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Effect of Seed-Blending of Rice Varieties on Grain Yield and Occurrence of Blast Disease[1]

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Abstract: Ten blends, formed from two blast (BL) and brown planthopper resistant varieties and one susceptible major variety of rice, were tested with their component varieties in BL free and also prevailing areas. The three varieties are closely related, therefore nearly isogenic. No significant yield difference existed among entries in two tests conducted at the BL free site, while the yield difference was highly significant in the BL nursery test. Those resistant varieties and their blends were all BL resistant. Many blends showed a decrease in observed disease rates from those expected from their component varieties in pure stands, at most by 27.0% to 48.8% in LBL and 44.1% in PBL. The percentage tended to be high at initial and recovering stages of the disease but low at the peak stage. The decrease of disease rates was made possible by a favorable cooperation effect of resistant varieties rendered to the susceptible variety.

The blast disease, including leaf blast (LBL) and panicle blast (PBL), has been one of the menaces to rice culture in Taiwan since the adoption of Japonica rice varieties more than 60 years ago. The LBL attacked mostly the first rice crop with 6 peaks of diseased area occurred during past 20 years in Taiwan. The first 5 peaks, occurred in the period of major variety Tainan 5, reached mostly about 50,000-70,000 ha/season, while the last peak in the Tainung 67 period was about 30,000 ha. The major races of the blast fungus identified with international differentials were IG-1, IG-2 and IH-1 about 20 years ago (Chien, 1967), 10 years ago (Wu, *et. al.* 1979) and seemed to be so last year but not this year or 1987 (Chiayi AES/TARI, 1987). Changes in major races of the fungus were detected between different seasons and localities, when local differentials were used (Chien and Huang, unpublished).

Kameji, NC No. 4, Li-chi-hung, Kanto 55 and CI 5309 were the major sources of BL resistance used in varietal improvement in the past. The resistance of developed varieties quickly broke down in a couple of years after the release. Tainung 70 that received the resistance from CI 5309 is now used widely in BL prevailing areas of the island in addition to other sources. The LBL diseased area dropped to about 5,000 ha

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in the first crop and 1,000 ha in the second in 1986, the lowest ever reported, indicating that the resistance(s) mostly from CI 5309 was functioning. Howerer, apparently this resistance broke down in the 1987 frist crop two years after its official extension.

To tackle with the quick change in pathogenic races of the blast fungus, resistance genes existed in various Indica varieties have been transferred to Tainung 67 by a series of backcross breeding. Tainung 67, a Japonica rice variety that is related with the Indica semidwarf, has been grown in about 60-70% of the total rice area in Taiwan. This variety raised the rice yield per unit area of Taiwan to a new level , although it is susceptible to the blast disease. The resistant lines developed from the backcross breeding can be used either to form multiline or composite varieties or to further pyramid resistance genes, or can be used as an instant germplasm reservoir package which may be quickly put into use.

Multiline varieties of cereal crops, established from isogenic lines, are considered more stable in their performances with an equal or higher yielding ability and the greater protection from disease than the average of their component lines in pure stands (Borlaug, 1959, Murphy, *et. al.*, 1982). Tainung 69 and 70 resistant to BL and brown planthopper (BPH) are the improved types of Tainung 67, having the agronomic traits practically similar one another. Since they are nearly isogenic, multiline varieties can be established from them for testing. In this study, the effects of their seed-mixed varieties on grain yield and the occurrence of BL disease are investigated.

Material and Methods

Three Japonica rice varieties, Tainung 67, 69 and 70 were used in the experiment. Tainung 69 is supposed to receive the BL resistance from CI 5309 through Tainung 62 and also the brown planthopper (BPH) resistance from a wild rice, *Oryza rufipogon* (IRRI Acc. No. 100923), while Tainung 70 inherited the blast resistance from CI 5309 and the BPH resistance from Mudgo. Seed of the three varieties were blended to establish 10 seed-mixed varieties including one trinary blends in a seed-ratio of 1 : 1 : 1, three binary mixtures, respectively, in 1 : 1, 1 : 3 and 3 : 1 ratios, all on a seed weight basis (Table 1). The differences in seed-weight among three component varieties are almost negligible, which served as the controls in these experiments.

