International Journal of Horticulture and Food Science 2022; 4(1): 187-191

E-ISSN: 2663-1067
P-ISSN: 2663-1075
https://www.hortijournal.com
IJHFS 2022; 4(1): 187-191
Received: 18-01-2022
Accepted: 19-02-2022

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Using Agumented design for screening of cold tolerant rice genotypes for seedling traits under low temperature condition [*Oryza sativa* (L.)]

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DOI: https://doi.org/10.33545/26631067.2022.v4.i1c.146

Abstract
An experiment was conducted with the objective of determining the magnitude of association between seedling survival rate and its component characters. 100 MAGIC indica population rice genotypes were evaluated in Augmented Randomized complete Block Design (RCBD) with two replications. Correlation coefficient analysis of seedling survival rate showed positive and significant association with seedling height, root length; shoot length, under cold condition. Thus, these traits could play pivotal role in developing cold tolerant rice. Separation of correlation coefficients into direct and indirect effects of component traits for seedling survival rate revealed that the traits seedling height and shoot length exerted maximum positive direct and indirect effect respectively on seedling survival under cold stress conditions. These characters, therefore, are required to be considered during selection for seedling survival improvement in rice under cold conditions.

Keywords: Seedling survival rate, augmented block design, correlation

Introduction

Rice (*Oryza sativa* L.), is a model plant that feeds more than half of the world's population, (Sasaki and Burr, 2000) [18], evolved in tropical and subtropical climates and is sensitive to chilling stress With a total cultivated area of about 162.06 million hectares, an annual harvest of more than 700 million tonnes (495.78 million tonnes of milled rice) and 3.9 tonnes ha-1 productivity, serving over 3.5 billion people in the world and accounting for 20% of total daily calorie intake. In India, rice is cultivated in 43.77 million hectares with production of 118.9 million tonnes and productivity of 2722 kg ha-1(Indiastat, 2019-2020). The Food and Agriculture Organization of the United Nations (FAO) estimates that global food production must be increased by 70% by 2050 to meet the food requirements for the estimated global population of ~ 9.7 billion in 2050.

Low temperature (cold) is one of the main abiotic stresses in rice cultivation and improvement of cold tolerance in the Seedling Stage (CTSS) in rice is a difficult trait controlled by several genes (Dilday 1990 and Glaszmannand Khush 1990) [10]. Cold stress in rice delays germination and emergence, however, the successive stages of germination (*i.e.*, growth of coleoptiles and radical) are the most vulnerable phases to cold spell (Yoshida, 1981) [21].

For breeding cold tolerant rice variety, selection for tolerance requires a suitable and cost effective evaluation and screening technology. Evaluation for cold tolerance during seedling stage under field conditions is limited by environmental variation, which makes it difficult to identify genetically superior lines. Evaluation of cold tolerance during germination and seedling stage under controlled temperature conditions may be performed by assessing percentage of reduction in coleoptile length and coleoptile growth. Even after three decades of the release of variety Tellahamsa, a ruling variety for its cold tolerance during seedling stage, no other varieties developed are able to replace it and progress was inadequate in this direction to tackle this problem. This may be attributed to lack of basic information on the nature and degree of cold tolerance, especially during seedling to vegetative stage.

Correlation and path analysis establish the extent of association between the traits of interest and its attributes and brings out the relative importance of direct and indirect effects, giving an obvious understanding of their association with the interested traits (Babu *et al*. 2012). The advantage of path analysis is that it permits the partitioning of the correlation coefficient into its components. One component is the path coefficient (or standardized partial regression coefficient) that measures the direct effect of a predictor variable upon its response variable. The other component is the indirect effect (s) of a predictor variable on the response variable through the predictor variables (Dewey and Lu 1959). Considering the above facts a laboratory study was conducted to identify best performing rice genotypes under cold stress at seedling stage as high seed and seedling vigour are good indicators for a successful crop.

