

Full Length Research Paper

Relationship between coleoptile and mesocotyl elongation of upland rice (*Oryza sativa* L.) seedlings under submergence and soil-sand culture

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In rice, the coleoptile and mesocotyl are two morphologically important structures for seedling emergence. After germination, coleoptile and or mesocotyl elongation projects the seedling tip out of the soil and water in which it is planted, allowing the leaves to make contact with the atmosphere thus enabling the seedling to develop into a normal rice plant. Rice genotypes with inherently longer coleoptiles and or mesocotyls are thought to emerge better than those with shorter ones. However, the patterns of elongation under submergence and in soil-sand culture remain unclear especially for upland rice. This study examined coleoptile and mesocotyl elongation in 63 upland rice genotypes comparing elongation patterns under submergence to elongation in soil-sand culture. Coleoptile and mesocotyl elongation in soil-sand culture differed from elongation under submergence. Coleoptile lengths were enhanced more under submergence and mesocotyls grew longer in soil-sand culture. This appeared to link seedling emergence under submergence more to coleoptile elongation, and emergence in drained soil-sand culture largely to mesocotyl elongation. We found no obvious relationship between coleoptile and mesocotyl elongation under submergence. But in soil-sand culture, increases in coleoptile length corresponded to increases in mesocotyl length, suggesting that simultaneously coleoptile and mesocotyl elongation contribute to seedling emergence in drained soils.

Key words: Seedling emergence, mesocotyl elongation, coleoptile elongation, upland rice.

INTRODUCTION

In young rice (*Oryza sativa* L.) seedlings, the coleoptile is the tapered cylindrical structure that encloses the young leaves and is joined at its basal end to the mesocotyl. It varies in color from colorless, pale green to green, or pale purple to purple. The mesocotyl on the other hand is the length of the axis between the coleoptile and the point of union of the root and culm (Chang et al., 1965). The coleoptile and mesocotyl are reported to be morphologically important structures for seedling emergence. Under field conditions, elongation of the mesocotyl

elevates the coleoptile to a point within a few millimetres of the soil surface. The coleoptile then extends outwards, making contact with the atmosphere and allows the primary leaves to emerge. Seedlings may fail to emerge with deep seed placement due to either inability of the mesocotyl to raise the coleoptile near the soil surface or failure of the coleoptile to reach the soil surface. In this event the leaves may unfurl beneath the soil resulting in seedling death. The combined force associated with coleoptile and mesocotyl elongation is essential for successful seedling emergence (Turner et al., 1981; Luo et al., 2007 and Dilday et al., 1988). Seedlings with long coleoptiles and mesocotyls may be able to emerge through the soil better than those with short ones. Coleoptile and mesocotyl lengths are genetically controlled,

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but coleoptile and mesocotyl elongation are influenced by seeding depth and environmental factors (Turner et al., 1982). Changes in aeration, light and temperature have varied effects on coleoptile and mesocotyl elongation of rice seedlings. Takahashi (1978) established that removing carbon dioxide (CO₂) from the developing seedling inhibited both coleoptile and mesocotyl elongation, while Biswas and Yamauchi (1997) found that anaerobic conditions of water seeding promoted coleoptile growth but inhibited mesocotyl elongation. Wu et al. (2005) showed that mesocotyl elongation only occurs in the dark and hence measurements of mesocotyl length can only be done when seedlings are grown in a dark environment, such as a growth chamber or underground. Thus by adjusting seedling growth conditions, it is possible to alter patterns of coleoptile and or mesocotyl elongation. It may also be possible to directly and precisely determine potential and inherent mesocotyl and coleoptile elongation for different rice genotypes. Little is known about the effect of genotypic selection for mesocotyl and coleoptile length for enhancing seedling emergence and establishment in direct seeded upland rice. Seedling mesocotyl and coleoptile lengths may be potential measurement criteria to select genotypes with improved seedling emergence, vigor and subsequent growth and development. The objectives of this study were therefore to; a) Evaluate the potential coleoptile and mesocotyl lengths of various upland rice genotypes in seedlings grown under water (submergence) and in soil-sand culture b) Examine how coleoptile and mesocotyl elongation under submergence relates to coleoptile and mesocotyl elongation in soil-sand culture.