A total of 13 entries were tested in a randomized complete block with four replications for two crop seasons at TARI in 1986 and at the Kuohsin BL nursery in the first crop. Each plot consisted of five rows in an area of $4.48m^2$ planted at a hill spacing of 28×16cm at TARI. In the BL nursery, a $4.32m^2$ area planted at a 24×18cm spacing was used. As a rule, heavy nitrogenous fertilizers (320kg/ha) were applied to the nursery for easy occurrence of the disease. The rates of LBL disease infection were recorded on 4 dates at an interval of 7-10 days starting from 45 days after transplanting (DAT) and also for PBL before harvest. Five hills from the central three rows of each plot were sampled at random to rate BL disease of five tillers per hill. Finally, the average disease rate of 25 tillers or panicles represented the disease rate of each plot. The IRRI's system of disease rating for rice blast was used in the investigation.

The grain yield and the related traits of each plot were also recorded.

Table 1. Component varieties and their proportions in seed-blended populations of rice.

Blends	Component varieties	Proportion
1	Tainung67 : Tainung69 : Tainung70 (67 : 69 : 70)	1 : 1 : 1
2	Tainung67 : Tainung69 (67 : 69)	1 : 1
3	Tainung67 : Tainung70 (67 : 70)	1 : 1
4	Tainung69 : Tainung70 (69 : 70)	1 : 1
5	Tainung67 : Tainung69 (67 : 69)	1 : 3
6	Tainung67 : Tainung70 (67 : 70)	1 : 3
7	Tainung69 : Tainung70 (69 : 70)	1 : 3
8	Tainung67 : Tainung69 (67 : 69)	3 : 1
9	Tainung67 : Tainung70 (67 : 70)	3 : 1
10	Tainung69 : Tainung70 (69 : 70)	3 : 1
11	Tainung67 (67) BL, BPH susceptible	1
12	Tainung69 (69) BL, BPH resistant	1
13	Tainung70 (70) BL, BPH resistant	1

Results

Grain yield

The 1986 crop weather was considered favorable to rice growth, particularly in the first rice crop, although the second crop was seriously attacked by typhoons. Fortunately, the damage in the plots of this study was almost negligible. The grain yield per hectare generally reached 6 tons or more.

In variance analyses, no significant yield difference was found among all entries in the two experiments conducted at TARI, however the yield difference in the test at the Kuohsin BL nursery was highly significant (Table 2). The three component varieties

Table 2. Analysis of variacne for grain yield of seed-blended varieties from Tainung 67, 69 and 70.

S. V.	D. F.	Year, season and site of testing					
		1986-I, TARI		1986-I, BLN		1986-II, TARI	
		M. S.	F	M. S.	F	M. S.	F
Blocks	3	494.6		7143.2		781.2	
Blends	12	334.6	1.46ns	1252.3	3.10**	160.5	0.96ns
Error	36	229.3		404.1		166.6	
Total	51						

TARI=Taiwan Agricultural Research Institute, Wufeng, Taichung,
BLN=Blast disease nursery at Kuohsin, Nantou Hsien.

were so similar in agronomic traits that entire field plots looked like one single variety growing there. Except severe LBL and PBL disease occurred in the Kuohsin BL nursery test, no any other disease or insect affected the tests. An attempt to study the effect of seed-blending on the occurrence of BPH in the second crop failed of success, because no BPH damage occurred.

Although no significant yield difference existed in the two tests conducted at TARI (Table 2), Taiunug 69 was found to be the lowest yielder in the mean comparison. In the provincial regional trials, this variety yielded lower than Tainung 67 by about 5%. In the BL nursery test, all entries of 1 : 1 and 1 : 3 blends gave the same yield as that of Tainung 70 except the blend of 67 : 69=1 : 1 (Table 3). Lodging was another limiting factor for grain yield in addition to rice blast in the BL nursery trial.

