CHARACTERIZATION OF RICE (*Oryza sativa* L.) GERMPLASM FOR EARLY SEEDLING VIGOUR-RELATED TRAITS UNDER DIRECT SEEDED CONDITION

Abstract

In pursuit of boosting rice production while maintaining economic feasibility within resource constraints, the shift from the traditional puddled-transplanted rice system to direct-seeded rice is gaining significance. Early seedling vigour (ESV) is an important character crucial for direct-seeded rice to outcompete and suppress weed growth. This study evaluated hundred rice germplasm accessions for their early seedling vigour-related traits. Mesocotyl and coleoptile lengths,chlorophyll 'a', chlorophyll 'b', total chlorophyll, alpha-amylase, speed of emergence, shoot length, root length, total seedling length, number of nodal roots, fresh and dry weights of both shoot and root, root network area and volume, and vigour indices exhibited notable variability as indicated by their high PCV and GCV. Moreover, they were predominantly under the influence of additive geneeffects, as illustrated by their high heritability and genetic advance as per cent of mean. This suggests that these traits are heritable and could be effectively exploited through selective breeding efforts.Positive correlations were found between seedling vigour and traits like emergence (%), speed of emergence, chlorophyll content, and alpha-amylase activity. Based on PCA,Vigour index-I, II, total seedling length, and chlorophyll contents were regarded as major discriminators in rice germplasm for direct seeded traits. The superior genotypes for seedling vigour could be used as donors in the rice breeding programs to develop direct seeded rice cultivars.

Key words: Direct seeded rice, Seedling vigour, correlation, variability, PCA

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops, serving as the primary dietary staplefor half of the global population[1]. With the ever-increasing global demandfor rice, maintaining rice production is of utmost importance.Worldwide, different systems ofrice cultivation are in vogue, among which the puddled-transplanted rice (TPR)has led to an increase in rice crop productivity [2]. Apart from the benefits of TPR, it consumes enormous water, labour and energy.As these resources are becoming scarce, rice production has become less profitable [3]. Consequently, it is important to enhance crop productivity while ensuring economic viability. Thus, the situation calls for a substantial change in the rice cultivation system, shifting from puddled-transplanted rice to direct-seeded rice.

Rice is extensively grown under irrigated as well as rainfed conditions. Among the rice ecosystems, irrigated rice holds the dominant position in terms of both cultivated area and production[1]. By 2025, approximately 17 million hectares of irrigated rice fields in Asia could face physical water scarcity, while 22 million hectares might encounter economic water scarcity [4]. Hence, optimizing water use in rice cultivation is imperative.As an alternative to the traditional transplanted system, direct-seeded rice (DSR) offers advantages such as reduced labour and water needs, enables crops to mature 7–14 days earlier, and results in reduced methane emissions from the field [5].

Despite these advantages, one of the major devastating threats associated with DSR is weed growth[6], and more than 20 % of attainable yield or even total losses occurwhen the situation is not controlled [7]. A crop plant exhibiting faster growth holds a competitive advantage over weed plants, leading to the inhibition of weed growth. The advancement of DSR continues to be impeded by challenges, and a significant one among them is the limited presence of suitable cultivars [8]. In practice, DSR cultivation employs cultivars that were originally developed and chosen for transplanting methods, which are semi dwarf and having poor early vigour. This mismatch leads to yield inconsistencies across diverse cultivation sites, primarily due to inadequate crop establishment and a high infestation of weeds[9]. The evaluation of appropriate characters for DSR cultivars becomes indispensable for the strategic development of future rice breeding programs.Thus, it is important for rice breeders to exploit the genetic variability present among the rice germplasm in terms of seedling vigor.In this scenario, the present study was conducted to evaluate hundred rice germplasm accessions for their early seedling vigour-related characters.

