Physiological Mechanism of Heterosis in Seedling Growth of Indica F_1 Rice Hybrids*

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Received January 22, 1990

Abstract : To determine the possible cause of higher seedling vigor in F_1 rice (*Oryza sativa* L.) hybrids, embryo weight, endosperm weight, leaf area, and seedling growth performance of 16 F_1 indica crosses and their parental lines were evaluated. The embryos of 16 F_1 rice hybrids showed midparent heterosis and those of 11 F_1 rice hybrids showed better parent heterosis (heterobeltiosis). Lower heterosis in the F_2 embryos was also observed. Midparent heterosis in endosperm weight did not always appear. Seedling growth of F_1 rice hybrids grown in standard nutrient solution for 16 days showed midparent heterosis and relative growth rate in each four-day interval had little heterosis. Heterosis in embryo weight and leaf area were closely related with that in seedling weight.

Higher leaf area expansion related with bigger embryo was considered the primary factor for heterosis in seedling growth of indica-indica F_1 rice hybrids.

Key words : Embryo, Endosperm, F_1 rice hybrids, Heterosis, Indica, Seedling.

インディカ F_1 雑種水稻の幼苗生長における雑種強勢の生理的機構について：秋田重誠・リブラダ ブランコ・片山勝之（国際稲研究所）

要 旨： F_1 雑種水稻の高い初期生長の原因を明らかにするために、16 系統のインディカ F_1 雑種水稻とその両親の胚重、胚乳重と葉面積が幼苗生長に及ぼす影響について検討を行った。16 系統の F_1 雑種水稻の胚重は両親の中間値よりも大きかった。この内、11 系統の F_1 雑種水稻の胚重は優れた親の胚重よりも雑種強勢を示した。雑種第 2 代目の水稻 (F_2 水稻) の胚重にみられるヘテロシスは F_1 雑種水稻よりも小さかった。胚乳重については、必ずしも雑種強勢がみられなかった。発芽後 16 日間、水耕栽培した F_1 雑種水稻の生長量 (幼苗重) は雑種強勢をしめしたが、4 日毎の相対生長率にはほとんど雑種強勢がみられなかった。

これらのことから、インディカを両親とする F_1 雑種水稻の幼苗生長にみられる雑種強勢は、主として大きい胚重及び高い葉面生長によると推察された。

キーワード： インド型稲、 F_1 雑種水稻、雑種強勢、胚、胚乳、幼苗。

Growth analysis of field-grown F_1 rice hybrids showed little heterosis in relative growth rate (RGR) and net assimilation rate when measured with field-grown materials³⁾. Also, Yamauchi and Yoshida²⁰⁾ reported little heterosis in photosynthetic activity of rice cultivars. These results indicate that what triggers higher growth of F_1 rice hybrids is higher growth in the seedling stage or even before.

The existence of heterosis in F_1 embryos has long been controversial but it may provide clues for the elucidation of the physiological mechanism of heterosis.

Ashby^{1,2)} observed positive heterosis in embryo weight and little heterosis in relative

growth rate in the succeeding growth of F_1 maize hybrids. Based on these observations, he proposed the so-called "initial capital theory" for heterosis: F_1 rice hybrids have an initial advantage over the inbreds due to their bigger embryo size and more "initial capital" is maintained throughout the whole period of growth. Urano and Sakaguchi¹⁷⁾ observed a higher number of leaf primordia of F_1 maize embryos showing significant heterosis in embryo weight. Faster germination of F_1 seeds was also reported in maize and other crops^{1,8,10,14,18)}.

However, Ashby's theory could not be substantiated by other workers. Among others, East⁶⁾ reported that hybrid maize embryos did not always exceed those of parent inbreds in size, and Sprague¹⁶⁾ showed that hybrid embryos may be smaller than, equal to, or larger than those of the maternal parent. It was also found that the weight of maize hybrid em-

* The outline of this paper was presented in the 183rd meeting of the Crop Science Society of Japan, Tsukuba, Apr., 1987.

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bryos was not consistently greater than that of inbreds when these are compared on the basis of dry weight in later stages of embryo development.

