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Screening of rice varieties for direct seeding method

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Abstract

Direct seeding method of rice planting offers several advantages. However, all rice cultivars are not suitable for this practice. In this report forty-three high yield rice varieties and an anoxia/hypoxia tolerant control land race were studied to verify their suitability to establish seedling under anaerobic conditions. The study was conducted in controlled field conditions. The percentage of seedling establishment, first leaf, plant height, root length, leaf number and seeding depth parameters were observed. The varieties were grouped into six different clusters following cluster and discriminant function analysis (DFA). As hierarchical clustering was done in the analysis, the genotypes clustered mainly on the basis of higher value of discriminating variables contributing to the first two principal components. The clusters 3, 5 and 1 located at the positive side of the Y-ordinate represented function 1. The best option from the selected cluster 3 was variety number 6 (BR6) showing the highest seedling establishment percentage (85%) and longest first leaf (1.97) and seedling height (2.86), seeding depth 0.5, root length 1.65 at low land condition. The other varieties from the same clusters were variety 9, 15 and 17. There was a negative relationship (0.756) between seeding depth and seedling establishment. The surviving seedlings showed a tendency to push them towards the soil surface, which might be a morphological response for getting oxygen through their coleoptile tip. The screening revealed that BR6 is the best variety for direct seeding in lowland conditions.

Keywords: Anaerobic stress, coleoptiles, directs seeding, lowland rice, mesocotyl, seedling establishment.
Abbreviation: BRRI_Bangladesh Rice Research Institute; DFA_Discriminant function analysis; D^2_pairwise mahalanobis distance; OM_ organic matter.

Introduction

Rice (*Oryza sativa* L.) is an important food crop for over half of the world's population (Li et al., 2011; Juraimi et al., 2013). Transplantation of rice seedlings is the most common practice in intensive rice cultivation. Alternatively, various pattern of direct seeding method are also used depending on the agroecological conditions. Transplanting rice has some drawbacks, including increased labor requirement and exposure of 'transplant shock' that delays rice harvesting for 7-15 days (Khan et al., 1992). The adoption of the direct-seeding method would significantly reduce the cost of rice production in the lowland cultivation system in South East Asia and some other parts of the world (Flinn and Mandac, 1986). Faster and easier planting, reduced requirements for water and labor makes the direct seeding method economically profitable (Bhushan et al., 2007; Jehangir et al., 2005). Direct seeding can be applied on either dry or wet soils. Dry seeding is a method of putting dry seeds into dry soils or soils where moisture is below the field capacity. In contrast, wet seeding is a method of spreading onto puddled soils. Yamauchi (1995) suggested an alternative method of direct seeding related to conventional direct seeding under lowland conditions. Water seeding is a modified method of wet seeding, where pre-germinated seeds are broadcast into standing water (Biswas et al., 1991; DeDatta, 1981; Pathinayake et al., 1991). Some reports suggest that wet direct seeding could increase grain yield by 10-15% (Hussain et al., 2003). With the introduction of mechanical seeding equipment, such as a drum seeder, wet-direct seeding is gaining momentum (Hossain et al., 2003). Therefore, this method is gaining increasing popularity in South East Asia, especially in a low land cultivation system. Despite its economic benefits, all rice cultivars are not suitable for wet direct seeding. Unstable seedling establishment resulting from abiotic and biotic stresses limit the success in lowland conditions. Low oxygen stress

Table 1. Pairwise Mahalanobis distance (D^2) between 6 clusters of 44 varieties.

Average linkage (between groups)	1	2	3	4	5
1	-	-	-	-	-
2	21.874***	-	-	-	-
3	174.559***	222.376***	-	-	-
4	158.916***	22.040***	558.334***	-	-
5	33.828***	77.074***	32.015***	234.912***	-
6	338.648***	85.266***	797.360***	38.804***	389.159***

