J. ISSAAS Vol. 16, No. 1:40-47 (2010)

VARIETAL DIFFERENCE OF NEGATIVE GRAVITROPISM IN RICE SEEDLINGS AND INVOLVEMENT OF ETHYLENE PRODUCTION IN ITS MECHANISM

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(Received: December 24, 2009; Accepted: April 21, 2010)

ABSTRACT

This study aimed to investigate the varietal difference in lodging recovery of rice seedling (negative gravitropism), which might be related to the suitability for seedling establishment in direct-seeding cultivation. Characteristics of general features of rice varieties which were collected from different countries were studied, focusing on the comparison of lodging recovery. The results obtained are as follows:

1. The 18 rice varieties tested in this experiment (17 Asian rice and one African rice) showed a wide range of variation in grain shape, phenol reaction and alkali degradability according to their ecotypes such as Japonica, Indica, and Javanica. African rice exhibited the Indica like characteristics in general.
2. A wide variation in negative gravitropism was found when tested with seedlings sown on agar media. The degree of negative gravitropism is not common within the ecotypes.
3. Kasalath (Indica) and Shinriki (Japonica) showed very strong lodging recovery and they were considered to be desirable breeding materials to introduce high negative gravitropism to direct-seeding oriented new varieties.
4. Calrose76 and Nongan, which are semi-dwarf varieties bred for direct seeding in USA and Korea respectively, were found to exert high lodging recovery.
5. The degree of lodging recovery of seedlings was observed to be well correlated with ethylene production rate of horizontally placed seedlings when tested with Nongan, WO492 and Daorenqiao.

Key words: direct sowing, seedling establishment, semi-dwarf variety, lodging recovery

INTRODUCTION

Since the practice of rice cropping is now decreasing under an aging agricultural society and outflow of rural population in Asian countries including Japan, it is highly important to produce rice of high quality and high value by developing new technologies for extending operation scale or saving labour and cost. The monsoon Asian countries, for instance, are starting to introduce the direct-seeding technology to extend operation scales although they have preferred transplantation for weed control (Savary *et al.*, 2005). The establishment of the direct-seeding technique calls for urgent attention in Japan as well, especially after the GATT Uruguay Round agreements, to decrease labour with lower cost of seedling raising and transplanting (Fujimaki, 1997).

There are two types of direct-seeding techniques; the seeding on well-drained paddy fields

followed by water logging; and the seeding on submerged paddy fields. The former has a number of advantages such as the high efficiency of seeding operation, but this technique can be applied only in warm-temperature regions and seedlings can be damaged by birds. The latter, on the other hand, can be applied in broader areas but the seedling height can be unequal, and due to bad rooting, seedlings can be easily suffered from lodging. Although there is a technique using Calper-coated seeds for stabilization of emergence and seedling erection, establishment of such technologies is not yet sufficient for steady spread of the direct-seeding techniques. It is therefore essential to develop varieties suitable for direct-seeding. Such varieties are expected to excel in the following characteristics: low-temperature germination, low-temperature emergence/seedling direction, hypogeal germination, and resistance against lodging (Miura, 2003).

Under submerged conditions, the lodging recovery of a seedling plays an important role. Mishima (1938) found that the characteristics of seedlings to re-rise vertically after being kept horizontal differ according to varieties. He also suggested that this difference might be related with the recovery ability of rice plants after lodging. However, to date, few studies have been done on varietal difference for lodging recovery in rice seedlings among ecotypes such as Indica, Japonica and Javanica. Thus, we carried out research on varietal difference of the lodging recovery ability of rice seedlings in relation to the negative gravitropism.

In this study, we investigated the difference in lodging recovery among rice varieties using 17 *Oryza sativa* varieties including the ecotypes of Indica, Japonica, and Javanica and one *Oryza glaberrima* variety, and the involvement of ethylene production in its mechanism was also discussed.

MATERIALS AND METHODS

At first, the characteristics of 17 varieties of Asian rice (*Oryza sativa*) and one variety of African rice (*Oryza glaberrima*) which have the potential to head panicles in the Kanto region were checked for their phenol reaction, alkali digestibility, husk shape and color, and grain color, shape, and size. The original place of origin for *Oryza sativa* tested in this experiment varies from India, China, Laos, Thailand, USA, Japan, to Korea and consisted of 8 Indica, 2 Javanica, and 7 Japonica ecotypes.

