Rice Science, 2007, 14(1): 27-32

Analysis on Factors Affecting Seedling Establishment in Rice

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Abstract: Elongations of coleoptile and mesocotyl are related directly to rice seedling establishment in soil and height of plant is related to lodging in rice production. Twelve typical rice cultivars with different lengths of coleoptile and mesocotyl (long, medium and short) were selected by screening the lengths of coleoptile and mesocotyl in 1500 accessions. The seedling establishments of these typical cultivars were compared under the combinations of different sowing depths and flooding durations, and two semi-dwarf varieties (G140, Zhong 96-21) with good seedling establishments and optimum mesocotyl lengths were found. The length of mesocotyl was completely fitted negative binomial distribution and the length of coleoptile was nearly fitted lognormal distribution. Analysis of the relationships among mesocotyl, coleoptile, seeding depth, flooding duration, and their interactions to seedling establishment percentage showed that there existed significant relations among mesocotyl, coleoptile, mesocotyl × coleoptile, seeding depth, flooding duration and mesocotyl × sowing depth in the experiment for seedling establishment.

Key words: direct seeding; seedling establishment; coleoptile; mesocotyl; rice

The direct-seeded planting in rice is getting more common due to simple farming, labour and time-saving. Nowadays, developed countries, such as USA, mainly engage in direct seeding rice production, while South-East Asian countries are adopting this technique [1]. However, low seedling establishment, weed infestation and lodging proneness are three main obstacles to popularization of this technique [2]. Studies on direct seeding rice in the past years revealed that the seedling establishment is related to the lengths of coleoptile and mesocotyl, i.e. the longer coleoptile and mesocotyl, the better the seedling establishment [1-4]. It has been observed that temperature, light, air (contents of O_2 and CO_2) and hormones affect the coleoptile and mesocotyl elongation, while the decreases in soil CO_2 concentration causes the decreases in lengths of coleoptile and mesocotyl [5-8]. The previous studies on plant height, lengths of coleoptile and mesocotyl of their filial generations of tall and semi-dwarf varieties indicated that the gene responsible for plant height is linked to the genes controlling coleoptile and mesocotyl elongation [9]. The rice breeders expect to obtain resources with optimum coleoptile and mesocotyl length to breed rice cultivars with good seedling establishment and lodging resistance for direct seeding.

The current study is focused on interrelationships among coleoptile, mesocotyl and seedling establishment by screening different rice genotypes to find related factors affecting seedling establishment in order to provide breeding resources.

MATERIALS AND METHODS

Plant materials

In this experiment, 1500 accessions with different genotypes were selected from China, Cuba, USA, Korea, Brazil, and IRRI, including indica, japonica, javanica, or glabrous, aromatic, and upland rice varieties.

Experiment 1: Measurement of the lengths of mesocotyl and coleoptile

Two hundred plump, dry and disease-free seeds per variety were selected and used in the experiment of seed germination. The following treatment was processed:

Soak seeds in distilled water for 42 h at 28℃ → incubate for 24 h → put germinated seeds into split platstic germination box on the aluminum plate →

Received: 16 August 2006; **Accepted:** 27 December 2006

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transfer aluminum plate into growth chamber for 72 h at 28℃ and 95％ of relative humidity under darkness → measure the lengths of mescocotyl and coleoptile.

Five layers of absorbent paper was laid at the bottom of plastic germination box and saturated with distilled water before adding germinated seeds. The experiment was designed with 20 seeds each variety, one variety each plastic germination box, eighteen plastic box each plate and with five replications.

Experiment 2: Treatments of different sowing depth and flooding duration

Typical rice varieties with different lengths of coleoptile and mesocotyl (long, medium and short) were selected by measuring the length of coleoptile and mesocotyl of 1500 accessions and used in this experiment. The germinated seeds were sown into a 60 cm×100 cm×30 cm plastic pot, at 2 cm, 4 cm, and 6 cm of seeding depth, and for 1 d, 3 d, and 6 d flooding duration, with 50 seeds per accession as one replication and four replications for each accession, and the seedling numbers were recorded every day at 3-6 days after sowing.

Statistics analysis methods

Experimental data were analyzed with χ^2 method of distribution matching test [10] and generalized linear analysis method [11].