Materials and Methods

The experiment was conducted at College farm, PJTSAU, Rajendranagar, Hyderabad, India, during *Rabi*, 2021, to evaluate rice genotypes for cold tolerance based on seedling parameters as per Cruz and Milach (2004) [4]. The experimental material comprised of 100 MAGIC *indica* population with 5 checks (Resistant-Tellahamsa, SR3. Susceptiblechecks-BPT5204, RNR2465 and RNR15048) presented in (Table1) laid out in Augmented Randomized complete Block Design (RCBD). The design consisted of 5 blocks containing 20 genotypes in each with 105 test entries and five check entries with two sets. In each block the checks were allotted randomly. Maintain a temperature at 4 ºC for cold stress and 26 ºC for recovery period to create appropriate environment to phenotypic screening. Five representative plants for each population were randomly selected to record observations on the quantitative characters under study.

Table 1: List of 105 MAGIC *indica* population used in the study

S .No	Name of Genotype
1	TELLAHAMSA
2	SR3
3	JGL1798
4	RNR15048
5	BPT5204
6	IR 93327:1-B-7-21-25-1RGA-2RGA-1-B-B
7	IR 93334:8-B-10-8-4-1RGA-2RGA-1-B-B
8	IR 93333:4-B-8-19-8-1RGA-2RGA-1-B-B
9	IR 93358:3-B-3-12-11-1RGA-2RGA-1-B-B
10	IR 93329:11-B-18-22-16-1RGA-2RGA-1-B-B
11	IR 93331:33-B-19-20-17-1RGA-2RGA-1-B-B
12	IR 93348:15-B-16-20-21-1RGA-2RGA-1-B-B
13	IR 93329:54-B-10-20-23-1RGA-2RGA-1-B-B
14	IR 93356:36-B-3-5-19-1RGA-2RGA-1-B-B
15	IR 93329:44-B-8-20-4-1RGA-2RGA-1-B-B
16	IR 93331:9-B-10-10-12-1RGA-2RGA-1-B-B
17	IR 93329:10-B-15-15-2-1RGA-2RGA-1-B-B
18	IR 93335:21-B-20-23-8-1RGA-2RGA-1-B-B
19	IR 93331:33-B-19-20-17-1RGA-2RGA-1-B-B
20	IR 93330:37-B-9-15-16-1RGA-2RGA-1-B-B
21	IR 93327:3-B-13-7-5-1RGA-2RGA-1-B-B
22	IR 93343:14-B-12-5-20-1RGA-2RGA-1-B-B
23	IR 93347:12-B-3-5-8-1RGA-2RGA-1-B-B
24	IR 93327:17-B-5-13-12-1RGA-2RGA-1-B-B
25	IR 93334:47-B-23-16-12-1RGA-2RGA-1-B-B
26	IR 93328:21-B-13-15-18-1RGA-2RGA-1-B-B
27	IR 93329:53-B-4-21-23-1RGA-2RGA-1-B-B
28	IR 93327:42-B-17-17-13-1RGA-2RGA-1-B-B
29	IR 93326:25-B-6-13-22-1RGA-2RGA-1-B-B
30	IR 93334:1-B-20-12-9-1RGA-2RGA-1-B-B
31	IR 93330:15-B-16-11-18-1RGA-2RGA-1-B-B
32	IR 93328:27-B-19-8-21-1RGA-2RGA-1-B-B
33	IR 93327:43-B-16-24-8-1RGA-2RGA-1-B-B
34	IR 93329:45-B-14-13-10-1RGA-2RGA-1-B-B
35	IR 93327:36-B-23-8-15-1RGA-2RGA-1-B-B
36	IR 93332:23-B-9-8-18-1RGA-2RGA-1-B-B
37	IR 93328:39-B-15-23-14-1RGA-2RGA-1-B-B
