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GENETIC AND PLANT GROWTH REGULATOR MANIPULATION OF RICE (*ORYZA SATIVA* L.) MESOCOTYL AND COLEOPTILE LENGTHS

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ABSTRACT

Significant differences in mesocotyl lengths of semidwarf and non-semidwarf rice (*Oryza sativa* L.) cultivars were observed. However, the relationship between plant height and mesocotyl length was found to be due to linkage rather than pleiotropism. Seed treatments of gibberellic acid (GA_3) significantly increased mesocotyl and coleoptile lengths in the laboratory study. The GA_3 seed treatments significantly increased stand density compared to the untreated control in the field study. However, no significant differences were observed for plant height at maturity or grain yields among the GA_3 treatments or the untreated control.

INTRODUCTION

Semidwarf rice (*Oryza sativa* L.) cultivars are short in stature (80 to 120 cm in height at maturity), have short and more upright leaves, greater nitrogen (N) responsiveness without lodging, and higher grain yields relative to taller traditional rice cultivars (Turner *et al.*, 1982). Semidwarf rice cultivars are becoming more popular with rice producers in the southern states of Arkansas, Louisiana, Texas, and Mississippi. Semidwarf rice cultivars were seeded on 790,365 hectares in these states in 1988 (Matlick, 1988). Stansel (1984) stated that there may be problems with seedling emergence of semidwarf rice cultivars and changes in management practices may be required to maximize economic productivity of the semidwarfs.

Rice seedling emergence is a function of mesocotyl and coleoptile elongation. The mesocotyl in the rice seedling is the internode between the coleoptile node and the point of union of the root and the culm. The coleoptile is the cylinder-like protective covering that encloses the young plumule (DeDatta, 1981). Turner *et al.*, (1982) reported that poor stand establishment of semidwarf cultivars is largely attributable to short mesocotyl length, and further stated that the semidwarfs have shorter mesocotyls than traditional, non-semidwarf rice cultivars.

Mesocotyl and coleoptile lengths are genetically controlled but mesocotyl and coleoptile elongation are also influenced by seeding depth and environmental factors (Turner *et al.*, 1982). Kordan (1974) concluded that coleoptile elongation was enhanced by low oxygen (O_2) levels while Chang and Bardenas (1965) reported that light inhibits mesocotyl elongation. Takahashi (1978) found that removing carbon dioxide (CO_2) from the developing seedling decreased both coleoptile and mesocotyl elongation.

Turner *et al.* (1982) examined mesocotyl and coleoptile elongation of six rice cultivars at five seeding depths. Variability among cultivars existed for the mesocotyl and coleoptile elongation especially at different seeding depths. In general, the sum of the mesocotyl and coleoptile length equalled the seeding depth. However, the semidwarf cultivars 'Bellemont' and 'M101' did not emerge at the 10 cm seeding depth. Reduced emergence of these and other semidwarf rice cultivars at seeding depths exceeding 2.5 cm had led some researchers to theorize that there was a possible pleiotropic relationship between mesocotyl and coleoptile elongation and plant height.

Sunderman (1964) reported positive correlations between plant height and coleoptile lengths of winter wheat in three of five tests and coleoptile length was positively correlated with seedling emergence in one of two tests. Takahashi (1946) also found a positive correlation between coleoptile and culm length in barley but found was no relationship between coleoptile length and seedling emergence.

Stowe and Yamaki (1957) postulated that since maize dwarfs are caused by single gene defects, the dwarfness may be interpreted as a block in a biosynthetic pathway that ultimately leads to the production of gibberellin-like compound. They suggested that by adding gibberellic acid (GA_3), these blocks may be overcome and the requirement for the growth substance satisfied. However, Allan *et al.* (1962) found that dwarf and semidwarf wheat cultivars were not induced to grow to normal height by injections of GA_3.

Bird and Ergle (1961) found significant increases in seedling emergence and seedling height of cotton when cotton seed was treated with potassium gibberellate (75% GA_3). The positive influence of the GA_3 was thought to be associated with the increased rate of hypocotyl elongation.