Procedure of χ^2 method of distribution matching test

Step 1: The frequency distribution of the samples was divided into S sections, i.e., $(-\infty, a_1)$, $[a_{s-1}, a_2]$,…, $[a_{s-1}, \infty]$. If there were 'm' unknown parameters ($0 \leqslant m < s$) in the distribution function of $F_0(x)$, the parameters were estimated by samples and the estimated values were substituted in $F_0(x)$.

Step 2: Under H_0, the theoretical probability (p_i) and theoretical frequency were calculated [$p_i=P(a_{i-1}< X \leqslant a_i = F_0(a_i)-F_0(a_{i-1})$, $i=1, 2,\ldots, s$; $a_0=-\infty$, $a_s=\infty$)].

Step 3: If 'α' was specified, the Chi-square statistic was obtained from χ^2 distribution chart. If $\chi^2 < \chi^2_{s-m-1}(\alpha)$, the original assumption that the sample tested belonged to this distribution type was acceptable.

Step 4: The following distributions, including five continuous distributions (normal distribution, lognormal distribution, Γ distribution, Weibull distribution, exponential distribution), and five discrete distributions (Poisson distribution, binomial distribution, negative binomial distribution, geometric distribution, composite Poisson distribution) were estimated by the maximum-likelihood method. The density function of lognormal distribution was:

$$f_N(x,\mu,\sigma) = \frac{1}{\sqrt{x\sigma^2}} e^{-\frac{[\log(x)-\mu]^2}{2\delta^2}}$$

Where, μ is mathematic expectation, and σ^2 is variance.

The density function of negative binomial distribution was:

$$NP_r = \frac{(k+r-1)!}{r!(k-1)!} p^r q^{-k-r}$$

Where, p is occurrence probability of individual, q is non-occurrence probability of individual, k is characteristic parameter of negative binomial distribution.

Generalized linear analysis

The mesocotyl and coleoptile were classified into three groups by lengths respectively: mesocotyl <4 mm, 4－10 mm, >10 mm, and coleoptile <18 mm, 18－23 mm, > 23 mm. The screening was conditioned by the significance of F value in variance analysis [11] and the determination of the best regression subsets [12].

RESULTS

Length distributions of mesocotyl and coleoptile

The initial screening on germination of 1500 accessions indicated that the differentiation of mesocotyl length ranged from 0 to 19 mm, while the length distribution strictly met the negative binomial distribution. Under $\chi^2=20.6<\chi^2_{(15,\ 0.05)}=25$, its density function formula was:

$$NP_r = \frac{(r-0.6114)!}{r!(0.3886-1)!} 0.2261^r \times 1.2261^{-(0.3886+r)}$$

$r=0, 1, 2, 3, \ldots\ldots, n$

The result showed that the probability of mesocotyl length < 2 mm was $> 70\%$ and the probability of mesocotyl length >10 mm was $<1\%$ (Fig. 1).

The screening on germination of 1500 accessions showed that the differentiation of coleoptile length ranged from 1 to 35 mm. The length distribution approximated lognormal distribution under the condition of $\chi^2=36.2>\chi^2_{(15,\,0.05)}=25$ (Fig. 2). The result showed that 80% of coleoptile length was at 9 – 17 mm, while few were at >25 mm and <7 mm. The function for coleoptile length was:

$$f=\frac{1}{0.06292\sqrt{x}}e^{-\frac{[\log(x)-2.5993]^2}{0.1258}}$$

Where, x is coleoptile length.

Relationship between the lengths of mesocotyl and coleoptile

The data revealed no obvious correlation between the length of mesocotyl and coleoptile, but a significant correlation was noted between the maximum length of mesocotyl and coleoptile (Fig. 3): $y=18.57\times\exp[-(x-16.29)^2/117.66]$, $F_{(2,15)}=1123.82$, $R^2=0.96$. This demonstrated that the varieties with long mesocotyl had medium coleoptile, and rarely had extreme long or short coleoptile.

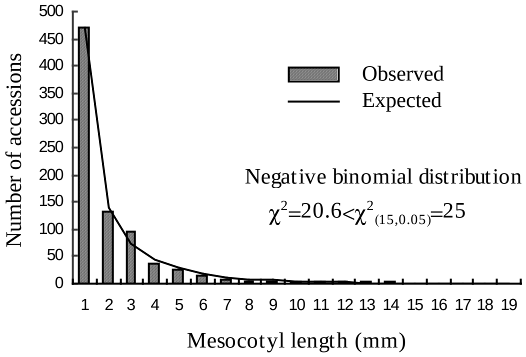

Fig. 1. Frequency distribution of mesocotyl length in 1500 rice accessions.