Materials and methods

A total of one hundred rice germplasm accessions (Table 1), obtained from Dr. Ramiah Gene bank, Department of Plant Genetic Resources, TNAU, Coimbatore, along with four check varieties suitable for irrigated ecosystem were evaluated for early seedling vigour-related traits under direct-seeded conditions at the Department of Plant Genetic Resources, TNAU, Coimbatore. The experiment was laid out in a completely randomized design with three replications. Polybags of size 30x28x25cm were filled with field soil and recommended basal dose of fertilizer was applied. Ten seeds of each genotype were direct sown in it, at a spacing of 10×5 cm and maintained weed free throughout the growing period by hand weeding. To investigate the variability among genotypes for early seedling vigour-related traits, various vegetative characteristics were examined. The emerged seedlings were counted from second day of sowing till the fourteenth day and emergence (%) and speed of emergence [10]were calculated.

$$\text{Speed of emergence} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \cdots + \frac{X_n - X_{n-1}}{Y_n}$$

Where Xn is the number of germinated seeds on nth day and Yn is the number of days from the first experimental day.

On 15 DAS (days after sowing), five seedlings from each replication were chosen for analyzing traits such as number of leaves, shoot length (cm), root length (cm), total seedling length (cm),number of nodal roots, shoot fresh and dry weights (g), root fresh and dry weights (g). Root network area (cm^2) and network volume (cm^3) were recorded through GiA Roots software (General Image Analysis of Roots) [11]. The same set of traits was also recorded on the 30th DAS, along with parameters like the number of tillers per plant and seedling vigour[12](scoring basedon standard evaluation system (SES) recommended by IRRI., 2013). Vigour index I and vigour index II were calculated to assess seedling vigour as suggested by Abdul-Baki and Anderson (1973)[13]

Vigour index I = Germination (%) × Total seedling length(cm)

Vigour index II = Germination (%) ×Seedling dry weight(g)

Chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll were extracted using DMSO from 30 days old seedlings[14,15] andcalculations were made based on Arnon’s equation.

Chlorophyll ‘a’ (mg/g fresh weight) = ((12.7*A_{663})- (2.69*A_{645})) *(V/1000*W)

Chlorophyll ‘b’ (mg/g fresh weight) = ((22.9*A_{645}) –(4.68*A_{663})) *(V/1000*W)

Total chlorophylls (mg/g fresh weight) = ((20.08*A_{645}) + (8.02*A_{663})) *(V/1000*W)

Alpha amylase activity was estimated from pre-germinated seeds [16]. Analysis of variance was carried out using R software (R version 4.3.1) and variability parameters such PCV, GCV[17], broad sense heritability[18]and genetic advance as percentage of mean[19]were calculated. Simple correlation among the different parameters contributing to the seedling vigour was performed. Principal Component Analysis (PCA) was done to comprehend how each trait contributes to the variation and how the genotypes are distributed along the axes of principal components usingGRAPES software (version 1.1.0)[20]

Table1. List of rice germplasm evaluated under the present study.