The seeds were germinated for 2 days under dark conditions at 30 ^{o}C, and sown on a 1/5000a Wargner's pot on May 5, 1998. The pot was filled with 3 kg red Kanto loam soil, mixed with 2 g ammonium sulfate, 7 g superphosphate lime, and 1.2 g potassium chloride, as N, P, K fertilizer, respectively. Two seeds were planted and grown in a pot for each variety with two replications in the net field of the Tropical Crop Science laboratory, Tokyo University of Agriculture located in Setagaya ward. The seeds were harvested in the end of October, 1998 and the characteristics were investigated for phenol reaction, alkali digestibility, husk shape and color, and grain color, shape and size. The phenol reaction was carried out by soaking the seeds in 1.5% phenol solution for 24 hours, and the results were described as positive or negative, when grain was dyed black or not, respectively. The alkali digestibility was evaluated 24 hours after soaking in 1.7% KOH solution, using 10-grade decomposition index of Ebara's method (Ebara, 1968).

Negative gravitropism of the tested 18 rice varieties were compared by a newly developed method using an agar medium as follows. First, seeds of the 18 varieties were cultured at 30^{o}C under dark conditions for two days to stimulate germination. Then fifteen germinated seeds were sown on the 1% agar medium filled in a plate and were cultured at 30^{o}C under bright conditions in a climate chamber for two days. Thereafter, the plates were inclined at a 90 ^{o} angle so that the first leaf would bend down its head and whole plants would be kept horizontal. Then, as an indicator of negative gravitropism, the angle of the uprising plant body was measured with a protractor for 48 hours at every 3 hours interval. The typical three varieties that is, Nong-an for its high negative gravitropism, WO492 (*Oryza glaberrima*) for its moderate negative gravitropism and Daorenqiao for its low negative gravitropism were selected to measure their lodging recovery ability and ethylene evolution

pattern during uprising process at 30 °C.

After one day incubation at dark condition (30 °C), five seeds were placed on 1% agar medium in 100 ml Erlenmeyer flask and kept at 30 °C under dark condition after sealed with silicon cap. The half was kept vertically, and the other half was placed horizontally. The lodging recovery pattern was measured with a protractor for 48 hours at every 3 hours interval and ethylene evolution in the atmosphere of the flask was analyzed after 24 hour incubation using GC14A (Shimadzu) equipped with flame ionization detector (FID). The analysis conditions were as follows: carrier gas was N_2 (6kg/cm^2), column: Sunpack A (porous polybeads), and the temperatures of injector, column, and detector were 120 °C, 80 °C, and 140 °C, respectively. The experiment was carried out on April 30, 2002 with three replications.

RESULTS

The 18 rice varieties were classified as Japonica, Indica, and Javanica based on their phenol reaction and alkali digestibility (Table 1). The grain shape showed a wide range of variation and it was found a typical characteristics according to the ecotypes; relatively round in Japonica type, long in Indica type, and big and wide in Javanica type. As for phenol reaction, all the Indica except Surjamkhi showed a positive reaction and all the Japonica and Javanica except Owarihatamochi showed a negative reaction. WO492, an African rice of *Oryza glaberrima*, indicated a positive phenol reaction as same as most of the Indica varieties.

In the case of alkali digestibility, Japonica varieties showed relatively higher digestibility. Indica varieties indicated lower alkali digestibility comparing to Japonica varieties and Javanica varieties showed intermediate values. The alkali digestibility of WO492 was rather low, indicating a similarity with the Indica type. This variety exerts Indica like characteristics in general, except for the peculiar trait of *Oryza glaberrima* such as short and round ligule, lesser secondary spikelets, and rarely observed pubescence of glumes and leaves.

The varietal difference of negative gravitropism which was indicated by the time to rise up to 50, 70 and 90 degree angle from horizontal position, was shown in Table 2. Observing the ability to rise to a 30 degree angle, Kasalath (Indica), Hunanxian (Indica), and Shinriki (Japonica) showed strong lodging recovery.