When our study was initiated, the association of semidwarf rice cultivars and inadequate stand establishment were known. Furthermore, the proposed theory was that the mesocotyl and coleoptile were pleiotropic with plant height. The objectives of the study were to: (a) evaluate the potential mesocotyl and coleoptile elongation of semidwarf and standard cultivars, (b) determine the relationship between mesocotyl and coleoptile elongation and plant height in parental rice germplasm, (c) evaluate enhanced germplasm developed by hybridization and selection to determine the relationship of mesocotyl and coleoptile elongation to plant height, and (d) evaluate GA_3 as seed treatments on rice for mesocotyl and coleoptile elongation, stand establishment, plant height, and grain yield.

MATERIALS AND METHODS

Laboratory and field experiments were conducted from 1982 through 1988 to determine the mesocotyl, coleoptile, and total (mesocotyl + coleoptile) lengths of rice cultivars to determine the association of these parameters to plant height. Studies were also initiated to examine the effects of GA_3 at rates of 10, 50, and 100 mg ai/kg seed on seedling morphology, stand establishment, plant height, and grain yield. One kilogram of seed was treated with solutions of GA_3. The seed lots were then dried to inhibit germination.

The rice germplasm utilized in the mesocotyl/coleoptile and plant height study were the very-short-season cultivars 'M101', 'L201', and 'Labelle', and the experimental line RU7703008 (NWRX//CI9881/PI331581). The semidwarf germplasm was represented by M101 (an early maturing California cultivar) and RU7703008 (a sister line of USDA-Texas Agricultural Experiment Station midseason cultivar, Bellemont). The comparatively taller statured germplasm was represented by Labelle, developed by USDA-Texas Agricultural Experiment Station and L201, developed by California Cooperative Rice Research Foundation.

The rice cultivars used in the GA_3 seed treatment study included 'Lemont' and 'Rexmont', two semidwarf cultivars developed by USDA-Texas Agricultural Experiment Station and 'Mercury', a semidwarf cultivar developed by the Louisiana Agricultural Experiment Station. The data from the studies were statistically analyzed using the General Linear Mean Model (GLM) procedures provided by the Statistical Analysis System (SAS Institute, 1982).

Laboratory Experiments

The laboratory test was designed to determine the potential length of the mesocotyl and coleoptile of the germplasm sources when grown in darkness. The slantboard technique described by Jones and Peterson (1976) was used. Moistened blotters (13 X 18 cm) were placed on acrylic plates of the same size and 20 seeds were placed in a horizontal line, germ-end down, 1 cm apart and 4 cm from the bottom of the plate. The seeds were held in position by placing a single thickness of germination paper over the seeds. The plates that were holding the seed were moistened with distilled water. Plates, blotters, and adhering seed were placed in slotted acrylic racks which held 12 acrylic plates. The entire assembly was placed in a glass tray containing 1,000 ml of distilled water. The assembly was wrapped in two black polyethylene bags to maintain high humidity and to assure a uniform dark environment. The assembly was placed in a temperature controlled germinator in total darkness at 100% RH for 10 days at 25°C, after which the mesocotyl and coleoptile of each seedling were measured. The sum of the mesocotyl and coleoptile measurements comprised the total length. Each acrylic plate contained 20 uniform, mature seeds (five seeds/germplasm entry) which represented one replication. Also, the previously described technique was used in the GA_3 seed treatment study. However, a separate glass tray was used for each concentration of the GA_3.

Germplasm Enhancement - Field Study

The four germplasm sources (M101, L201, Labelle and RU7703008) were hybridized in all possible combinations to determine if semidwarf recombinant plants which produce long mesocotyls and coleoptiles could be recovered. Parent and F_1 seed were hand planted in 4.7 m rows with seed being placed 15 cm apart within a row and 20 cm between rows on Crowley silt loam (Typic Albaqualf) at the Rice Research and Extension Center at Stuttgart, AR. Seeds were hand harvested from F_1 plants and individual F_2 seed were hand planted in the same design that was used for the parent and F_1 seed. Seeds from 340 randomly selected F_2 plants from each cross combination were hand harvested, threshed, and stored at 10°C and about 50% RH. These seeds were sown in two replications of 1 m rows using 19 cm row spacing. Heading date, percent lodging and average plant height (five plants/row) were measured and uniform, mature seeds from individual plants of the F_3 rows were evaluated as described in the materials and methods for mesocotyl and coleoptile length. Twenty-three semidwarf selections that produced long mesocotyls and coleoptiles were evaluated at two locations (four replications/location) in 1986 and 1987 for plant height, and mesocotyl/coleoptile length.

