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Mechanized Production of F_1 Seeds in Rice by Mixed Planting

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Abstract
F_1 seed production of hybrid rice is practiced in Chine by planting a male sterile line and a pollinator line in alternative rows. This planting method is rather complicated and needs a great manpower. By mixing the seeds of a male sterile line and a pollinator line, mechanized cultivation is feasible for seed production. This method ensures a higher ratio of seed-set on the male sterile plants because the average distance between the pollinators and male sterile flowers becomes closer than that under the alternative-row method. The seeds are harvested in a mixed from of pollinator seeds and F1 seeds. Separation of the F1 seed from a mixed harvest could be made, if the F1 seeds are different from the pollinator seeds in color, size or other traits. If a female sterile pollinator is made available, F1 seeds are only harvested. Another possible method for separation is to incorporate a herbicide-sensitive gene to a pollinator, which should be subjected to a spray of the specific herbicide just after pollination: then all the harvests are F1 seeds only.

Discipline: Crop production
Additional key words: female sterility, herbicide-sensitive gene, male sterility, pollination

Introduction

In China, hybrid rice grown to more than 13 million ha was harvested in 1989. Hybrid rice has never been dealt with on a commercial basis in producing its F_1 seeds. As rice is a self-pollinating plant with a pistil and stamens in a floret, commercial seed production has been materialized only after the use of male sterility (MS).

F_1 seeds are produced by planting an MS line and a pollinator line in alternative rows in China. This system requires a great number of laborers for transplanting and harvesting. It is very difficult in Japan to use much hand-labor to produce F_1 seeds. With the purpose of improving the production system of F_1 seeds of rice hybrid, the present paper attempts to discuss the possibility of mechanization for F_1 seed production by a mixed planting. In this system, seeds of a parental MS line and a pollinator are mixed at the sowing time and are grown under a usual mechanized transplanting system. The F_1 seeds on the MS plants are separated by some special devices.

Mixed planting for F_1 seed production in other crops is also proposed in wheat[1,2,13], onion[3], alfalfa[5] or sugar beet[4]. Differences in grain color or grain size are suggested to separate the F_1 seeds from the pollinator seeds in these reports.

Current method by alternative-row planting

Cytoplasmic male sterile (CMS) line is used for a seed parent, and a line with a restorer gene is used for a pollinator. A maintainer line to multiply the seeds of CMS line is also needed in this system. Accordingly, this system is called a three-line method. The alternative-row planting is effective in producing F_1 seeds and multiplying CMS seeds because rice plant is an anemophilae.

F_1 seed production by gamatecides is practiced in a small scale in Guangdong Province of China[16]. Environment-sensitive genic male sterility (EGMS) is recently used for hybrid rice breeding[10,12,17]. The effective environment for EGMS is related to day-length and/or temperature. Maternal MS seeds are easily multiplied in the EGMS system.

Transplanting of one MS line accompanied by one pollinator line gives a high seed-set ratio on the MS plants, but does not give a high yield, because total number of the MS plants is not large enough. On the other hand, if the number of pollinator lines is extremely limited, then the seed-set ratio may be so low as to affect F_1 seed yield. The seed-set ratio depends on the distance from the pollinator and the direction of wind as well[7,8] (Fig. 1). For indica F_1 seed production, a ratio of 8 MS : 1 pollinator to 12 MS : 1 pollinator rows give the highest yield [19]. In japonica hybrid varieties, an alternative planting of 6 rows of MS lines and 2 rows of pollinator lines gives the highest F_1 yield[14]. This 6–2 row ratio can be easily transplanted by an 8-row transplanting machine.

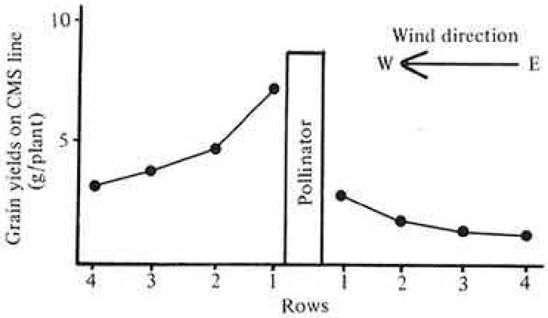


Fig. 1. Grain yields of CMS lines in relation to distance from pollinators and wind direction
Four rows of (cms-bo) Nekken 2 were planted along both sides of two rows of pollinator (Nekken 2).
Twenty plants per row with 20 cm distance between hills and 30 cm row-spacing were transplanted.