S. No.	Breeder assigned name	Vernacular name		S. No.	Breeder assigned name	Vernacular name
1	T 3446	Malagit		51	T 3985	Sang Do
2	T 3448	Milyang 23		52	T 3986	Sayllebon
3	T 3477	Mehr		53	T 3999	Sunbonnet
4	T 3488	Arabi		54	T 4014	Tox 1011-4-4
5	T 3489	Assey Y Pung		55	T 4023	Victoria
6	T 3497	Boa Vista		56	T 4030	WIR 1372-1
7	T 3501	British Honduras crede		57	T 4040	Yu nong 1
8	T 3508	Carolina gold		58	T 4046	Calady 40
9	T 3509	Caucasica		59	T 4054	Labelle
10	T 3523	Cuba 65		60	T 4058	Matuyamaomachi
11	T 3546	Gharib		61	T 4078	Bicotorto
12	T 3552	Honduras		62	T 4086	EL Paso L227
13	T 3580	Lemont		63	T 4092	Jing XI17
14	T 3598	Osogovka		64	T 4098	Lumbini
15	T 3615	Rathuwee		65	T 4100	Navolato
16	T 3631	Sufaid		66	T 4114	K.Haopahk maw
17	T 3649	Vavilovi		67	T 4125	Sambegeury
18	T 3658	Yodanya		68	T 4187	Sahelika
19	T 3667	Agami M1		69	T 4191	Tokambany
20	T 3738	Pihatuwee		70	T 4199	WAB 706-3-4-K4-KB1
21	T 3747	Seenetti		71	T 4207	WAS 207-B-B-3-I-I
22	T 3764	Weda heenati		72	T 4215	Malagkit
23	T 3778	Assay-Pung		73	T 4254	Jorge valladres
24	T 3787	Gunja		74	T 4256	Kaduma
25	T 3790	Khao Do ngoi		75	T 4319	Harbhoondi
26	T 3804	JagliBoro		76	T 4331	Surmaniya
27	T 3821	Ausjhari		77	T 4350	ARC 12451
28	T 3822	Bagananasalao		78	T 4372	Ram Tulasi
29	T 3829	Belle patna		79	T 4392	ARC 15872
30	T 3836	Calrose		80	CB-T 1509	Kuruva 24. valasumundon
31	T 3844	Cirad		81	CB-T 1351	Cheerachampam
32	T 3855	Drago		82	CB-T 1399	Puthuvithu
33	T 3858	Elvo		83	CB-T 1405	Thovan brown
34	T 3868	Guatemala 1021		84	CB-T 1770	Kalar palai
35	T 3875	Hong jeong		85	CB-T 7	Chirumalairayan
36	T 3885	IRAT 170		86	CB-T 34	Krishna neelabhatta

37	T 3889	Jamaica 3		87	CB-T 51	Sakulathisannabhatta
38	T 3892	Jibo ya		88	CB-T 1464	Thekkan
39	T 3900	K. Hao kap sang		89	CB-T 1929	Kinthu samba
40	T 3904	Komal bhog		90	CB-T 1937	Kanaka samba
41	T 3911	Kullu		91	ADT-T 216	White sirumani
42	T 3912	Kuruwee		92	ADT-T 355	Nalla konamani
43	T 3922	Lichuan Da Bai Gu		93	ADT-T 366	Omachi
44	T 3928	Mahsuri		94	ADT-T 1157	Perunkar
45	T 3935	Mat merah		95	ADT-T 1915	BT 154 Baramani
46	T 3948	Muttu samba		96	ADT-T 1967	Jayporesannam
47	T 3957	Nungyu 1511		97	ADT-T 2060	Radhuripagal
48	T 3964	Pecos		98	ADT-T 2221	Bhasamanick
49	T 3969	Popong		99	ADT-T 183	Kodai kulathan
50.	T 3976	Rikuto norin 264		100	ADT-T 1340	Rajah kazhama
Checks						
101.	CO 52					
102.	CO 54					
103.	ADT54					
104.	CR1009 Sub 1					

Results and discussion

Analysis of variance showed significant differences among the genotypes for all the charactersstudied, illustrating the presence of high variability among the germplasm lines for seedling vigour related traits in turn signifying ample scope for selection. Seedlings were scored for ESV on 30 DAS. Out of the 100 genotypes studied, nearly half (52 genotypes) of them recorded ESV score of 1 which is classified as highly vigorous. Twenty four genotypes were normal withfour leaves and no tillers. Genotypes 86 (*Krishna Neela bhatta*) and 93 (*Omachi*) were very weak with stunted growth and yellowing. All the check varieties were normal with a score of 5. Genotype 11 (*Gharib*) showed the highest value for number of tillers, leaves, nodal roots and root network area at 30 DAS. At the same day, longest shoot length and root length were recorded by genotype 12 (*Honduras*) (16.09 cm and 39.08 cm respectively) and shortest (4.52 cm) shoot length by 93 (*Omachi*) and root length (5.11 cm) by 86 (*Krishna Neela bhatta*). Highest (0.71 g) and lowest (0.03 g) shoot dry weights at 30 DAS were recorded by genotype 18 (*Agami M1*) and 36 (*Jamaica 3*) respectively. The parameters vigour index I and II at 30 DAS were highest (5148.9 and 74.41 respectively) in genotype 12 (*Honduras*), whereas VI-I was lowest (652.4) in genotype 93 (*Omachi*) and VI-II (4.73) in genotype 36 (*Jamaica 3*).

