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Evaluation of rice germplasm under *jhum* cultivation in North East India and breeding for Aluminium tolerance

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Abstract

In North Eastern (NE) region, Aluminium (Al) toxicity is a major problem due to acidic soils. Shifting (*Jhum*) cultivation of rice is a primitive mode of agriculture prevailing in the hills of the NE region. The present study was carried out to screen a set of 67 genotypes for agronomic traits, yield and characters related to Al tolerance. The components of variability revealed high magnitude and highly heritable nature of variation. Heritability was found high for ear bearing tillers, panicle length and yield per ha. Genetic advance over mean was also high for all the traits except for days to 50% flowering. Significant correlations were observed for traits evaluated in this study. The clustering pattern as revealed by dendrogram, based on average distance classified the accessions into three groups. Principal component analysis revealed that the first three most informative components accounted for 56% of the variance. Several high performing genotypes like, Lengja and Koyabo were identified suitable for acidic soil conditions of *Jhum* areas.

Key words: *Jhum* rice, soil acidity, aluminium tolerance, variability, correlation

Introduction

North Eastern (NE) region of India is considered as a center of diversity of rice. For this region rice is the principal food crop accounting for more than 80% of the food grain production and is extensively cultivated (72% of the total cultivated area) in upland, lowland and deep-water conditions [1]. While settled agriculture primarily takes place in narrow valleys in the hills giving average yield, shifting cultivation locally known as *jhum*, is practiced on hill slopes with a very less yield [2]. *Jhum* cultivation is the most traditional and dominant land use system in NE region. On an average, 3,869?km^2 area is put under shifting cultivation every year [3]. Shifting cultivation is an economically viable system of agriculture as long as population densities are low and *jhum* cycles are long enough to maintain soil fertility. The shifting cultivation became unsustainable today primarily due to the increase in population that led to increase in food demand. Jhuming cycle in the same land, which extended to 20-30 years in earlier days [4], has now been reduced to 3-6 years [5]. *Jhum* cultivation in NE region resulted in indiscriminate destruction of forests in the *jhum* land, coupled with high rainfall, has resulted in heavy soil erosion and consequent silting of rivers causing floods in the lower reaches.

Excessive rainfall in NE region is responsible for removing basic cations over a long period of time (thousands of years). Wet climates have a greater potential for acidic soils. In time, excessive rainfall leaches the soil profile's basic elements (calcium, magnesium, sodium, and potassium) that prevent soil acidity. Thus Aluminum (Al) toxicity is a major problem for crop production on acid soils. Under highly acidic soil conditions (pH, 5.0), Al^{3+} is solubilized into the soil solution and is highly phytotoxic. Al^{3+} causes a rapid inhibition of root growth that leads to a reduced and stunted root system, thus having a direct effect on the ability of a plant to acquire both water and nutrients [6].

Although crop production on acid soils can be sustained by application of lime, runoff pollution is an undesirable effect. Liming is often not economic or practical because of the slow movement of lime especially in the deeper layers of sub-soils [7, 8]. Furthermore, heavy application of lime may have

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adverse effects on some crops in the rotation or cause deficiencies of certain nutrients [9]. Thus, developing cultivars with improved tolerance to acid soil stress may be a solution to address this problem [10]. There are several screening methods for aluminum tolerance such as solution, sand and soil cultures and field screening. However, reliable ranking of tolerance in the field screening is difficult because of the temporal and spatial variation in acidic soils. Selection of seedlings in hydroponic assay has been used as a rapid screening method to screen for aluminum tolerance in several crops. Also the results obtained with solution culture screening methods correlate positively with those obtained using field screening [11]. Al tolerance screening is typically conducted by comparing root growth of seedlings grown in hydroponic solutions, with and without Al as done by Pineros and Kochian, [12] in maize and Sasaki *et al.* [13] in wheat. Two concentrations of Al *viz.*, 2.5 and 20 μM $AlCl_3$ were used for comparative studies on aluminum tolerance screening techniques for sorghum, soybean and maize in simple solution culture [14]. Rice (*Oryza sativa*) has been reported to be the most Al-tolerant cereal crop (6-10 times more tolerant than other cereals) under field conditions and capable of withstanding significantly higher concentrations of Al than other major cereals [15]. Considerable genetic variability exists for Al tolerance within *jhum* rice genotypes which can be exploited to develop a tolerant genotype. Therefore, the present study has been conducted to identify aluminium tolerant genotypes suitable for the *jhum* conditions which can be utilized to get more profit and reducing the *jhum* cycle.