38	IR 93327:27-B-8-19-6-1RGA-2RGA-1-B-B
39	IR 93327:19-B-21-15-23-1RGA-2RGA-1-B-B
40	IR 93327:7-B-16-4-9-1RGA-2RGA-1-B-B
41	IR 93333:42-B-17-20-20-1RGA-2RGA-1-B-B
42	IR 93327:19-B-21-15-23-1RGA-2RGA-1-B-B
43	IR 93331:24-B-17-16-18-1RGA-2RGA-1-B-B
44	IR 93331:24-B-17-16-18-1RGA-2RGA-1-B-B
45	IR 93329:61-B-21-12-21-1RGA-2RGA-1-B-B
46	IR 93326:23-B-4-8-12-1RGA-2RGA-1-B-B
47	IR 93330:49-B-14-19-16-1RGA-2RGA-1-B-B
48	IR 93328:13-B-8-6-23-1RGA-2RGA-1-B-B
49	IR 93327:24-B-7-12-12-1RGA-2RGA-1-B-B
50	IR 93328:39-B-15-23-14-1RGA-2RGA-1-B-B
51	IR 93326:18-B-12-7-15-1RGA-2RGA-1-B-B
52	IR 93327:34-B-12-5-22-1RGA-2RGA-1-B-B
53	IR 93329:7-B-11-6-14-1RGA-2RGA-1-B-B
54	IR 93334:45-B-22-7-15-1RGA-2RGA-1-B-B
55	IR 93330:46-B-16-18-17-1RGA-2RGA-1-B-B
56	IR 93337:10-B-20-6-19-1RGA-2RGA-1-B-B
57	IR 93334:61-B-13-16-19-1RGA-2RGA-1-B-B
58	IR 93329:61-B-21-12-21-1RGA-2RGA-1-B-B
59	IR 93335:30-B-13-3-11-1RGA-2RGA-1-B-B
60	IR 93328:28-B-23-13-21-1RGA-2RGA-1-B-B
61	IR 93331:17-B-12-14-20-1RGA-2RGA-1-B-B
62	IR 93327:3-B-13-7-5-1RGA-2RGA-1-B-B
63	IR 93329:36-B-21-9-14-1RGA-2RGA-1-B-B
64	IR 93335:37-B-3-3-13-1RGA-2RGA-1-B-B
65	IR 93327:36-B-23-8-15-1RGA-2RGA-1-B-B
66	IR 93328:25-B-19-12-15-1RGA-2RGA-1-B-B
67	IR 93330:47-B-13-9-5-1RGA-2RGA-1-B-B
68	IR 93328:19-B-11-12-6-1RGA-2RGA-1-B-B
69	IR 93326:24-B-11-8-16-1RGA-2RGA-1-B-B
70	IR 93327:27-B-8-19-6-1RGA-2RGA-1-B-B
71	IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B
72	IR 93332:26-B-22-17-18-1RGA-2RGA-1-B-B
73	IR 93326:9-B-21-3-4-1RGA-2RGA-1-B-B
74	IR 93330:36-B-25-23-15-1RGA-2RGA-1-B-B
75	IR 93328:22-B-14-15-6-1RGA-2RGA-1-B-B
76	IR 93329:39-B-12-16-8-1RGA-2RGA-1-B-B
77	IR 93331:25-B-20-16-19-1RGA-2RGA-1-B-B
78	IR 93327:11-B-8-8-17-1RGA-2RGA-1-B-B
79	IR 93335:26-B-9-20-3-1RGA-2RGA-1-B-B
80	IR 93328:23-B-23-11-17-1RGA-2RGA-1-B-B
81	IR 93329:51-B-18-1-21-1RGA-2RGA-1-B-B
82	IR 93326:18-B-12-7-15-1RGA-2RGA-1-B-B
83	IR 93336:50-B-5-13-20-1RGA-2RGA-1-B-B
84	IR 93327:1-B-7-21-25-1RGA-2RGA-1-B-B
85	IR 93328:40-B-15-25-16-1RGA-2RGA-1-B-B
86	IR 93326:17-B-8-5-7-1RGA-2RGA-1-B-B
87	IR 93336:15-B-10-9-18-1RGA-2RGA-1-B-B
88	IR 93337:4-B-19-10-19-1RGA-2RGA-1-B-B
89	IR 93334:45-B-22-7-15-1RGA-2RGA-1-B-B
90	IR 93335:32-B-16-20-13-1RGA-2RGA-1-B-B
91	IR 93329:59-B-14-25-20-1RGA-2RGA-1-B-B
92	IR 93328:27-B-19-8-21-1RGA-2RGA-1-B-B
93	IR 93326:24-B-11-8-16-1RGA-2RGA-1-B-B
94	IR 93333:40-B-12-12-22-1RGA-2RGA-1-B-B
95	IR 93335:48-B-15-5-15-1RGA-2RGA-1-B-B