MATERIALS AND METHODS

Plant materials

In this study 63 upland rice genotypes of diverse origins, most from Africa (34) and Japan (25), and a few from Taiwan (3) and Myanmar (1) were used. The genotypes and their sources of origin are contained in Table 1.

All 25 of the genotypes from Japan including 19 genotypes from other Asian countries and Africa were obtained from the seed bank maintained by Prof. Kenji Irie in the Tropical Crop Science Laboratory at Tokyo University of Agriculture. The additional 19 foreign genotypes were secured from the National Institute of Agro biological Sciences (NIAS) gene bank of Japan.

Mesocotyl and coleoptile elongation under submergence

In this experiment, fifteen seeds were used per genotype. The experiment was laid out in completely randomised design (CRD) with three replications. Five seeds were glued embryo end up on a plastic screen placed in a glass tube (2.5 cm in diameter and 15 cm in height) and the glass tube was filled up with distilled water, completely submerging the seeds (Figure 1). The assembly was kept in a growth chamber under dark conditions at 30°C and 95%

relative humidity without changing water. After 10 days, coleoptile and mesocotyl were measured.

Mesocotyl and coleoptile elongation in sand-soil culture

The experiment was laid out in a randomized complete block design (RCBD) with two replications. Seeds were sown in a mixture of sand and *akadama* soil (1:1 ratio). *Akadama* is inert inorganic volcanic clay with good water retention abilities used as a potting medium in Japan. The soil-sand mixture was added to plastic seedling trays (68 cm length, 42 cm width and 14 cm height) and uniformly spread to provide a thin layer (4 cm) onto which the seeds were sowed. Six rows (10 cm apart) were marked on either side of each tray and the genotypes randomly assigned to the rows. Five seeds were sowed per row (2 cm apart) and buried to a depth of 8cm with the same soil-sand mixture. Water was added at regular intervals (when the top soil appeared noticeably dry) to maintain adequate soil moisture for germination and seedling growth. On the tenth day after sowing, the seedlings were carefully excavated and washed, and coleoptile and mesocotyl lengths measured.

Data from the two experiments was subjected to a series of linear contrasts to compare coleoptile and mesocotyl elongation in soil-sand culture and under submergence. The data was subjected to analysis of variance using GENSTAT Seventh Edition DE 3(2008) and relationships between the measured parameters were determined by Pearson's correlation coefficient.

RESULTS

Coleoptile and mesocotyl elongation in soil-sand and water

Among the genotypes tested, there was a significant variation in final coleoptile and mesocotyl length attained under submergence and in the soil-sand culture. Wider variations in both coleoptile and mesocotyl elongation were recorded in the soil-sand culture than under submergence. The coefficients of variation (CV) for coleoptile and mesocotyl elongation were 10.5 and 27.3 respectively under submergence, and 19.8 and 35.5 respectively for the soil-sand culture. In both circumstances, variations in mesocotyl elongation were larger than variation in coleoptile elongation. Whereas wider variation in mesocotyl elongation was observed in seedlings grown in soil-sand, coleoptile elongation was more varied under submergence (Figure 2).

Except in a few genotypes, longer combined coleoptile and mesocotyl growth were observed in seedlings grown under submergence as opposed to seedlings grown in soil-sand culture. Combined coleoptile and mesocotyl elongation under submergence ranged from 3.7 to 6.4 cm with an average of 4.9 cm while the range in soil sand culture was 2.2 to 7.6 cm with an average of 3.9 cm. In both conditions, coleoptile elongation contributed proportionately more to the combined length; but contributed even more under submergence. On average, coleoptile elongation alone contributed 91% of total length (coleoptile + mesocotyl) under submergence compared to just 74% in the soil-sand culture. Length

Table 1. Complete List of upland rice genotypes used in experiment and their sources of origin.