One of the possible causes of inconsistent results on heterosis in F_1 embryos would be the sample size used. In a cultivar, the weight of the embryo usually shows big variation. In rice, the variation in embryo weight was quite high even within a cultivar. The differences within cultivars or lines become significant only when sample size is more than 110 seeds (Akita, et al. unpublished data).

Recently, Yamada¹⁹⁾ reported significant heterosis of F_1 maize embryos and reconfirmed Ashby's observation by using special marker genes to identify true F_1 seeds. Matsuba et al.¹²⁾ observed less dormancy of primary leaf tiller primordia in three F_1 rice hybrids tested compared to those of parent genotypes. This would explain the fast and strong tiller appearance after germination in F_1 rice hybrids. These findings support the view that hybrid vigor occurs at the early growth stage, even at the embryo stage.

When embryo weight of F_1 rice hybrids is discussed, the effect of extremely low seed setting of F_1 seeds on their embryo weight has to be considered when cytoplasmic male sterile (CMS) lines are used. Seed setting is poor in F_1 hybrid seeds obtained by using CMS lines. In this paper, heterosis in embryo weight of F_1 rice hybrid obtained from indica-indica crosses were examined using an improved method. Then, factors affecting heterosis in embryo weight and seedling growth were investigated.

Materials and Methods

Experiment I. Determination of Embryo and Endosperm Weights

Eighteen indica F_1 rice hybrids obtained by using cytoplasmic male sterile (CMS) lines²²⁾, their parental lines, and five selected F_2 rice hybrids (seeds harvested from F_1) were used (Table 1). Seeds were soaked in 1.06 specific gravity NaCl solution to remove poor grains and dried in an oven at about 45–50°C. The embryo of 200 seeds in each cultivar was separated manually from the endosperm using a modified dissecting needle (Thomas Scientific, Type 3881—D50) and dried at 105–110°C overnight and weighed. The weight of each embryo and each endosperm were deter-

mined using an electronic balance (Mettler AE163).

Experiment II. Effect of Lower Setting of F_1 Seeds on Embryo Weight

The embryo weights of cytoplasmic male sterile (CMS) and isogenic lines of IR54753 grown in the field by the Plant Breeding Department of IRRI was compared. One hill of each line was sampled at maturity. Seeds were dried in the oven and embryo and endosperm weights were determined by the method in *Experiment I*.

Experiment III. Seedling Growth Analysis

Seeds were dehulled and surface-sterilized with 0.1% $HgCl_2$ solution for 1 minute and washed with demineralized water. Here, two experiments were conducted. In the first seedling growth experiment, 200 seeds each of 4 F_1 rice hybrids (out of the 16 F_1 rice hybrids) and their parental lines were used. Seeds were soaked in distilled water for 24 hours and sown in styrofoam seedling beds lined with nylon net. They were grown for 16 days inside the glasshouse room of the IRRI phytotron (day/night temperatures, 29/21°C) in plastic trays containing standard nutrient solution²¹⁾. One hundred fifty seedlings were sampled every 4 days and dried in a draft oven at about 80°C for 3 days. Dry weight was recorded.

In the Second seedling growth experiment, 3 F_1 rice hybrid combinations and their corresponding F_2 seeds were soaked in distilled water for 24 hours and germinated until the shoots were about 1 mm. Using 200 seeds per cultivar, 50 germinated seeds were sown in a seedling bed in 4 replications. Each seedling bed had a capacity of 200 seedlings so that for each replication, seedlings of F_1 , F_2 , and parental lines were accommodated in one bed. All cultivars were grown in standard nutrient solution contained in 220-liter-capacity plastic bins for 21 days inside the glasshouse room. Culture solutions were prepared following the modified Yoshida method²¹⁾. The pH was adjusted to about 5.0 everyday and culture solution was renewed on the 15th day after seedling. Dry weight was recorded and leaf area of 10 random seedlings was measured in an automatic area meter (AMM-7 Hayashi Denko Co.).

Heterosis in growth parameters was assessed. Percent heterosis was expressed over the mean parental value using the formula^{15,22)}.

Table 1. Heterosis in embryo and endosperm weight of F₁ rice hybrids.