96	IR 93327:24-B-7-12-12-1RGA-2RGA-1-B-B		101	IR 93328:20-B-8-22-7-1RGA-2RGA-1-B-B
97	IR 93336:23-B-15-21-6-1RGA-2RGA-1-B-B		102	IR 93327:5-B-15-3-8-1RGA-2RGA-1-B-B
98	IR 93333:18-B-19-7-16-1RGA-2RGA-1-B-B		103	IR 93327:43-B-16-24-8-1RGA-2RGA-1-B-B
99	IR 93327:6-B-17-5-20-1RGA-2RGA-1-B-B		104	IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B
100	IR 93329:51-B-18-1-21-1RGA-2RGA-1-B-B		105	IR 93328:15-B-8-12-14-1RGA-2RGA-1-B-B

Resistant checks -Tellahamsa, SR3 Susceptible checks - JGL1798, BPT 5204

Statistical analysis

The analysis of variance was done using R software. We considered seven quantitative traits related to seedling survival rate namely cold tolerance scoring, chlorophyll content, root length, shoot length, seedling height, seedling survival rate and cold stress tolerance index. Correlation coefficients were calculated at genotypic and phenotypic level using the formulae suggested by Falconer (1964) [7].

Results and Discussion

Analysis of augmented design

Augmented randomized complete block design (Federer, 1956) [22] is a method of choice to undertake initial evaluation of a large set of germplasm accessions to select genotypes suitable for different aspects of crop breeding. This is all the more important in cases where initial seed is limited in quantity to undertake replicated experiments as well as our failure to ensure comparably homogenous experimental units which is a basic requirement of standard field designs. The design makes use of a procedure wherein a large number of test entries to be evaluated are evaluated along with standard checks, with the checks being replicated randomly in all blocks. The data from checks is used to adjust mean values of test entries to make them comparable and also provide an estimate of experimental error.

In the present study, 100 test entries along with 5 checks were evaluated in an augmented block design for seedling survival rate and its component traits. The mean values (Table 2) of cold tolerance scoring, chlorophyll content, root length (cm),shoot length(cm), seedling height(cm), seedling survival rate (%) and cold stress tolerance index were 3.28+1.15, 20.71+2.88, 5.3 +, 1.29, 12.31+0.96,17.4+1.18, 76.9+6.93 and 18.06+3.54, respectively.

The range for these traits was 0.88-6, 11.8-26.06, 3.63-13.93, 10.12-15.12, 14.08-20.48, 52.991.6 and 10.26-25.65, respectively. The highest value of co-efficient of variation (C.V) was found in case of cold tolerance scoring (13.37%) followed by seedling survival rate (3.26%) and root length (2.84%) while as seedling height (1.41%) and shoot length (0.92%) and chlorophyll content (0.8%) had lower C.V values. Rana *et al*. (2015) [17] evaluated 4274 germplasm accession of common bean from 58 countries and observed substantial variability for the 22 traits studied.

Table 2: Descriptive statistics of seedling stage related traits in 100 genotypes of magic indica population Trait Mean ± SD Range CV (%)

Trait	Mean±SD	Range	CV%
Cold tolerance scale	3.17±1.24	0.88-6	13.37
Chlorophyll content	20.71±2.88	11.8-26.06	0.8
Root length	5.3±1.29	3.63-13.93	2.56
Shoot length	12.31±0.96	10.12-15.12	0.92
Seedling height	17.4±1.18	14.08-20.48	1.41
Cold stress tolerance index	18.06±3.54	10.26 -25.65	0.96
Seedling survival rate	76.9±6.93	52.9 -91.6	3.26

The Analysis of variance (Table 3) revealed significant mean sum of squares for all traits for different sources of variation. The Block effect (unadjusted) and the treatment effects (adjusted as well as unadjusted) were significant for all the traits. Similarly the effects due to checks and varieties were significant. However, the adjusted block effects were nonsignificant for all traits indicating homogeneity of evaluation blocks. Similarly, the mean square due to checks v/s varieties was significant for all the traits indicating thereby that the test entries were significantly different from checks.

