Variations in Morpho-Physiological Traits Relating to Seedling Vigor and Heterosis in Reciprocal Crosses of Rice

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Heterosis has been mainly studied to improve yield. However, heterosis is differentially expressed depending on the growth stages (Yamauchi 1994), and hybrid performance for yield may not be related to that for seedling growth. The following traits, which are closely associated with seedling growth, vigor, and/or heterosis in rice (*Oryza sativa* L.), have been studied in inbreds and some hybrids: seed size, coleoptile and mesocotyl length, leaf primordia size, embryo and endosperm weight and/or size, speed of germination, relative growth rate (RGR), net assimilation rate (NAR) and alpha-amylase activity (Jones and Peterson 1976, Sasahara *et al.* 1986, Akita 1988, Akita *et al.* 1990, Redoña and Mackill 1996).

According to Revilla *et al.* (1999), information on how seedling traits influence each other after the heterotrophic period (or during the early autotrophic growth phase) is limited. The present experiments were carried out to examine the expression of morpho-physiological traits of young rice seedlings using a set of reciprocal crosses and parental cultivars with varying degrees of genetic similarities. In this study, the definition of seedling vigor by Glewen and Vogel (1984) was adopted. Thus, seedling vigor refers to all the components of seedling growth, and consists of physiological processes at the heterotrophic, transitional, and autotrophic stages.

Experimental materials, design and data analysis

In mid- to late August of 1998, reciprocal crosses between the Japonica (J) cultivars Akihikari (AK), Koshihikari (KO), Todorokiwase (TO) and the Indica (I) cultivar IR50 (IR) were made by the clipping method in a greenhouse of Yamagata University. The F₁ and parental seeds were harvested one month after the crosses were performed. Seeds were stored at -5°C for 7 months and then kept at room temperature for one month prior to use. Genetic lineage similarities between Japonica cultivars were previously esti-

mated as: AK-TO < AK-KO < KO-TO. IR50 is a check cultivar commonly used in Indica yield trials, and it matures early like AK, KO, and TO.

On May 28, 1999 dehulled F₁ and parental seeds were incubated at 30°C. The number of germinated seeds, when the tips of both the coleoptile and radicle appeared, were counted at 24, 36, and 48 h after incubation to determine the germination index (GI). GI was calculated as $GI = \sum X_n (t-n)/t$, where X_n is the number of germinated seeds in the n^{th} count, n is the number of counts, and t is the total number of counts (Jones and Peterson 1976). Three days after incubation, radicle length (RL) and shoot length (SL) were measured according to the method of Newman (Yoshida 1981). Ten representative seedlings of the 12 F₁ and 4 parental cultivars were then sown to their respective "plots" (5 cm × 5 cm in size) in each of 2 sectioned wooden frames with a nylon screen underlay. A completely randomized design with 2 replications was adopted in which one frame was equivalent to one complete replication. Each of the wooden frames with the seedlings was then placed in a separate plastic tray (47 cm × 38 cm × 15 cm) that contained the culture solution. The materials were maintained in a greenhouse until 23 days after sowing (DAS). The culture solution was renewed every 2 days.

Observations for the second leaf emergence index (2nd LEI) were recorded following the transfer of the seedlings to their 'plots'. The 2ndLEI was determined when the tip of the 3rd leaf became visible. The calculation of the 2ndLEI was similar to that of the GI. At 16 and 23 DAS, 4 seedlings per plot were sampled and the fresh weight (FW) was determined. Seedling height (SH) and root length (RTL) were also measured according to Newman's method. Leaves and roots were then separated and leaf areas (2ndLA, 3rdLA, 4thLA and 5thLA) were measured individually using an infrared area meter (Hayashi-Denko, Co. LTD, Tokyo, AAM-7 type). Dry weight of shoot and root (SDW and RDW, respectively) was measured 3 days after drying of the materials in a ventilated oven at 80°C. Leaf area ratio (LAR), RGR and NAR during 16 to 23 DAS were calculated. Least significant difference (LSD) test and linear correlation (Spearman) were performed on all the traits. Mid-parent heterosis was estimated as $MPH (\%) = [(F_1 - ((P_1 + P_2)/2)) / ((P_1 + P_2)/2) \times 100\%]$, where F_1 , P_1 and P_2 represent the mean performance of the hybrids and their parents, respectively.

