

## Phenotypic evaluation of seedling vigour-related traits in a set of rice lines

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### Abstract

Under the changing climatic conditions, depleting nutrients and water scarcity, the dry-direct seeded rice (DDSR) with an aerobic system of cultivation is gaining ground in India. The uniform establishment of seedlings is critically dependent on the seedling vigour and high seedling vigour provides better uptake of nutrients with uniform plant growth. With an aim to identify rice lines exhibiting high seedling vigour traits, we phenotyped the seedling vigour index-related traits *viz.*, germination percentage (GP), mesocotyl length (ML), coleoptile length (CL), root and shoot fresh and dry weights in a set of rice lines consisting of introgression lines, landraces, mutant lines and popular varieties using the paper towel method. ANOVA revealed high significant variation for all the traits including seedling vigour index-I (SVI-I) and seedling vigour index-II (SVI-II). Significant positive intercorrelation was recorded between two sub-traits for establishment of seedling *viz.* coleoptile length and mesocotyl length. The rice lines *viz.*, ATR-486, ATR-473, ATR-385, ATR-279, ATR-472, ATR-397, ATR-275, KK-12 and ATR-387 exhibited comparatively higher CL, ML, SVI-I and SVI-II than all the checks used in the present study. The identified lines exhibiting better seedling vigour traits are promising genetic resources that can be deployed in breeding programs for improving adaptability under DDSR conditions.

**Keywords:** Mesocotyl, coleoptile, early seedling vigour, seedling vigour index-I (SVI-I), seedling vigour index-II (SVI-II)

### Introduction

The uniform establishment of a crop is critically dependent on seedling vigour and seedling development (Singh *et al.*, 2017). Seedling vigour is determined by various traits *viz.*, coleoptile length, germination rate and early germination, so that weed competitiveness in the crop can be enhanced with the help of seedling vigour and associated traits (Okami *et al.*, 2011). Varieties having the maximum mesocotyl length are preferred for improving rice seedling emergence rate, predominantly under deep sowing and submergence, because mesocotyl length has the beneficial effect of vigour enhancement by pushing the buds from the rhizosphere soil.

Mesocotyl (an organ between the coleoptile node and the basal part of the seminal root) and coleoptile elongation are vital traits influencing seedling emergence and establishment in direct-seeded rice cultivation (Alibu *et al.*, 2012, Wang *et al.*, 2021). The rice lines with long mesocotyl have stronger germination and a higher rate of seedling emergence than those with short ones. Such rice lines have a better chance of overcoming the problems faced during direct seeding of rice like weed competitiveness, emergence rate, and poor seedling establishment (Zhang *et al.*, 2005; Alibi *et al.*, 2011, Zhan *et al.*, 2020). Rice lines with inherently longer coleoptile and mesocotyl perform better than those with shorter ones.



In the present study, we have screened phenotypically a set of rice lines (introgressed lines, mutant lines, landraces, and varieties) for seedling vigour index-related traits.

## Materials and Methods

The experimental material comprised of introgression lines, mutant lines, landraces and varieties (**Table 1**). The seeds were kept at 50°C for 48 hours to overcome possible dormancy, followed by rinsing with sterile water to ensure aseptic conditions and surface sterilized with 1% sodium hypochlorite solution for 15 minutes and later rinsed three times with sterile

water to ensure aseptic conditions. The brown paper towels, size (30 × 20 cm) were autoclaved and made wet with sterile distilled water. Ten surface-sterilized seeds were placed at equidistance on the wet brown paper towels in two replications (three plants in each replication) with a completely randomized design (CRD). The paper towels with seeds were covered with a polythene sheet to avoid wear and tear of paper towels and to avoid moisture loss. The paper towels with polythene cover were folded from both ends towards the centre and rubber bands were tied to ensure unfolding. Each setup was properly labelled

