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Variation in Seedling Growth within a Rice (*Oryza sativa* L.) Cultivar and Its Effect on Seedling Salt Tolerance Evaluation

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Abstract : The variation of seedling growth, which may affect seedling salt tolerance, of 12 rice cultivars was examined based on the pattern of seedling fresh weight histogram. Some cultivars showed a normal distribution pattern while other cultivars exhibited an irregular or multimodal histogram. The variability in seedling growth histogram almost coincided with that in the embryo weight histogram. After one cycle of selection from a major peak in the seedling fresh weight histogram, the number of peaks in the seedling growth histogram of the next generation plants was considerably reduced. When seedling salt tolerance within the rice cultivar was studied (by growing one-week-old seedlings for 30 days under salinized and control culture solutions), the seedling dry weight histogram of the cultivar in salinized condition coincided well with that of the control. Larger seedling had higher relative weights under salinized conditions, lower sodium ion content, and lower Na/K ratio than smaller plants. Variability in seedling growth within a cultivar, therefore, would affect the quantitative evaluation of salt tolerance (e. g., survival ratio and growth rate) of rice seedling. The possibility of selecting salt-tolerant materials from a given cultivar was also examined. The results were not consistent because of various factors involved in establishing seedling salt tolerance. It is suggested that cultivars should be genetically purified first before they are used in salinity response studies.
Key words : Embryo weight, Rice cultivar/line, Salt tolerance, Seedling growth.

稲幼植物生体重の品種内個体変異とその変異が品種の幼植物耐塩性評価に及ぼす影響 : CABUSLAY, G.S.・BLANCO, L.C.・秋田重誠（国際稲研究所）

要　旨 : 既存の稲品種・系統について幼植物の耐塩性評価に影響を及ぼす幼植物の生体重の頻度分布（ヒストグラム）を求めた結果，ある品種の幼植物の生体重ヒストグラムはほぼ正規分布に近い形を示すのに対し，他のあるものは複数のピークを持つヒストグラムを示した．この幼植物生体重のヒストグラムは胚重のそれと類似していた．また，生体重ヒストグラムの各ピークごとに個体をとり圃場に移して出穂期を観察したところ，各々異なる出穂日を示す場合もみられた．このことは生体重ヒストグラムの変異に遺伝的要因も関与する可能性を示すと考えられた．稲種子を播種後1週間標準的な水耕液中にて育てた後，幼植物を標準および5，10 ds/m と塩濃度の異なる培養液中に移し30日間育て，各処理条件での生長量および植物体内のナトリウムおよびカリウム濃度を測定した．処理30日後の標準水耕液中で育てた材料の生体重のヒストグラムと塩処理下で育てられた材料の生体重ヒストグラムは平均値の値は異なるが分布のパターンはお互いに似ていた．また，既存の品種を用いて塩処理条件下で生存する個体を選抜採種し，次世代の個体の耐塩性を評価した場合，品種によりもとの材料の耐塩性より強くなるもの，弱くなるものおよびほとんど変化しないものがみられた．これらの結果は稲幼植物の耐塩性に関与する要因は複数あり，耐塩性の比較試験を行うにあたっては少なくとも幼植物生体重についての遺伝的変異をできるだけ小さくした材料について評価がなされるべきであることを示すものと考えられた．
キーワード : 稲品種/系統，耐塩性，ナトリウム，胚，幼植物．

Salt tolerance in rice is a complex character that depends on many plant, soil, water, and environmental factors [1,7]. Although a strong negative relationship exists between leaf Na concentration and survival, leaf Na concentration accounts for only 65% of the variability[8]. One of the factors contributing to variability in rice response to salinity might be the high variation in growth within a rice cultivar. In our preliminary experiments, seedling growth of a rice cultivar showed a significant variation. Flowers and Yeo[6] observed considerable variability in the survival of individual rice seedling within a cultivar or breeding line after treatment with NaCl.