Acc.	Variety	Acc.	Variety	Acc.	Variety
081	Yoshino Mochi Zairai (JPN)	179	Okabo (JPN)	125	NERICA-18 (CIV)
082	Gaisen Mochi (JPN)	180	Rosette (Yokoyama) (JPN)		IS- 280 (CIV)
083	No Shinriki Mochi (JPN)	181	Rikutou Rikuun (JPN)		IS-452 (CIV)
089	Sankanka (JPN)	185	Raiden (JPN)		Tos Mori (BEN)
094	Kouchi Zairai (JPN)	108	NERICA-1 (CIV)		Kom Aushin (EGY)
139	Gaisen Mochi (JPN)	109	NERICA-2 (CIV)		Kaaby Village (EGY)
140	Hinode (JPN)	110	NERICA-3 (CIV)		Zebila (GHA)
141	Senshou (JPN)	111	NERICA-4 (CIV)		Bawku (GHA)
142	Yamadabake (JPN)	112	NERICA-5 (CIV)		Plu Go (LBR)
143	Kaneko-B (JPN)	113	NERICA-6 (CIV)		Bo-Blah IV (LBR)
144	Iruma Nishiki (JPN)	114	NERICA-7 (CIV)		SS-361 (Sen)
145	Okka Modoshi (JPN)	115	NERICA-8 (CIV)		Mala (SLE)
146	Hirayama (JPN)	116	NERICA-9 (CIV)		Bengbete (SLE)
147	Kaheimoshi (JPN)	117	NERICA-10 (CIV)		Bogutti (SLE)
148	Oiran (JPN)	118	NERICA-11 (CIV)		Ex Dar Es Salam (TZA)
149	Meguromochi (JPN)	119	NERICA-12 (CIV)		ES-10 (TZA)
151	Hassokuho (JPN)	120	NERICA-13 (CIV)		ACC-1697 (ZMB)
158	Dango (JPN)	121	NERICA-14 (CIV)	069	Yar-2 (MMR)
162	Shichimenchou Mochi (JPN)	122	NERICA-15 (CIV)		Nutsurikui (TWN)
173	Akamai (JPN)	123	NERICA-16 (CIV)		Nakabo (TWN)
176	Akamai (JPN)	124	NERICA-17 (CIV)		Tarupatumochi (TWN)

Acc. = Accession number. Letters in Parenthesis () signify the ISO 3-letter codes identifying countries where the genotypes originated.

distribution of coleoptile under submergence and in soil-sand culture ranged from 3.4 to 6.0 cm and 1.9 to 4.3 cm respectively. Hence coleoptiles grew longer under submergence. Mesocotyl elongation on the other hand seemed to be enhanced in the soil-sand culture averaging 0.46 cm with a range of 0.15 to 4.4 cm against an average of 1.08 cm and range of 0.2 to 1.2 cm under submergence (Figure 3).

Results of the analysis of variance (ANOVA) and numerical data on coleoptile and mesocotyl lengths for the different upland rice genotypes is contained in Supplementary Table 1.

Relationship between coleoptile/mesocotyl elongation in soil and under submergence

Under submergence, there was no obvious correlation between coleoptile elongation and mesocotyl elongation. In the soil-sand culture however, coleoptile elongation occurred in a pattern similar to that of mesocotyl elongation that is, as coleoptiles elongated, so did the mesocotyls (Figure 4).

In the soil-sand culture, one genotype - Nutsurikui grew an exceptionally long mesocotyl (4.4 cm), which had a profound effect on the slope of the regression line and consequently the value of the correlation. Nutsurikui alone

was responsible for the moderate correlation ($r = 0.39$) that would have otherwise been stronger ($r = 0.48$) with Nutsurikui excluded.

Results relating coleoptile elongation in soil-sand culture to that under submerged conditions showed a very weak correlation (Figure 5) that gave no apparent indication of the predictability of one variable based on knowledge of the other. A significantly moderate positive correlation ($r = 0.48$) was however found between mesocotyl elongation in soil-sand and elongation under submergence. Once more, the coefficient of correlation was unduly influenced by the overly elongated mesocotyl of the genotype Nutsurikui, without which a stronger correlation ($r = 0.52$) is realized.

To evaluate potential inherent coleoptile and mesocotyl elongation using soil-sand culture, seeds were sown 8 cm deep, which was considered the maximum possible depth from which normal seedling emergence would occur. After 10 days, most genotypes had not emerged, but still a few like Nutsurikui, Plu go, Tos mori, ACC-1697 Malagasy, Bawku Market, Ex Dar Es Salaam, Bogutti, IS-280, and Tarupatumochi had successfully emerged. A notable distinction between the genotypes that either emerged or came close to emergence (successful emergers) and those that failed to emerge and were far off from emergence (failed emergers) was in the final length of mesocotyl attained. Despite the comparative



Figure 1. Experiment 1 setup.