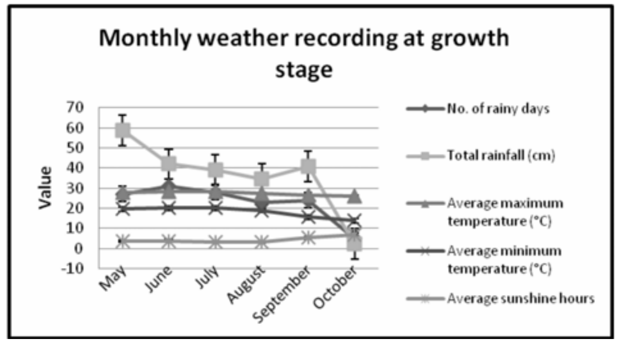
Materials and methods

A set of 67 genotypes collected from *Jhum* areas of Nagaland were screened for agronomic traits, yield and characters related to Al tolerance. Four released varieties were used as checks due to their stable and superior performances.

Climate and weather

The experiment was carried out at upland experimental farm of Division of Plant Breeding, Indian Council of Agricultural Research ICAR Research Complex for NEH Region, Barapani, Meghalaya, India (latitude 25° 39' North, longitude 91° 54' East and elevation 1010 m asl). Weather conditions during the crop growth period is presented in Fig. 1.

The soil of the experimental field was highly acidic (pH 4.7) and consisted of a sandy loam with 15.4 g/l organic carbon, 126 mg/kg available N, 97.40 mg/kg available K, 6.11 mg/kg available P and 5.57 mg/kg available S. The fertilizers, NPK were applied at the rate of 60:60:40 kg/ha.



Experimental layout

The field experiment was set up as an Randomized Block Design (RBD) design with four checks. Plots consisted of five rows each 4 m long. Plants were spaced 20 x 15 cm apart. The recommended agronomic practices and plant protection measures were followed to ensure normal crop growth. At field level measurements were recorded on number of days to mid-flowering, number of days to maturity, plant height, ear bearing tillers, panicle length and yield. Measurements for days to mid-flowering and days to maturity were recorded on per plot basis; days to mid-flowering were calculated as number of days required for 50 % of the plants to flower.

The lab experiment was conducted in RBD with three replications each for control and Al solution. Amount of Al in hydroponic solution was maintained by $AlCl_3.6H_2O$ and its concentration in solution (100μM) was determined based on the level of injury found in the roots of plants in earlier experiments (data not published yet). The actual concentration of Al^{3+} in the solution was 11.462 μ molar. At lower concentration, discrimination between susceptible and tolerant genotypes was not possible as rice crop tolerate significantly higher concentrations of Al as compared to other cereals and pulses [6]. Chuan-zao *et al*. [16] also used 0 or 183 μM $AlCl_3$ as with and without Al solution for their studies in rice.

The genotypes were grown in hydroponics in laboratory conditions with and without Al for seven days to screen for root and shoot characters. Amount of Al in hydroponic solution was maintained by $AlCl_3$ and pH of the solution was maintained (4.5) by addition of HCl and NaOH. Temperature was maintained at

25°C and proper sunshine was provided to all the treatments. Seeds were germinated on filter paper moistened with double distilled water in a petri dish overnight in the dark for 4 days. The germinated seeds of each genotype of an age of 4 days with an emerging root were shifted to hydroponic solution with and without Al.