Based on the definition of a cultivar, certain

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agronomic characteristics must be maintained when propagated[4]. Usually breeders observe several important qualitative and quantitative characterisitics to determine the uniformity of the cultivar before its release. However, the significant genetic impurity of seeds of most rice cultivars is known[5]. The existing level of seed impurity may be acceptable for practical use in farmer's field and for breeding purposes. However, for physiological and genetic work, this impurity often becomes a hindrance in identifying responsible physiological characteristics and in analyzing the inheritance of such characteristics. There is no assurance of uniformity in other characteristics such as seedling growth other than those observed by breeders.

This study was done to determine the variability in seedling growth of selected rice cultivars and to determine how variation in seedling growth within cultivars affects the response of rice seedling to salt stress. It likewise investigated the possibility of selecting salt-tolerant materials from a given cultivar because selection under salt stress conditions should identify variants with higher salt tolerance if variation within cultivar is genetic in nature.

Materials and Methods

Experiment I

Around 400 pregrminated seeds each of six IR and six traditional cultivars were sown on a styrofoam seedbed and floated over a culture solution prepared following modified Yoshida's recommendation[2]. After three weeks, the fresh weights of individual plants were determined. The seedling fresh weight histogram was obtained by counting the number of seedlings falling under a certain interval of fresh weight. To know the cause of the variability in seedling growth histogram, the embryo dry weight (determined by oven-drying overnight at 105—110°C) histogram of selected cultivars was also obtained using 200 seeds per cultivar after manually separating the embryo from the endosperm with a modified dissecting needle[3]. Representative seedlings from each peak of the seedling growth histogram were planted in the field and grown until maturity for seed production during the wet season. The fresh weight histogram of the next generation plants from each peak in the seedling growth curve of the former generation was obtained using 200 seedling to know the variability after one generation of selection.

Experment II

To determine how variation in seedling growth within cultivars affects the response of rice seedlings to salt stress, four cultivars/lines-Pokkali (Acc. No. 26869), IR4595—4—1—13, KR1—24, and IR8-were used. The experiment was conducted inside the Phytotron glasshouse maintained at 29/21°C (day/night), at about 70% relative humidity, and under natural light intensity. Three sets of seeds (45 per cultivar) were grown on a styrofoam seedbed and floated in normal culture solution prepared as described previously. The pH of the solution was transferred to a culture solution salinized at an EC of 5 dS/m while another set was salinized at 10 dS/m. After 30 days of treatment, the dry weight of each plant was determined and a seedling dry weight histogram was obtained for each cultivar. The number of peaks in the seedling growth histogram of the cultivar under salinized condition was compared with that of the control. Seedlings belonging to one peak within a cultivar were combined and relative dry weight was obtained by dividing the averge dry weight in each peak in salinized condition by the averge dry weight in the corresponding peak in the control. The materials belonging to each peak were ground, acid-extracted, and analyzed for Na and K ion contents by atomic absorption spectrometry[9].

Experiment III

The possibility of increasing the level of salinity tolerance by selection within the cultivar was examined. About 60 germinated seeds of 22 cultivars (Table 3) were grown in culture solution in the screenhouse and salinized after one week at 15 dS/m. Salinization of a cultivar was discontinued when only a few plants remained alive. The surviving seedlings were transferred to a normal culture solution for recovery and then planted pots to produce seeds. To determine salinity tolerance, harvested seeds were bulked and the survival ratio (percentage of surviving plants to total treated plants) of R_2 seedlings (R_2 are plants which have undergone one cycle of selection) was compared with that of the original population (R_1) in the salinized culture solution (15 dS/

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m).

Results and Discussion

Experiment I

A number of peaks appeared on the seedling fresh weight histogram, ranging from 3 for IR30 to 13 for Nona Bokra. IR64 exhibited 5 peaks (Fig. 1) To determine whether this irregular distribution pattern is related to genetic impurity, seedling of IR64 obtained from different peaks of the seedling distribution curve were transplanted to the feild and heading time was observed. Plants obtained from different peaks had different heading times (Fig. 2) Seddling from the next generation obtained from a major peak of original cultivar showed a marked reduction in the number of peaks in all cultivars studied (Table 1). This result indicate the possibility of selecting plants with less genetic variability in seedling growth from already released cultivars.