Advantages of mixed planting

As mentioned earlier, the direct advantage of mixed planting is related to possibility of mechanization for cultivation. First, F_1 seed producers do not have to deal with two kinds of seedling. The optimum rate of MS seed and pollinator seed could be decided empirically or by simulation. Second, transplanting becomes easier. In the case of the alternative-row planting method, F_1 seed producers have to install two types of seedlings of parental lines on a transplanting machine.

The greatest advantage of the mixed planting could be brought about in harvesting. In China, F_1 seed producers harvest the two parental lines separately by hand. Machine harvesting of alternative rows may possibly cause a mixture with pollinator seeds, which is not generally acceptable in Japan, because purity of seeds is strictly required. In the mixed planting, both of the MS and pollinator lines can be harvested at the same time with a combine harvester. Therefore, the harvested seeds come out as a mixture which is subjected to separation of F_1 seeds from pollinator seeds as referred to later.

As the seed-set ratio heavily depends on the distance between MS and pollinator flowers, there would be a great benefit in mixed planting. When the MS and pollinator plants are grown in a mixed form within a hill, the distance of those flowers become close, and a higher seed-set ratio could be expected as compared with the case in separate planting.

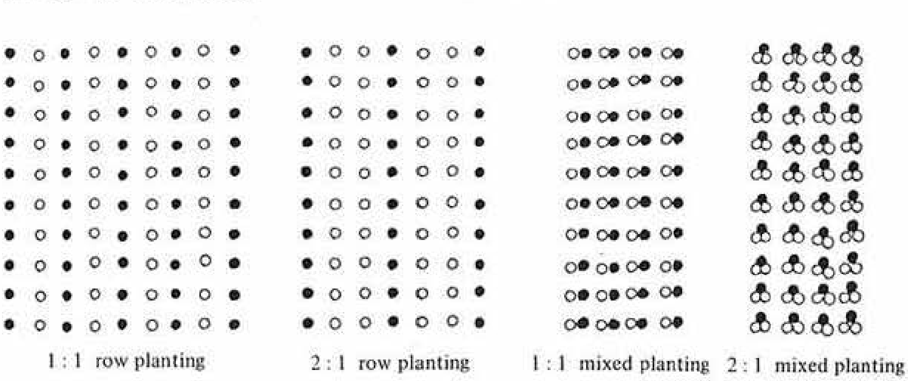


Fig. 2. An experimental design to test the effects of mixed planting on hybridization
Two kinds each of a row planting and a mixed planting were set. (cms-bo) Akihikari (○) was used as a CMS and Tatsumimochi (●) was used as a pollinator. Distances between rows and hills were 30 cm and 20 cm, respectively.

Table 1. Comparison of seed set ratios and yield in alternative-row planting and mixed planting[a)]

Plot type	Heading date (in August)	Culm length (cm)	Seed set ratio (%)	Number of seed set per hill	F_1 seed yield (kg/ha)
Alternative-row planting					
1 CMS : 1 Pollinator	14.1 (13.6)[b)]	82 (100)[b)]	16.6	304	68
2 CMS : 1 Pollinator	13.6 (14.3)	84 (99)	13.8	244	73
Mixed planting					
1 CMS : 1 Pollinator	13.5 (13.3)	83 (98)	23.9	234	105
2 CMS : 1 Pollinator	10.8 (11.5)	81 (96)	23.5	269	121

a): Refer to Fig. 2.
b): Heading date and culm length of the pollinator, Tatsumimochi.