Measurements were recorded on root length, shoot length, root dry weight and shoot dry weight in both control and Al solutions. Observations from ten plants were used for each replication of both treatments *viz.*, control (without Al) and Al solution. Initial measurement for root length and shoot length was taken at the time of shifting genotypes to hydroponic nutrient solution. After seven days plants were taken out and final measurements for root and shoot length were taken for both control and Al solution. Then the relative root and shoot length was computed using both the initial and final values. The plants were kept in oven at 60°C for drying and measurement was taken for root dry weight and shoot dry weight of genotypes grown in both control and Al solutions.

$$\text{Relative Difference} = \frac{[(\text{Final reading with Al} - \text{initial reading with Al}) * 100]}{[(\text{Final reading without Al} - \text{initial reading without Al})]}$$

Statistical analysis

Genotypic and phenotypic coefficient of variability was computed according to Burton and Devane [17]. Broad sense heritability was estimated based on the ratio of genotypic variance to the phenotypic variance and was expressed in percentage [12]. Genetic advance (GA) was computed according to the formula given by Johnson *et al*. [19]. Higher estimates of heritability coupled with better genetic advance confirm the scope of selection in developing new genotypes with desirable characteristics. The correlation coefficients [14] were calculated to determine the degree of association of characters with yield. The estimates of direct and indirect effects of quantitative traits on seed yield were calculated through path co-efficient analysis suggested by Wright [21] and elaborated by Dewey and Lu [22].

Result and discussion

Mean performance

The detailed evaluation of sixty seven genotypes along with four checks *viz.*, Bhalum-1, Bhalum-2, Bhalum-3 and Bhalum-4 were performed in solution culture for variation in Al related and morphological traits in hydroponics and field respectively. For Aluminium related traits, such as differences for shoot length, root length, root weight and shoot weight of the genotypes, namely, Konpemo (108.76), Dhao Tipnuakulong (106.36), Khatighi Kumnypu (100.39), Chuk Melen (94.29), Hahsho (90.44), Lengja and Koyabo (79.98) excelled for relative difference for root length. Among these Hahsho (130.41) and Khatighi Kumnypu (127.42) were also superior for relative difference for root weight. As far as yield per ha is concerned Tsaknak (14.93 q/ha) was superior to Lengja (12.45 q/ha), Koyabo (10.80q/ha) and Zotsok (12.39 q/ha). However, the performance with respect to yield of these genotypes is quite less as compared to national average (22.40 q/ha). The performance of new genotype needs to be improved through breeding for the upliftment of farmers and tribes practicing *Jhum* cultivation under acidic soils.

Genetic variability, heritability and genetic advance

Analysis of variance revealed that genotypic differences were significant for all the characters. Among Aluminium related traits, range of variation was the highest for relative difference for shoot length followed by relative difference for shoot weight (Table 1). Alvim *et al*. [23] also reported that at Al^{3+} concentrations of 40, 80 and 120 μM, differences between rice cultivars were significant (P = 0.05) for root growth inhibition whereas Osman *et al*. [24] reported a wide range of variability among the genotypes for most of the morphological traits studied. Range on plant height was highest among agro-morphological traits followed by ear bearing tillers. The nature and magnitude of variation as assessed by the components of variation *viz.*, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H) and genetic advance over mean for the Al related and morphological traits are presented in (Table 1) and discussed as under.