The embryo weight histogram of seeds harvested from one hill grown from one seed showed a normal distribution pattern (Fig. 3). On the other hand, an irregular or a multimodal distribution pattern of embryo weight was ovserved almost similar to that of the seedling weight histogrom (Fig. 4) This indicates that the variability in seedling growth within a cultivar can probably be attributed to differences in embryo weight. The embryo weight distribution pattern of IR8 seeds obtained from the International Rice Germplasm Center (IRGC) and the Plant Breeding Department of IRRI (PB) differed remarkably (Fig. 5). This difference in the pattern of embryo weight histogrom depending on seed source may be attributed to differences in seed multiplication conditions. Usually seeds in IRGC are multiplied at long-term intervals. The more frequent cultivation done at PB may have caused the elimination of one peak having a smaller embryo weight which might have been lost due to some recent selection pressure like tungro disease. These results clearly indicate that the irregular seedling growth histogrom is closely related to the embryo weight histogrom and is partly due to genetic variability; selecting plants with less variation in seedling growth would be necessary for the precise evaluation of seedling salt tolerance of a given cultivar.

Experiment II

Pokkali showed a normal frequency distribution pattern of seedling dry weight in nonsaline condition. On the other hand, the growth pattern of other cultivars often showed a multimodal pattern in nonsaline condition. The frequency distribution curves of seedling growth in salinzed condition coincided well with those of the control (Figs. 6—8). Furthermore, within the same cultivar, plants obtained in the heavier portion of the distribution curve had higher relative dry weights at 5

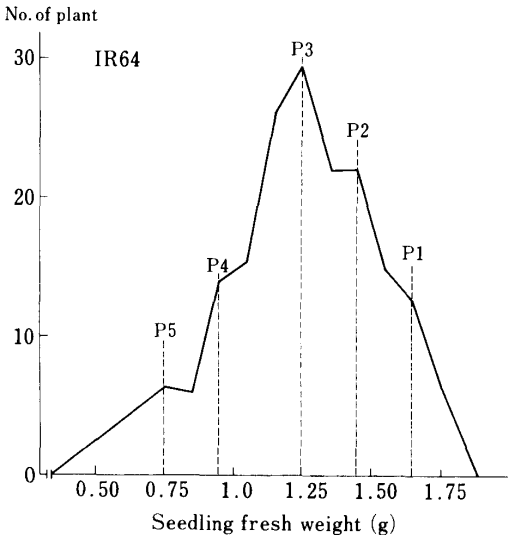


Fig. 1. Distribution curve of seedling fresh weight of IR64.

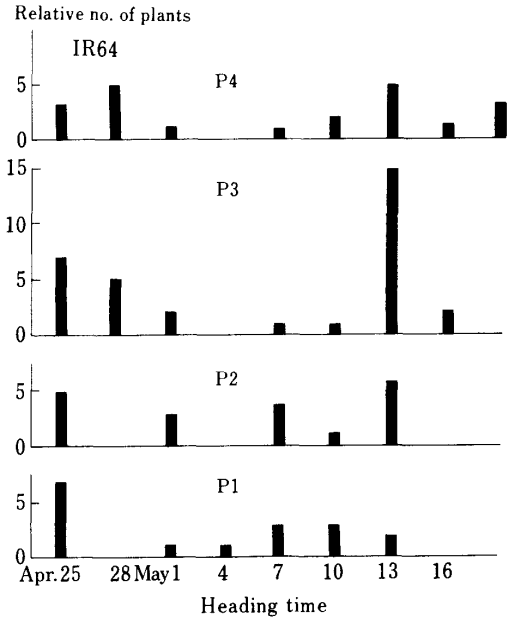


Fig. 2. Distribution curve of heading time of plants obtained from each peak of IR64 as shown in Figure 1.