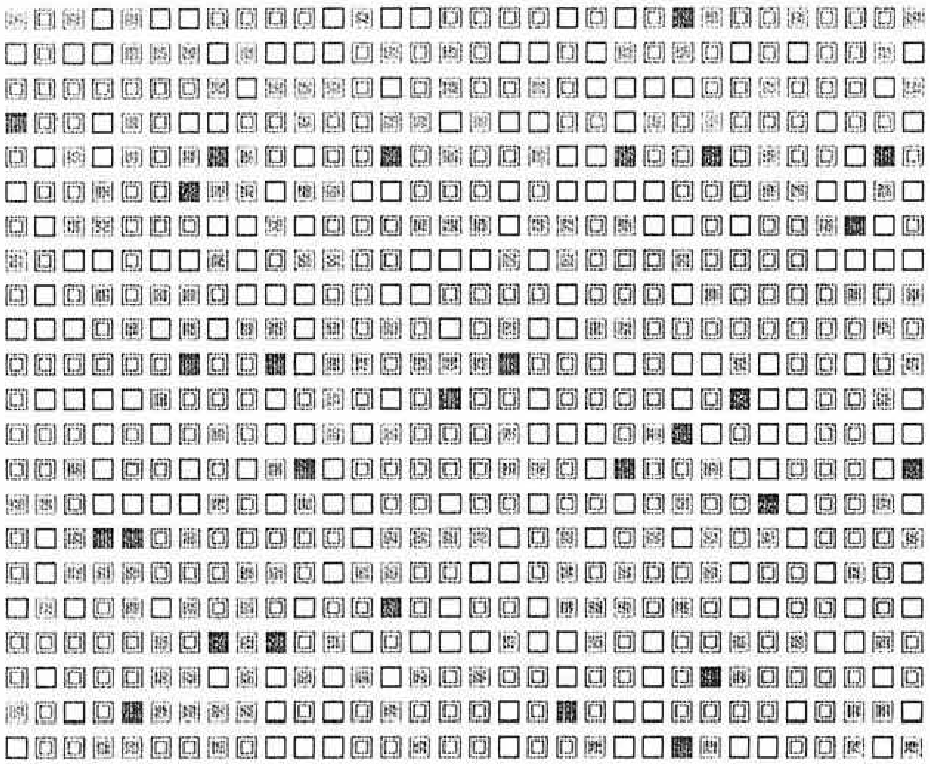
Table 2. Effects of dosages of pollinator in mixed planting

Plot[a)]	Seed set ratio (%)	F_1 seed yield (kg/ha)	Hill constitution and their number			
			3CMS	2CMS+1P and 1CMS+2P	3P	Total
10% mixed plot 1	12.3	894	146	54	0	200
10% mixed plot 2	11.6	594	168	32	0	200
Average	12.0	744	157	43	0	200
20% mixed plot 1	14.9	754	138	58	4	200
20% mixed plot 2	18.0	869	127	70	3	200
Average	16.5	812	132.5	64	3.5	200
30% mixed plot 1	15.8	753	104	88	7	199
30% mixed plot 2	22.4	767	87	107	6	200
Average	19.1	760	95.5	97.5	5.5	199.5

a): 10, 20 and 30% of pollinator seeds were mixed to CMS seeds. In transplanting, 3 seedlings were put in each hill.
Source: Zennoh, unpublished.

An experiment was designed to evaluate the effect of mixed planting as shown in Fig. 2. Seed-set ratios of the mixed planting plots comprising 1 MS + 1 pollinator and 2 MS + 1 pollinator were 23.9% and 23.5%, whereas those of alternative-row planting of 1 MS : 1 pollinator rows and 2 MS : 1 pollinator rows were 16.6% and 13.8%, respectively[15] (Table 1). When seedlings composed of 2 MS + 1 pollinator are transplanted with 3 seedlings per hill, the following patterns of a planting structure could be expected: 30% hills are MSs only, 44% are 2 MS + 1 pollinator, 22% are 1 MS + 2 pollinator, and 4% are pollinators only. An example of the simulated composition is shown in Fig. 3. An average seed-set ratio was estimated on the basis of the data shown in Table 1 and some assumptions regarding the effect of neighboring hills: the result was 20.8%.

Another experiment was conducted to estimate an



Assumption: A volume ratio of mixing CMS seeds with pollinator seeds is 2 : 1.
Three seedlings randomly sampled are transplanted in each hill.

□	Hills of 3CMS plants	28.7%
▣	Hills of 2CMS+1 pollinator plants	43.6%
▦	Hills of 1CMS+2 pollinator plants	23.7%
■	Hills of 3 pollinator plants	4.0%

On the basis of F_1 seed yields estimated from the data presented in Table 1, the total grain production of all the hills is 1,109 kg/ha.

