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Identification of superior recombinant inbred lines for early seedling vigor traits in bread wheat (*Triticum aestivum* L.)

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Abstract
Wheat (*Triticum aestivum* L.) is a globally important cereal crop, and early seedling vigour plays a crucial role in ensuring rapid crop establishment, resource use efficiency, and yield stability. The present study evaluated 280 recombinant inbred lines (RILs) derived from a cross between Raj-3765 (HTG) and HD-2329 (HSG) for early seedling vigour traits. Germination percentage, coleoptile length, seedling length, and seedling dry weight using an augmented design during the *Rabi* season of 2023-24 at VNMKV, Parbhani. Germination percentage was uniformly high ranging 97-100% with negligible variability of 0.011 indicating limited scope for selection. Coleoptile length ranging 4.89-20.59 cm showed the greatest variability 3.65 followed by seedling length in the range of 8.58-29.08 cm with variability 2.61 and seedling dry weight ranging from 20.15-49.35 mg with showing variability of 0.85. Several RILs exhibited transgressive segregation, outperforming both parents in key vigor traits. Notably, lines L-27, L-141, L-165, L-172, L-209, L-210, and L-211 demonstrated superior coleoptile length, seedling growth and biomass accumulation, identifying them as promising donors for breeding programs aimed at enhancing early vigour and establishment under diverse environmental conditions. The findings underscore the potential of these RILs for genetic improvement of wheat to achieve better emergence, early growth and yield stability.

Keywords: Coleoptile length, germination percentage, seedling dry weight, seedling length

1. Introduction
Wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally serving as a staple food for a large portion of the world's population. Successful crop establishment is a key determinant of final yield and early seedling vigour plays a crucial role in this process. Early seedling vigour refers to the rapid and uniform germination and growth of seedlings, which allows plants to efficiently utilize soil nutrients, water, and light, while also enhancing competition with weeds and tolerance to environmental stresses. (Rebetzke *et al*., 2007a) [15].
Wheat is grown worldwide on an estimated 221.82 million hectares. Projections from the FAO and USDA for 2024 indicate that global wheat production will reach approximately 793.24 million tonnes, representing an increase of 2.09 million tonnes compared to the previous year. China remains the largest producer, contributing about 136.59 million tonnes, followed by India, where wheat ranks second only to rice in importance. In India, the crop occupies around 31.8 million hectares, with an average productivity of 3.56 tonnes per hectare. For the 2024-25 season, national production is expected to reach a record 113.29 million tonnes, underscoring both the agronomic importance of wheat and the ongoing need for strategies to further enhance yield potential (FAS-USDA, 2023-24).
Traits such as germination percentage, coleoptile length, seedling length and seedling dry weight are commonly used to evaluate early seedling vigour. Longer coleoptiles facilitate seedling emergence from deeper sowing depths, while higher seedling biomass is associated with better establishment and early growth. Deeper sowing can help minimize the phytotoxic effects that are sometimes caused by pre-emergent herbicides (O'Sullivan *et al*., 1985) [12] and can also decrease the likelihood of seed predation by birds and rodents during germination (Brown *et al*., 2003) [1]. However, planting seeds at greater depths often leads to fewer plants emerging, and those that do emerge tend to appear later (Kirby, 1993) [10].

Seedlings produced under these conditions typically exhibit lower biomass and slower relative growth rates, which delays leaf area development (Hadjichristodoulou *et al.*, 1977; Huang and Taylor, 1993) [8, 9]. Consequently, the reduced leaf area during the early growth stages can make wheat crops less competitive against weeds (Coleman *et al.*, 2001) [2]. The expression of these traits like coleoptile and germination percentage is influenced by both genetic and environmental factors making it necessary to screen diverse populations under controlled conditions to identify superior genotypes. Augmented designs allow efficient evaluation of large populations with minimal replication enabling the identification of superior genotypes while maintaining experimental rigor (Federer, 1975) [6].

Successful crop establishment is a critical stage in achieving high yields in cereal production (Rebetzke *et al.*, 2007a) [15]. The ability of seedlings to elongate and emerge from deeper sowing depths is essential for attaining optimal plant populations and early leaf area expansion-key traits in breeding programs aimed at enhancing adaptation to water-limited environments (Richards, 1992) [19]. With increasing climate variability and shifts in the timing of seasonal rainfall, strong establishment and early vigor are becoming even more significant, particularly where crops depend on stored soil moisture from summer precipitation (Flohr *et al.*, 2021) [7]. Enhanced seedling vigor has also been identified as an important trait for improving a crop's competitiveness against weeds (Coleman *et al.*, 2001; Zerner *et al.*, 2016) [2, 21] and for promoting efficient nutrient uptake (Pang *et al.*, 2014; Ryan *et al.*, 2015) [13, 20].