Cultivar ^a	Embryo		Endosperm	
	Weight (mg)	Midparent heterosis (%)	Weight (mg)	Midparent heterosis (%)
IR46830B	0.40		16.12	
IR9761-19-1R	0.30		16.01	
IR46830A/IR9761R	0.38	109	15.82	99
IR21845-90-3B	0.25		17.95	
IR54R	0.32		17.74	
IR21845A/IR54R	0.33	116**	17.83	100
97B	0.38		18.48	
Milyang 54R	0.39		17.59	
97A/Milyang 54R	0.46	119**	17.78	101
IR46830B	0.40		16.18	
IR50R	0.31		13.18	
IR46830A/IR50R	0.44	124**	14.38	102
IR46830B	0.37		16.05	
Milyang 54R	0.38		17.24	
IR46830A/Milyang 54R	0.45	120**	15.84	95
V20B	0.37		19.99	
Milyang 54R	0.34		17.25	
V20A/Milyang 54R	0.44	117**	21.51	116**
V20B	0.37		19.99	
IR1347R	0.34		18.05	
V20A/IR1347R	0.40	112*	22.49	118**
MR365B	0.35		16.31	
IR36R	0.28		16.27	
MR365A/IR36R	0.39	124**	17.37	107**
IR46830B	0.37		16.05	
IR1347R	0.34		18.05	
IR46830A/IR1347R	0.37	104	15.34	90
IR46828B	0.28		13.29	
IR54R	0.32		17.06	
IR46828A/IR54R	0.32	107	13.90	92
IR46828B	0.28		13.29	
IR135242-21-2-3-3-2R	0.30		12.03	
IR46828A/IR135242R	0.34	117**	14.49	114**
IR46830B	0.37		16.05	
Milyang 46R	0.38		19.02	
IR46830A/Milyang 46R	0.38	101	15.01	86

Cont. Table 1.

Cultivar ^a	Embryo		Endosperm	
	Weight (mg)	Midparent heterosis (%)	Weight (mg)	Midparent heterosis (%)
IR46830B	0.37		16.05	
Suweon 294R	0.31		14.44	
IR46830A/Suweon 294R	0.36	106	14.77	97
V20B	0.48		21.83	
IR9761-19-IR	0.33		16.78	
V20A/IR9761R	0.52	128**	23.36	121**
IR747B	0.38		13.00	
IR50R	0.33		12.89	
IR747A/IR50R	0.41	115**	12.27	95
IR21845B	0.35		18.90	
IR29512-81-2-IR	0.34		19.20	
IR21845A/IR29512R	0.40	116**	17.98	94
CV %		6.62		1.58
LSD (1%)		14		3
LSD (5%)		11		2

a : A and B show the isogenic male sterile line and fertile line, respectively. In F_1 rice hybrids, labels for restorer lines were abbreviated to fit into the space available. For LSD, standard value to compare is 100.

Table 2. Heterosis (%) in embryo weight of F_1 and F_2 rice hybrids.

Hybrid	F_1	F_2
IR747A/IR50R	116	101
MR365A/IR36R	126	98
V20A/IR9761-19-1R	128	108
IR46830A/IR9761-19-1R	112	108
IR21845A/IR29512R	116	104

$$\text{Heterosis} = F_1 \times 100 / (P_1 + P_2) / 2$$

Where F_1 = hybrid

P_1 , P_2 = female and male parent, respectively.

The correlation of seedling dry weight to leaf area, embryo, and endosperm was also calculated.

Results and Discussion

Experiment I

The endosperm weight of F_1 rice hybrids was not always higher than the midparent values (Table 1). However, all 16 F_1 rice hybrids showed higher embryo weight than midparents. Eleven of 16 F_1 rice hybrids showed significantly heavier embryos than midparents. F_2 embryo weight of five crosses tested showed almost midparent values but lower than in F_1 's (Table 2). F_1 and F_2 plants were not grown at the same time in the same field. However, all 5 crosses used in this experiment were consistently showing the same tendency. The negligible heterosis in F_2 embryo was consistent with the lower heterosis observed in the growth of F_2 generations^{6,12}.

Experiment II

Table 3. Difference in embryo weight between the isogenic male sterile line and fertile line of IR54753.

Cultivar	Embryo ^a weight (mg)	Seed setting (%)	Spikelets observed (no.)
IR54753A (male sterile line)	0.336 ± 0.0042	4	83
IR54753B (fertile line)	0.304 ± 0.0040	79	317

^aValues represent the means $\pm$ SE.