Fig. 3. A simulation of randomly mixed planting

Table 3. Adjustment of heading date by varied transplantings[a)]

Planting density (cm)	Number of plant per hill	Heading date (in August)	Variance in heading date
	1	27.2	7.50
30 × 40	3	26.5	7.03
	7	25.1	7.44
	1	26.4	6.03
30 × 20	3	25.3	5.98
	7	25.1	5.55
	1	26.1	5.27
30 × 10	3	25.0	5.39
	7	25.4	5.69

a): Variety Nekken 2 was transplanted on June 20.

optimum ratio for F_1 seed production by randomly mixed pollinator and MS seeds. The seed-set ratio on MS plants augmented as the rate of pollinater plants increased. The maximum F_1 seed yield was obtained in case where pollinator plants accounted for 20% of the total population (Table 2).

Disadvantages of mixed planting

Synchronous flowering in both parents is vital for F_1 seed production. In case of hand transplanting, the heading date could be adequately adjusted by transplanting either pollinator or MS line lately, or by changing a planting density within a hill[8)] (Table 3). In case of mixed planting, however, the

Before treatment 12 days after treatment

Plate 1. Application of herbicide to pollinator
An induced mutant, Norin 8 M, is susceptible to bentazon, a herbicide. Norin 8 M (left) died completely in 12 days after application of bentazon, 10 ppm solution, whereas it did not affect Nipponbare.

difference in heading date between the parents has to be within one or two days for practical use[19]. Therefore, the F_1 combination adaptable to the mixed planting system is limited.

The seed-set ratio could be effectively enhanced by an appropriate pollination treatment at the flowering time. Among various treatments, pulling a rope over rice plants by two men on both sides of the field is a simple and effective method. Wind blowing by a duster or a helicopter may also be effective, but the costs incurred are yet to be evaluated. The proposed mixed planting may not benefit from such operations.

Separation methods of F_1 seeds in mixed planting

Separation of the F_1 seeds from a mixed harvest is prerequisite for practical use of a mixed planting system. The method for separation would be a combination of genetic devices and some additional treatments. The devices are evaluated on the basis of easiness in breeding procedures, feasibility to maintain seed purity, and costs for application. The devices are classified into the following three types according to the principles employed.

(1) Biological method

Female sterility is already identified in rice[18]. One of the authors proposed to utilize a female sterile line as a pollinator for F_1 seed production[9]. This method naturally requires to accompany another step: i.e. multiplication of female sterile plants. But no additional treatment is needed, since these types of rice plants are dioecious. In practice, however, more adequate materials, such as a facultative female sterile mutant, will have to be exploited. That

Table 4. Separation of mixture of seeds by circle pored sieves[a)]

Smaller variety	Larger variety	Case I: To eliminate larger grain		Case II: To eliminate larger grain	
		Contamination[b)] (%)	Loss[c)] (%)	Contamination (%)	Loss (%)
Koshihikari (3.35)[d)]	Taiho (3.98)	0.17	11.95	10.69	0.15
Akihikari (3.28)	Taiho (3.98)	0.10	3.99	3.84	0.10
Musashikogane (3.28)	Taiho (3.98)	0.66	3.15	3.07	0.64
IR36 (2.55)	Taiho (3.98)	0.00	0.00	0.00	0.00
IR36 (2.55)	Musashikogane (3.28)	1.03	0.00	0.00	1.04
CP-SLO (2.49)	Musashikogane (3.28)	1.14	0.00	0.00	1.15

a): An equal amount (50 g) of seeds of two varieties were mixed and filtrated by circle pored sieves with various pore sizes.
b): Percentage of non-targeted seeds mixed among targeted seeds.
c): Percentage of targeted seeds remained among the non-targeted seeds.
d): Grain width in mm.

mutant should possess female fertility under a special environmental condition, and at the same time, female sterility under a normal environmental condition in the F_1 seed production fields. A demerit of this method is that the pollinator gives no grain yield.

(2) Chemical method

Rice varieties respond diversely to chemicals. A herbicide-sensitive mutant was found by Mori[11]. This mutant sharply respond to bentazone (3-isopropyl-2, 1, 3-benzothiadiadin-4-one-2, 2-dioxide) and is controlled by a recessive gene. Bentazon has to be applied after pollination and before maturing of the pollinator plants (Plate 1). This gene is presently utilized by the authors in the breeding of pollinators. Demerits are: no grain yield from the pollinators used and additional costs for bentazon as well.