Aluminium related traits

Genotypic coefficient of variation measures the variability of any trait. The extent of the environmental influence on any trait is indicated by the magnitude of the differences between the genotypic and phenotypic coefficients of variation. Large differences reflect high environmental influence, while small differences reveal high genetic influence. Phenotypic coefficients of variation were slightly higher than the genotypic coefficients of variation for all the traits studied. This

Table 1. Components of variation in *Jhum* paddy genotypes for agronomic and Aluminium related traits

Character	Mean	Range	GCV	PCV	H^2b	GAM
RDRL	58.12	-10.34-206.25	6.42	26.72	5.81	24.66
RDSL	108.18	28.52-341.67	4.57	24.48	3.53	20.32
RDRW	84.12	9.95-183.96	12.06	18.02	44.80	38.32
RDSW	113.52	53.13-303.75	8.90	20.19	19.52	36.84
DFF	104.94	94.00-121.00	2.55	4.04	39.81	12.29
PH	152.09	85.60-183.40	6.34	34.07	3.54	31.23
EBT	8.50	2.60-73.50	34.78	35.81	74.32	72.74
PL	25.81	17.60-36.80	10.13	12.20	69.05	21.44
YPH	5.05	0.50-15.67	73.75	73.76	68.36	49.50

RDRL = Relative difference for root length, RDSL = Relative difference for shoot length, RDRW = Relative difference for root weight, RDSW = Relative difference for shoot weight, DFF = Days to fifty percent flowering, PH = Plant height, EBT = Ear bearing tillers, PL = Panicle length, YPH = Yield per ha (q)

indicated the presence of environmental influence to some degree in the phenotypic expression of the characters. Akinwale *et al.* [25] reported similar results in African rice genotypes. The PCV for relative difference for root length, shoot length and shoot dry weight was high (>20%) but medium for relative difference for root dry weight (18.02%). The GCV was medium for relative difference for root dry weight (12.06%). For other traits the value was found low (<9 %). The amount of genetic variability observed for Al related traits indicate the scope for breeding Al tolerant genotype(s). Reliable breeding methodology needs to be developed for conversing the laboratory results into practical plant breeding. Heritability was moderate (44.80%) for relative difference for root dry weight (18.02%). For other traits the value of heritability was found low. Likewise GAM was also found to be high (>20) for all the traits.

Agronomic traits

The PCV for plant height (34.07 %), ear bearing tillers (35.81 %) and yield per ha (73.75 %) was high (>20%), medium for panicle length (12.20 %) and low for days to fifty percent flowering (4.04 %). A similar trend of distribution was recorded for genotypic coefficient of variability. High magnitude of GCV recorded for ear bearing tillers indicated that there is ample scope of enhancing number of tillers, which may have positive influence on yield.

The value of heritability was found high (>60%) for all traits except for plant height (3.54 %) and days to fifty percent flowering (39.81 %). Sadeghi [26] also reported high broad sense heritability for panicle length (93.15%), ear bearing tillers (70.2%) and yield (68.19%). Likewise GAM was also found to be high (>20) for all the traits except for days to fifty percent flowering (12.29). These results were supported by earlier findings of Akinwale *et al.* [25] and Sadeghi [26]. The relatively high value of heritability and genetic advance observed in the present study indicated that superior genotype could be selected in segregating population derived from hybridization between selective parents.

Character association and path coefficient analysis

Understanding of the relationship between the traits, for the selection of the important traits, is the utmost importance. Determination of correlation coefficients between various characters helps to obtain best combinations of attributes in crop for obtaining higher return per unit area. Correlations between traits *viz.*, Al related root and biomass traits and morphological traits were calculated and are presented in (Table 2). Significant correlations were found between relative difference for root length and agronomic traits *viz.*, ear bearing tillers (0.308), panicle length (0.169), days to fifty percent flowering (0.257) and yield per ha (0.258). Relative difference for root dry weight (0.268) and relative difference for shoot dry weights (0.433) were also significantly correlated with yield per ha. Significant correlation was not found between relative difference for root length and relative difference for shoot length (0.115) indicating that selection of genotypes based on relative difference for shoot length won't be a fruitful approach. This can be justified by the negative

Table 2. Correlations for agronomic and Aluminium related traits in *Jhum* paddy genotypes

