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Early Germination Traits - A Comprehension of the Relations with other Early Seedling Vigour Traits in Rice

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ABSTRACT

The early emergence and uniform establishment of seedlings are crucial in direct seeded rice cultivation (DSR). The process of seedling emergence is influenced by complex factors and characteristics that operate during both seed germination and heterotrophic seedling growth phases. Therefore, it is important to identify and understand the traits specific to these early phases. In this study, we report on the early germination traits, including time to 50 percent lemma rupture (TFLR), time to 50 percent coleoptile emergence (TFCE), and time to 50 percent radicle emergence (TFRE), and their variability among rice genotypes. Although TFRE has been previously associated with other ESV traits, comprehensive studies of TFLR and TFCE have not been conducted. Additionally, previous studies utilized rice seed batches of varying aging periods to examine the impact of seed aging on ESV traits, making it challenging to draw definitive conclusions about freshly harvested seeds of different rice genotypes. Therefore, we assessed the relationship between the early germination traits and other ESV traits in rice and found that TFLR, TFCE, and TFRE have highly significant positive correlations with each other, but significant negative correlations with all other ESV traits like shoot length, root length, seedling length, shoot fresh weight, root fresh weight, seedling fresh weight, germination percentage, and Vigour index-I. Further the importance of early germination traits in seedling emergence and DSR system are discussed.

Key words: *DSR, Early seedling vigour, Early germination traits, Lemma rupture, Coleoptile emergence, Radicle emergence.*

Introduction

In India, rice is mainly taken up as a transplanted crop under stagnant water conditions, which require large quantities of water, labour, and energy. In the near future, these may become limiting factors for rice cultivation (Tuong and Bouman, 2003) and put the present favorablesituation of positive average annual growth rates at stake. The increased use of groundwater for rice cultivation in the Indo-Gangetic Plains (especially in Punjab and Haryana) has led to a decreased water table, which in turn results in water scarcity and an increased cost of cultivation (Hira, 2009; Rodell *et al.*, 2009), causing farmers to shift from transplanted rice (TPR) to direct-seeded rice (DSR) cultivation. Although TPR aids in high rice yields, it leads to other associated problems, such as groundwater over-exploitation (Singh and Kasana, 2017) and increased soil and groundwater pollution with pesticides (Chauhan *et al.*, 2012). Considering this, the state governments of Punjab and Haryana have announced incentives for DSR (Anonymous, 2022a; Anonymous, 2022b), which has further motivated farmers to shift from TPR to DSR in the recent past. However, present yield levels under the DSR system are comparatively low, which is mainly attributed to poor seedling establishment and non-availability of genotypes suitable for the DSR system (Mahender *et al.*, 2015). To overcome this challenge, it is crucial to breed rice genotypes tailored for DSR. Among the traits to consider for DSR-focused rice breeding, early seedling vigour (ESV) traits are essential for ensuring rapid and uniform seedling emergence and establishment, enabling the early dominance of DSR genotypes.

Like in any crop, seedling emergence is crucial for the success of DSR cultivationas it provides emerging seedlings with a competitive advantage in above and below ground resource(sunlight, water and nutrients) utilization. In fields, seedling emergence is affected by many factors like soil moisture, soil mechanical impedance (soil crust), soil temperature, available oxygen, nutrient status, soil toxicity level, allelopathic effect of other vegetations, etc. Similarly, it also depends on the inherent genetic composition of the seed, influence of maternal environment, status of stored reserves, seed ageing, seed handling conditions, etc. Thus, seedling emergence is a complex trait influenced by as-yet-unknown factors and characteristics that operate during both seed germination and heterotrophic seedling growth phases (Finch-Savage *et al.*, 2016). Therefore, the identification and comprehension of traits specific to these early phases will not only facilitatein breeding of rice varieties better suited to the challenges and opportunities of DSR farming but also for better agronomic/seed technological interventions to facilitate the better early seedling emergence and establishment.

Previous studies in our lab reported three quantifiable early germination traits, time required for 50 percent lemma rupture (TFLR), time for 50 percent coleoptile emergence (TFCE), and time for 50 percent radicle emergence (TFLR), which showed wide variations among the rice genotypes studied (Gupta *et al.*, 2013) and showed significant positive correlation with galactomannan hydrolysing enzymes (*viz.*, β-mannanase, β-mannosidase and α-galactosidase) that were reported to be involved in cell wall loosening and reserve mobilization during seed germination in different crops (Nonagaki, 2006). Although TFRE has been reported to be associated with other ESV traits (Onwimol *et al.*, 2016; Luo *et al.*, 2017; Chinnasamy *et al.*, 2021; Yimpin *et al.*, 2022), no comprehensive studies have been conducted with respect to TFLR and TFCE. In addition, previous studies utilised rice seed batches of varying aging periods to examine the impact of seed aging on ESV traits. Consequently, it is challenging to draw definitive conclusions regarding whether the same relationships hold true for freshly harvested seeds of different rice genotypes. Hence, the study was conducted to assess the relationship between TFLR, TFCE, and TFRE and other ESV traits, such as shoot length, root length, seedling length, shoot fresh weight, root fresh weight, seedling fresh weight, shoot dry weight, root dry weight, seedling dry weight, germination percentage, Vigour index-I, and Vigour index-II in rice.