*** = distance differing from zero at 0.001 level of significance

is the foremost. It severely restricts the growth of roots and leaves of rice seedlings, while allowing the growth of the coleoptile and mesocotyl of the shoot. A number of reports evidenced the availability of genotypic variation in the sensitivity to oxygen deprivation (Yamauchi et al., 1993; Turner et al., 1982). A positive relationship between anoxia tolerance and seedling establishment in submerged soil has been documented. Chemical measures, such as coating sprouted seeds with calcium peroxide which provide oxygen around the seed, provided some limited success in improving overall seedling establishment. However, expenditure for chemicals, machinery, and expertise in seed coating may outweigh the benefits of direct seeding. Seed germination and seedling growth under flooded conditions necessitate normal tolerance of the low-oxygen conditions usually experienced during flooding (Ismail et al., 2009). Anaerobic seeding using anoxia-tolerant rice genotypes appears to be a more convenient method than the other methods (Yamauchi and Choung, 1995). Thus, selection of low oxygen stress-tolerant genotypes would be more practical solution. Although earlier studies have generated general information on the pattern of seedling establishment, very specific data are necessary to allow the development and selection of varieties giving a uniformly high rate of establishment. Globally, Bangladesh ranked fourth in terms of rice production. Despite its clear benefit in many places, the practice of the direct seeding method is insignificant. Research reports on screening and selecting for specifically suited varieties for this purpose are scarce. At the same time, the existing varieties used for lowland culture do not appear to be well-adapted for seedling growth in an initially oxygen-depleted micro-environment. The objective of this study was to test major high-yielding rice cultivars for their suitability for direct seeding. We noticed several high-yielding varieties successfully establish seedlings in anaerobic conditions and are suitable for direct seeding.

Results and discussion

Grouping of varieties and cluster analysis

Hierarchical cluster analysis produces a dendogram (Fig.1) that sketches a preliminary idea about the number of clusters to be done using five variables on 44 varieties. Accordingly, clustering and discriminate function analysis (DFA) was repeated until an appropriate number of clusters were obtained. Finally, the varieties were grouped into six by cluster analysis followed by DFA to verify the precision of the groups originally made by cluster analysis.

Establishment of seedling under anaerobic conditions

Forty-four varieties were grouped into six clusters with the maximum possible precision level. Based on Mahalanobis distance (D^2) the clusters were distinct from each other at

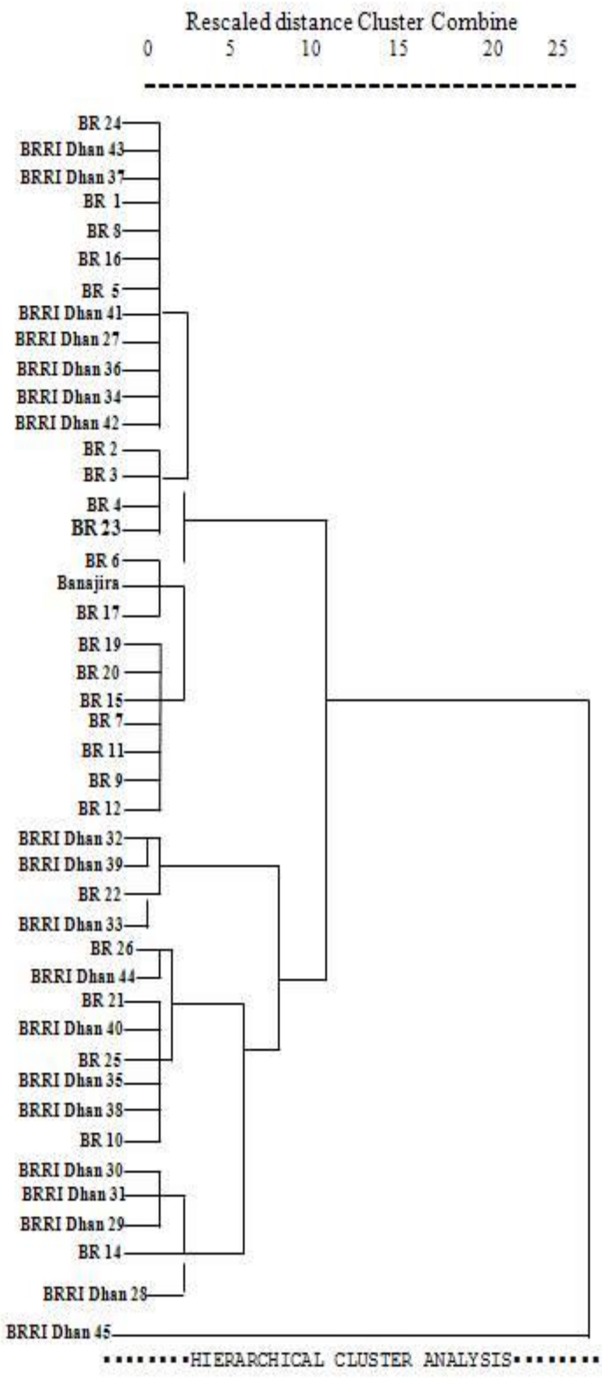

Fig 1. Dendrogram using average linkage (Between Groups) based on variables under consideration. The dendrogram is showing the overall similarity/dissimilarity between varieties.