GA_3 Seed Treatment - Field Study

In 1988, field studies to examine the effects of GA_3 seed treatments on stand establishment, plant height, and grain yield were initiated at the Southeast Branch Experiment Station, Rohwer, AR. The semidwarf, Lemont, was seeded at 2.5 and 7.5 cm depths at a rate of 112 kg/ha in Desha silt loam (Vertic Haplaudolls). Plots were nine drill rows wide (15 cm spacing) and 4.6 m in length.

A split-plot experimental design was used with seeding depth as main plot and GA_3 seed treatments as subplots. Each treatment combination was replicated four times. Stand counts were taken in two 0.09 m^2 areas prior to flooding. At maturity, plant height was measured from the soil level to the top of the extended panicles at two locations in each plot. The plots were combine harvested and grain yields are expressed as 12% moisture.

RESULTS AND DISCUSSION

Laboratory Experiment

I. Mesocotyl/Coleoptile Evaluation of Parental Germplasm

The mesocotyl and total lengths of the two semidwarf genotypes, M101 and RU7703008, differed significantly from those of the taller genotypes, L201 and Labelle (Table 1). These data are similar to results reported by Turner *et al.* (1982), who found that semidwarf cultivars have shorter mesocotyl and total lengths than taller rice cultivars. Furthermore, the coleoptile length of L201 was significantly longer than the coleoptiles of RU7703008 and Labelle, but not that of M101.

Table 1. Mesocotyl, coleoptile and total (mesocotyl + coleoptile) lengths of four rice cultivars (laboratory experiment).

	Lengths in mm		
Cultivar	Mesocotyl	Coleoptile	Total
M101	2.1	26.7	28.9
7703008	1.6	13.4	15.0
L201	16.9	31.6	48.5
Labelle	15.9	20.7	36.7
LSD 0.05	2.9	7.6	6.2

The germplasm in this study exhibited genetic differences in mesocotyl and coleoptile length which could be advantageous in the subsequent development of semidwarf cultivars. Furthermore, the slantboard technique previously described would be useful in determining mesocotyl/coleoptile lengths in large segregating populations because the technique would permit the saving and transplanting of individual seedlings that possess the desired seedling vigor traits.

II. GA_3 Seed Treatments

The GA_3 seed treatments significantly increased mesocotyl, coleoptile and total lengths of the three cultivars compared to the untreated control (Table 2). The mesocotyl lengths of Rexmont at the GA_3 10 mg/kg rate were significantly less than mesocotyl lengths at the GA_3 50 and 100 mg/kg rate. No significant differences were observed among the GA_3 rates for mesocotyl lengths of Lemont or Mercury. Additionally, no significant differences were observed among the GA_3 rates for coleoptile or total lengths of any cultivar.

Table 2. Influence of GA_3 seed treatments on rice mesocotyl, coleoptile and total (mesocotyl + coleoptile) length of three rice cultivars (laboratory experiment).

	Length in mm								
	LEMONT			REXMONT			MERCURY		
Seed Treatment mg/ha	Meso	Cole	Tot	Meso	Cole	Tot	Meso	Cole	Tot
Untreated Control	1.7	9.4	11.1	1.9	10.9	12.8	1.7	15.3	17.0
GA_3 10	16.3	26.7	43.0	6.9	23.8	30.7	6.9	33.4	40.3
GA_3 50	12.4	24.8	37.2	11.6	27.1	38.7	8.0	32.6	40.6
GA_3 100	15.8	22.6	38.4	9.5	19.8	29.3	8.3	33.0	41.3
LSD 0.05	3.6	5.9	7.7	2.5	7.8	8.7	2.6	10.6	12.7

Germplasm Enhancement

This part of the study was designed only to identify semidwarf germplasm with mesocotyls and coleoptiles that were equal to the mesocotyl/coleoptile length of normal height germplasm. The test was not designed to determine the inheritance of mesocotyl or coleoptile elongation. Approximately 600 semidwarf plants (< 80 cm in height) from each of the six cross combinations were tested for mesocotyl/coleoptile elongation (Table 3). The greatest frequency of the desired plant type was identified in the RU7703008 X L201 (eight plants) and M101 X L201 (six plants) population (Table 3).