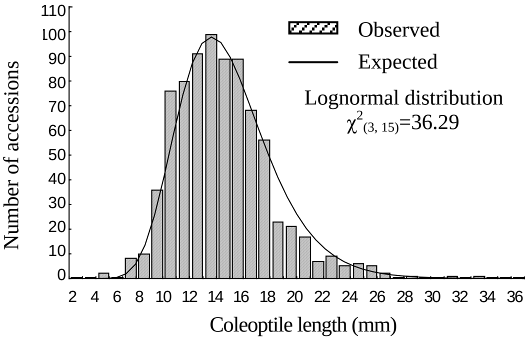

Fig. 2. Frequency distribution of coleoptile length in 1500 rice accessions.

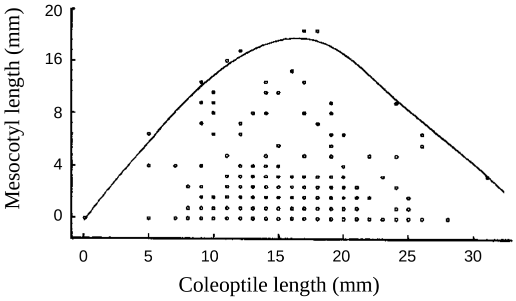

Fig. 3. Relationship between mesocotyl length and coleoptile length of rice.

Effects of environments on the lengths of mesocotyl and coleoptile and seedling establishment

Among the twelve typical varieties (lines) there was a close relationship between the seedling establishment percentage and the factors including the lengths of mesocotyl and coleoptile, sowing depth, and flooding duration (Table 1). The best performing varieties, G140 and Zhong 96-21 with semi-dwarf plant type and good lodging resistance, showed over 10% seedling emergence percentage when grew at 6 cm seeding depth and 6-day flooding duration. Therefore, these two cultivars were the first candidates for direct seeding. G140 was obtained by crossing a US glabrous rice with a Chinese early indica rice, while Zhong 96-21 was an early indica cultivar developed by China National Rice Research Institute (CNRRI). However, it has been noted that the total seedling establishment of Hongdonggu was only 45% in 1 d flooding duration and 2 cm seeding depth, thus it was not suitable for direct seeding.

The analysis of generalized variance indicated mesocotyl length (MES), coleoptile length (COL), flooding duration (FLD), seeding depth (SD), SD×MES and MES×COL had significant effect on seedling establishment (Table 2). Their variance contributions were 19%, 17%, 26%, 5%, 16%, and 22%, respectively, all of which accounted for 85% of the total variance. There was no significant difference between seedling establishment of small and medium COL, and both small and medium COL had no significant effect on seedling establishment; but in

Table 1. Seedling establishments of cultivars at various sowing depth and flooding duration.

Cultivar	Length (mm)		Seedling emergence rate (%)								
	Mesocotyl	Coleoptile	1 d flooding			3 d flooding			6 d flooding		
			2 cm	4 cm	6 cm	2 cm	4 cm	6 cm	2 cm	4 cm	6 cm
IACUBA19	14.55	19.63	68.00	53.00	33.50	66.50	43.00	20.50	43.50	23.00	8.50
IACUBA20	17.70	14.38	80.00	62.50	43.50	71.50	57.00	32.00	52.00	28.50	6.50
PERLA	1.20	14.38	78.00	50.00	16.00	76.50	48.00	6.00	54.00	6.50	0.00
J-104	0.00	19.13	91.00	38.00	11.50	81.50	28.00	0.00	60.50	1.50	0.00
LP7	9.00	15.25	68.00	21.50	3.50	58.00	18.00	3.50	37.00	2.50	0.00
LP9	15.45	17.13	80.00	48.00	35.00	65.00	41.50	30.00	56.50	17.00	3.50
Lemont	2.70	27.50	90.00	15.00	7.00	83.00	36.50	1.50	63.50	0.00	0.00
Shanhe	1.20	21.25	72.00	30.00	18.50	40.00	35.00	6.50	17.00	1.50	0.00
Hongdonggu	0.00	21.00	45.00	22.50	10.50	34.00	6.50	0.00	13.50	4.50	0.00
G140	17.80	26.88	93.00	78.00	60.00	91.50	70.50	50.50	73.50	44.50	11.50
Katy/9091	0.00	15.63	91.50	13.00	0.00	85.00	8.00	0.00	56.50	0.00	0.00
Zhong 96-21	12.45	24.38	95.00	78.00	56.50	87.00	51.50	45.00	71.50	43.00	10.00

2 cm, 4 cm, and 6 cm indicate the sowing depth.