		Genotypic correlation								
	Character	RDRL	RDSL	RDRW	RDSW	DFF	PH	EBT	PL	YPH
Phenotypic correlation	RDRL	**1**	-0.087	0.245**	0.109**	0.257**	-0.025	0.308**	0.169**	0.258**
	RDSL	0.115	**1**	0.034	0.039	0.052	0.015	-0.120	-0.041	-0.011
	RDRW	0.397**	0.046	**1**	0.038	0.569**	0.118	0.354**	0.318**	0.268**
	RDSW	0.148*	0.053	0.050	**1**	-0.035	0.129*	0.353**	0.258**	0.433**
	DFF	0.332**	0.070	0.391**	0.010	**1**	0.019	0.351**	0.347**	0.356**
	PH	-0.101	0.026	0.121	0.151*	0.028	**1**	0.038	0.097	0.152*
	EBT	0.409**	-0.052	0.484**	0.434**	0.426**	0.056	**1**	0.741**	0.814**
	PL	0.296**	-0.110	0.453**	0.337**	0.473**	0.129	0.809**	**1**	0.658**
	YPH	0.370**	-0.022	0.393	0.467**	0.419**	0.197**	0.866**	0.775**	**1**

*,**Significance level at 0.05%, 0.01%, respectively
RDRL= Relative difference for root length, RDSL = Relative difference for shoot length, RDRW= Relative difference for root weight, RDSW= Relative difference for shoot weight, DFF= Days to fifty percent flowering, PH= Plant height, EBT= Ear bearing tillers, PL= Panicle length, YPH= Yield per ha (q)

correlation with plant height (–0.025) also. Thus taller genotypes seem to be intolerant to Aluminium toxicity. However biomass related traits *viz.*, relative difference for root dry weight (0.245) and relative difference for shoot dry weight (0.109) have significant correlation with relative difference for root length. Agronomic traits *viz.*, ear bearing tillers (0.814), panicle length (0.658), days to fifty percent flowering (0.356) and plant height (0.152) were also significantly correlated with yield per ha. Similar values of correlations were found in Bangladesh and Sudanese upland rice genotypes for agronomic traits in the studies carried out by Ullah *et al.* [27] and Osman *et al.* [24]. These studies have thus demostrated associations between Al related and agronomic traits contributing towards yield are useful for facilitating breeding of improved rice genotypes. This association proves the parallelism between Al related traits and yield related traits grown in hydroponic solution and field respectively enabling breeder to select genotypes for high yield.

Path-coefficient analysis is a cause-and-effect relationship among traits. Further compartmentalization of correlation coefficients into direct and indirect effects discerned the true nature of association between two traits. In this study, eight predictor variables were used to describe the response variable rice yield per ha. Among the predictor variables four were Aluminium related variables *viz.*, relative differences for root length, shoot length, root dry weight and shoot dry weight and four were agronomic variables *viz.*, days to fifty percent flowering, plant height, ear bearing tillers and panicle length. Except for relative difference for root dry weight (–0.060) all other characters exhibited positive direct effect on grain yield (Table 3). Ear bearing tillers exhibited maximum direct effect (0.620) followed by panicle length (0.217). Panicle length has shown maximum indirect effect through ear bearing tillers (0.502). Oad *et al.* [28] and Osman *et al.* [24] also observed similar results in a study of quantitative characters in rice ratoon cultivars and advance lines. Since Aluminium related traits have taken at very early stage of plant development they might have not contributed much to yield per ha. Plant height has very less direct as well as indirect effect on yield. This implies that there won't be increase in grain yield on selection of taller genotypes. This finding is contradicting to many other earlier findings which may be due to the taller nature of *jhum* paddy genotypes where height has a negative impact on grain yield. This can also be visualized by negative association of relative difference for shoot length with yield (–0.022). Selection based on panicle length and ear bearing tillers will be rewarding for breeders as far as yield is concerned. Results of the correlation and path analysis suggest that all the characters having positive association with yield are also directly contributing towards grain yield and selection of genotypes may reliably be done through these characters.