The mean and variability parameters are given in Table 2. Traits *viz.*, mesocotyl and coleoptile lengths, chlorophyll ‘b’, total chlorophyll, alpha amylase, speed of emergence, shoot length, root length, total seedling length, number of nodal roots, fresh and dry weights of both shoot and root, root network area and volume, and vigour indices, irrespective of their days of observation, and number of leaves, tillers and ESV at 30 DAS exhibited considerable amount of variation as suggested by their high PCV and GCV values. Furthermore, these traits were predominantly controlled by additive gene effects as they had a high heritability coupled with high genetic advance as percentage of mean. These results were consistent with the findings of [1] and [21]. Seedling length had high heritability and GAM as per the observations of [22]. All the traits studied had higher PCV than GCV indicating the influence of environment. Number of leaves at 15 DAS and emergence (%) showed low GCV and moderate PCV which implied less variation and high influence of environment on these traits.

Table 2. Mean, range and genetic parameters for seedling vigour related traits among rice germplasm

S.No.	Character	Mean	Min.	Max.	PCV (%)	GCV (%)	H(%)	GAM
1.	Mesocotyl length (cm)	0.22	0	2.63	124.98	121.66	94.75	243.96
2.	Coleoptile length (cm)	1.29	0.44	2.39	29.86	25.33	71.92	44.25
3.	Chlorophyll ‘a’ (mg/g fresh wt.)	2.43	0.68	3.69	21.02	19.82	88.92	38.5
4.	Chlorophyll ‘b’(mg/g fresh wt.)	0.85	0.32	1.42	29.33	25.57	76	45.92
5.	Total chlorophyll (mg/g fresh wt.)	3.28	0.99	5.18	21.01	20.56	95.75	41.44
6.	Alpha amylase (mg maltose min^{-1})	5.49	0.2	11.07	31.21	30.86	97.75	62.85
7.	Emergence %	83.46	60	96.67	12.22	7.81	40.83	10.28
8.	Speed of emergence	1.65	0.65	3.2	23.17	22.09	90.88	43.38
15 DAS								
9.	No. of leaves	2.7	2.2	3.67	13.27	8.92	45.21	12.36
10.	Shoot length (cm)	5.66	3.26	9.28	22.07	21.18	92.11	41.87
11.	Root length (cm)	7.18	3.06	12.22	26.48	25.18	90.4	49.31
12.	Total seedling length (cm)	12.85	6.68	19.21	20.07	19.34	92.9	38.4
13.	No. of nodal roots	4.46	1.47	8	33.76	31.32	86.07	59.85
14.	Fresh shoot wt. (g)	0.104	0.042	0.616	67.53	66.46	96.85	134.72
15.	Dry shoot wt. (g)	0.019	0.01	0.088	56.1	55.17	96.69	111.75
16.	Fresh root wt. (g)	0.033	0.012	0.275	83.59	82.79	98.08	168.89
17.	Dry root wt. (g)	0.007	0.002	0.04	71.52	65.4	83.61	123.19
18.	Root network area (cm^2)	2.57	0.76	8.30	51.12	50.77	98.62	103.86
19.	Root network volume (cm^3)	0.14	0.02	0.94	112.53	111.88	98.85	229.15
20.	Vigour index-I	1070.12	580.55	1664.87	21.82	21.16	94.03	42.27
21.	Vigour index-II	2.16	0.815	7.25	52.94	52.31	97.6	106.45
30 DAS								
22.	No. of leaves	6.62	2.6	16.27	41.17	40.8	98.2	83.29