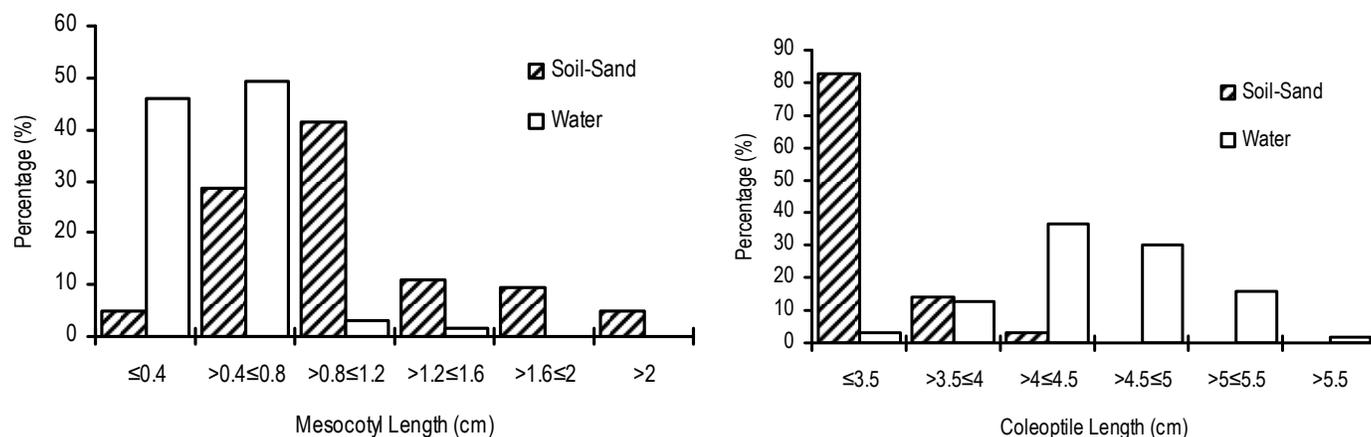


Figure 2. Distribution of the tested upland rice genotypes by mesocotyl and coleoptile length determined on seedlings grown in soil - sand and under submergence or in water.

similarity in coleoptile lengths between the successful emergers and failed emergers, there was enhanced mesocotyl elongation in the successful emergers. Thus increased mesocotyl elongation appeared to enhance seedling emergence from deep sowing (Figure 6).

DISCUSSION

Poor seedling emergence and establishment are among the most important factors limiting productivity of direct seeded rice. The coleoptile and mesocotyl are morphologically important structures said to enhance emergence of direct seeded rice (Turner et al., 1981). These seedling structures (coleoptile and mesocotyl) have been extensively studied in lowland rice in attempts to overcome the problem of low seedling emergence in flood conditions, and it has led to identification of cultivars with a superior ability to germinate under anaerobic conditions. Such cultivars have developed coleoptiles that are longer and elongate faster than others (Biswas and Yamauchi, 1997). In direct seeded upland rice however, the interactive contribution to seedling emergence of coleoptile and mesocotyl elongation remains to be explored. This study compared coleoptile and mesocotyl elongation under submergence and in soil-sand culture in seedlings of upland rice genotypes. Our results revealed clear differences between coleoptile and mesocotyl elongation in soil-sand culture and under submergence. Whereas coleoptile elongation was enhanced under submergence, mesocotyls grew longer in the soil-sand culture. Similar findings were reported by Biswas and Yamauchi (1997) who observed that coleoptiles of superior emerging cultivars elongated more in flooded soil than in drained soil while the mesocotyl elongated along with sowing depth in drained soil. Takahashi (1978) also observed stimulation of coleoptile growth under submergence, although there was no