Table 2. Precision level of two (2) clusters of 44 rice varieties by Discriminant Function Analysis (DFA) amplify based on four parameters –rows being observed category and columns predicted category.

Cluster number	Correction %	*1*	*2*	*3*	*4*	*5*	*6*	Total observation
1	100	10	-	-	-	-	-	10
2	100	-	4	-	-	-	-	4
3	100	-	-	5	-	-	-	5
4	100	-	-	-	11	-	-	11
5	100	-	-	-	-	4	-	4
6	100	-	-	-	-	-	10	10

100% of original grouped cases correctly classified.

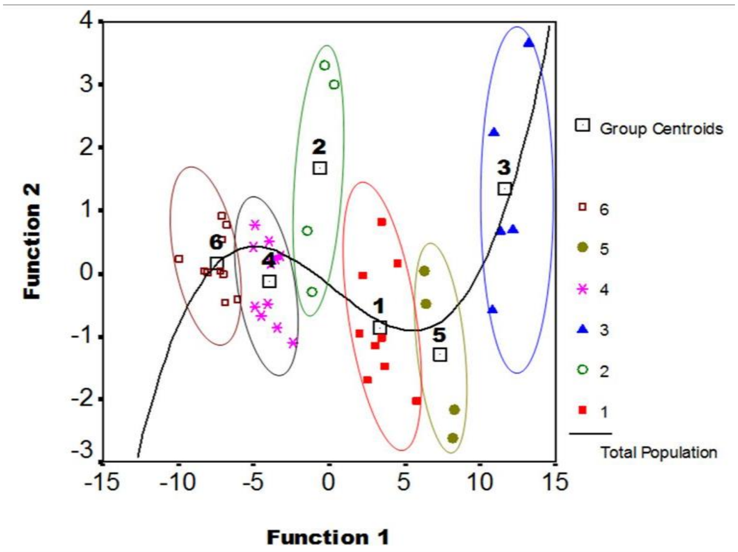

Fig 2. Cubic regression relationship between function 1 and function 2. The clusters were oriented in different position in comparison to the origin of X and Y ordinates. Graphic illustration of discriminatory analysis of 6 groups/clusters of 44 varieties based on different parameters evaluated.

the 0.001 significance level (Table 1). In stepwise statistics, seedling establishment (%), plant height, first leaf length, root length and leaf number entered the analysis. At each step the variable that maximizes the Mahalanobis distance between the closest clusters was entered into the analysis (Table 1 and Table 2). The hierarchical cluster and stepwise DFA showed a maximum correlation between function 1 (Table 3) and seedling establishment (0.926). Accordingly, first leaf length and plant height were correlated to function 2. The corresponding correlation values were 0.895 and 0.668. Root length and leaf number were related to function 3 and 5, respectively. The contribution of each of five canonical discriminant functions explains the variance, along with their corresponding eigenvalues and canonical correction coefficients. Eigenvalues and canonical correction coefficients values were significantly higher for function 1, the first principal component (Table 4). Function 1 (the first principal component)explained 97.5% of total variance, while function 2 (the second principal component), explained 1.9% of total variance and function 3 (the third principal component) explained only 0.5% of the total variance. The cumulative effective of function 1 and function 2 were 99.5% of the total variance. The remaining function was negligible. Singh et al. (1996) showed the contribution of different principal components in grouping of rice genotypes in their studies. The standardized coefficients of variables explain the contribution of variables in the functions. The coefficient for seedling establishment was the highest in function 1 (1.256). In function 2, these values for first leaf length and leaf number were 0.929 and 0.510, respectively (Table 5). Fig. 2 presents the graphical illustrations of all clusters. The clusters were oriented in different positions compared to the origin of X and Y ordinates. As hierarchical clustering was done in the analysis, the genotypes clustered mainly on the basis of higher value of discriminating variables contributing in function 1 and 2. The variables that contributed more in function 2, played a more dominant role in clusters 2, 4, and 6 but less compared to others. Function 1 was highly dominant over function 2 and played a decisive role in specifying the cluster location. The cluster located on the positive side of the Y- ordinate representing function 1. These clusters were cluster 3, 5 and 1. The prime objective of this study was to determine the variety with superior seedling establishment ability under lowland conditions. The number of genotypes was quite large to get a valid conclusion from ANOVA. Therefore, cluster and discriminated function analysis was executed following the method used by Yadav et al., (2008). Cluster analysis is quite satisfactory to make homogeneous groupings based on the variables under consideration. Discriminate function analysis is employed to pinpoint the worthiness of a particular set of variables in the previously delineated groups. Discriminate function analysis could be used to ascertain the precision level of clusters. In this study, the first objective is to ascertain superior seedling establishment with good first leaf length and plant height. Cluster 3 should have superior seedling establishment as well as elongated first leaf and seedling height, according to the detailed analysis. Cluster 5 and 1