Fig. 1. Seedling growth of indica F_1 rice hybrids and parental lines from 4 days to 16 days after seeding in standard nutrient solution. a: $\square$ IR46830B, $\square$ IR9761-19-1, $\square$ IR46830A/IR9761 b: $\square$ IR21845B, $\square$ IR54, $\square$ IR21845A/IR54 c: $\square$ IR46830B, $\square$ IR50, $\square$ IR46830A/IR50 d: $\square$ 97B, $\square$ Milyang54, $\square$ 97A/Milyang54. The vertical bar indicates the LSD(5%) value for the F_1 rice hybrid and the midparent.

Embryo weight of IR54753 CMS line was 10% higher than that of the isogenic fertile line (Table 3). The preliminary survey of artificial thinning of spikelets on embryo weight (data is not shown) also suggested that the embryo weight of materials with limited number of seed set may slightly showed higher embryo weight. Thus, low setting of F_1 seeds affected embryo weight to some extent. These

results indicated that higher embryo weight of F_1 rice hybrids obtained by utilizing CMS lines may partly be involved due to limited seed setting. This effect has to be studied further.

The growth of F_1 generation plant starts just after fertilization and the initial stage is embryo growth. With the maturity of F_1 seeds, the growth of F_1 plants stops temporarily but

Table 4. Relative growth rate (g/g/day) of four F₁ rice hybrids and their parents when grown in standard nutrient solution at 29/21 °C IRRI phytotron.

Cultivar [±]	Days after seeding		
	4-8	8-12	12-16
IR21845B	0.38	0.22	0.15
IR54	0.33	0.23	0.14
IR21845A/IR54R	0.39	0.24	0.16
LSD (5%)	0.03	0.02	0.03
97B	0.41	0.21	0.16
Milyang 54	0.38	0.27	0.11
97A/Milyang 54R	0.35	0.26	0.14
LSD (5%)	0.01	0.02	0.01
IR46830B	0.44	0.23	0.16
IR9761-19-1R	0.44	0.27	0.18
IR46830A/IR9761R	0.74	0.27	0.16
LSD (5%)	ns	ns	ns
IR46830B	0.46	0.21	0.14
IR50R	0.49	0.23	0.17
IR46830A/IR50R	0.45	0.25	0.18
LSD (5%)	0.03	0.02	0.02

[±] ; the same as in Table 1.

Table 5. Seedling growth of F₁ and F₂ rice hybrids, 21 days after seeding in nutrient solution.

Cultivar [±]	Seedling dry weight (mg/plant)	Leaf area (cm ² /plant)
V20B	161 ^b	30.7 ^c
IR9761-19-1R	154 ^b	32.4 ^{bc}
V20A/IR9761R F ₁	211 ^a	44.6 ^a
V20A/IR9761R F ₂	200 ^a	36.3 ^b
IR46830B	181 ^b	36.9 ^b
IR9761-19-1R	177 ^b	35.6 ^b
IR46830A/IR9761R F ₁	214 ^a	40.8 ^a
IR46830A/IR9761R F ₂	193 ^{ab}	38.2 ^{ab}
IR21845B	184 ^c	30.5 ^c
IR29512-81-2-1R	173 ^c	29.1 ^c
IR21845A/IR29512R F ₁	248 ^a	42.2 ^a
IR21845A/IR29512R F ₂	215 ^c	36.4 ^b

In a column for each combination, means followed by a common letter are not significantly different at the 5% level.

[±] ; the same as in Table 1.

starts again after sowing. The embryo itself is one growth stage of F_1 plants which does not involve photosynthesis for its growth. Heterosis in embryo weight indicates higher initial growth independent from photosynthesis and the mechanism involved in the initial growth process like embryo growth stage would be one crucial criterion in knowing the mechanism of vigorous growth of F_1 rice hybrids.

Experiment III.