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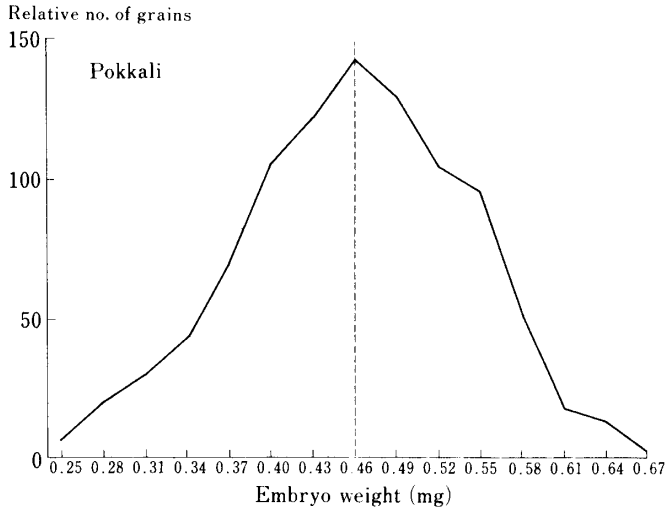


Fig. 3. Distribution curve of embryo weight of Pokkali grown from one seed.

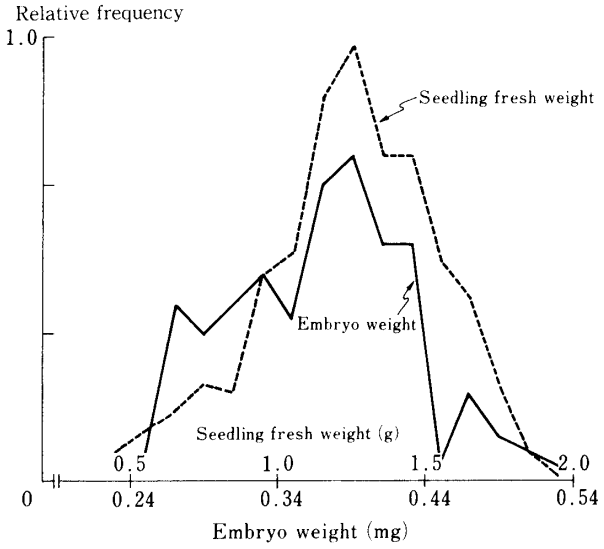


Fig. 4. Distribution curve of embryo dry weight and seedling fresh weight of IR64.

and 10 dS/m (Table 2) and lower sodium content in the shoots and roots (Table 3) than smaller plants. Table 2 also shows that bigger plants had lower Na/K ratios in the shoot, indicating less substitution for K by Na in the shoot.

The presence of plants with different seedling weights will produce inconsistent results in the evaluation of survival ratio and growth rate since bigger plants absorb less Na and have higher chances of survival than do smaller plants. Variability in the seedling survival within a cultivar or breeding line after treatment with NaCl was also observed by Flowers and Yeo[6]. In studies dealing with response of rice cultivar to salinity, it is therefore necessary

Table 1. Number of peaks in rice seedling growth histogram before and after selection

Cultivar	Number of peaks	
	Before selection	After selection*
IR64	5	2
IR48	7	2
IR44	7	2
IR40	10	1
IR30	3	2
IR26	7	2
IR8	7	4
Cheriveruppu	6	2
KR1-24	13	2
M1-48	5	3
Nona Bokra	13	2
Pokkali	9	2

* Seeds of selected plants from major peak of seedling growth histogram of original cultivar was used.

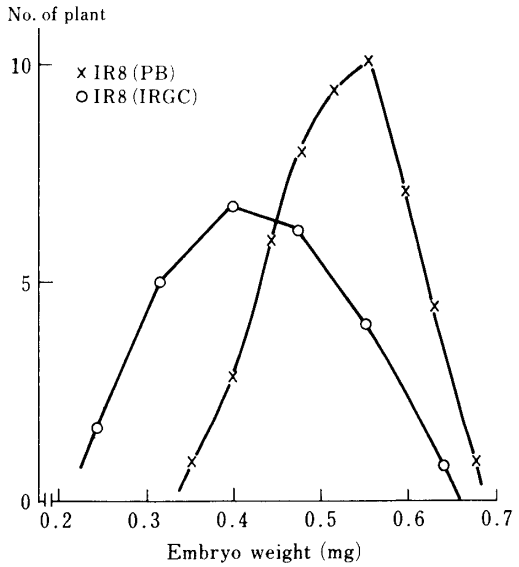


Fig. 5. Comparison of histogram of embryo weight of IR8 obtained from two seed sources. One is from the International Rice Germplasm Center (IRGC) and the other is from the Plant Breeding Department (PB) of IRRI.

to select seeds for uniform seedling growth first ; otherwise, data obtained from already released cultivars without genetic purification will show high variability. In addition, at least

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100 seedlings per selected cultivar have to be used for the margin of error to be within 3 —10% of the true mean value. This is because even after proper selection, the seedlings will still exhibit a normal distribution curve caused by environmental and/or morphological factors.