**Table 1. Rice lines used in the study**

Sl. No.	Rice lines	Biological status of accession
1	ATR-284	Azucena × KMR3
2	ATR-286	
3	ATR-373	
4	ATR378	
5	ATR-380	
6	ATR-385	
7	ATR-387	
8	ATR-391	
9	ATR-393	
10	ATR-394	
11	ATR-397	
12	KK-6	
13	KK-7	
14	KK-8	
15	KK-9	
16	KK-10	
17	KK-11	
18	KK-15	
19	KK-12	Azucena × IR20
20	KK-13	Azucena × IR20
21	ATR-472	Azucena × Bala
22	ATR-473	
23	ATR-275	Azucena × BPT-5204
24	ATR-279	
25	KK-25	
26	KK-2	BPT 5204 × Azucena
27	KK-3	
28	KK-4	

Sl. No.	Rice lines	Biological status of accession
29	KK-16	Azucena × RPHR 1005
30	KK-17	
31	KK-18	
32	KK-23	
33	KK-24	
34	ATR-486	Azucena × Dular
35	PUP1	MTU 1010 × Vandana
36	PUP2	
37	PUP3	
38	PUP4	
39	KK-14	MTU 1010 × MDU 5
40	Rasi	TN1 × C029
41	Sahbhagi Dhan	IR74371-70-1-1(IR5541-04 × WayRarem)
42	NH-1	N22 EMS mutant
43	NH-2	
44	NH-4	
45	Azucena	Landrace
46	Swarna	Vasista/ Mahsur
47	N22	Aus landrace
48	IR-64	IR5657-33-2-1/IR2061-465-1-5-5
49	KMR-3	IR 78937-B-4-B-B-B

with the rice line name and was placed in a water container in a slanting position.

Seedling vigour traits *viz.*, germination percentage (GP) was calculated as the total number of normal seedlings counted to the total number of seeds kept for germination and expressed in percentage; mesocotyl length (ML), coleoptile length (CL), shoot length (SL), root length (RL) and total seedling length (TSL) were measured manually using a centimetre scale. Shoot fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW) and total dry weight (TDW) ratios were calculated using an electronic balance (IGene, India). The SVI-I and SVI-II were obtained using the formulae mentioned by Addanki *et al.*, (2019) as SVI-I = Germination percentage  $\times$  seedling

length, SVI-II = germination percentage  $\times$  total dry weight (g). The SVI-I and SVI-II were recorded at 14<sup>th</sup> days after sowing (DAS) in two replications. The CRD ANOVA analysis of variance was carried out in R studio (*version* 3.5.2) using R-scripts for statistical analysis (Aravind *et al.*, 2019) and correlation analysis was carried out in past-3 using Spearman correlation coefficient (<http://folk.uio.no/ohammer/past>).

## Results and Discussion

### Analysis of variance (ANOVA) for seedling vigour traits

The mean sum of squares for all the seventeen seedling vigour traits has been presented in **Table 2**. The ANOVA revealed a significant mean sum of square (MSS) at  $p < 0.01$  and  $p < 0.05$  for all the traits except

**Table 2. Analysis of variance for seedling vigour traits in rice lines**

Traits	Mean sum of squares	Error	C.D.	C.V. (%)
	Lines			
<i>Degrees of freedom</i>	48	49		
GP	190.07**	50.94	14.21	7.65
CL	0.30**	0.09	0.60	28.75
ML	0.90**	0.09	0.62	7.65
SL	19.98**	1.70	2.62	10.03
RL	19.78**	3.37	3.69	19.48
RSLR	0.142**	0.023	0.30	20.06
TSL	50.65**	6.43	5.09	11.29
SFW	1134.30**	21.43	9.30	8.16
RFW	356.38**	9.35	6.14	9.96
RSFWR	0.19NS	0.12	NA	59.60
TFW	2409.80**	30.69	11.13	6.30
SDW	80.82NS	52.00	NA	77.79
RDW	110.94**	28.29	10.69	93.80
RSDWR	4.05**	0.08	0.57	32.43
TDW	218.95*	126.05	22.57	75.12
SVI-I	56.23**	7.44	5.48	13.13
SVI-II	193.78**	97.46	19.84	71.54

The \* and \*\* indicate the mean sum of squares are significant at  $P < 0.05$  and  $P < 0.01$  respectively, NS indicates non-significance