Recombinant inbred lines (RILs), developed through repeated selfing of F2 individuals, provide a permanent resource for genetic evaluation due to their homozygosity and stability across generations. RIL populations derived from contrasting parents allow the detection of genetic variability and transgressive segregation, which can result in lines outperforming both parents.

Given the importance of early seedling vigour in crop establishment and productivity, identifying superior RILs with enhanced germination, coleoptile length, seedling growth and biomass is of high priority for wheat breeding programs. The present study aimed to evaluate a RIL population for these traits and to identify promising lines for future breeding efforts.

2. Materials and Methods

2.1 Experimental material

A set of 280 recombinant inbred lines (RILs) derived from a cross between two contrasting wheat parents Raj-3765 (HTG) and HD-2329 (HSG) was used for the study as standard checks which are acquired from ICAR-National Institute for Plant Biotechnology, Pusa campus, New Delhi.

2.2 Experimental design and growing conditions

The experiment was conducted on the research farm of the Wheat and Maize Research Unit, VNMKV, Parbhani during the rabi season of 2023-24. The study followed an augmented design with 280 unreplicated RILs and two checks replicated across all 8 blocks. Standard agronomic practices were followed to ensure uniform seedbed preparation and moisture conditions.

2.3 Traits measurement

Data were recorded on the following early seedling traits:

2.3.1 Germination percentage (%): Calculated as the proportion of germinated seeds out of total seeds sown after 7 days of sowing.

$$\text{Germination (\%)} = \frac{\text{Germinated Seed count}}{\text{Total seeds}} \times 100$$

2.3.2 Coleoptile length (cm): Measured from the base of the seed to the tip of the coleoptile in five randomly selected seedlings per RIL at 7th day of sowing. Coleoptile lengths were measured from the scutellum to the tip of the coleoptile, following the method outlined by Rebetzke *et al.*, (2004) [17].

2.3.3 Seedling length (cm): Total seedling length measured from the base to the tip of the longest leaf at 7th day of sowing from five randomly selected seedlings per RIL.

2.3.4 Seedling dry weight (mg): Five seedlings per line were sun-dried weighed to determine dry biomass.

2.4 Statistical analysis

Data were analyzed using the Augmented design model as described by Federer (1956). Adjusted means for RILs were estimated after accounting for block and check effects. Descriptive statistics including range, mean and coefficient of variation (CV%) were computed to quantify variability. RILs showing performance superior to the best check were classified as superior lines for that particular trait. All computations were carried out using GRAPS Agri1 (2021) soft-ware.

3. Results

3.1 Genetic Variability

3.1.1 Germination percentage

The germination percentages ranging from 97 to 100 per cent, with an average of 98.38 per cent. Notably, all the RILs exhibited germination percentages that were higher than that of IMSCS. The coefficient of variation (CV) was extremely low (0.011), indicating very little variability among the lines. All RILs showed consistently high germination, suggesting that the population is highly uniform for this trait. Therefore, germination percentage may not be a critical selection criterion in this population as almost all lines performed near the maximum (Table 1).

However, among the total RILs ten higher and ten lower values of germination percentages which is presented in (Table 2), highest germination percentage which is 100 per cent, measuring in line number L-1, L-104, L-133, L-134, L-138, L-147, L-148, L-152, L-154, L-155 which is similar to both the parents *i.e.* Raj-3765 (100) and HD-2329 (100), then had the lowest germination percentage which is 97 per cent, measuring in line number L-46, L-59, L-60, L-7, L-70, L-74, L-79, L-83, L-85, L-9 which is lowest than both the parents. Germination percentage is critical for uniform crop establishment. In this study, several RILs exhibited higher germination rates than the parental lines and checks, suggesting the presence of favourable alleles controlling seed viability and early growth. High germination ensures rapid stand establishment, which is particularly important under suboptimal conditions, such as limited moisture or low soil temperatures (Rebetzke *et al.*, 2007a) [15].