23.	No. of tillers	1.68	0	7.6	84.58	82.79	95.81	166.93
24.	ESV (SES score)	8.24	9	1	25.4	22.44	78.03	40.83
25.	Shoot length (cm)	9.13	4.52	16.09	24.82	24.61	98.35	50.28
26.	Root length (cm)	14.64	5.11	39.08	39.89	39.7	99.09	81.41
27.	Total seedling length (cm)	23.77	9.93	55.17	31.45	31.33	99.27	64.31
28.	No. of nodal roots	7.26	2	15.33	36.29	35.57	96.07	71.81
29.	Fresh shoot wt. (g)	0.781	0.11	2.48	64.26	64.21	99.85	132.17
30.	Dry shoot wt. (g)	0.225	0.03	0.71	64.25	64.15	99.68	131.94
31.	Fresh root wt. (g)	0.236	0.03	1.32	87.27	87.19	99.81	179.44
32.	Dry root wt. (g)	0.041	0	0.22	86.3	86.16	99.66	177.18
33.	Root network area (cm^2)	13.49	4.75	70.48	71.02	70.67	99.02	144.86
34.	Root network volume (cm^3)	1.25	0.2	3.52	63.68	63.42	99.16	130.09
35.	Vigour index-I	1998.4	652.4	5148.8 9	35.73	35.63	99.42	73.18
36.	Vigour index-II	22.65	4.73	74.41	66.96	66.88	99.77	137.6

Figure 1. depicts the correlation among the ESV and related traits. There was significant positive correlation between ESV and all the traits, except mesocotyl and coleoptile lengths. In the study by [23] also, coleoptile length was not significantly correlated with emergence (%) which is indeed a contributing factor to ESV. Vigour index- I showed significant positive correlation with all traits except mesocotyl length while vigour index- II was correlated with all other traits except mesocotyl length, coleoptile length and chlorophyll ‘b’ content. As observed in the present study, [24] reported significant and positive correlation of Vigor Index -I and Vigor Index -II with germination percentage, seedling shoot length, root length, fresh weight, dry weight, number of leaves and total seedling length. Mesocotyl length exhibited no correlation with any of the traits. Emergence percentage, speed of emergence, alpha amylase activity and total chlorophyll content were found to be significantly correlated with ESV, Vigour index-I and Vigour index-II in positive direction. As per the report of [25], amylase activity was a reliable indicator of rice seed’s germination and seedling vigour. Germination rate, amylase activity, and chlorophyll content were significantly correlated with ESV in rice according to [26,27,28]. The absence of significant correlation of coleoptile length with ESV, emergence (%) or speed of emergence agreed with the findings of [29].

Traits such as number of leaves and tillers, ESV, shoot, root and total seedling lengths, number of nodal roots, fresh and dry shoot weights, dry root weight, root network area, network volume, VI-I and VI-II, were inter correlated in a significantly positive manner which