Table 3. Grain yield of seed-blended varieties from Tainung 67, 69 and 70 at the Kuohsin BL nursery (1st crop, 1986).

Blends	Mix-proportion	kg/ha	LSD, p=5%
1	67 : 69 : 70=1 : 1 : 1	3,445	bc
2	67 : 69=1 : 1	3,291	c
3	67 : 70=1 : 1	4,155	ab
4	69 : 70=1 : 1	3,634	abc
5	67 : 69=1 : 3	3,628	abc
6	67 : 70=1 : 3	4,332	a
7	69 : 70=1 : 3	4,119	ab
8	67 : 69=3 : 1	3,208	c
9	67 : 70=3 : 1	3,551	bc
10	69 : 70=3 : 1	2,983	c
11	Tainung 67 (67)	3,232	c
12	Tainung 69 (69)	3,137	c
13	Tainung 70 (70)	3,610	abc

Within the three varieties tested, it seemed not possible to select a blend of particular combination that produced a favorable cooperation effect so that its grain yield increased significantly. When serious BL disease occurred, the blends produced a yield at most equal to the level of the resistant high yielding component variety. This result contradicts with other reports (Chiu and Teng, 1976; Chang and Hsieh, 1986), in which some composites or multilines gave a significantly higher yield than the pure stands and also a favorable performance in blast disease resistance. None of their strains was recommended for registration or used for further testing.

Blast disease resistance

The blast disease occurred in our BL nursery was serious, but not as serious as expected, since the disease rates of Tainung 67 reached at most 6.13, and the plant did not die (Table 4). The differences in disease rates among entries were highly significant in variance analyses on all dates of investigation. The two resistant varieties, Tainung 69 and 70, and all blends between them showed a consistent resistance during experiment. The disease rates of different blends seemed to be in an order of S : R=3 : 1 >

Table 4. Rates of blast disease in seed-mixed varieties of rice on different date at Kuohsin nursery (transplanted on March 13 1986).

Blends/DAT	45	52	59	69	PBL (%)
1, 67 : 69 : 70	1.77 cd*	2.34 b	3.12 c	1.83 ab	20 def
2, 67 : 69=1 : 1	2.37 de	3.37 cd	3.25 c	1.9 bc	23 f
3, 67 : 70=1 : 1	2.32 de	2.88 bc	3.12 c	1.83 ab	21 ef
4, 69 : 70=1 : 1	0.87 ab	0.98 a	1.72 ab	1.5 ab	13 bcd
5, 67 : 69=1 : 3	0.93 abc	1.09 a	1.97 ab	1.42 a	13 bcd
6, 67 : 70=1 : 3	1.59 bcd	2.52 bc	2.64 bc	1.74 ab	15 cde
7, 69 : 70=1 : 3	0.77 ab	1.02 a	1.76 ab	1.5 ab	4 a
8, 67 : 69=3 : 1	3.62 d	4.11 de	4.44 d	2.59 d	33 g
9, 67 : 70=3 : 1	3.14 ed	4.53 e	4.68 d	2.35 cd	56 h
10, 69 : 70=3 : 1	0.64 a	0.9 a	1.9 ab	1.6 ab	8 abc
11, TNG67	4.99 f	5.92 f	6.13 e	3.61 e	66 i
12, TNG69	0.76 ab	0.81 a	1.59 a	1.39 a	9 abc
13, TNG70	0.89 abc	1.04 a	1.74 ab	1.49 a	6 ab

*=LSD, significant at p=5%. DAT=Days after transplanting.