increase in mesocotyl length. Hence the observations appear to link seedling emergence under submergence more to coleoptile elongation and emergence in drained soils largely to mesocotyl elongation. Alpi and Beevers (1982) showed that rice coleoptiles elongate more under anoxic conditions with decreasing oxygen concentration, and elongate at a steady rate for about 2 weeks while coleoptile growth in air ceases at just about 7 days. Alpi and Beevers noted however that the anaerobic coleoptile was quite thin and fragile despite its greater length as compared to the aerobic coleoptile. Similarly we found that coleoptiles grown under submergence were markedly thinner and weaker compared to coleoptiles in the soil sand culture. This suggests that coleoptile elongation may be an adaptation to overcome conditions of low oxygen as may be the case with low dissolved oxygen in water. According to Yamauchi et al., (1995), rapid coleoptile elongation hastens shoot emergence from the soil or water leading to rapid oxygen transport to the apical meristem.

Moreover, inherent differences in genotypic coleoptile elongation were manifested more under submergence as opposed to differences in mesocotyl elongation which were clearer in soil-sand culture. While coleoptile and mesocotyl elongation progressed similarly ($r = 0.39$) in the soil-sand culture, we found no apparent correlation ($r = -0.06$) between the two parameters under submergence. In the same way, Takahashi (1978) observed corresponding increases of mesocotyl and coleoptile length in seedlings grown in a vermiculite seedbed with low soil moisture (under 100% moisture content) while Ju et al. (2007) found no obvious correlation between mesocotyl and coleoptile lengths under submergence. This suggests that mesocotyl and coleoptile elongation are both important for emergence of seedlings in drained soils, and the same may be said for emergence of seedlings under submergence, except that under submergence, the role of mesocotyl elongation remains