Table 2. MANOVA results for seedling establishment.

Influence factor	*SS*	*MS*	*F*	*P*
Mesocostyl length (MES)***	1.08	1.08	51.70	0.00
Coleptile length (COL) ***	0.55	0.55	26.55	0.00
Flooding duration (FLD) ***	1.83	0.91	42.13	0.00
Seedling depth (SD) ***	5.74	2.87	132.36	0.00
MES×COL***	0.46	0.15	7.29	0.00
MES×FLD	0.01	0.00	0.15	0.86
COL×FLD	0.00	0.00	0.06	0.94
MES×SD***	0.43	0.22	10.33	0.00
COL×SD	0.01	0.00	0.22	0.80
SD×FLD	0.06	0.02	0.92	0.52
MES×COL×FLD	0.03	0.01	0.25	0.96
MES×COL×SD	0.09	0.02	0.75	0.61
MES×SD×FLD	0.11	0.03	1.27	0.30
COL×FLD×SD	0.01	0.00	0.16	0.96
COL×MES×FLD×SD	0.06	0.00	0.23	1.00
Error	0.75	0.02		

*** Significant at 0.001 level.

extreme large COL, the seedling establishment percentage increased remarkably (Fig. 4-A). Moreover, the change of MES from small to large led to the sharp rise of seedling establishment percentage (Fig. 4-F), while MES×COL significantly affected the seedling establishment: when small MES, COL controlled seedling establishment; when large MES, MES controlled seedling establishment (Fig. 4-B). The SD×MES showed a significant effect on seedling establishment with additive effect between SD and MES. The seedling establishment percentage was higher in large MES and small SD, increasingly affected by MES along with increasing SD, and nearly zero with small MES and large SD (Fig. 4-E). Both SD and FLD showed significant effects on seedling establishment, and the seedling establishment percentage suffered sharp decrease with the increase in SD or FLD. There was no interaction between SD and FLD (Fig. 4-C, D).

According to results from a multiple regression analysis, the estimated equation for seedling establishment percentage is $Y = -0.39\text{FLD} - 0.87\text{SD} - 0.41(\text{MES}\times\text{SD}) + 0.84(\text{MES}\times\text{COL}\times\text{SD})$, with *t* values of the best regression subsets (FLD, SD, MES × SD, MES × COL × SD) at -9.65***, -19.68***, 2.89**, and 5.88***, respectively.

DISCUSSION

The linkage among the genes responsible for the mesocotyl and coleoptile elongation, and plant height was the major restriction in breeding semi-dwarf variety with long mesocotyl and coleoptile. The seedling establishment was closely related to the lengths of mesocotyl and coleoptile. The result of the present experiment indicated that the common cause of the low screening frequency for long mesocotyl varieties are the negative binomial distribution of mesocotyl length and the nearly lognormal distribution of coleoptile length. We observed no obvious correlation between the mesocotyl and