S : R=1 : 1 or 1 : 1 : 1 > S : R=1 : 3. Chang and Hsieh (1986) also reported the same tendency, but only when the disease rates were increasing. In the R : S=1 : 1 and =3 : 1 groups, the 67 : 69 and 67 : 70 blends had a similar disease rate; but in the S : R=1 : 3 group, the 67 : 70 blend always showed a higher disease rate than the 67 : 69 (Table 4). Mixing the seed of a susceptible variety in the same ratio with different resistant varieties not necessarily produces the similar cooperation effect in disease infection. Both Tainung 69 and 70 received the blast resistance from PI 5309 which was originated from the China mainland and introduced from the United States to Taiwan.

The effect of seed-blending on blast infection can be evaluated by estimating the percent difference of the observed disease rate of a blend substracted from the average of observed disease rates of its component varieties in pure stands (Table 5) as shown bellow.

Difference (%) for blend (67 : 69 : 70)=

[{(Obs67+Obs69+Obs70)/3-Obs(67 : 69 : 70)}/(Obs67+Obs69+Obs70)/3]×100

A positive difference indicates a decrease in disease infection due to blending, hence is advantageous for the disease control, while a negative value indicates disadvantage. In this experiment, most of the differences were positive (Table 5), indicating the effectiveness of seed-blending for the disease control, although they ranged from −11.5%

Table 5. Percent differences of observed BL disease rates from expected rates in seed-mixed varieties at the Kuohsin nursery (tranplanted on March 13, 1986).

Blends/DAT	45	52	59	69	PBL (%)
1, 67 : 69 : 70	20.0	9.7	1.1	15.4	25.9*
2, 67 : 69=1 : 1	17.6	− 0.1	15.8	24.0*	38.7*
3, 67 : 70=1 : 1	21.1	17.2	20.7	28.2*	41.7*
4, 69 : 70=1 : 1	− 5.5	− 5.9	− 3.3	− 4.2	− 73.3
5, 67 : 69=1 : 3	48.8*	47.8*	27.7	27.0*	44.1*
6, 67 : 70=1 : 3	17.0	− 11.5	7.0	13.9	28.6
7, 69 : 70=1 : 3	10.2	− 3.8	− 3.4	− 2.4	40.7
8, 67 : 69=3 : 1	7.9	11.5	11.1	15.2*	36.2*
9, 67 : 70=3 : 1	20.8*	3.6	7.0	23.7*	− 9.8
10, 69 : 70=3 : 1	19.2	− 3.7	− 16.7	− 13.1	3.0

Difference (%) for blend 1=[{(Obs67+Obs69+Obs70)/3−Obs(67 : 69 : 70)}/(Obs67+Obs69+Obs70)/3]×100. DAT=Days after transplanting.

to +48.8% in LBL and from −73.3% to +44.1% in PBL. The difference tended to be higher at the initial and recovering stages and diminish at the raging or peak stage of disease. The advantage of using composite variety seemed to be lower when the disease was very raging. The blends, composed of only resistant varieties such as Tainung 69 and 70, tended to have a negative but small percentage of the difference. Blend (67 : 69=1 : 3) seemed to be most advantageous for the disease control by seed-blending, since the differences were high and fairly consistent (27.0−48.8% in LBL and 44.1% in PBL). Blend (67 : 70=1 : 1) was the next (Table 5).

Resistance of component varieties in blended stands.

Another way to evaluate the effect of seed-blending on disease control is to check the difference between the observed disease rate of a variety in pure stand its estimated rate in blended stand. To estimate the disease rate of a component variety in a blend is not an easy task, if no marker gene can be used. Here, the disease rates of pure stands and their related blends are used for the estimation as shown below.

(1) Estimated disease rate of Tainung 67 in the blend (67 : 69=1 : 1).

$$\begin{aligned} E67(69) &= Obs.(67:69)\times 2 - Obs.\ 69 \\ &= (E67/2+E69/2)\times 2 - E69 \\ &= E67 \end{aligned}$$

(2) Estimated disease rate of Tainung 67 in the blend (67 : 69=3 : 1).