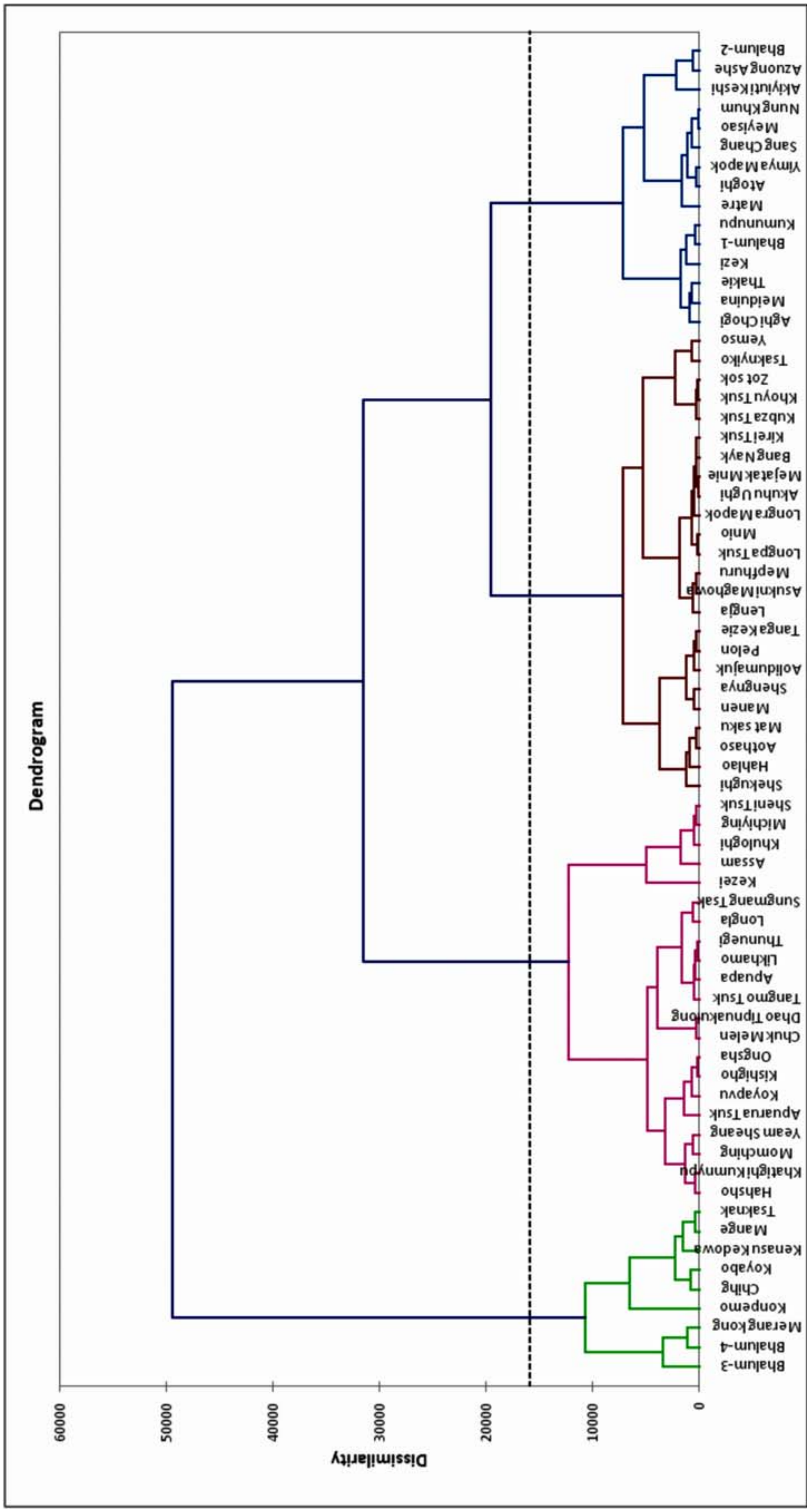


Fig. 1. A cluster analysis of *Jhum* paddy genotypes based on field and Aluminium related traits