conforms

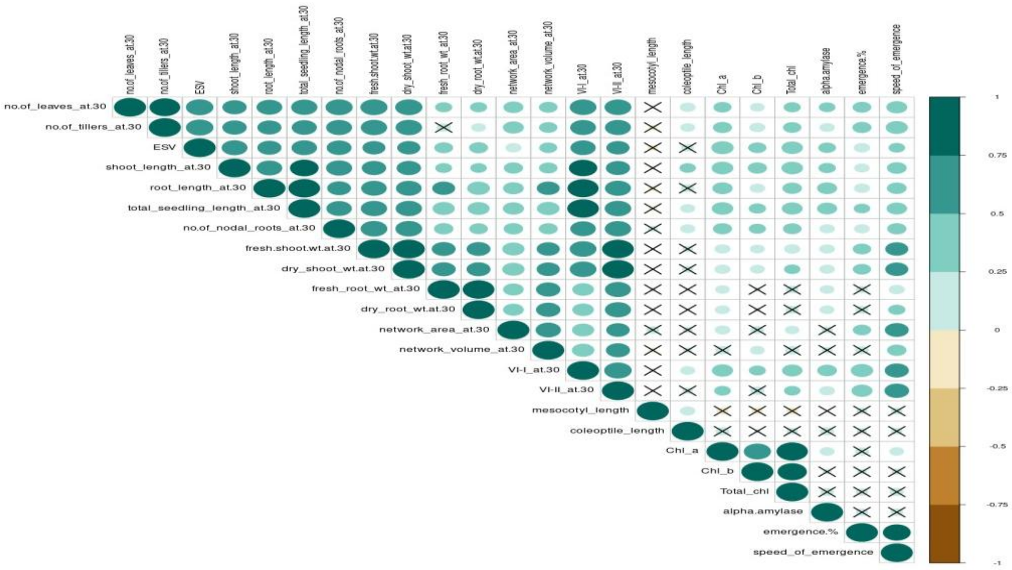
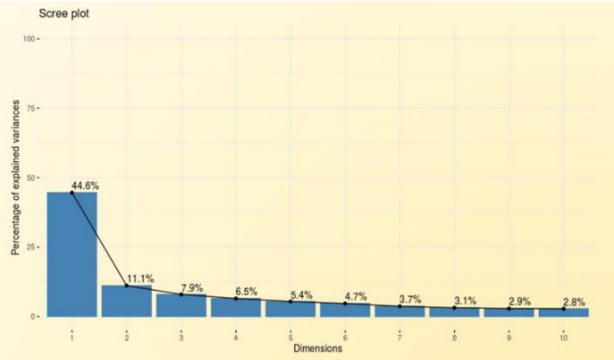


Figure1. Correlogram showing the correlation among the ESV traits studied. (Non-significant correlations

with the findings of [30]. None of the traits under study exhibited significant negative correlation. Hence, traits *viz.*, number of leaves and tillers, shoot, root and seedling lengths, number of nodal roots, fresh shoot and root weights, dry shoot and root weights, root network area and volume, chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll contents, alpha amylase activity, emergence (%) and speed of emergence can be used as selection indices for the improvement of early seedling vigour in rice as per correlation analysis.

A)



B)

Principal components	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC1	10.258	44.599	44.599
PC2	2.55	11.087	55.687
PC3	1.824	7.93	63.617
PC4	1.492	6.487	70.104
PC5	1.236	5.375	75.479
PC6	1.071	4.657	80.135

C)