Table 3. Structure matrix representing correlation between five variables and standardized pooled within groups correlations between discriminating variables and standardized canonical discriminant functions variables ordered by absolute size of correlation within function.

Variables	Function				
	1	*2*	*3*	*4*	*5*
Establishment frequency (%)	0.926*	0.063	0.289	0.233	0.018
First leaf length (cm)	0.313	0.895*	-0.047	-0.041	-0.313
Plant height (cm)	0.309	0.668*	0.251	-0.589	0.217
Root length (cm)	0.245	0.363	0.793*	0.319	-0.277
Leaf number	0.523	0.358	0.132	0.126	0.752*

*Largest absolute correlation between each variable and any discriminant function.

followed cluster 3. Further stepwise DFA of each cluster was done and spikes were drawn for the genotypes to the Y-reference line passing through the cluster-centroid (Fig. 3) to identify the genotypes better representing their respective clusters. The relative position of the genotypes indicated the cumulative response of the variables representing function 1 and 2. The cluster centroid represents the optimum values of function 1 and 2 resulting from the cumulative effects of the genotypes of the clusters based on the responses to the discriminatory variables. The genotypes oriented at or very near to the cluster with a relatively lower distance of the parameter to the Y-reference line refers to the deviation of the genotypes in response to discriminating variables. If it is very close to the cluster centroid it might be considered as the most representative one of that cluster. Accordingly, genotype number 9 and 17 from cluster 3 and genotype number 12 from cluster 5 might be considered as more representative genotypes (Fig. 3 a, b). It may be mentioned that the most representative variety of a selected cluster might not be the best option. Accordingly, the best choice from cluster 1 was variety number 6. It exhibited the highest seedling establishment percentage and elongation of the two other associated seedling organs, first leaf and seedling height (plant height) (Table 6) represent cluster 5, 3 and 1, respectively.

Physiological perspectives of seedling establishment under anaerobic conditions

Pairwise Mahalanobis distance (D^2) analysis (Table 3) is important to measure the distance among cluster to provide appropriate direction to the breeder to select the suitable parents in a breeding program. According to D^2 analysis the highest distant unit was observed between clusters 6 and cluster 3 (797.36). There is a negative relationship between seeding depth and seedling establishment (Fig. 4 a). This suggests that with an increase of seeding depth, the seedling establishment decreased significantly. The coefficient of determination (R^2) is 75.66%. Genotypes with no tolerance to anoxia might have a very limited possibility of surviving in these conditions. However, genotype Banajira, (relatively tolerant to anaerobic conditions) was able to sustain the stress. Similarly variety BR 6 (Variety # 6) showed a similar tolerance level (Table 6). Generally, a genotype could sustain a similar stress through an avoidance strategy. First the coleoptile elongates to reach the tip at the oxygenated soil layer, withholding root growth to conserve energy during the early phase of the seedling development and initiate first leaf growth (Biswas and Yamauchi, 1997). However, this phenomenon varies with the genotypes and a new phenomenon has been recognized in this study. Seedling establishment is characterized by the ability of a genotype to produce first leaf, while coleoptile emergence was characterized by the appearance of a coleoptile tip above the soil. Most varieties could not establish seedlings in this study. Only a few varieties with good seedling organs could be sustained from the cluster. The interdependence of the seedling organs assisting seedling establishment was studied for a few genotypes (Yamauchi et al., 1993; Biswas and Yamauchi, 1997; Yamauchi and Biswas, 1997; Biswas et al., 2001, 2002). However, traditional land race genotypes