Genetic divergence studies

The clustering pattern as revealed by dendrogram, based on agronomic, yield and Al related traits, classified the genotypes into four groups (Fig. 1). Cluster II was the largest group which comprised 24 genotypes while 21 present in cluster III. Mean and range were calculated for each cluster (data not given). Cluster III has shown highest value for Aluminium related traits *viz.*, relative difference for root length, relative difference for shoot length and relative difference for root dry weight. Cluster I has highest rage of expression for relative difference for root length and relative difference for shoot length. Highest range for agronomic and yield related traits *viz.*, plant height, ear bearing tillers, panicle length and yield per ha was shown by cluster II. Based on divergence studies further hybridization programme needs to be started so as to assemble yield with Aluminium tolerance.

Principal components analysis, performed on agronomic, yield and Al related traits, revealed that the first three most informative

Table 3. Path Matrix of YPH in *Jhum* paddy genotypes for agronomic and Aluminium related traits

Character	RDRL	RDSL	RDRW	RDSW	DFF	PH	EBT	PL
RDRL	**0.0449**	0.005	0.018	0.007	0.015	–0.004	0.018	0.013
RDSL	0.0024	**0.020**	0.001	0.001	0.001	0.001	–0.001	–0.002
RDRW	–0.0238	–0.003	**–0.060**	–0.003	–0.023	–0.007	–0.029	–0.027
RDSW	0.0162	0.006	0.005	**0.109**	0.001	0.017	0.047	0.037
DFF	0.0186	0.004	0.022	0.001	**0.056**	0.001	0.024	0.026
PH	–0.0067	0.002	0.008	0.010	0.002	**0.066**	0.010	0.008
EBT	0.254	–0.032	0.300	0.269	0.264	0.096	**0.620**	0.502
PL	0.064	–0.024	0.098	0.073	0.102	0.028	0.175	**0.217**
YPH	0.370	–0.022	0.393	0.467	0.419	0.197	0.566	0.7745
Partial R^2	0.0166	–0.0005	–0.0235	0.0512	0.0235	0.0129	0.5373	0.1679

R Square = 0.7854, Residual Effect = 0.4633

RDRL= Relative difference for root length, RDSL= Relative difference for shoot length, RDRW= Relative difference for root weight, RDSW= Relative difference for shoot weight, DFF= Days to fifty percent flowering, PH= Plant height, EBT= Ear bearing tillers, PL= Panicle length, YPH= Yield per ha (q)

