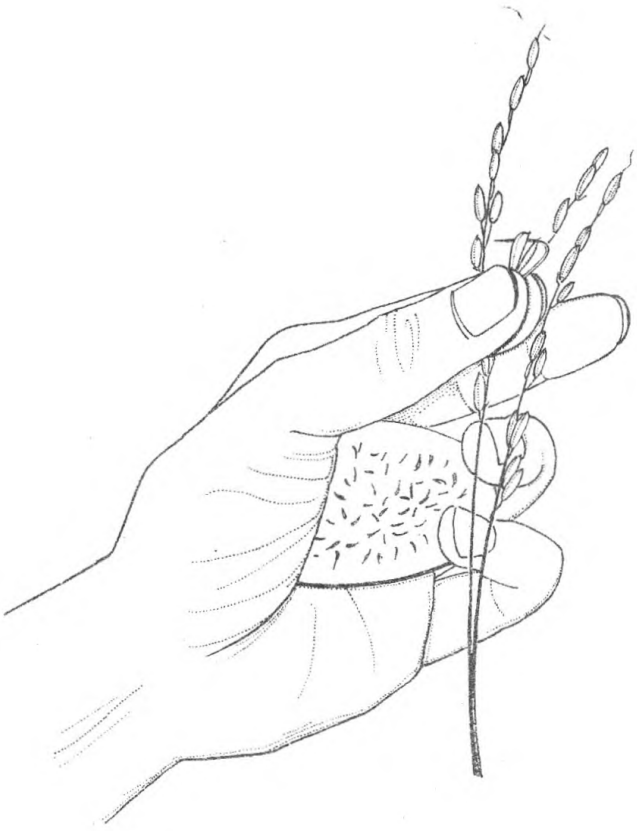
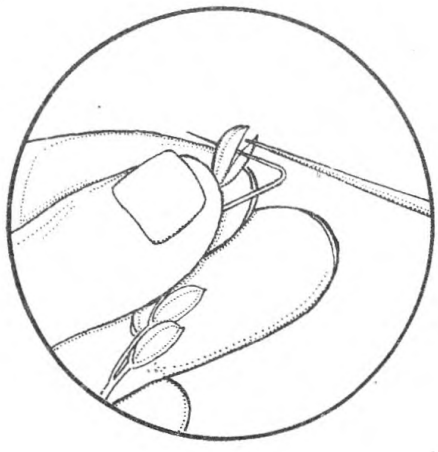


Fig. 2.



GERMINATION OF F_1 SEED

As the ordinary methods of germinating seed in porous dishes leaves much to be desired, the following method based on Mendiola (1926) was adopted. The grains after the removal of the glumes, are steeped in a 0·1 per cent. solution of Mercuric chloride for 15 minutes, and then washed 3 times in sterile water. Each naked kernel is then transferred into a test tube of sterilized Agar containing the following :—

Calcium nitrate	0·100	per cent.
Potassium biphosphate	0·025	,,
Magnesium sulphate	0·225	,,
Ferric phosphate	0·005	,,
Ammonium sulphate	0·100	,,
Agar	Two	,,

The Agar medium is autoclaved in test tubes for 15 minutes under 15 atmospheres of pressure.

This method gives nearly 100 per cent. germination, even in the case of the mutilated seed obtained by the writer's new technique of emasculation. The germination of the unhusked grain in Agar is found to be comparatively poor. The naked rice, on the other hand, germinates within 2 days and the seedlings grow to a height of 7 in. within 10 days. When they reach this stage they are planted out in pots.

SUMMARY

The results of studies in hybridization technique with paddy are presented.

Records made at Peradeniya of the anthesis of paddy indicate that the period 7.30 a.m. to 9.15 a.m. is the most suitable time to emasculate.

The results of the writer's tests of emasculation methods in use in other countries are described.

Details of an emasculation technique developed by the writer are given. The technique entails the clipping-off in one operation of all the anthers and the upper half of the glumes.

A method of germinating F_1 seed is described.

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Paddy Yields

Season, Year	Paddy—Extents Sown Yields				Rice Production
		Yield–Paddy			
	Extent Sown (acres)	Bushels	Long Tons	Metric Tons	Rice available for consumption. Metric Tons (1)
Maha 1943–44	680,919	9,119,013	182,380	185,275	103,309
Yala 1944	349,313	4,447,192	88,944	90,356	50,382
Total 1944	1,030,232	13,566,205	271,324	275,631	153,691
Maha 1944–45	633,563	7,900,411	158,008	160,516	89,504
Yala 1945	262,097	2,900,059	58,001	58,922	32,855
Total 1945	895,600	10,800,470	216,009	219,438	122,359
Maha 1945–46	610,070	7,847,668	156,953	159,444	88,906
Yala 1946	317,008	3,502,851	70,057	71,169	39,684
Total 1946	927,078	11,350,519	227,010	230,613	128,590
Maha 1946–47	616,507	8,013,348	160,267	162,811	90,783
Yala 1947	310,176	3,506,982	70,140	71,253	39,731
Total 1947	926,683	11,520,330	230,407	234,064	130,514
Maha 1947–48	—	—	—	—	—
Yala 1948	343,055	4,344,562	86,891	88,270	49,210
Total 1948	—	—	—	—	—
Maha 1948–49	635,987	9,007,580	180,152	183,012	102,047
Yala 1949	436,816	6,227,667	124,553	126,530	70,553
Total 1949	1,072,803	15,235,247	304,705	309,542	172,600
Maha 1949–50	671,916	9,432,925	188,659	191,653	106,865
Yala 1950 (2)	409,700	5,567,505	111,350	113,118	63,075
Total 1950 (2)	1,081,616	15,000,430	300,009	304,771	169,940

(1) Total production of paddy (less 15 per cent. seed allowed and 3 per cent. waste) converted into rice. Extraction rate 68 per cent.
(2) Revised estimate.