(3) Mechanical method

It would be convenient if any traits or a group of traits could be used to separate F_1 and pollinator seeds. One of the potential methods for separation is to utilize a trichome-related charactor of rice husks. Some cultivars have short trichomes on palea and lemma, but others not. The former easily stick to clothes, but the latter does not. Such glabrousness might be applicable for a rough separation. In practice, however, a more precise separation is required. The other potential criteria relate to grain size and grain color. Grain size consists of three dimensions: length, width and thickness. Among these components, grain length is difficult in filtration based on its difference. As far as grain width is concerned, it is rather easy to separate rice grain into several groups from wide to narrow, by a circle-pored sieve. It is also possible to separate thin grains from thick ones by passing them through parallely expanded wires like a harp. However, grain thickness is not a stable trait, depending on maturity. Therefore, grain width could only be available in practical use. The result of the study undertaken by the authors indicates difference of 0.7 mm is large enough for needed separation (Table 4). Applicability of this method is limited by a grain shape, however.

Color of rice grains is generally brownish white, but some are pigmented of red, purple, brown or black. Red and purple colors are given by anthocyanin pigments and brown and black color are due to polyphenolic compounds. However, grain colors are not always uniform even in the same cultivar and easily fade out in the course of ripening. They are not fully reliable in practical use, accordingly. Some varieties, however, possess a *Ph* gene with which rice grains are dyed firmly and uniformly in the solutions of some phenolic compounds. The degree of staining by phenolic compounds is shown in Table 5. These varieties are most sensitive to phenol, catechol and pyrogallol. It is not necessary to dip the grains into the solution. After incubating for 24 hr under 27°C, 1 kg of rice grains in a plastic bag can be stained completely with 100 m*l* of 0.5–1% phenol solution. Staining under a high content, however, deteriorates viability of seeds (Table 6). After dyeing treatment, the mixture of brownish white- and black-colored grains can be separated by a photosensing sorter, as stated below.

Table 5. Staining reaction of rice seeds to phenolic compounds[a)]

Compound name	Degree of staining
Phenol	+ +
Catechol	+ + + +
Resorcinol	–
Hydroquinone	+
Pyrogallol	+ +
o-Cresol	–
m-Cresol	±
p-Cresol	±
α-Naphthol	+ (basal part only)
β-Naphthol	–
Phenylalanine	–
Tyrosine	+ + (basal part only)
Coumaline	–
p-Hydroxy-benzoic acid	–
Gallic acid	–
Vanillic acid	–
Vanillin	–
Ferulic acid	–

a): Seven to eight grains of Rikuto Norin Mochi 4 were incubated in 5 m*l* of 0.2 M of phenolic compounds under 30°C for 4 days.

Breeding of Kanto Cross 1

A new hybrid line, Kanto Cross 1, was released in 1990 to test local adaptability. Seed parent is CMS Nekken 2 with a male sterile cytoplasm originated from Chinsurah Boro II. Nekken 2, the nuclear parent of CMS line, was derived from Akihikari/3/Akihikari//(Ketan Nangka/Nihonmasari F_4). A wide compatibility gene, $S\text{-}5^n$, was incorporated from an Indonesian variety, Ketan Nangka, into a japonica genetic background and it did not cause hybrid sterility in most japonica/indica crosses[6]. Pollinator, H87-53, posseessing a *Ph* gene is a line selected from a japonica/indica cross. The agronomic traits of the parents and Kanto Cross 1 are shown in Tables 7 and 8, respectively. Heading

Table 6. Viability of phenol treated seeds

Concentration (%)	Amount of solution (m*l*)	Days of incubation (days)	Compounds and variety			
			Phenol		Catechol	
			Sasanishiki (%)	IR 26 (%)	Sasanishiki (%)	IR 26 (%)
1	5 m	1	83	90	99	84
		2	89	88	96	81
		3	88	91	92	64
	15 m	1	33	79	66	85
		2	34	78	56	77
		3	4	32	33	68
3	5 m	1	43	82	86	66
		2	31	83	83	79
		3	7	66	77	63
	15 m	1	0	0	0	29
		2	0	1	0	15
		3	0	0	0	12

Two solutions (1 and 3%) of 5 or 15 m*l* each of phenolic compounds (phenol and catechol) were poured into a polyethelene bag containing 50 g of seeds (Sasanishiki or IR 26). They were incubated for 1 to 3 days under 27°C, and dried. The viabilities of seeds are expressed by germination rates.