E67(69/4) = Obs. (67 : 69 = 3 : 1) × 2 − Obs. (67 : 69 = 1 : 1)
= (E67 × 3/4 + E69/4) × 2 − (E67/2 + E69/2)
= E67 × 3/2 + E69/2 − E67/2 − E69/2
= E67

(3) Estimated disease rate of Tainung 67 in the blend (67 : 69 : 70 = 1 : 1 : 1).
E67(69, 70) = Obs. (67 : 69 : 70 = 1 : 1 : 1) × 3 − Obs. (69 : 70 = 1 : 1) × 2
= (E67/3 + E69/3 + E70/3) × 3 − (E69/2 + E70/2) × 2
= E67

The t-test was used in testing the significance for differences between the observed and expected disease rates. For this, SQRT(2/N) used in ordinary LSD computation was substituted by SQRT(6/N) for (1) and (2) and SQRT(14/N) for (3).

If a variety receives no effect in disease infection from the neighboring variety mixing within hills, the estimated disease rate of that variety in the blend should be the same with its observed rate in pure stand. A positive difference of the observed rate from the estimated rate indicates a reduction in the disease infection due to seed-blending, while negative one indicates an increase.

Susceptible variety Tainung 67, blended with resistant varieties in a ratio of 1 : 1, always showed a reduced disease rate from that of Tainung 67 in pure stand particularly in blend (67 : 70 = 1 : 1) (Table 6). The magnitude of the reduction in disease rate was about 1.2-1.4 in LBL and about 30% in PBL. In the blend composed of only resistant varieties, the difference between observed and estimated rates were small, negative and not significant (Table 6). In the blends of 1 : 3 and 3 : 1 groups, the differences

Table 6. Differences between the observed BL disease rates of component varieties in pured stands and their estimated rates in various blends.

Component var. /DAT	45	52	59	69	PBL (%)
O67-E67 (69) or O69-E69 (67)	1.01	− 0.01	1.22	1.2 *	29*
O67-E67 (70) or O70-E70 (67)	1.24	1.2	1.63	1.44*	30*
O69-E69 (70) or O70-E70 (69)	− 0.09	− 0.11	− 0.11	− 0.12	−11
O67-E67 (69/4)	0.12	1.07	0.5	0.33	23*
O67-E67 (70/4)	1.03	− 0.26	− 0.11	0.74	−25*
O69-E69 (67/4)	1.27	2	0.9	0.45	6
O69-E69 (70/4)	0.35	− 0.01	− 0.49	− 0.31	6
O70-E70 (67/4)	0.03	− 1.12	− 0.42	− 0.16	− 3
O70-E70 (69/4)	0.22	− 0.02	− 0.06	− 0.01	11
O67-E67 (69, 70)	1.42	0.86	0.21	1.12	32*
O69-E69 (67, 70)	0.09	− 0.45	− 1.53	− 0.44	− 9
O70-E70 (67, 69)	0.32	0.76	− 1.12	− 0.2	− 8

O67 = Observed BL rate of Tanung 67 in pure stand. DAT = Days after transplanting.
E67(69) = Expected disease rate of Tainung 67 blended with Tainung 69 in 1 : 1 ratio. For its estimation, refer to the text.

were inconsistent and not significant except some in the PBL. Tainung 67 in trinary seed-mixture with Tainung 69 and 70 had its disease rate reduced by 0.21-1.42 in the LBL and 32% in the PBL, when compared with Tainung 67 in pure stand. For Tainung 69 and 70, such reduction in disease rate due to blending was relatively small and not significant (Table 6).

Discussion

Rice culture in Taiwan has been in monocultural situation since 1970 after the release of Tainan 5. The situation became worse when Tainung 67 took the leading position in 1980. The main criticism against Tainung 67 has been the occurrence of BL and bacterial leaf blight (BLB) diseases, even though the total BL diseased area dropped to about a half of that in the previous Tainan 5 period. The BL disease has been almost controlled by the improved type of Tainung 67 or the BL resistant Tainung 70 in recent years. The first sign of resistance breakdown in this variety appeared in 1986-87, two years after its official release and cultivation in BL prevailing areas. Since breeding to pyramid BL resistance genes in a variety is a longterm project, we have to look for other ways to tackle with its possible breakdown. Here our approach is the use of composite varieties.