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Reciprocal F_1 performance, heterosis and trait correlations

Significant reciprocal cross effects on seedling vigor were observed. Most of the significantly lower performance was noted in reciprocal F_1 s with AK as female plant, i.e., AK/TO, AK/KO and AK/IR. However, the reciprocal cross effect appeared to be less conspicuous towards the 23 DAS stage, especially in AK/KO (Table 1). It is interesting to note that no significant differences in the 2ndLA at 23 DAS were detected between reciprocal crosses while highly significant differences were noted in the 2ndLEI (LSD = 1.655, $P < 0.001$). The absence of significant differences in the 2ndLA at 23 DAS across all the reciprocal combinations may imply that the variations between fully expanded second leaves of reciprocal crosses were negligible. The degree of negative reciprocal cross effect on hybrid performance for the GI and 2ndLEI also paralleled the level of performance of the seed parents (Fig. 1). This is somewhat analogous to the findings of Ikehashi (1972) on the germination characteristics of Koshihikari, Ginmasari and their reciprocal crosses.

The above results may be ascribed to a maternal effect of plant in which the F_1 seed matured, or to interactions between cytoplasmic factors and nuclear genes (Miller *et al.* 1996). In the present experiments, we removed the seed husks or hulls, which predominantly determine the level of seed dormancy (Ikehashi 1972), prior to the germination treatment. Thus, the removal of the husks was considered to be an efficient way to break seed dormancy, or to promote

germination (Ikehashi 1972). Furthermore, F_1 and parental seeds were produced within about two weeks, i.e., from mid- to late August, and used 8 months later for the experiments. This implied that temperature effects from flowering to ripening on seed dormancy (Ikehashi 1972) could have been partly reduced. However, the pericarp, seed coat and aleurone layer of hybrids were identical with those of their maternal parents. It remains to be determined, therefore, whether the genetic and/or physiological characters of the covering tissues of brown rice had actually affected the performance of the hybrids.

The indicator of hybrid vigor or heterosis, MPH, showed superior values for the $I \times J$ crosses compared to the $J \times J$ combinations, except for the AK/IR hybrids, which might have experienced an adverse maternal effect (Table 2). These results are consistent with those reported by Yuan (1994) and Kuroda *et al.* (1998). All these findings suggest that rice breeders should aim at the development of inter-subspecific hybrids. Akita (1988) observed a limited heterosis for RGR and NAR in rice after transplanting, and heterosis estimated for these parameters in the current study seemed consistent with his findings. The MPH results also confirmed the depressive maternal effects of AK on hybrid seeds which matured on the plants. At 23 DAS, however, the seedlings of AK/KO and AK/IR exhibited a positive MPH for several traits, suggesting that such depressive effects were partly alleviated at this stage. The maternal effect of the AK plant appears to have prevented such hybrids,

Table 1. Test of significance of reciprocal cross mean differences by LSD

Reciprocal Cross	Trait Means															
	Initial stage		16 DAS stage						23 DAS stage							
	SL	RL	SH	2ndLA	3rdLA	4thLA	FW (x10 ⁻¹)	SDW (x10 ⁻²)	SH	2ndLA	3rdLA	4thLA	5thLA	FW (x10 ⁻²)	SDW (x10 ⁻²)	RDW (x10 ⁻³)
J × J																
AK/TO	0.4 *	1.7 ns	13.7 **	0.1 **	1.0 **	1.6 **	1.5 ***	1.1 **	23.2 ***	0.2 ns	1.0 *	1.9 **	3.6 ***	23.9 ***	2.9 ***	5.9 ***
TO/AK	0.7	2.4	18.1	0.3	1.7	2.6	3.0	2.1	32.2	0.2	1.6	2.9	6.0	54.7	5.4	12.0
AK/KO	0.5 ns	0.7 ***	12.4 **	0.1 ns	0.8 *	1.1 *	1.3 *	0.9 **	26.0 **	0.2 ns	1.4 ns	2.4 ns	4.4 ns	33.9 **	3.7 ns	8.3 ns
KO/AK	0.6	2.1	16.9	0.1	1.3	1.8	2.0	1.7	29.3	0.1	1.5	2.3	4.8	38.8	3.9	8.2
TO/KO	0.8 **	2.3 *	20.3 ns	0.2 ns	1.5 ns	2.4 ns	2.4 ns	2.0 ns	32.6 ns	0.2 ns	1.5 ns	2.5 ns	5.4 ns	48.1 ***	5.1 ns	10.9 *
KO/TO	0.4	1.4	18.4	0.2	1.2	1.9	2.1	1.7	30.8	0.2	1.3	2.5	5.2	38.6	4.1	8.1
I × J																
AK/IR	1.1 ns	1.9 *	14.8 **	0.4 *	1.1 **	2.1 **	2.1 ns	1.5 *	24.2 ***	0.3 ns	1.0 **	2.2 *	4.2 **	36.9 ***	3.4 **	6.9 *
IR/AK	1.2	2.8	19.1	0.3	1.7	3.0	2.5	2.1	28.8	0.4	1.7	2.9	6.0	46.1	5.0	9.5
TO/IR	1.1 ns	2.5 ns	21.3 ns	0.4 ns	1.4 ns	3.0 ns	3.0 ns	2.3 ns	30.6 ns	0.4 ns	1.6 ns	3.1 ns	6.1 ns	52.7 *	6.1 ns	12.4 ns
IR/TO	1.1	2.5	18.5	0.4	1.5	2.7	2.3	1.9	29.2	0.4	1.3	2.6	5.4	48.9	5.1	10.4
KO/IR	1.2 ns	2.5 ns	20.7 ns	0.5 ns	1.4 ns	2.9 ns	3.3 ns	2.5 ns	30.7 ns	0.5 ns	1.4 ns	2.8 ns	5.7 ns	61.2 ***	6.6 ns	13.7 *
IR/KO	1.2	2.5	20.0	0.4	1.6	3.0	3.0	2.4	29.7	0.4	1.6	2.8	5.8	50.9	5.8	10.9
LSD 5%	0.3	0.7	2.9	0.1	0.4	0.6	0.7	0.5	2.4	0.1	0.4	0.6	1.2	3.5	1.2	2.2
LSD 1%	0.4	1.0	4.0	0.1	0.5	0.9	0.9	0.8	3.2	0.2	0.6	0.8	1.6	4.8	1.6	3.1
LSD 0.1%	0.5	1.4	5.5	0.2	0.7	1.2	1.3	1.0	4.5	0.3	0.9	1.1	2.2	6.5	2.3	4.3