Table 3: Analysis of variance of augmented block design for seven quantitative traits in genotypes of MAGIC indica population

Source of variation	d.f	CTS	CC	RL	SL	SH	CSTI	SSR
Block (ignoring Treatments)	4	0.97**	5.04**	1.22**	1.1**	2**	18.81**	38.29**
Treatment (eliminating Blocks)	104	2.08**	9.22**	1.62**	1.36**	2.05**	12.28**	58.93**
Checks+ varieties vs. varieties	100	1.4	8.08	1.67	0.94**	1.2**	12.24**	42.15**
Block (eliminating Check+ var.) (adj)	4	0.26	0.09	0.05	0.03	0.03	0.04	4.4
Entries (ignoring Blocks) (adj)	104	2.11**	9.41**	1.66**	1.4**	2.12**	13**	60.23**
Checks	4	19.06**	37.7**	0.33**	11.86**	23.13**	13.26**	478.4**
Varieties	99	1.42**	8.32**	1.73**	0.81**	1.22**	13.03**	43.92**
Checks vs. Varieties	1	2.18**	4.36**	0.05**	17.6**	7.03**	9.12**	2.05*
Error	16	0.19	0.03	0.02	0.01	0.06	0.03	6.3

CTS - Cold tolerance scoring, CC- Chlorophyll content, RL- Root length, SL- Shoot length, CSTI -Cold stress tolerance index, SSR- Seedling survival rate

The results on character associations between seedling survival rate, seedling survival rate components characters are presented in Table 4. A perusal of these results revealed positive and significant association of seedling survival rate with seedling height indicating scope for simultaneous improvement of these traits. The results are in agreement with the reports of Prasad *et al*. (2013) [16] and Ji *et al.* (2008) [13] for seedling height under stress conditions.

Positive and significant associations were also noticed for cold tolerance scoring with root length and cold stress tolerance index Cruz and Milach (2013) [5], chlorophyll content with seedling height and shoot length Farzin *et al.* (2013) [8], shoot length with seedling height Siddi *et al.* (2020) [20].

Negative and significant associations were in contrast observed for cold tolerance scale with chlorophyll content

Biswas *et al.* (2017) [3], chlorophyll content with cold stress tolerance index Kim *et al*. (2009) [14], root length with shoot length. A perusal of these results indicated the need for balanced selection while effecting simultaneous improvement for these traits.

Selections based on the results of correlation coefficient analysis may not often produce the desired result and hence the study of path coefficient analysis for the estimates of degree of relationship is necessary. Path coefficient analysis allows the separation of the correlation coefficients into direct as well as indirect effects. The results on path analysis of trait of interest components are presented in Table 3.Shoot length had the greatest positive direct effect on seedling survival rate Sharif *et al*. (2010) [19], followed by seedling height Allen and Ort (2001) [1], indicating that selection for these characters was likely to result in a direct increase trait of interest. The trait root length had also recorded high positive and significant association with seedling survival rate, indicating the effectiveness of direct selection for this trait in improvement of trait of interest.

Table 4: Correlation coefficient analysis of seedling stage cold tolerance related traits under cold conditions

Character	CTS	CC	RL	SL	SH	CSTI	SSR
CTS	1.0000	-0.7888	0.0182	-0.5311**	-0.5628**	0.5972**	-0.7294
CC		1.0000	-0.013	0.5041**	0.5289**	-0.6066**	0.7258
RL			1.0000	-0.0507	0.2916***	-0.0583	0.0251
SL				1.0000	0.8817***	-0.4134**	0.5010
SH					1.0000	-0.4177**	0.5271
CTS						1.0000	-0.4833
SSR	-	-	-	-	-	-	-

CTS - Cold tolerance scoring, CC- Chlorophyll content, RL- Root length, SL- Shoot length, CSTI -Cold stress tolerance index, SSR-Seedling survival rate.

In current research, the residual effect is 0.514 showing that the characters involved in present study contributed only 10% of variability influencing to the dependent variable i.e., seedling survival rate. The higher residual value of in the study indicates that apart from the traits considered, there could be several other morphometric traits which could have a significant influence in expression of trait of interest.

Conclusion

All traits are exhibited significant association. Due to a significant and positive association with seedling survival rate, seedling height, is considered as a major character while selecting the genotypes for trait of interest. Direct positive association towards seedling survival rate was contributed by the traits chlorophyll content, root length, shoot length and seedling height indicating the importance of these traits as selection criteria for enhancing trait of interest.

Acknowledgements

The authors are thankful to IBT- Institute of Biotechnology and Prof. Jayashankar Telangana State Agricultural University, Hyderabad for providing the facilities and financial assistance for carrying out the present study.

This study is part of M.Sc. student thesis work of first author.

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