In the seedling growth experiment, the dry weight of 4 F_1 rice hybrids at 16 days after seeding (DAS) in standard nutrient solution was significantly higher than midparent values (Fig. 1). Three F_1 rice hybrids showed significantly higher dry weight than better parents. At earlier growth period (4, 8, and 12 DAS) in most cases the dry weight of the hybrids was significantly higher than the midparent values. No significant difference on the RGR at four-day interval between F_1 rice hybrids and better parents was observed (Table 4). In some cases, RGR of the hybrids was significantly higher only to lower parents. Also, in one of the hybrid, IR46830A/IR9761-19-1, RGR did not differ significantly between parents in all-growth period interval.

In another seedling growth experiment, total seedling dry weight at 21 DAS was significantly higher in F_1 rice hybrids than in better parents (Table 5). Heterosis in seedling dry weight of F_2 rice hybrids was less than that of F_1 rice hybrids. Greater leaf area was also observed in all three F_1 rice hybrids used. Heterosis in leaf area ranged from 113 to 142%. Also, leaf area was significantly correlated to seedling dry weight ($r=0.759^{**}$, $n=12$), followed by embryo weight ($r=0.293$). Correlation coefficient relating endosperm weight to seedling dry weight was very low

($r=0.055$).

Heterosis of seedling weight at 16 and 21 DAS was closely related with heterosis of embryo weight but endosperm weight heterosis was not related to that of embryo weight or seedling weight (Table 6). Frequency distributions of embryo weight and seedling dry weight in F_1 rice hybrids showed the same tendencies (Fig. 2).

Cooper and Brink⁴⁾ and Grozman and Sprague⁷⁾ reported large endosperm of F_1 seeds in alfalfa (*Medicago sativa* L.) and maize, respectively. They claimed that endosperm weight is the main cause of heterosis. However, the low correlation between endosperm weight of F_1 rice hybrids and initial seedling growth observed in the present study shows that endosperm weight is not a major factor for vigorous growth in F_1 rice hybrids. Faster growth of F_1 rice hybrids observed in this experiment support the observation by Ashby^{1,2)}.

These observations suggest that heterosis in embryo weight of F_1 rice hybrids may exist even in self-pollinating species such as rice. Heterosis in embryo weight may indicate heterosis in vegetative growth especially in F_1 rice hybrids obtained from indica parents with relatively similar genetic backgrounds. The primary factor responsible for seedling vigor of F_1 rice hybrids was greater leaf area. Higher leaf area expansion rate could be related with embryo weight. A bigger embryo may indicate bigger initial capital for growth as well as higher ability for leaf area expansion. High heterosis in leaf area in rice were also reported^{9,13,20)}. These results indicate that heterosis in embryo weight can possibly trigger higher initial growth in F_1 rice hybrids obtained from indica-indica crosses.

Table 6. Heterosis (%) in embryo, endosperm, and seedling dry weight in F_1 rice hybrid.

F_1 rice hybrid	Embryo	Endosperm	Seedling growth
IR21845A/IR54R*	116	100	117
IR21845A/IR29512R	116	94	139
IR46830A/IR9761R	112	94	119
IR46830A/IR50R*	124	98	122
97A/Milyang 54R*	119	99	119
V20A/IR9761R	128	121	134

* Determined at 16 DAS ; others at 21 DAS.

Fig. 2. Frequency distribution of plant dry weight (a, b, c) and embryo weight (d, e, f) in 3 indica F_1 , F_2 rice hybrids and parental lines. In figure b, P_1 and P_2 have the same mean value; also in e, P_1 have the same mean value. In figures a and d: ● V20B(P_1), ○ IR9761(P_2), ▲ V20A/IR9761 F_1 , △ V20A/IR9761 F_2 . In figures b and e: ● IR46830B(P_1), ○ IR9761(P_2), ▲ IR46830A/IR9761 F_1 , △ IR46830A/IR9761 F_2 . In figures c and f: ● IR21845B(P_1), ○ IR29512(P_2), ▲ IR21845A/IR29512 F_1 , △ IR21845A/IR29512 F_2 .

In this experiment, only within the indica-indica F_1 rice hybrids were used. Observation on F_1 rice hybrids crossed between genetically divergent genotypes (Such as indica and japonica) may be helpful to better understand this phenomenon. Also, the mechanism involved in the ability of F_1 rice hybrids to have bigger embryo and higher leaf area expansion should be further investigated.

Acknowledgement

The authors thank Dr. S. S. Virmani, plant breeder, IRRI, for providing critical review of this paper and seeds of F_1 rice hybrids and their parental lines.