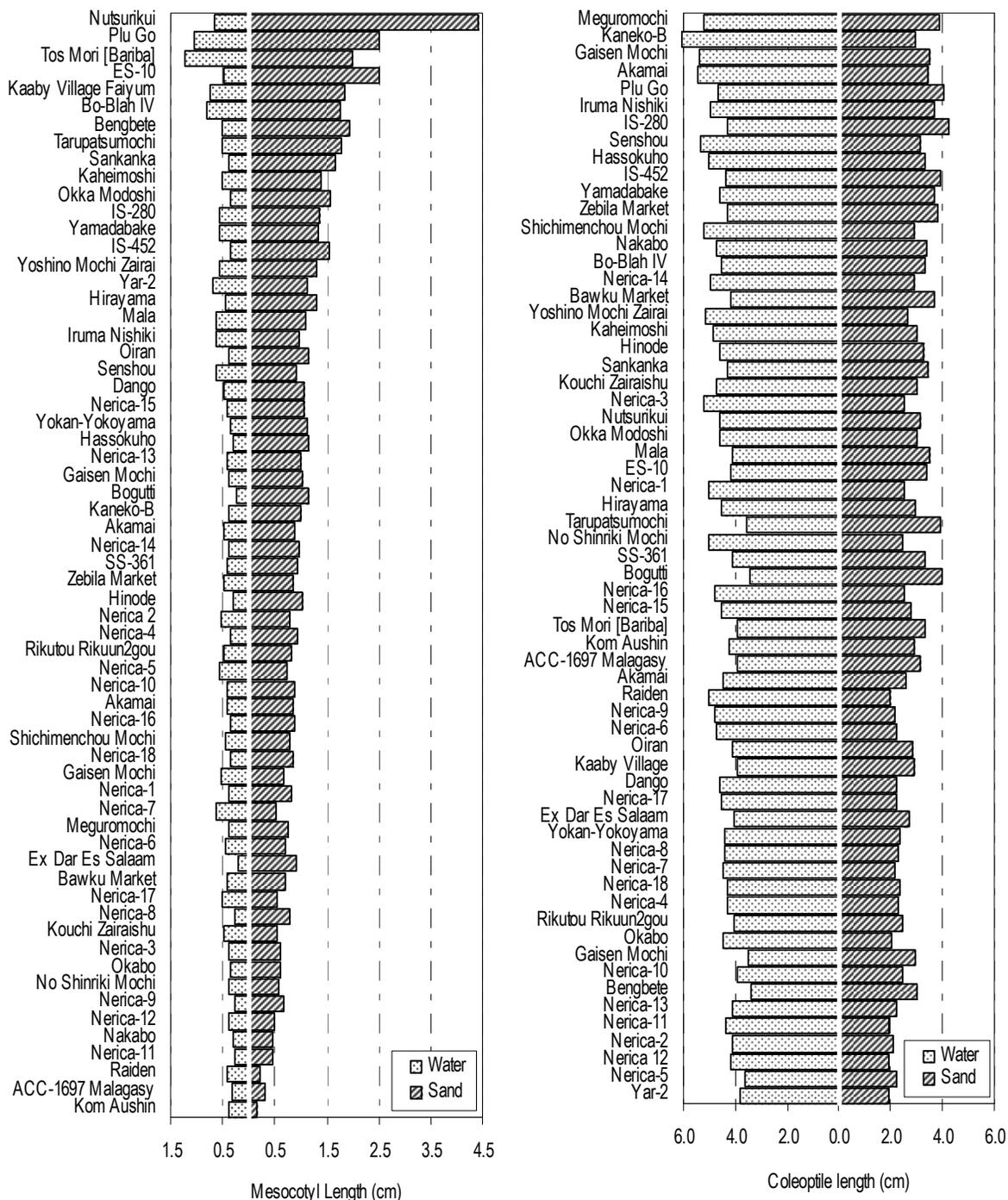


Figure 3. Mesocotyl and coleoptile lengths of 63 upland rice genotypes for 10 day old seedlings grown in soil-sand culture and in water (under submergence).

unclear. The lack of a significant correlation between coleoptile and mesocotyl lengths under submergence also suggests that selection for the two parameters may be conducted independently if the interest is to find genotypes with either enhanced coleoptile or mesocotyl elongation adapted for submerged conditions. Our results

also revealed a very weak relationship between coleoptile elongation in soil-sand and under submergence, implying that coleoptile elongation in soil-sand cannot be used to predict elongation under submergence since the pattern of elongation changes with the conditions in which seedlings are grown. We however found the pattern of

Supplementary Table 1. List of the 63 upland rice genotypes and their corresponding coleoptile and mesocotyl lengths.

Genotype	Mesocotyl length (cm)		Coleoptile length (cm)		Mesocotyl + Coleoptile (cm)	
	Submergence	Soil-sand	Submergence	Soil-sand	Submergence	Soil-sand
Yoshino Mochi Zairai	0.6	1.3	5.2	2.7	5.7	4.0
Gaisen Mochi	0.5	0.7	5.4	3.5	5.9	4.2
No Shinriki Mochi	0.4	0.6	5.0	2.5	5.4	3.1
Sankanka	0.4	1.7	4.3	3.5	4.7	5.1
Kouchi Zairaishu	0.5	0.5	4.7	3.0	5.2	3.6
Gaisen Mochi	0.4	1.0	3.5	2.9	3.9	3.9
Hinode	0.3	1.0	4.6	3.2	4.9	4.3
Senshou	0.6	0.9	5.3	3.1	6.0	4.0
Yamadabake	0.6	1.3	4.6	3.7	5.2	5.0
Kaneko B	0.4	1.0	6.0	2.9	6.4	3.9
Iruma Nishiki	0.6	1.0	4.9	3.7	5.6	4.7
Okka Modoshi	0.3	1.6	4.6	3.0	4.9	4.6
Hirayama	0.4	1.3	4.6	3.0	5.0	4.3
Kaheimoshi	0.5	1.4	4.8	3.0	5.3	4.4
Oiran	0.4	1.2	4.1	2.8	4.5	4.0
Meguromochi	0.4	0.8	5.2	3.9	5.6	4.6
Hassokuho	0.3	1.2	5.0	3.3	5.3	4.5
Dango	0.5	1.1	4.6	2.2	5.1	3.3
Shichimenchou Mochi	0.4	0.8	5.2	2.9	5.7	3.7
Akamai	0.5	0.9	5.4	3.4	5.9	4.3
Akamai	0.4	0.9	4.4	2.6	4.9	3.5
Okabo	0.4	0.6	4.4	2.1	4.8	2.6
Rosette (Yokoyama)	0.4	1.1	4.4	2.4	4.7	3.5
Rikutou Rikuun2gou	0.5	0.8	4.1	2.5	4.5	3.3
Raiden	0.4	0.2	5.0	2.0	5.4	2.2
NERICA-1	0.4	0.8	5.0	2.5	5.4	3.3
NERICA-2	0.5	0.8	4.1	2.1	4.6	2.9
NERICA-3	0.4	0.6	5.2	2.5	5.6	3.1
NERICA-4	0.3	1.0	4.3	2.3	4.6	3.3
NERICA-5	0.6	0.7	3.6	2.2	4.2	2.9
NERICA-6	0.5	0.7	4.7	2.2	5.2	2.9
NERICA-7	0.6	0.5	4.5	2.2	5.1	2.7
NERICA-8	0.3	0.8	4.4	2.3	4.7	3.0
NERICA-9	0.3	0.7	4.8	2.2	5.1	2.9
NERICA-10	0.4	0.9	3.9	2.5	4.3	3.4
NERICA-11	0.3	0.5	4.3	1.9	4.6	2.4
NERICA-12	0.4	0.5	4.2	1.9	4.6	2.4
NERICA-13	0.4	1.0	4.1	2.2	4.5	3.2
NERICA-14	0.4	1.0	5.0	2.9	5.4	3.9
NERICA-15	0.4	1.1	4.5	2.8	5.0	3.8
NERICA-16	0.4	0.9	4.8	2.5	5.2	3.4
NERICA-17	0.5	0.5	4.6	2.2	5.1	2.8
NERICA-18	0.4	0.9	4.3	2.3	4.6	3.2
Yar-2	0.7	1.1	3.8	1.9	4.5	3.0
IS-280	0.6	1.4	4.3	4.3	4.9	5.6
IS 452	0.3	1.6	4.4	3.9	4.7	5.5
Plu Go	1.0	2.5	4.6	4.0	5.7	6.5
Bo-Blah Iv	0.8	1.8	4.6	3.3	5.4	5.1
Bogutti	0.2	1.2	3.5	4.0	3.7	5.1
Acc-1697 Malagasy	0.3	0.3	3.9	3.1	4.2	3.4