CV: Co-efficient of variation; CD: Critical difference; GP: Germination (%); ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSFWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II

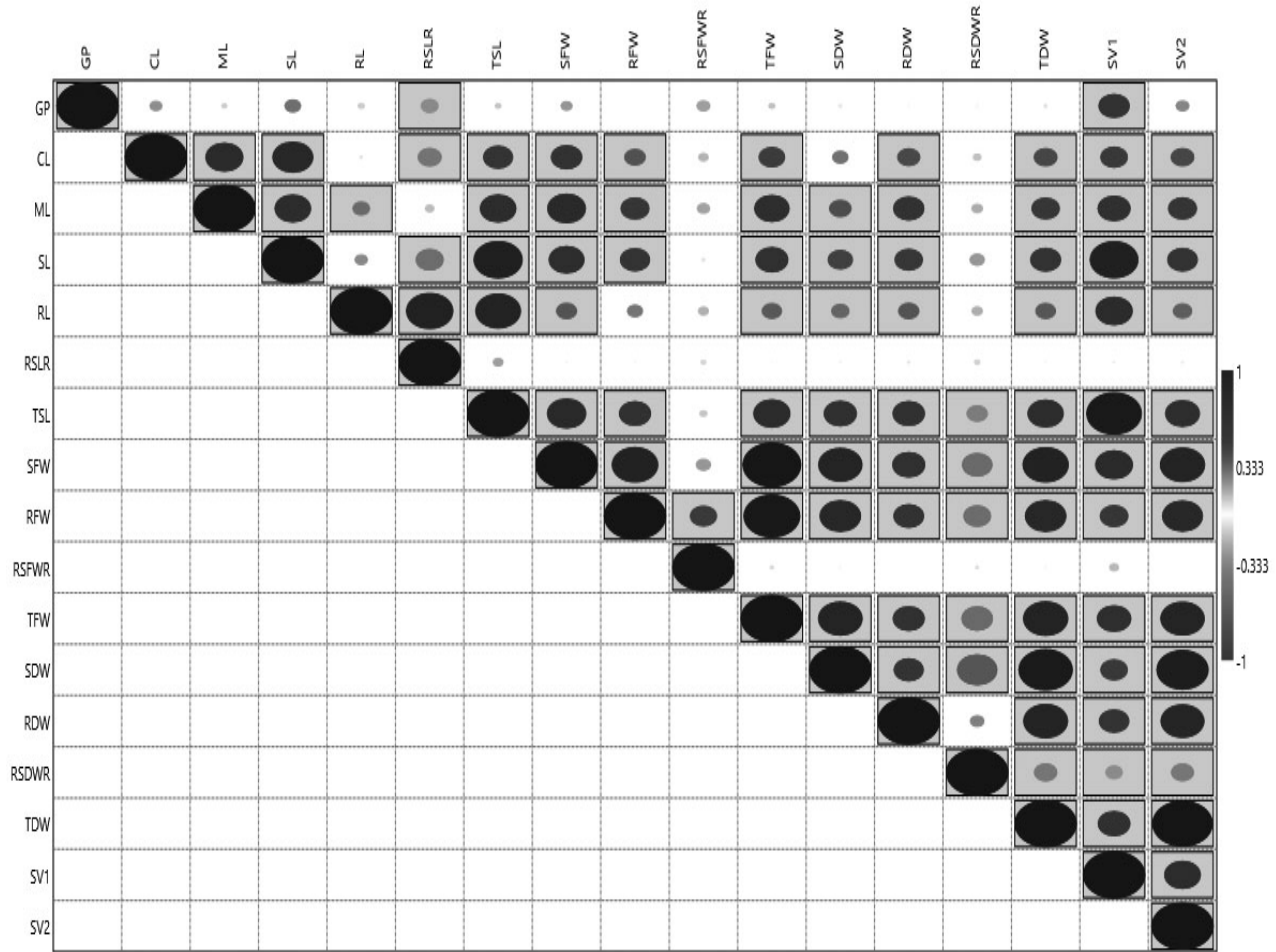


for root to shoot fresh weight ratio and shoot dry weight which was highly variable for the traits under study for seedling vigour traits.