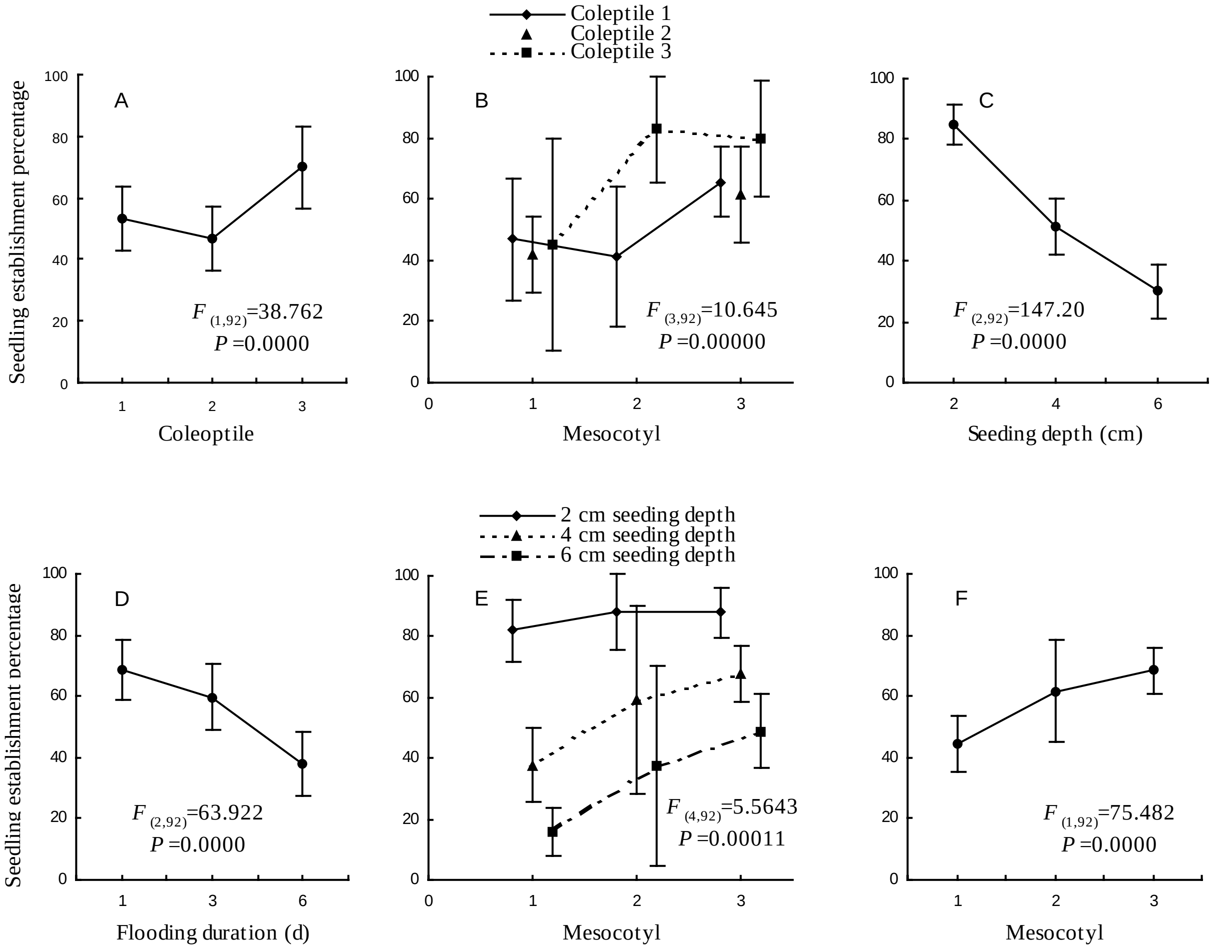

Fig. 4. Relationship between seedling establishment percentage, and lengths of coleptile and mesocotyl, flooding duration and seeding depth.

Coleptile 1, 2, and 3 indicate the coleptile length <18 mm, 18 – 23 mm, and >23 mm, and mesocotyl 1, 2, and 3 indicate mesocotyl length < 4 mm, 4 – 10 mm, and >10 mm, respectively.

coleoptile lengths, but a significant correlation was noted between the marginal distribution of the maximum value of the length of mesocotyl and coleoptile, which was of importance for breeding of direct seeding rice. Therefore, the two semi-dwarf and long mesocotyl materials, G140 and Zhong 96-21, screened from the 1500 accessions were of importance for direct seeding rice breeding.

The analysis of variance indicated that the seedling establishment was significantly affected by the factors of mesocotyl length, coleoptile length, and mesocotyl length × coleoptile length; meanwhile the seedling establishment was obviously inhibited by flooding duration and seeding depth, and the interactions between environment and varieties was demonstrated at mescocotyl length and seeding depth.

ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (Grant No. 2002CCA04100), the Key Science and Technology Program of Zhejiang Province, China (Grant No. 2003C34012), China Rice Science Foundation (Grant No. CRSF0003338), and International Atomic Energy Agency (IAEA) Training Program.

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