The rising pattern shown in Figure 1, indicates the relationship between the time taken to rise to a 50 degree angle and the one from a 50 degree angle to a 70 degree angle. Most of the varieties rise quickly to a 50 degree angle, and thereafter shows relatively slow pace of getting upward to a 70 degree angle, as typically shown in Dular (Indica), Kasalath (Indica), Liuzhoubaoyacao (Indica), Calrose76 (Japonica), Shinriki (Japonica) and Nong-an (Japonica). On the other hand, the varieties such as Hunanxian (Indica), Dam Ngo (Javanica), Asahi (Japonica), and WO492 (*Oryza glaberrima*) showed a rather constant rising speed, and in case of Daorenqiao, it showed a very slow rising pattern.

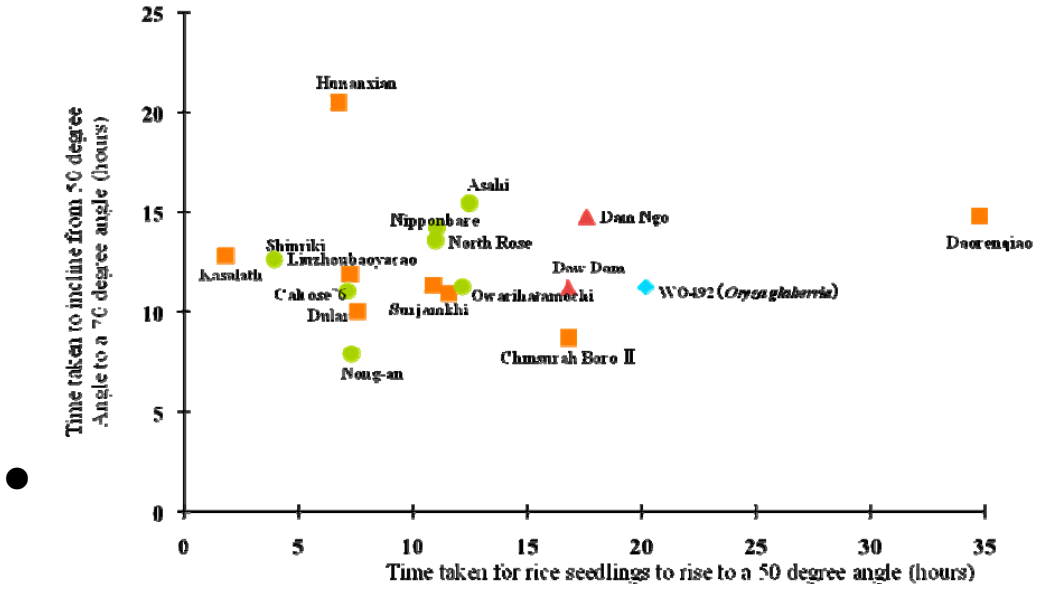
The lodging recovery pattern of typical three varieties (Nong-an for strong, WO492 for intermediate, and Daurenqiao for week variety) and their ethylene evolution were well coordinated; the stronger the lodging recovery ability, the higher the ethylene evolution, especially when placed horizontally (Fig. 2 and 3).

Table 1. Selected chemical and morphological characteristics of husks and grains of 18 rice varieties of various origins and ecotypes.