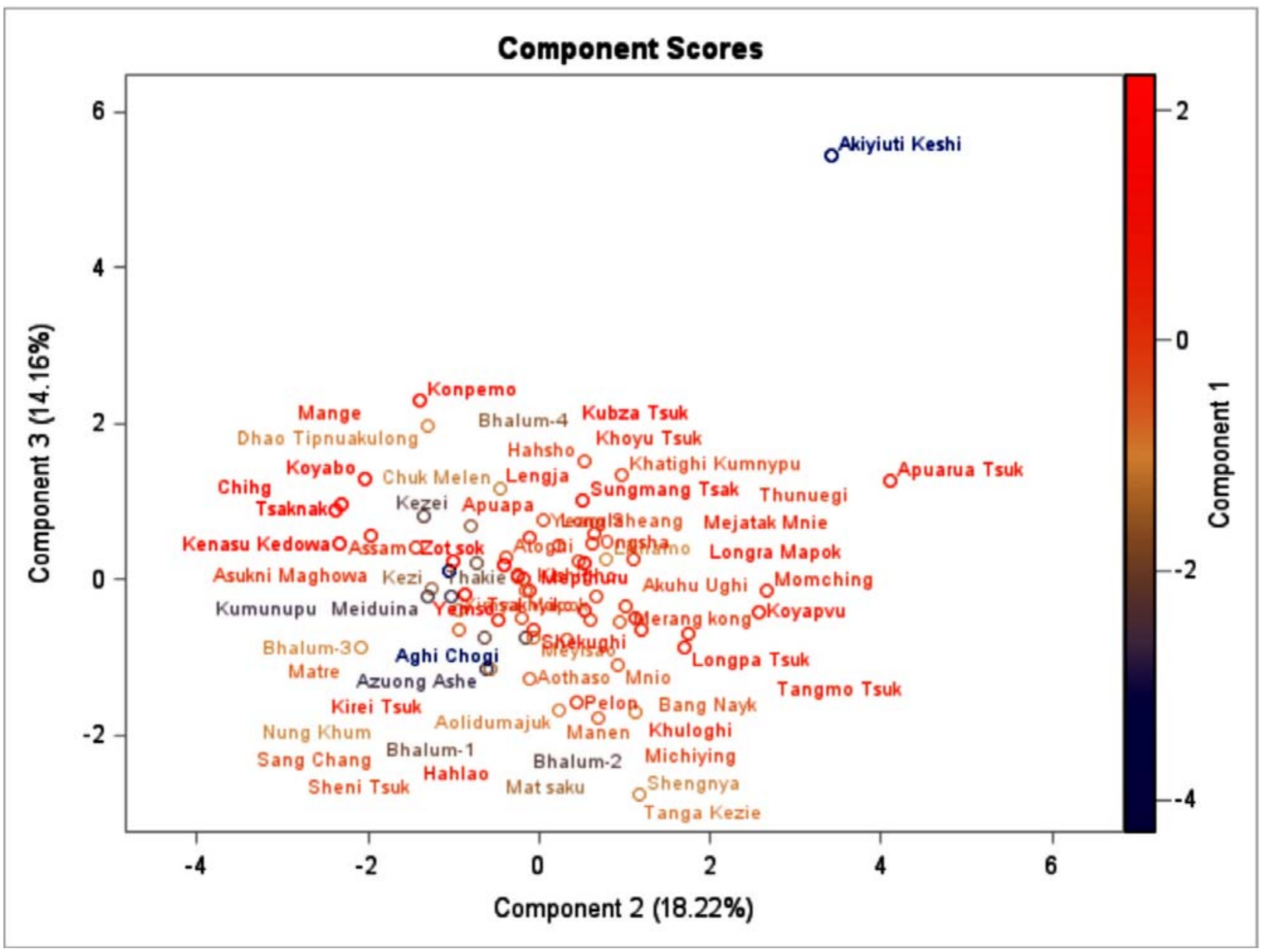


Fig. 2. A representation of *Jhum* paddy genotypes based on first three principal component scores

components accounted for 56.00% of the variance (Fig. 2). First PCA explained 23.62 % of the variation while second explained 18.22% of the variation. This could also be visualised by Scree plot (not given) indicating the significant components. Agronomic and yield related characteristics *viz*., plant height (0.58), panicle length (0.46), yield per ha (0.35) and days to fifty percent flowering (0.35) have greater weighting in principal component axis I while aluminium related characteristics like relative difference for root length (0.64), relative difference for shoot length (0.53), relative difference for shoot dry weight (0.32) and relative difference for root dry weight (0.21) have more weighting in principal component axis III. Ashfaq *et al.* [29] also found that the first PC was more related to plant height, panicle length, plant yield, heading days and maturity days in their studies with rice. Principal component II has similar trend of weightage for both agronomic and yield related characteristics (panicle length, 0.47 and ear bearing tillers, 0.35) and Aluminium related characteristics (relative difference for root length, 0.47 and relative difference for shoot length, 0.25). The principal components analysis in general confirmed the groupings obtained through cluster analysis.

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