### Estimates of correlation coefficients for seedling vigour traits

Among the seventeen seedling vigour traits studied, SVI-I reported the highest significant positive correlation with total seedling length, followed by shoot length, shoot fresh weight, root length, total fresh weight, mesocotyl length, total dry weight, germination

percentage, root dry weight, root fresh weight, shoot dry weight and coleoptile length, similarly SVI-II exhibited positive significant association with total dry weight followed by shoot dry weight, shoot fresh weight, total fresh weight, root dry weight, root fresh weight, SVI-I, total seedling length, shoot length, mesocotyl length, coleoptile length and root length (Table 3 and Figure 1). Further, inter-correlation among the important traits, such as coleoptile length recorded a significant positive correlation with shoot length, mesocotyl length, shoot fresh weight, total



**Figure 1: Correlation among seedling vigour traits in the rice lines**

GP: Germination (%);ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSPWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II

**Boxed rectangles indicate significant correlation. Red box:** Indicates negative correlation and **Blue box:** Indicates positive correlation; the clockwise direction of the boxes indicates the intensity of positive correlation, and the anticlockwise direction of the boxes indicates the intensity of negative correlation

**Table 3. Estimation of correlation coefficients for seedling vigour traits in rice lines**

Traits	GP	CL	ML	SL	RL	RSLR	TSL	SFW	RFW	RSFWR	TFW	SDW	RDW	RSDWR	TDW	SVI-I	SVI-II
GP	1	0.196	0.084	0.259	-0.103	-0.277*	0.095	0.181	-0.008	-0.213	0.101	-0.051	0.020	-0.027	0.050	0.493**	0.212
CL		1	0.601**	0.650**	0.047	-0.375**	0.472**	0.495**	0.335*	-0.157	0.416**	0.255	0.362**	-0.128	0.370**	0.431**	0.370**
ML			1	0.578**	0.274*	-0.141	0.578**	0.614**	0.454**	-0.202	0.558**	0.350*	0.492**	-0.176	0.446**	0.522**	0.456**
SL				1	0.204	-0.438**	0.783**	0.567**	0.475**	-0.054	0.520**	0.397**	0.446**	-0.237	0.487**	0.777**	0.482**
RL					1	0.754**	0.728**	0.327*	0.248	-0.164	0.315*	0.285*	0.329*	-0.172	0.320*	0.591**	0.303*
RSLR						1	0.162	-0.021	-0.019	-0.082	0.013	0.022	-0.045	-0.090	-0.019	0.028	-0.037
TSL							1	0.624**	0.507**	-0.117	0.588**	0.524**	0.511**	-0.331*	0.575**	0.888**	0.551**
SFW								1	0.741**	-0.235	0.940**	0.704**	0.523**	-0.478**	0.737**	0.598**	0.721**
RFW									1	0.425**	0.908**	0.659**	0.484**	-0.433**	0.659**	0.448**	0.650**
RSFWR										1	0.061	0.020	-0.007	0.053	-0.021	-0.147	-0.012
TFW											1	0.711**	0.506**	-0.496**	0.720**	0.545**	0.706**
SDW												1	0.473**	-0.641**	0.870**	0.432**	0.834**
RDW													1	0.224	0.714**	0.479**	0.698**
RSDWR														1	-0.364**	-0.271	-0.357**
TDW															1	0.516**	0.971**
SVI-I																1	0.582**