Since no significant yield difference among entries was found in the two tests conducted at TARI, the merit from growing composite variety in BL free areas seems to be limited. Other workers (Chang and Oka, 1986) also reported the same result. On the other hand, the yield difference was highly significant in the Kuohsin BL nursery, indicating that composite varieties behave differently under blast prevailing conditions. Seed-blending frequently reduced the disease infection from those expected from their component varieties in pure stands. For examples, blend(67 : 69=1 : 1) got a disease reduction of 27.0-48.8% in LBL and 44.1% in PBL, and another blend (67 : 70=1 : 1) had a 17.2-28.2% reduction in LBL and 41.7% in PBL (Table 5). In rust disease of wheat, 33% to 100% were reported (Suneson, 1960) ; and in stem rust disease of oats it was 20% (Leonard, 1969). The decrease of disease rate in many blends was made possible by a favorable cooperation effect between the susceptible variety and the resistant variety in mixed stands. In blends (67 : 69=1 : 1), (67 : 70=1 : 1) and (67 : 69 : 70=1 : 1 : 1), Tainung 67 received a favorable effect in various degrees rendered by the resistant Tainung 69 and 70 (Table 6).

Several blends like (67 : 70=1 : 1), (67 : 70=1 : 3) and (69 : 70=1 : 3) seem to be desirable in grain yield and blast resistance. As mentioned in previous paragraphs, the reduction of disease rate due to seed-mixing tended to be low at the peak stage of BL disease. In this stage, rapid propagation of synchronized race(s) of the blast fungus took place due to predominated host, Tainung 67. Resistant varieties, Tainung 69 and 70, could controlled it but probably not long. As an alternative measure, we may use composite varieties or rotationally grow a series of varieties resistant to new race(s) that come up in each race cycle. To control completely the blast disease by seed-mixed varieties is not an easy task, because some of their component varieties may become susceptible to predominated race(s).

Composite varieties are commonly grown in Europe and registered in the USA (Chang and Oka, 1986). Considering the worsened monocultural situation of rice in Taiwan, it is probably the time to practice this type of rice culture. For general acceptance of composite varieties by farmers, there needs to amend the seed production and inspection system as proposed by Chang and Oka (1986). They further suggested to establish seed lots of a blend from separately harvested stock seed of different component varieties to produce its extension seed. If this system is employed, the extension seed should not be included in seed-testing. About 70 % of extension seed growers of rice in Taiwan are now simultaneously the operators of rice nursery centers. They produce rice seed they need and raise seedlings in trays as the final product for sale. Raising rice seedlings in trays is a "must" work for mechanical transplanting by powered transplantors. They receive stock seed once every two years in average. How much genetic change occured in a blend population due to the interaction between host and pathogen and due to the competition between component varieties in four generations of two years, needs investigation. Another problem which this system may encounter is that farmers may complain for the nonuniformity of rice quality of multiline particularly the so-called dirty multiline variety.

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水稻混合品種對稻穀產量及稻熱病之反應[1]

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摘　要

從二個抗稻熱病及抗褐飛蝨之水稻品種及一個極感主要品種，成立10個混合品種，在易及不易發病兩處，以成分品種爲對照做產量比較試驗。此等三品種有密切的親緣關係，有如類似擬基因品系。在不易發病處的二次試驗，產量差異均不顯著，但在易發病處有顯著的產量差異。多數混合品種的罹病率均比其成分品種在純種時的罹病率之平均減少。葉稻熱病最高減少27.0%至48.8%，而穗頸稻熱病減少44.1%。此百分率在發病初期及末期較高，但在發病盛期卻較低。上述混合品種罹病率之減少乃抗病品種所顯示有益的合作反應所致。

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