Experiment III

Table 4 shows the survival percentage of parent (R_1) and the next generation (R_2) seedlings from seeds from seeds of few selected plants that survived in salinized culture solution. When survival ratio of the plants from seeds of the surviving plants under salt stress condition is compared with that of the original materials of a given cultivar, the ratio was not always improved. After one generation of selection, a marked increase in survival was observed in such cultivars as IR8, Jhona349, IR31375-3-3-1-1, KR1-24, IR4563-52-1-3-6, and Kharai Ganja. Five other cultivas-

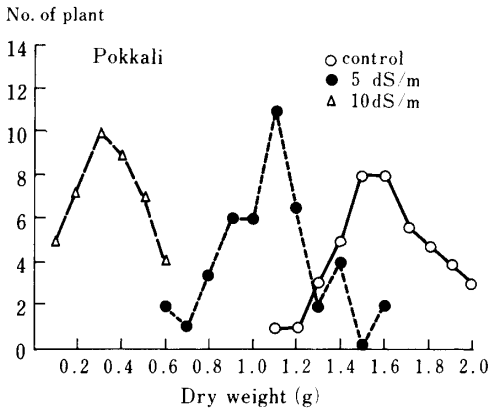


Fig. 6. Frequency distribution of dry weight of IR8 at 0 and 5 dS/m.

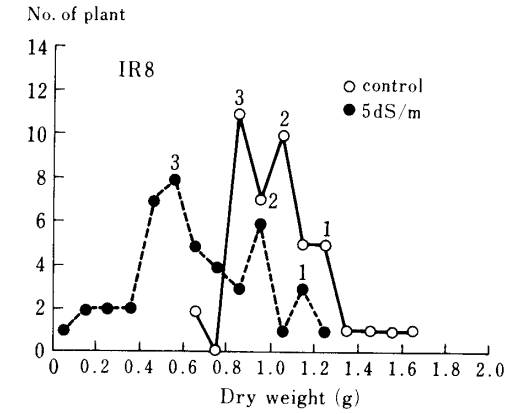


Fig. 7. Frequency distribution of dry weights of IR8 at 0 and 5 dS/m.

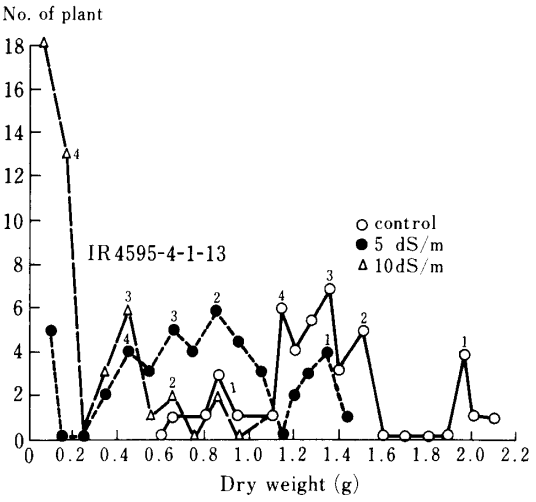


Fig. 8. Frequency distribution of dry weights of IR4595-4-1-13 at 0, 5, and 10 dS/m.

Table 2. Relative total dry weights of plants located in each peaks[a] of the distribution curve. IRRI, 1985.