**Note: \*and \*\* indicate the mean sum of squares were significant at P<0.05 and P<0.01 respectively**

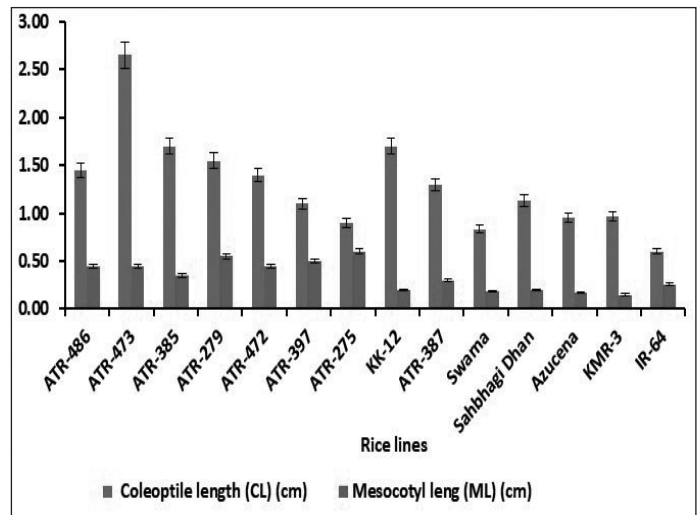
GP: Germination (%);ML: Mesocotyl length (cm); CL: Coleoptile length (cm); SL: Shoot length (cm); RL: Root length (cm); TSL: Total seedling length (cm); RSLR: Root to shoot length ratio; SFW: Shoot fresh weight (mg); RFW: Root fresh weight (mg); TFW: Total fresh weight (mg); RSFWR: Root to shoot fresh weight ratio; SDW: Shoot dry weight (mg); RDW: Root dry weight (mg); TDW: Total dry weight (mg); RSDWR: Root to shoot dry weight ratio; SVI-I: Seedling vigour index-I and SVI-II: Seedling vigour index-II



seedling length, total fresh weight, total dry weight, root dry weight, and root fresh weight. The mesocotyl length exhibited a significant positive correlation with shoot fresh weight, coleoptile length, shoot length, total seedling length, total fresh weight, root dry weight, root fresh weight, total dry weight, shoot dry weight and root length, similar correlation study was reported earlier (Yang *et al.*, 2020, Dilday *et al.*, 1990). Root length showed a significant positive correlation with mesocotyl length, root to shoot length ratio, total seedling length, shoot fresh weight, total fresh weight, shoot dry weight, root dry weight, total dry weight, SVI-I, and SVI-II. Similarly, total dry weight exhibited a significant correlation with coleoptile length, mesocotyl length, shoot length, root length, total seedling length, shoot fresh weight, root fresh weight, total fresh weight, shoot dry weight, root dry weight, SVI-I, and SVI-II. The positive and significant correlation among component traits indicated that these related traits could be used in combination for selection or initial phenotyping for seedling vigour.

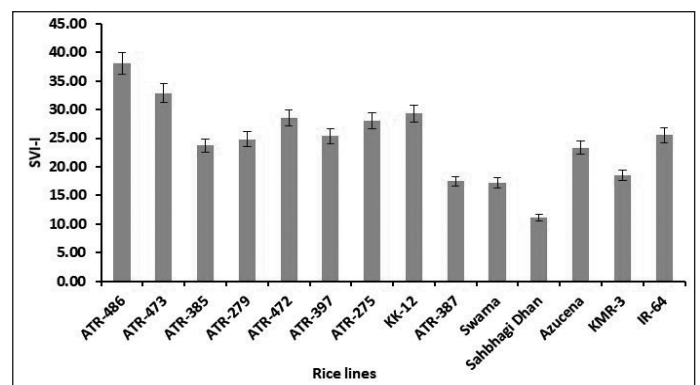
The rice lines ATR-473, ATR-385, ATR-279, ATR-472, and ATR-486, recorded higher CL, ML, SVI-I, and SVI-II than the checks Swarna, Sahbhagi Dhan, Azucena, KMR-3 and IR-64. KK-12 reported higher CL, SVI-I, and SVI-II, ATR-397 and ATR-275 recorded higher ML, SVI-I, and SVI-II. The line, ATR-387 exhibited the highest CL and SVI-II (Figures 2, 3 and 4), whereas the N22 mutant NH-4 and rice line KK-6 showed higher ML, SVI-II, and SVI-I respectively than the checks. These lines with the superior potential of ML and CL have a strong inherent genetic ability for seedling emergence (Turner, *et al.*, 1982, Yang *et al.*, 2020, Dilday *et al.*, 1990). Longer mesocotyl is an important characteristic for the breeder to select as it is beneficial for germination under deep sown conditions and primarily responsible for seedling emergence under DDSR (Gao *et al.*, 2012; Hu *et al.*, 2010; Watanabe *et al.*, 2001; Zhang *et al.*, 2005, Zhou *et al.*, 2006; Finch-Savage *et al.*, 2010, Wu *et al.*, 2015). Luo *et al.*, (2007) reported a significant positive interactive effect of mesocotyl elongation and sowing depth on seedling emergence and showed that mesocotyl elongation increasingly

influenced seedling establishment when sowing depth was increased, but only extremely long coleoptiles affected seedling establishment.