		Variety Name	Origin	Ecotype	Phenol reaction	Alkali digestibility	Grain length (mm)	Grain width (mm)	Grain length/ width
O. sativa	1	Surjamkhi	India	Indica	−	0	8.77	2.64	3.3
	2	Dular	India	Indica	+	0	8.83	2.82	3.1
	3	Kasalath	India	Indica	+	1	7.5	2.7	2.8
	4	Chinsurah Boro II	India	Indica	+	1	7.9	3.01	2.6
	5	Liuzhoubaoyacao	China	Indica	+	3	8.01	3.23	2.5
	6	Hongxuenuo	China	Indica	+	1	9.07	3.16	2.9
	7	Hunanxian	China	Indica	+	2	7.55	3.12	2.4
	8	Daorenqiao	China	Indica	+	0	7.41	3.35	2.2
	9	Dam Ngo	Laos	Javanica	−	5	8.86	3.81	2.3
	10	Daw Dam	Thailand	Javanica	−	4	8.37	3.81	2.2
	11	North Rose	U.S.A	Japonica	−	5	7.84	3.08	2.5
	12	Calrose76	U.S.A	Japonica	−	5	6.96	3.34	2.1
	13	Asahi	Japan	Japonica	−	8	7.13	3.49	2
	14	Shinriki	Japan	Japonica	−	7	6.9	3.47	2
	15	Nipponbare	Japan	Japonica	−	6	6.85	3.3	2.1
	16	Owarihatamochi	Japan	Japonica	+	5	8.1	3.68	2.2
	17	Nong-an	Korea	Japonica	−	7	7.16	3.24	2.2
O. glaberrima	18	WO492	Africa		+	2	7.84	2.93	2.7

Table 2. The time taken for rice seedlings to rise up to 50, 70, and 90 degrees from the horizontal position.

		Variety Name	Time to rise to 50 degrees (hr)	Time to rise to 70 degrees (hr)	Time to rise to 90 degrees (hr)
O. sativa	1	Surjamkhi	11.6	22.5	42.9
	2	Dular	7.6	17.6	39.0
	3	Kasalath	1.8	14.7	41.6
	4	Chinsurah Boro	16.8	25.5	45.2
	5	Liuzhoubaoyacao	7.3	19.2	47.7
	6	Hongxuenuo	10.9	22.2	47.3
	7	Hunanxian	6.8	27.2	53.4
	8	Daorenqiao	34.7	49.6	60.7
	9	Dam Ngo	17.6	32.4	47.9
	10	Daw Dam	16.8	28.0	42.7
	11	North Rose	11.0	24.6	46.4
	12	Calrose76	7.2	18.2	42.9
	13	Asahi	12.5	27.9	49.4
	14	Shinriki	4.0	16.6	45.1
	15	Nipponbare	11.1	25.3	47.8
	16	Owarihatamochi	12.2	23.4	49.2
	17	Nong-an	7.3	15.2	29.7
O. glaberrima	18	WO492	20.2	31.4	44.6



=Japonica ▲ =Javanica ■=Indica ◆=Glaberrima

Fig. 1. Relationship between time taken for rice seedlings to rise from 0 to 50 degrees angle and from 50 to 70 degrees angle.

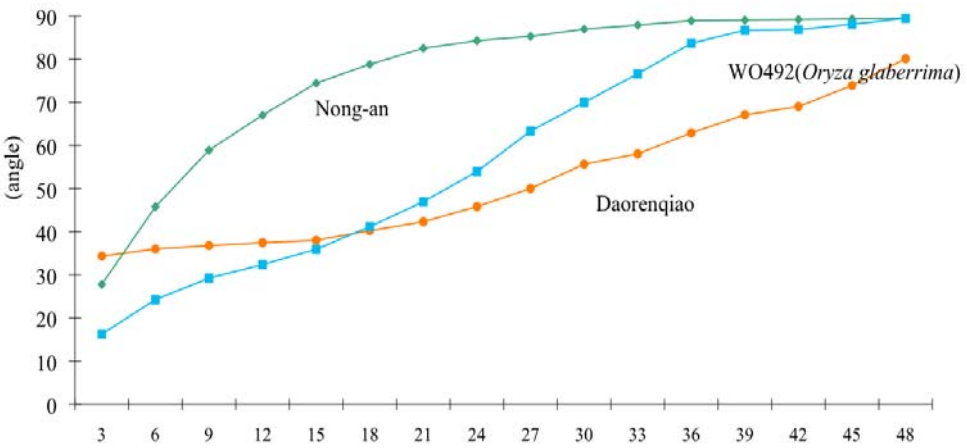


Fig. 2. Changes in rising pattern of horizontally placed rice seedlings on Nong-an, WO492 and Daorenqiao.