Supplementary Table 1. Contd.

Ex Dar Es Salaam	0.2	0.9	4.1	2.7	4.3	3.6
Kom Aushin	0.4	0.2	4.2	2.9	4.6	3.0
Zebila Market	0.5	0.9	4.3	3.8	4.8	4.7
Bawku Market	0.4	0.7	4.2	3.7	4.6	4.4
Es-10	0.5	2.5	4.2	3.4	4.7	5.9
Nutsurikui	0.7	4.4	4.6	3.2	5.2	7.6
Kaaby Village Faiyum City	0.8	1.9	4.0	2.9	4.7	4.8
Tos Mori (Bariba)	1.2	2.0	3.9	3.3	5.2	5.3
Nakabo	0.3	0.5	4.7	3.4	5.0	3.9
Ss-361	0.4	0.9	4.1	3.4	4.5	4.3
Mala	0.6	1.1	4.1	3.5	4.7	4.6
Tarupatumochi	0.5	1.8	3.6	3.9	4.1	5.7
Bengbete	0.5	1.9	3.4	3.0	3.9	5.0
LSD (5%)	0.20**	0.76**	0.77**	1.15**	0.85**	1.65**
CV (%)	27.3	35.5	10.5	19.8	10.6	20.8

At the bottom of the table are analysis of variance (ANOVA) values.