*, **, *** indicates significant difference between reciprocals at the 5%, 1% and 0.1% levels, respectively; ns: not significant.

Reciprocal crosses followed the female parent/male parent notation.

Units of measurement are as follows: cm for length, sq. cm for leaf area, and g for weight.

Trait	J × J						Reciprocal Crosses						I × J			
	TO/AK	AK/TO	TO/KO	KO/TO	KO/AK	AK/KO	TO/IR	IR/TO	AK/IR	IR/AK	KO/IR	IR/KO				
At initial stage																
SL	11.7	−47.4	1.5	−98.5	−18.6	−21.5	18.6	18.0	28.5	34.7	24.7	23.8				
RL	66.9	53.1	59.4	32.5	65.5	−7.7	16.5	15.9	2.3	32.2	20.1	20.3				
At 16 DAS stage																
SH	0.3	−31.3	9.1	−0.4	−1.8	−39.4	20.8	8.8	−5.9	18.2	22.1	19.7				
RTL	−3.1	−23.8	16.5	5.1	−31.8	−21.6	14.1	−0.4	−9.0	7.9	11.5	19.7				
2ndLA	19.4	−73.2	3.5	2.6	−57.5	−62.9	31.8	39.3	32.9	14.2	55.3	51.2				
3rdLA	28.3	−19.0	36.6	23.0	17.3	−37.0	19.5	25.3	−11.7	26.1	31.1	39.9				
4thLA	10.1	−116.6	8.8	−12.4	−15.0	−87.5	21.9	16.0	−5.4	25.7	29.5	30.5				
FW	15.7	−69.5	−2.3	−20.2	−24.1	−90.0	15.3	−8.1	−17.4	1.5	25.4	18.7				
SDW	6.1	−75.0	3.8	−16.7	−14.7	−129.4	20.7	1.4	−27.6	9.8	26.0	22.9				
RDW	4.2	−91.7	12.5	−31.3	−25.0	−100.0	14.3	−20.0	−43.8	−4.5	30.0	12.5				
At 23 DAS stage																
SH	15.1	−17.7	2.3	−3.4	8.1	−3.7	12.9	8.7	10.2	24.4	14.5	11.8				
RTL	22.6	0.5	−7.1	−15.6	16.6	21.0	6.3	1.1	8.5	25.5	8.3	5.5				
2ndLA	20.0	−14.6	13.6	−16.3	6.5	21.3	35.2	30.0	19.2	43.3	49.2	41.8				
3rdLA	19.9	−23.7	32.2	24.2	22.7	13.7	23.7	10.6	−40.4	18.5	21.3	31.7				
4thLA	19.1	−26.6	7.5	9.3	3.1	8.4	23.6	8.1	−7.9	20.7	21.5	21.1				
5thLA	11.8	−47.0	9.7	6.0	0.6	−8.3	26.7	16.8	−2.0	28.0	31.9	31.9				
FW	27.7	−65.3	4.1	−19.6	−3.0	−17.7	15.0	8.4	−4.4	16.3	26.2	11.2				
SDW	11.9	−62.0	2.9	−22.4	−13.1	−19.9	19.7	4.7	−27.8	14.4	31.3	22.5				
RDW	6.3	−90.7	−1.4	−37.3	−28.8	−26.5	8.5	−9.1	−57.7	−13.7	22.6	2.8				
LAR	10.2	10.6	14.3	24.2	14.7	27.6	5.7	17.3	24.8	20.3	2.4	14.2				
RGR	7.3	8.0	−4.4	−4.4	0.9	43.3	12.6	6.2	−0.9	4.2	4.7	−2.5				
NAR	−2.8	−6.7	−17.5	−36.7	−20.2	26.6	−0.8	−13.6	−26.8	−18.2	2.3	−19.8				

Positive heterosis is indicated in bold characters.