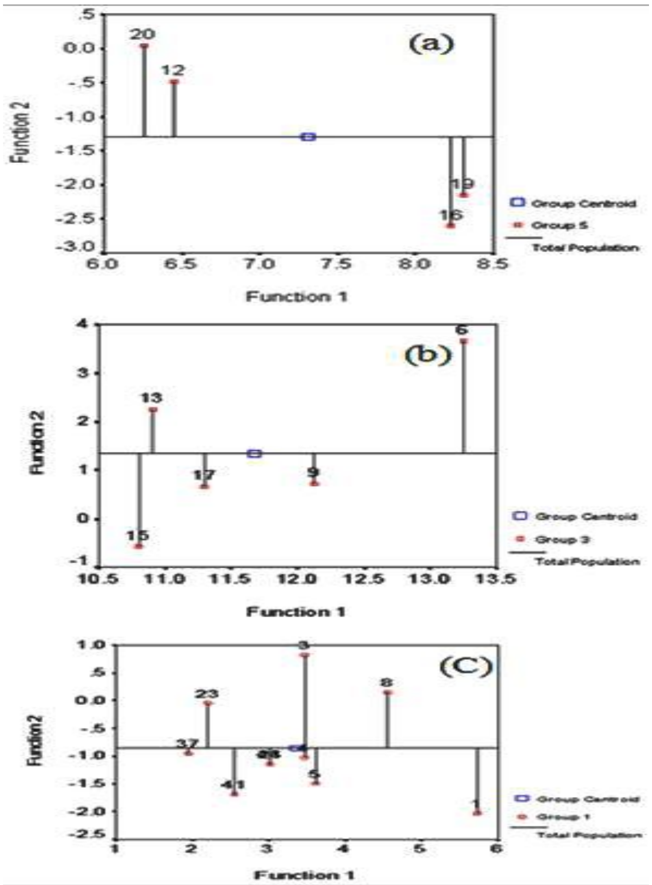

Fig 3. (a) Graphical illustrations of discriminatory analysis 6 group's 44 genotypes. Among 44 varieties, genotype number 9 and 17 from cluster 3 can be considered as more representative genotypes (b) Among 44 varieties, genotype number 12 from cluster 5 can be considered as more representative genotypes. (c) Accordingly, genotype number 12 from cluster 5 might be considered as more representative genotypes

Table 4. Percent contributed by each of discriminate functions in grouping of 44 rice varieties into 4 groups through stepwise discriminant functions analysis.

Function	Eigenvalue	% of variance	Cumulative %	Canonical correlation
1	45.858	97.5	97.5	0.989
2	0.913	1.9	99.5	0.691
3	0.215	0.5	99.9	0.420
4	0.028	0.1	100.0	0.165
5	0.002	0.0	100.0	0.044

All canonical discriminant functions were used in the analysis.