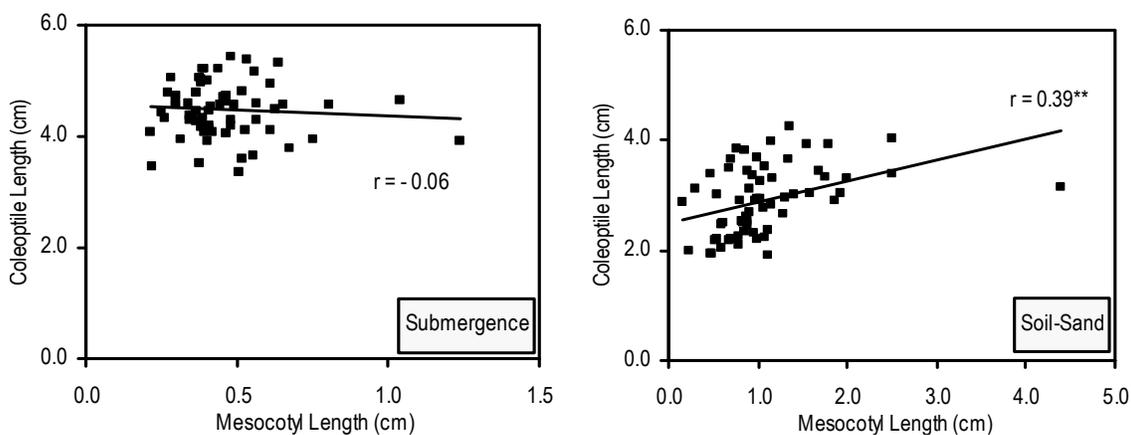


Figure 4. Relationship between coleoptile elongation and mesocotyl elongation in soil sand culture and submerged conditions. The symbol (**) signifies a statistically significant coefficient of correlation (r) at P = 0.01.

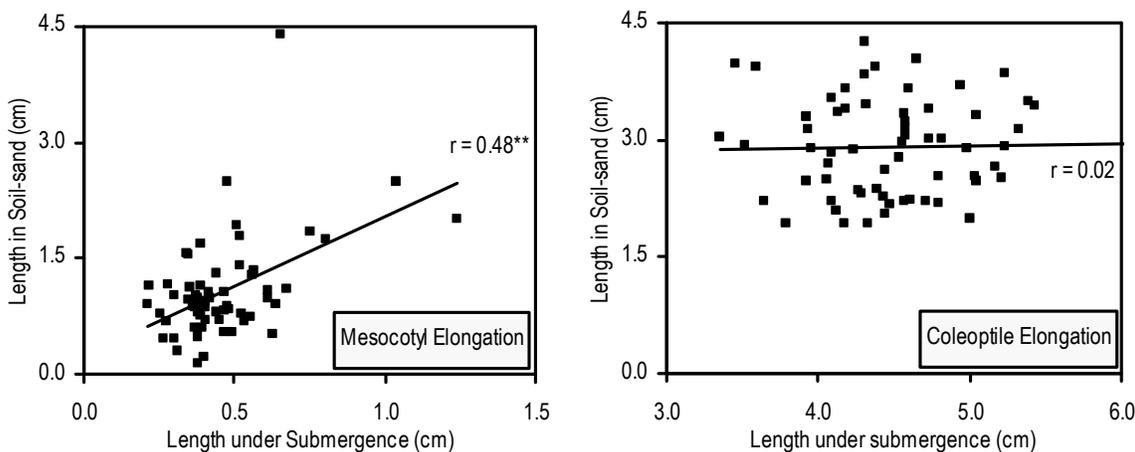


Figure 5. Relationship between mesocotyl or coleoptile elongation in soil-sand culture and under submerged conditions. The symbol (**) signifies a statistically significant coefficient of correlation (r) at P = 0.01.



Figure 6. Coleoptile/mesocotyl elongation in soil-sand culture of selected upland rice genotypes. The white line signifies the point at which seedlings emerged out of the soil to make contact with the atmosphere. The measuring tape is calibrated in centimetres.

mesocotyl elongation in soil-sand culture to be similar to that under submergence, which implies that mesocotyl elongation under submergence may give a clue on how mesocotyl elongation will proceed in soil-sand and vice versa, and may therefore be used as a criterion of estimating one parameter by measurement of the other. However, because inherent genotypic differences in coleoptile elongation were manifested most under submergence (owing to the wide variation), it implies that comparisons amongst genotypes for coleoptile elongation would be done best on seedlings grown under submergence. Conversely, genotypic differences in mesocotyl elongation would be clearer in seedlings grown in soil-sand culture.

Conclusion

The role played by coleoptile and or mesocotyl elongation to seedling emergence seems to change with the conditions under which rice seeds are sowed. While coleoptiles may grow longer under submergence, mesocotyls seem to lengthen more in soil-sand culture. Simultaneously, coleoptile and mesocotyl elongation seem to contribute to seedling emergence in soil-sand culture; under submergence however, the interaction

between coleoptile and mesocotyl elongation in effecting seedling emergence must be explored further.

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