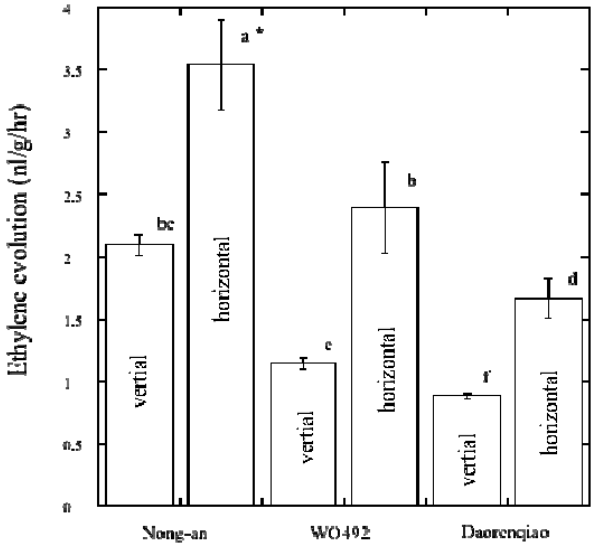


Fig. 3. Ethylene evolution of vertically or horizontally placed rice seedlings.
Different letters shows significant difference at 5% level by t-test.

DISCUSSION

The development of new varieties has supported the extension of direct seeding method of rice in USA, Italy, and Australia (Kushibuchi, 1997). The main characteristics noticed for breeding direct seeding rice varieties were germinability, seedling establishment ability and lodging tolerance, and the varietal differences or genetical characteristics have been studied on the elongation of mesocotyl and coleoptile (Katsuta, 1998). The study on varietal differences in lodging recovery of rice seedlings, which might well correlate with lodging recovery of lodged seedlings, however, are very rare.

In the present study, a wide range of lodging recovery of rice seedlings was observed, and there seemed no clear tendency within and among Japonica, Indica, and Javanica ecotypes. The strongest lodging recovery performance was observed in Kasalath (Indica) and Shinriki (Japonica). Though they are not presently applicable for direct-seeding since they have a longer stem, they can be

useful as breeding materials to produce direct-seeding oriented varieties.

Interestingly, it was found that the semi-dwarf varieties, such as Calrose76 (American) or Nong-an (Korean), which were both bred for direct-seeding, showed high negative gravitropism. The result indicates that the semi-dwarfing characteristics of these varieties are intentionally or unintentionally accompanied with high ability of negative gravitropism. Semi-dwarfing genes of practical use were clarified to be located on the identical locus (Kikuchi *et al*., 1985; Ashikari *et al*., 2002), and it was shown that they had no adverse effect on direct-seeding regardless of dried or submerged, Calper-coated or not-coated, deeply or shallowly sown conditions (Koshio *et al*., 2008). The fact that the horizontally placed Nong-an produced much ethylene compared with the other two tested varieties indicates the possibility that the semi-dwarfing gene has some effect both on ethylene production and lodging recovery. It was also found that Daorenqiao, a Chinese-origin Indica rice, has extremely low negative gravitropism accompanied with low ethylene production. Mesocotyl elongation of this variety was shown extremely low (Katsuta, 1998), and here also suggested the involvement of the lower ethylene production both in the lower lodging recovery ability and the lower mesocotyle elongation. The characteristics of Daorenqiao can be useful as a crossing material for genetic analysis to clarify the effect of mesocotyl elongation or lodging recovery.

The involvement of ethylene production in expressing gravitropism of rice or other plants (Abe *et al*., 1998; Blancaflor and Masson, 2003, Horton R. F., 1993; Lu BW *et al*., 2001) has been reported as well as enhancing flooding tolerance (Fukao et al., 2006) and low temperature stress (Gao et al., 2008). We suggest that ethylene production ability can be an indicator of negative gravitropism which may contribute to improve rice seedling establishment in direct seeding, with enhanced tolerance against environmental stresses.

CONCLUSION

In this study, we investigated the difference of the lodging recovery of 17 *Oryza sativa* varieties including the ecotypes of Indica, Japonica, and Javanica and one *Oryza glaberrima* variety. A wide range of negative gravitropism was observed among tested varieties, and the involvement of ethylene production was suggested. The knowledge obtained in this paper may contribute to breeding new varieties which are suitable for direct sowing cultivation method in the future.

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