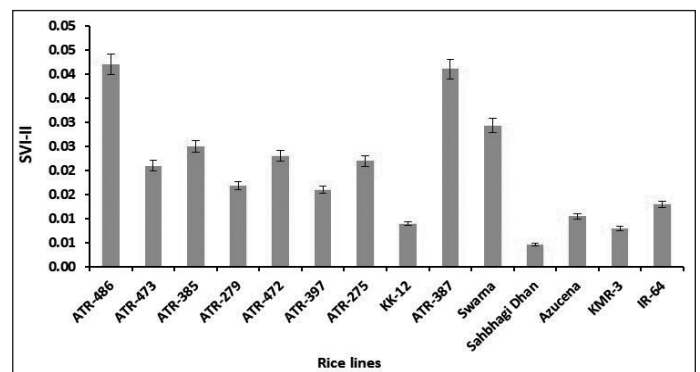
**Figure 2: Better performing rice lines for coleoptile length and mesocotyle length**

The bars on top of indicate standard deviation



**Figure 3: Better performing rice lines for seedling vigour-I**

The bars on top of indicate standard deviation



**Figure 4: Better performing rice lines for seedling vigour-II**

The bars on top of indicate standard deviation

In this study, the lines *viz.*, ATR-486, ATR-473, ATR-385, ATR-279, and ATR-472 had a better seedling

vigour index, mesocotyl, and coleoptile length. Our study revealed that SVI-I had the highest positive significant positive correlation with mesocotyl length and coleoptile length. Similar results were reported by Chung (2010) wherein a significant positive relationship between mesocotyl elongation in weedy rice and seedling emergence was recorded but found no correlation between coleoptile length and emergence. In literature Yang *et al.*, (2020) identified 20 QTLs for mesocotyl length by using GWAS on chromosomes 4, 6, 7, 8, 10, and 12. Mesocotyl and coleoptile are important for seedling emergence from deeper levels of soil and lines with the maximum mesocotyl length are preferable for improving rice seedling emergence rate, mostly under deep sowing and submergence (Lee *et al.*, 2017, Zhan *et al.*, 2020). It has been reported by Lu *et al.*, (2016) that natural genetic variation for mesocotyl elongation exists and provides the basis for developing rice varieties with long mesocotyl and the identification of genes through linkage mapping or genome-wide association studies (GWAS). Also, studies have reported that long mesocotyl is important for the uniformity of seedling emergence (Turner *et al.*, 1982; Mgonja *et al.*, 1993; Chung 2010). Thus, mesocotyl length has been projected as a key trait in developing rice varieties for DDSR cultivation. Yang *et al.*, (2020) reported a significant difference in the mesocotyl length of 290 rice accessions, and the trend of change in mesocotyl length for each accession is highly correlated to the sowing depths. The interaction between coleoptile and mesocotyl elongation in effective seedling emergence must be explored further for breeding (Zhan *et al.*, 2020). The introgressed lines identified as having comparatively higher mesocotyl and coleoptile lengths can be further evaluated in the field under DDSR conditions.

## Conclusion

The identified rice lines exhibiting the highest seedling vigour traits can be used for improving seedling vigour and adaptability under DDSR conditions. This method of phenotyping can be the first line of screening for large sets of germplasm before subjecting to field-level evaluation. The lines with high seedling vigour index can be registered as a novel genetic resource used in breeding programs.

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## Authors' contribution

The study was planned by KMB, phenotyping the panel and analysis was done by KMB, PR, NDM, AP, H; supervision and timely inputs were given by DB, PS, VM; critically edited the manuscript MSM, AMS, CG, LR.

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