Table 7. Agronomic traits of parental lines of Kanto Cross 1, an F_1 hybrid line[a)]

	Heading date	Culm length (cm)	Panicle length (cm)	Panicle number per hill	Phenol reaction	Apiculus color
Nekken 2 (Seed parent)	Jul. 29	63	19.0	12.8	–	Purple
H87-53 (Pollinator)	Jul. 28	49	16.8	10.6	+	Yellowish white
Akihikari (Check)	Jul. 27	69	17.7	9.8	–	Yellowish white

a): Transplanted on May 15, 1989.

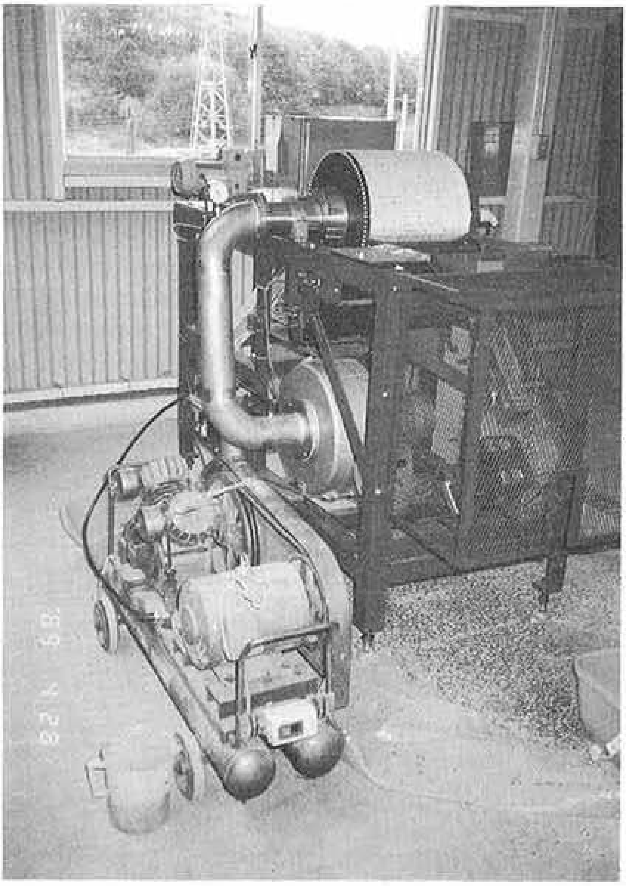


Plate 2. A sorting machine for F_1 seed production
A sorting machine with optical sensors connected with a microprocesser can separate colored and non-colored grains.
F_1 seeds can be selectively separated from the mixture of seeds harvested from mixed planting field where female and male parants are grown in a mixed form.

Table 8. Agronomic traits of Kanto Cross 1, an F_1 hybrid line[a)]

	Heading date	Culm length (cm)	Panicle length (cm)	Panicle number /m²	Grain number per panicle (main culm)	Percentage of ripened grain (%)	Total weight (kg/a)	Grain weight[b)] (kg/a)	Grain quality[c)]
Kanto Cross 1	Aug. 16	92	23.8	290	194	90.2	150.2	64.9	6
Akihikari (check)	Aug. 13	85	19.9	321	143	96.0	138.2	57.5	3
Nipponbare (check)	Aug. 25	82	21.4	383	112	91.2	152.3	50.6	3

a): Transplanted on June 8, 1989, N : P_2O_5 : K_2O = 1.1 kg/a each.
b): Brown rice.
c): 1; Highest, 9; Lowest.

dates of those parents are close each other, and the pollinator only possesses a *Ph* gene. The seeds of Kanto Cross 1, therefore, can be produced by mixed planting system, as proposed above. However, further improvement is required in Kanto Cross 1, since some agronomic traits of this line are not well suited to practical use.

A photosensing sorter to separate stained seed grains was developed by Kirin Brewery Company, Japan. The equipment consists of: a hopper to supply mixed seeds, guides to arrange grains, a drum with a number of pores to suck grains by vacuum operations which can switch on and off by an electromagnetic device, and an optical sensor connected with a small type of computer (Plate 2). Mixed grains of 10 kg can be treated within 30 min. Contamination of the stained seeds of pollinator with F_1 seeds can be minimized to a negligible level in practical use.

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