Cultivar	EC[b] (dS/m)	Relative dry weight			
		Peak 1[c]	Peak 2[c]	Peak 3[c]	Peak 4[c]
KRI-24	5	0.75	0.76	0.57	
	10	0.80	0.63	0.47	
IR4595-4-1-13	5	0.69	0.54	0.49	0.41
	10	0.44	0.41	0.32	0.12
IR8	5	0.93	0.92	0.66	

a) Weight of plants in decreasing order : Peak 1 > Peak 2 > Peak 3 > Peak 4

b) Electric conductivity

c) Peak number of IR8 and that of IR4595 corresponds to those in Fig. 7 and in Fig. 8 respectively. The procedure to get peak number of KRI-24 is not shown but was taken by the similar procedure with the materials mentioned above.

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Table 3. Sodium and potassium ion contents (%) of plants located in each peak[a] of the seedling growth histogram. IRRI, 1985.

Cultivar	EC[b] (dS/m)	Plant part	Peak 1[c]		Peak 2[c]		Peak 3[c]		Peak 4[c]	
			Na	Na/K	Na	Na/K	Na	Na/K	Na	Na/K
KR1-24	5	Shoot	2.27	1.23	2.29	1.33	2.83	1.59		
		Root	2.34	3.34	2.50	3.57	2.66	3.80		
	10	Shoot	3.63	1.32	2.96	1.13	4.22	1.43	4.33	2.11
IR4595-4-1-13	5	Shoot	1.27	0.64	1.52	0.77	1.69	0.92	2.02	1.15
		Root	2.30	2.42	2.29	2.16	2.33	2.08	2.64	2.56
	10	Shoot	3.70	1.38	4.31	1.61	4.83	1.84	5.05	3.30

a) Weight of plants in decreasing order : peak 1 > peak 2 > peak 3 > peak 4
b) Electric conductivity
c) Peak number corresponds to peaks that appeared in the histogram

Table 4. Percent survival of rice cultivars after one generation of selection using salinized culture solution. IRRI, 1986.

Cultivar	Number of days of salinization[a]	Survival (%)[b]	
		R_1 (Parent)	R_2
ADT 4	13	40	14
M1-48	13	18	18
IR36	16	13	18
IR56	16	20	28
Taichung 65	17	28	36
IR8	19	5	25
IR52	19	39	22
ADT 28	20	20	27
IR32	20	17	22
IR4595-4-1-13	20	47	27
IR9884-54-3	20	20	23
IR31375-3-3-1-1	20	20	42
Jhona 349	20	25	38
KRI-24	21	37	59
IR4563-52-1-3-6	22	11	36
BR4-10	23	52	16
Getu	23	27	25
IR4630-22-2-5-1-3	23	34	29
M242	23	33	29
Kharai Ganja	24	10	32
Nona Bokra	24	55	37
Pokkali	24	40	40

a) Concentration and duration of salinization : 15 dS/m for the first 10 days, 20 dS/m from 16th to 25th day.
b) Compared in salinized culture solution of 15 dS/m.

ADT4, IR52, IR4595-4-1-13, BR4-10, and Nona Bokra-showed a marked decrease in survival ratio. The other 11 cultivas either had little of no response to selection. The bigger seedlings tended to survive longer in salt stress condition as was shown in Experiment II ; however, seeds obtained from these bigger salt-tolerant seedlings did not always result in selection of plants with higher survival ratio.

The bigger plants did not absorb a higher amonut of Na ion as shown in Experiment II ; however, the results of this experiment indicate that seedling vigor is not always determined by genetic factor and that survival ratio

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at seedling stage may possibly be determined not only by the genetic factor but also by other factors. Blanco et al[3]. reported that embryo weight (which is highly correlated with seedling growth) shows a significant variation even within the panicle of a certain cultivar which may be caused by environmental factors. The evaluation of seedling salt tolerance after minimizing genetic variability in seedling growth by proper selection may give a better understanding of the salt tolerance mechaism in rice. These results do not exclude the possibility of selecting materials with slightly higher salt tolerance from ordinary existing cultivars. However, none of the modern high-yielding semidwarf cultivars-e.g. IR36, IR50, Taichung 65, IR8, IR52, and IR32-had a survival ratio similar to that of salt-tolerant cultivars like Pokkali and Nona Bokra. The probability of selecting highly tolerant plants from already released modern high-yielding IR cultivars through this method will be low.

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