Table 3. Linear correlation coefficients between seedling vigor traits

Trait	At 16 DAS										At 23 DAS									
	SL	RL	SH	RTL	2ndLA	3rdLA	4thLA	FW	SDW	RDW	SH	RTL	2ndLA	3rdLA	4thLA	5thLA	FW	SDW	RDW	
At initial stage																				
SL	–																			
RL	0.76	–																		
At 16DAS stage																				
SH	0.61	0.44	–																	
RTL	0.52	0.39	0.56	–																
2ndLA	0.79	0.69	0.50	0.56	–															
3rdLA	0.56	0.66	0.61	0.71	0.63	–														
4thLA	0.83	0.66	0.78	0.73	0.77	0.80	–													
FW	0.66	0.55	0.68	0.64	0.63	0.58	0.84	–												
SDW	0.67	0.53	0.81	0.73	0.54	0.72	0.86	0.90	–											
RDW	0.63	0.58	0.76	0.59	0.59	0.55	0.78	0.91	0.86	–										
At 23 DAS stage																				
SH	0.05	0.06	0.66	0.10	0.11	0.21	0.26	0.34	0.44	0.40	–									
RTL	0.34	0.29	0.23	0.01	0.04	0.28	0.34	0.30	0.36	0.17	0.29	–								
2ndLA	0.86	0.82	0.59	0.43	0.84	0.56	0.79	0.63	0.56	0.63	0.05	0.37	–							
3rdLA	0.39	0.52	0.46	0.60	0.33	0.81	0.63	0.47	0.63	0.45	0.11	0.52	0.41	–						
4thLA	0.55	0.59	0.74	0.60	0.59	0.76	0.83	0.66	0.70	0.65	0.37	0.48	0.75	0.72	–					
5thLA	0.48	0.39	0.82	0.60	0.46	0.75	0.82	0.70	0.79	0.65	0.56	0.48	0.57	0.74	0.91	–				
FW	0.64	0.61	0.79	0.42	0.55	0.65	0.76	0.77	0.83	0.78	0.60	0.50	0.60	0.47	0.74	0.74	–			
SDW	0.62	0.51	0.87	0.55	0.56	0.62	0.84	0.89	0.91	0.91	0.59	0.39	0.65	0.51	0.78	0.82	0.92	–		
RDW	0.48	0.41	0.67	0.45	0.47	0.44	0.69	0.86	0.79	0.94	0.45	0.30	0.53	0.42	0.63	0.65	0.78	0.91	–	

Critical values for comparison are 0.425 and 0.601 at the 5 % and 1 % probability levels, respectively, for n = 16.

Underlined and bold-faced numerals are significant at the 5 % and 1 % probability levels, respectively.

especially AK/TO, from consuming starch and protein stored in their endosperm, thereby limiting their energy and vigor for early seedling growth.

Interestingly, Matsuba *et al.* (1986) reported that under less dense seeding conditions, F₁s with the AK-cytoplasm could show higher rates of first node tillering than AK. Thus, this tillering ability was affected largely by the sowing density environment. However, such tillering was not observed in the present experiments, presumably due to the difference in the culture medium employed, i.e., hydroponic culture for this study and soil culture in seedling box in the cited experiment. The significant correlations exhibited by SL and RL with most of the 16 and 23 DAS traits (Table 3) suggested that seedling growth at the heterotrophic stage is substantially related to seedling development at the autotrophic stage. Traits observed after the heterotrophic stage, i.e., at 16 and 23 DAS, also exhibited close relationships with each other in general. The low and/or non-significant correlation of SH and RTL at 16 DAS with the other traits may be attributed to the fact that differentiation and growth of the 3rd and 4th leaves progressed rapidly at this stage. Consequently, these organs became large sinks that consumed nutrients for the whole seedling to grow. From these newly developed leaves, including the 5th leaf, assimilates may then be supplied to all the organs of the 23 DAS seedlings (transitional stage from heterotrophic to autotrophic stage). Thus, SH and RTL showed significant correlations with other traits at the 23 DAS.

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