References

1. Ashby, E. 1930. Studies in the inheritance of physiological characters. I. A Physiological investigation of the nature of hybrid vigor in maize. *Ann. Bot.* 44: 457—469.
2. Ashby, E. 1932. Studies in the inheritance of physiological characters. II. Further experiments upon the basis of hybrid vigor and upon the inheritance of efficiency index and respiration rate in maize. *Ann. Bot.* 46: 1001—1032.
3. Blanco, L. C., S. Akita and S.S. Virmani 1986. Growth and yield of F_1 rice hybrids in different levels of nitrogen. *Japan. Jour. Crop Sci.* 55 (Extra issue) 1: 12—13.
4. Cooper, D. C. and R. A. Brink 1940. Partial self incompatibility and the collapse of fertile ovules as factors affecting seed formation in alfalfa. *J. Agr. Res.* 60: 453—472.
5. Davis, M. D. and V. N. Rutger 1976. Yield of F_1 , F_2 and F_3 hybrids of rice (*Oryza sativa* L.). *Euphytica* 25: 587—595.
6. East, E.M. 1936. Heterosis. *Genetics* 121: 375—397.

7. Grozman, A. and G. F. Sprague 1948. Comparative growth rates in a reciprocal maize cross. I. The kernel and its component parts. J. Amer. Soc. Agron. 40 : 88—98.
8. Hageman, R. H., E. R. Leng and V. W. Dudley 1967. A biochemical approach to corn breeding. Adv. Agron. 19 : 45—86.
9. Kawano, K. and A. Tanaka 1986. Vegetative vigor in relation to yield and nitrogen response in the rice plant. Japan. J. Breed. 19 : 277—285*.
10. Kempton, V. H. and J. W. McLane 1942. Hybrid vigor and weight of germs in the seeds of maize. J. Agr. Res. 64 : 65—79.
11. Kim, K. H. and M.H. Heu 1979. A study on heterosis in crosses between semi-dwarf rice cultivars. Korean. J. Breed. 11 : 127—128.
12. Matsuba, K., H. Yoshida and F. Fujimaki 1986. Morphological analysis of tillering ability of F₁ hybrid rice. Japan. J. Breed. 36(Supp. 1): 164—165**.
13. Murayama, S., Y. Norihama, S. Miyasato and A. Nose 1982. Studies in dry matter production of F₁ rice hybrids. I. Heterosis in photosynthetic activity. Japan. Jour. Crop Sci. 51 (Extra issue 2) : 85—86**.
14. Sarkissian, I.V., M.A. Kessinger and W. Harris 1964. Differential rates of development of heterotic and nonheterotic young maize seedlings. I. Correlation of differential morphological development with physiological differences in germinating seeds. Proc. Natl. Acad. Sci. 51 : 212—218.
15. Sinha, S.K. and R. Khanna 1975. Physiological, Biochemical and genetic basis of heterosis. Adv. Agron. 27 : 123—174.
16. Sprague, G.F. 1936. Hybrid vigor and growth rates in maize cross and its reciprocal. J. Agr. Res. 53 : 819—830.
17. Urano, K. and S. Sakaguchi 1950. Development of the embryo in *Zea mays* L. Proc. Nagano Prefect. Agr. Exp. Stn. 3 : 50—56**.
18. Whaley, W.G. 1952. Physiology of gene action in hybrids. In: Heterosis. J.W. Gowen. ed. Iowa State College Press, Iowa. 98—113.
19. Yamada, M. 1985. Heterosis in embryo of mize. *Zea mays* L. Bull. Natl. Inst. Agrobiol. Res. 1 : 85—98*.
20. Yamauchi, M. and S. Yothida 1985. Heterosis in net photosynthetic rate, leaf area, tillering and some physiological characters of 35 F₁ rice hybrids. J. Expt. Bot. 36 : 274—280.
21. Yoshida, S., D. A. Forno, J. H. Cock, and K. Gomez 1976. Laboratory manual for physiological studies of rice. International Rice Research Institute, Manila, Philippines.
22. Yuan, Longping 1985. Heterosis in Rice. A Concise Course in Hybrid Rice. Handbook. 67—168.

* In English with Japanese summary.

** In Japanese.