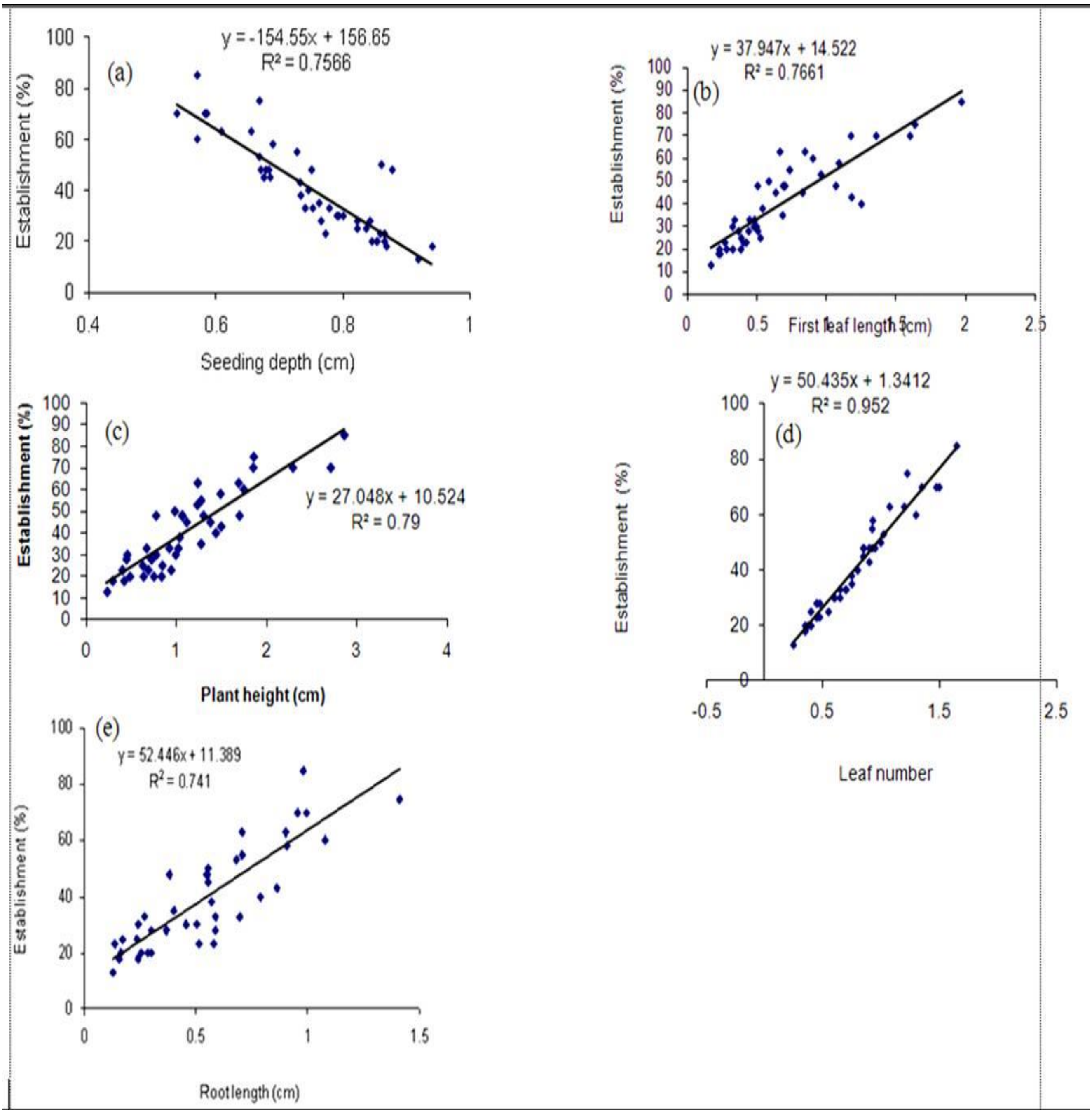

Fig 4. (a) Relationship between seeding depth (cm) height and seedling establishment,(b) Relationship between first leaf length and seedling establishment, (c) Relationship between seedling height/plant height and seedling establishment, (d) Relationship between leaf number and seedling establishment, (e) Relationship between root length (cm) and seedling establishment.

Table 5. Coefficient of variables of Standardized Canonical Discriminant Function mostly contributed in grouping of 7 genotypes by DFA.

Variables	Function				
	1	*2*	*3*	*4*	*5*
Plant height (cm)	0.186	0.084	0.575	1.431	0.050
First leaf length (cm)	0.058	0.929	-0.825	0.572	-0.434
Root length (cm)	-0.474	0.228	1.101	0.461	-0.049
Leaf number	-0.234	0.510	-0.100	0.828	1.129
Establishment frequency (%)	1.256	-0.634	-0.148	-0.305	-0.495

Table 6. Variety and their seedling parameter from different clusters.