Table 3. Recombinant semidwarf germplasm that produces normal length mesocotyls and coleoptiles from six hybrid populations (germplasm enhancement experiment).

Hybrid	Recombinant Plants	Total Population
RU7703008 X L201	8	609
M101 X L201	6	612
RU7703008 X M101	3	610
L201 X Labelle	0	597
M101 X Labelle	3	602
RU7703008 X Labelle	3	601

The new germplasm and their parents were evaluated for plant height versus mesocotyl/coleoptile elongation in the field in 1986 and 1987. Two of the semidwarf lines, designated (RU7703008 X L201)-512 and (RU7703008 X L201)-513, from the crosses produced significantly longer mesocotyls than the semidwarf parent, RU7703008 (Table 4). There was no significant difference in plant height between the new germplasm and RU7703008, a sister selection of Bellemont. However, these three genotypes were significantly shorter than L201, the normal height parent. There was no significant difference in coleoptile length of L201 or the new germplasm; however these three genotype sources have significantly longer coleoptiles than RU7703008 (Table 4). Therefore, these data verify that plant height and mesocotyl/coleoptile elongation in rice are not due to pleiotropism but rather these two plant characteristics are controlled by separate but closely linked loci.

Table 4. Mean separation of plant height, mesocotyl, coleoptile and total (mesocotyl + coleoptile) length of parental and new semidwarf germplasm that has normal length mesocotyls and coleoptiles (germplasm enhancement experiment).

Genotype	Plant Ht cm	Mesocotyl	Coleoptile	Total
L201	90.5 a	26.8 a	29.8 a	56.6 a
(7703008 X L201)-512	78.5 b	16.7 b	35.1 a	51.8 a
(7703008 X L201)-513	84.5 b	14.2 b	29.4 a	43.6 b
7703008	83.5 b	6.2 c	19.3 b	25.5 c

P = 0.05

GA_3 Seed Treatment - Field Study

Analysis of variance indicated there was a significantly greater stand density at the 2.5 cm seeding depth compared to the 7.5 cm seeding depth (Table 5). However, there was no difference between the seeding depths for plant height at maturity or grain yield.

Table 5. Influence of seeding depth on stand density, plant height, and grain yield of Lemont rice averaged over GA_3 seed treatments.

Seeding Depth cm	Stand Density plants/m^2	Plant Height cm	Grain Yield kg/ha
2.5	221	92.9	8110
7.5	137	91.3	7955
LSD = 0.05	32	NS	NS

The GA_3 seed treatments significantly increased stand density compared to the untreated control (Table 6). However, there was no difference among the GA_3 seed treatments for stand density. Furthermore, there was no difference for plant height at maturity or grain yield when the GA_3 seed treatments were compared to the untreated control. Although there were significant increases in mesocotyl, coleoptile, and total lengths from the GA_3 seed treatments as determined in the laboratory study, these seedling elongation effects were not observed at maturity.

Table 6. Influence of GA_3 seed treatments on stand density, plant height and grain yield of Lemont rice averaged over seeding depths.

Seed Treatment mg/kg	Stand Density plants/m^2	Plant Height cm	Grain Yield kg/ha
Untreated Control	127	92.3	7705
GA_3 10	191	92.8	8200
GA_3 50	191	91.3	7950
GA_3 100	201	92.1	8265
LSD 0.05	53	NS	NS

CONCLUSIONS

These results indicate that there are differences in mesocotyl/coleoptile lengths of semidwarf and taller rice cultivars, but no pleiotropic relationship between plant height and mesocotyl elongation was found. Furthermore, two sources of germplasm ([RU7703008 X L201]-512, [RU7703008 X L201]-513) have been identified which have semidwarf plant height and mesocotyl/coleoptile potential that is similar to taller cultivars which can be utilized in rice breeding programs.

Also, the use of the GA_3 seed treatments can enhance mesocotyl, coleoptile, and total elongation in present semidwarf rice cultivars. Field studies with Lemont demonstrated that stand establishment can be increased with the use of GA_3 seed treatments.

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