3.1.2 Coleoptile length (cm)

The coleoptile length ranged from 4.894 cm to 20.594 cm, with an average length of 12.72 cm. The CV of 3.65 indicates moderate variability, suggesting that coleoptile length is a highly segregating trait within the population. Several RILs, including L-27, L-141, L-165, L-172, L-209, L-210, and L-211, showed superior performance with significantly longer coleoptiles than the population mean. These lines could be important donors for breeding programs aiming to improve emergence from deeper sowing or under adverse soil conditions. which is presented in (Table 1). Among the lines, line number L-210 (20.594 cm) had recorded the longest coleoptile length. However, the shortest coleoptiles were observed in line number L-206 (4.894 cm), L-83 (5.59 cm), L-260 (5.64 cm), and L-13 (5.74 cm).

However, among the total RILs, ten higher and ten lower values of coleoptile lengths which is presented in (Table 2), where the highest coleoptile length ranging from 19.34 to 20.59 cm, measuring in line number L-210, L-189, L-209, L-141, L-150, L-165, L-172, L-71, L-211, L-274 which is maximum than both the parents *i.e.* Raj-3765 (19.44) and HD-2329 (13.75), then had the lowest coleoptile length ranging from 4.89 to 7.79 cm, measuring in line number L-205, L-245, L-20, L-85, L-21, L-84, L-13, L-260, L-83, L-206 which is lowest than both the parents. Previous studies have demonstrated significant genotypic variation in coleoptile length and width among wheat varieties (Ellis *et al*., 2004; Marais and Botma, 1987; Rebetzke *et al*., 1999, 2004, 2007b) [3, 11, 14, 17, 16]. Research suggests that coleoptile length and thickness are primarily governed by additive genetic effects (Rebetzke *et al*., 2004, 2007b) [17, 16], highlighting the potential for breeding programs to select genotypes with longer and thicker coleoptiles that exhibit improved seedling emergence.

3.1.3 Seedling length (cm)

The seedling length ranged from 8.58 cm to 29.08 cm, with an average length of 18.47 cm. The CV was 2.606 indicating low to moderate variation (Table 1). Although the mean is moderate, the maximum values suggest the presence of transgressive segregants that exhibit superior early growth. Seedling length is an important trait contributing to early vigour, which can improve stand establishment and competitiveness. Among the lines, line number L-27 (29.08 cm) and L-141 (28.68 cm), were recorded the maximum seedling length. however, line number L-13 (8.58 cm), L-83(10.52 cm), and L-206 (10.51 cm) were recorded minimum seedling lengths.

However, among the total RILs, ten higher and ten lower values of seedling length which is presented in (Table 2), where the highest seedling length ranging from 24.87 to 29.09 cm, measuring in line number L-27, L-141, L-165, L-209, L-210, L-211, L-26, L-179, L-218, L-234 which is maximum than both the parents *i.e.* Raj-3765 (27.10) and HD-2329 (22.34), then had the lowest seedling length ranging from 8.59 to 12.57 cm, measuring in line number L-245, L-261, L-57, L-275, L-205, L-260, L-20, L-206, L-83, L-13 which is lowest than both the parents.

3.1.4 Seedling dry weight (mg)

The seedling dry weight ranged from 20.15 to 49.35 mg, with an average length of 35.08 mg. Seedling dry weight showed considerable variation, ranging from 20.15 mg to 49.35 mg, with a mean of 35.08 mg. The CV was 0.854, indicating very low variability among the RILs (Table 1). Despite the low CV, the maximum value of 49.35 mg suggests that a few lines possess superior early biomass accumulation. These lines may be targeted for selection in breeding programs aimed at enhancing early vigour and nutrient uptake efficiency. Among the lines, line number L-141 (49.35 mg), L-165 (47.45 mg) and L-27 (46.65 mg) were recorded the highest seedling dry weight. however, line number L-16 (20.15 mg), L-191 (21 mg), L-226 (21.3 mg), L-83 (21.4 mg), and L-206 (21.6 mg) were recorded the lowest seedling dry weight.

However, among the total RILs, ten higher and ten lower values of seedling dry weight which is presented in (Table 2), where the highest seedling dry weight ranging from 43.35 to 49.35 mg, measuring in line number L-141, L-165, L-27, L-166, L-209, L-210, L-216, L-145, L-167, L-172 which is maximum than both the parents *i.e.* Raj-3765 (44.23) and HD-2329 (40.08), then had the lowest seedling dry weight ranging from 20.15 to 22.90 mg, measuring in line number L-245, L-15, L-202, L-222, L-20, L-206, L-83, L-226, L-191, L-13 which is lowest than both the parents. This variation indicates the potential of the population to select superior lines for early vigour traits.