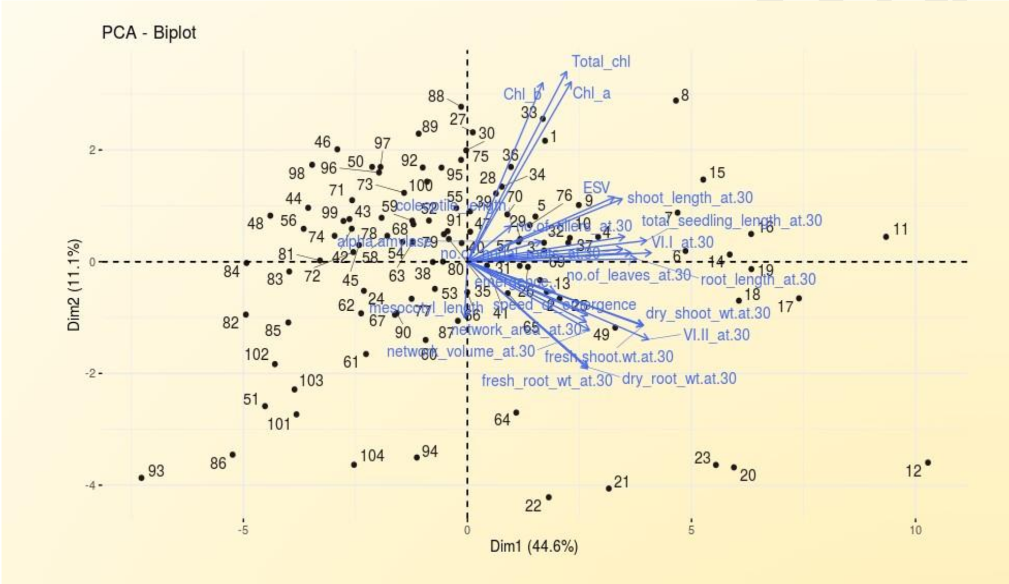


Figure 2. Principal component analysis (PCA)based on seedling vigour-related traits of rice germplasm. **A)**Scree plot representing the percentage contribution of PCs.**B)** Percentage and cumulative percentage contribution of principal components to the total variation. **C)** Projection of variables. (Genotypes are illustrated as numbers (black) given in table 1

In order to understand the trends among low and high early seedling vigour genotypes and the traits that are contributing to the observed variability, principal component analysis was performed for all 104 genotypes (Figure 2). On the cultivar-by-trait biplot based on first two principal components, rice genotypes were scattered as groups, with seedling vigour related traits shown as vectors.

The first six principal components with >1.0 Eigen value together accounted for the 80.13% of the total variation, of which PC1 had the most significant contribution of 44.6%. The other components in order *viz.*, PC2 to PC6 contributed for11.09%, 7.93%, 6.48%, 5.37%, and 4.66% of the variation individually. Variation in PC1 was mainly attributed to Vigour index-I, II and total seedling length which was in accordance with [1] for which the Eigen values were 7.96, 7.72 and 7.56 respectively. PC2 was highly related to total chlorophyll (22.07%), chlorophyll ‘a’ (19.74%) and chlorophyll ‘b’ (19.55%). Moreover, they were considered as the major discriminators due to long vector lengths. Similar to our findings, shoot length was identified as one of the major discriminators in the study of [30], and seedling vigour index-I and II were major contributors to the total variation as per [31]. Phenotypic diversity among the genotypes was illustrated by their distribution along the axes, where the genotypes that clustered together on the biplot exhibiteda similar diversity composition. Highest estimates were recorded in the genotypes 7,8,15 (*British Honduras crede*, *Carolina gold*, *Sufaid*) representing high ESV values. Minimum values of ESV were recorded by 93 (*Omachi*) and 86 (*Krishna neelabhatta*). Genotype 11(*Gharib*) had high values for the traits represented by PC1 and PC2 and it stands out, in terms of these traits and has a unique combination of trait values. Genotype 12 (*Honduras*) had greater values for Vigour index-I and II, shoot length and root length as the respective vectors point toward it and also farther from the origin. Genotype 93 and 86 (*Omachi* and *Krishna neelabhatta*) can be concluded as poor in terms of ESV as they were plotted farther in opposite direction of the ESV vector.

Conclusion

The study found significant variability among the germplasm for the evaluated traits*viz.*,mesocotyl and coleoptile lengths, chlorophyll 'b', total chlorophyll, alpha-amylase, speed of emergence, shoot length, root length, total seedling length, number of nodal roots, fresh and dry weights of both shoot and root, root network area and volume, and vigour indices indicating potential for selection to develop rice genotypes suitable for DSR condition. As these traits were predominantly controlled by additive gene effects, the germplasm with desired traits can be exploited in hybridization programs to fix the new recombinants with desirable traits. Positive correlations were observed between seedling vigour and various traits like emergence, speed of emergence, chlorophyll content and alpha-amylase activity.Based on PCA, certain traits like vigour index-I, II, total seedling length, and chlorophyll contents were identified as major discriminators in direct seeded rice germplasm. The research suggests that superior genotypes for seedling vigor could be used as donors in rice breeding programs to develop genotypes specifically suited to direct seeding in rice.

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