Variety	Plant height (cm)	First leaf length (cm)	Seeding depth (cm)	Root length (cm)	Leaf number	Establishment frequency (%)
Cluster mean of Cluster 3						
6	2.86	1.97	0.57	1.41	1.65	85
9	1.86	1.64	0.67	0.98	1.23	75
13	2.29	1.60	0.58	0.99	1.50	70
15	1.85	1.18	0.59	1.00	1.35	70
17	2.71	1.36	0.54	0.96	1.48	70
Cluster mean of Cluster 5						
12	1.49	1.09	0.69	0.91	0.93	58
16	1.24	0.67	0.66	0.71	1.20	63
19	1.69	0.85	0.61	0.90	1.08	63
20	1.75	0.90	0.57	1.08	1.30	60
Cluster mean of Cluster 1						
1	1.56	0.74	0.73	0.71	0.93	55
3	1.70	1.07	0.88	0.55	0.90	48
4	1.30	0.69	0.75	0.38	0.95	48
5	0.98	0.59	0.86	0.56	1.00	50
8	1.23	0.96	0.67	0.68	1.02	53
23	1.02	0.83	0.68	0.56	0.85	45
24	1.06	0.70	0.68	0.55	0.85	48
37	1.11	0.64	0.69	0.56	0.85	45
41	0.77	0.51	0.68	0.56	0.93	48
43	1.06	0.70	0.67	0.55	0.85	48

Were considered in those studies. This study was conducted using only the major high yielding varieties.

Materials and Methods

Seed source

Forty three high yielding rice cultivars developed by Bangladesh Rice Research Institute (BRRI) and a local low yielding variety Banajira (BRRI germplasm accession # 007) were used in this experiment. The selected cultivars for this study were BR1 to BR26 except BR13 and BR18, BRRI dhan 28 to BRRI dhan45 and Banajira. The variety Banajira is known to tolerate anaerobic conditions (Islam, 2005).

Experimental site and time

This research was conducted from July to September at the Bangladesh Rice Research Institute (BRRI) Gazipur, Bangladesh (22°21'N 90°20'E).

Germination test

A germination test was conducted before going to the field experiment. Only the materials showing more than 95% germination were used for the field experiment.

Seed germination before sowing

Fifty healthy seeds of each cultivar were sterilized by soaking in 0.1% sodium hypochlorite (NaClO) for one minute, washed thoroughly and allowed to imbibe incubated in 10 ml water in a petri dish on a filter paper at room condition to have more or less a prison breast condition.

Soil Collection and preparation

Soils (clay loam, Organic matter (OM) =0.9-1.5%, pH = 4.5-6) were collected from a paddy field of the BRRI farm.

Seedling tray preparation

The seeds were laid out in plastic tray (300 × 800 × 26-mm) that had 578 tiny compartments measuring 16 × 16 × 25-mm with 2-mm diameter drainage holes (Yamauchi et al. 1993). The compartments were filled to 1.25 cm with the soil and sprinkled with tap water to get sufficiently soaked. Sprouted seeds were placed on the soaked soil bed in the compartment and covered with soil to a depth of 1 cm. Ten sprouted (48 hours-old-seedling) seeds were sown for each variety. Finally, the trays were submerged in a paddy field with 2-3 cm of water above the surface and allowed to grow for seven days. A netted box was used to cover the submerged tray to protect it from rain-flash and bird damage.

Observation and measurement

Out of ten sown sprouted seedlings numbers of established seedlings were counted and percentage of seedling establishment was calculated using the following formula:

Establishment frequency (%) =

$$\frac{\text{Number of establishment plant}}{\text{Number of total seedlings}} \times 100$$

Seedling parameters and seeding depth parameters

The following parameters were measured to check the germination and seedling establishment performance: (i) first leaf (from first node to the tip of the first leaf), (ii) plant height (from first node to the tip of the nth leaf), (iii) root length (from first node to the tip of the longest root), (iv) leaf number and (v) seeding depth

Experimental design and Statistical Analysis

This experiment was arranged in randomized complete block design with four replications. Each tray was assigned for a replication and varieties were accommodated randomly in a tray. MS Excel was used to process raw data. Data were analyzed through Discriminate Function Analysis (DFA), using the analytical package SPSS (v12). We used Least Significant Difference (LSD) test for multiple comparison and p value 0.001was considered significantly different.

Conclusion

We observed a highly significant dependence of seedling establishment on first leaf length, plant height, leaf number and root length, separately. Seedling establishment is negatively correlated to seeding depth. The surviving seedlings showed a tendency to come up towards the soil surface either for survival, or to get oxygen or for both. Therefore, they might struggle to push up their coleoptiles. Thus, there is correlation between low-oxygen tolerance and success in survival in the direct seeding method.

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