Table 1: Descriptive statistics for early seedling vigour traits in wheat RILs

Sr. No	Parameter	Min	Max	GM	SE	CD (5%)	CV	Superior RILs
1	GP (%)	97	100	98.38	0.0184	0.0435	0.011	-
2	CL (cm)	4.89	20.59	12.728	0.816	1.93	3.65	L-27, L-141, L-165, L-172, L-209, L-210 L-211
3	SL (cm)	8.59	29.09	18.472	0.847	2.003	2.606	
4	SDW (mg)	20.15	49.35	35.08	0.524	1.238	0.854	

Table 2: Performance of best ten and lowest ten wheat RIL population for early seedling vigour traits

Sr. No.	GP (%)		CL (cm)		SL (cm)		SDW (mg)	
RILs with highest value								
1	L-1	100	L-210	20.59	L-27	29.09	L-141	49.35
2	L-104	100	L-189	19.79	L-141	28.69	L-165	47.45
3	L-133	100	L-209	19.79	L-165	27.59	L-27	46.65
4	L-134	100	L-141	19.44	L-209	26.82	L-166	45.45
5	L-138	100	L-150	19.44	L-210	26.32	L-209	44.30
6	L-147	100	L-165	19.44	L-211	25.37	L-210	43.60
7	L-148	100	L-172	19.44	L-26	25.09	L-216	43.40
8	L-152	100	L-71	19.39	L-179	25.02	L-145	43.35
9	L-154	100	L-211	19.34	L-218	24.87	L-167	43.35
10	L-155	100	L-274	19.34	L-234	24.87	L-172	43.35
RILs with lowest value								
1	L-46	97	L-205	7.79	L-245	12.57	L-245	22.90
2	L-59	97	L-245	7.74	L-261	12.17	L-15	22.75
3	L-60	97	L-20	7.54	L-57	11.89	L-202	22.40
4	L-7	97	L-85	7.39	L-275	11.87	L-222	22.10
5	L-70	97	L-21	6.94	L-205	11.62	L-20	22.05
6	L-74	97	L-84	6.79	L-260	11.07	L-206	21.60
7	L-79	97	L-13	5.74	L-20	10.69	L-83	21.40
8	L-83	97	L-260	5.64	L-206	10.52	L-226	21.30
9	L-85	97	L-83	5.59	L-83	10.51	L-191	21.00
10	L-9	97	L-206	4.89	L-13	8.59	L-13	20.15
Parents								
Raj-3765	100		19.44		27.10		44.23	
HD 2329	100		13.75		22.34		40.08	

Germination percentage (GP), Coleoptile length (CL), Seedling length (SL), Seedling dry weight (SWD).

3.2 Identification of Superior Line
The following RILs were identified as superior L-27, L-141, L-165, L-172, L-209, L-210 and L-211 based on coleoptile length, which showed the highest variability. These lines combine relatively higher coleoptile length with acceptable seedling length and dry weight, making them promising candidates for improving early seedling vigour in wheat breeding. Germination percentage, despite being an important trait, showed minimal variability and thus was not a distinguishing factor in this population.

5. Conclusion
The use of an augmented design allowed the evaluation of a large number of RILs efficiently while providing reliable comparisons with standard checks (Federer, 1975) [6]. This approach is particularly useful in segregating populations where replication of all entries may not be feasible. The identification of superior lines demonstrates the utility of augmented designs in preliminary screening for traits of agronomic importance. Overall, the observed genetic variation among RILs for early seedling vigour traits suggests substantial potential for selection. The superior RILs identified in this study can be exploited in wheat breeding programs to develop varieties with enhanced early vigour, contributing to improved crop establishment, higher yield stability, and resilience under diverse environmental conditions. Future studies should evaluate these lines under field conditions across multiple environments to validate their performance and determine their contribution to final grain yield.

Germination percentage was consistently high (97-100%) with negligible variability, indicating uniformity but limited use as a selection criterion. Coleoptile length exhibited the highest variability (CV - 3.65) and was the most effective trait for identifying superior RILs for early vigour. Seedling length and dry weight showed moderate to low variability but complemented coleoptile length in selecting lines with superior early growth. Seven RILs L-27, L-141, L-165, L-172, L-209, L-210, L-211 were identified as superior for early seedling vigour and can serve as valuable donors in wheat breeding programs aimed at improving emergence, stand establishment and early biomass accumulation.

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