Materials and Methods

Plant materials: We collected genetically pure seeds of twenty rice genotypes belonging to 3KRGP panel from the Rice section, Division of Genetics, ICAR-IARI, New Delhi. The details are given in Table 1. All the genotypes included in the present study were initially collected from different parts of India and were maintained at by International Rice Research Institute (IRRI), Philippines (Figure 1).

Phenotyping for early germination traits: To assess the relationship between the germination traits and other ESV traits, we generated the respective trait values for 20 rice genotypes as detailed below.

Time to fifty per cent lemma rupture (TFLR): TFLR values for the rice accessions (as presented in Table 2) were derived using the methodology outlined in ISTA (2018) with some modifications. In this process, a Complete Randomized Design (CRD) was followed utilizing three replicates of each genotype, with 100 seeds placed in circular Petri dishes measuring 15 cm in diameter. These Petri dishes contained two layers of filter paper moistened with 20 ml of distilled water. The assembled plates were then placed in a controlled germination chamber with a temperature of 25 ± 2 °C, 80% relative humidity, and diffused light from a white fluorescent lamp with an intensity ranging from 750 to 1250

Fig. 1. The geographical coordinates for the locations where rice genotypes were collected by the IRRI, Philippines. Geolocation map was generated using google earth Pro (Version 7.3.6.9345).

lux.Starting from 10 hours after incubation, seeds that reached the lemma rupture stage (Figure 2B) were periodically counted and removed from the plates every four hours until the last seed reached the lemma rupture stage. Subsequently, the acquired data underwent germinationmetrics analysis to calculate TFLR values, in accordance with the software's instruction manual (Aravind *et al.,* 2020). Later the curves fitted to derive the TFLR values for each 20 genotypes with three replicates in each.

Time to fifty per cent coleoptile emergence (TFCE): The TFCE values for the rice accessions listed in Table 1 were determined using an approach similar to that described for TFLR. This involved the process of removing and counting seeds that had progressed to the coleoptile emergence stage (Figure 2C) from the plate, continuing until the final seed had reached the coleoptile emergence stage. Subsequently, the TFCE values were computed using germinationmetrics.

Time to fifty per cent radicle emergence (TFRE): The TFRE trait values for the rice accessions listed in Table 1 were obtained using a method consistent with the procedure detailed for TFLR. This method involved the removal and tallying of seeds that had progressed to the radicle emergence stage (Figure 2D) from the plate, continuing until the final seed reached the radicle emergence stage. Subsequently, TFRE values were computed using germination metrics.

Other seedling specific ESV traits: To assess additional ESV (Endosperm Storage Protein) traits, a set of randomly chosen genotypes from Table 1 underwent a germination test following the 'between paper' method as per the ISTA (2018) guidelines. For this procedure, 300 seeds from each genotype were selected and organised into three replicates, each containing 100 seeds. These seeds were then placed between moist germination papers, rolled, and wrapped in butter paper to minimise moisture loss during the incubation process. The wrapped seeds

Table 1. List of rice accessions used for studying the relation between the early germination traits and other ESV traits

S. No.	Name	IRIS No.	Acc. No.
1	BHATA PYAGI	IRIS 313-11597	IRGC 127222
2	CUTTACK 29	IRIS 313-11409	IRGC 127289
3	KARUNJEERAGA SAMBA	IRIS 313-11414	IRGC 127495
4	LOCAL BHAT	IRIS 313-11452	IRGC 127571
5	NCS 458	IRIS 313-11640	IRGC 127658
6	NCS 809 A	IRIS 313-11647	IRGC 127666
7	NCS 901 A	IRIS 313-11650	IRGC 127668
8	VELLA PERUVAZHA 0-68-12	IRIS 313-10836	IRGC 127887
9	ZINYA KOLAMBA	IRIS 313-11453	IRGC 127916
10	ARC 11571	IRIS 313-10868	IRGC 127939
11	KHEJURTHUPI	IRIS 313-11363	IRGC 128089
12	ARC 15862	IRIS 313-11309	IRGC 128229
13	KALIPINCH	IRIS 313-11476	IRGC 128329
14	KALISAL	IRIS 313-11451	IRGC 128330
15	ARC 11768	IRIS 313-10870	IRGC 131966
16	ARC 15505	IRIS 313-11281	IRGC 131967
17	ARC 12655	IRIS 313-10886	IRGC 132240
18	ARC 10296	IRIS 313-10670	IRGC 132317
19	SACHI	IRIS 313-11370	IRGC 132368
20	ARC 5756	IRIS 313-10845	IRGC 132418

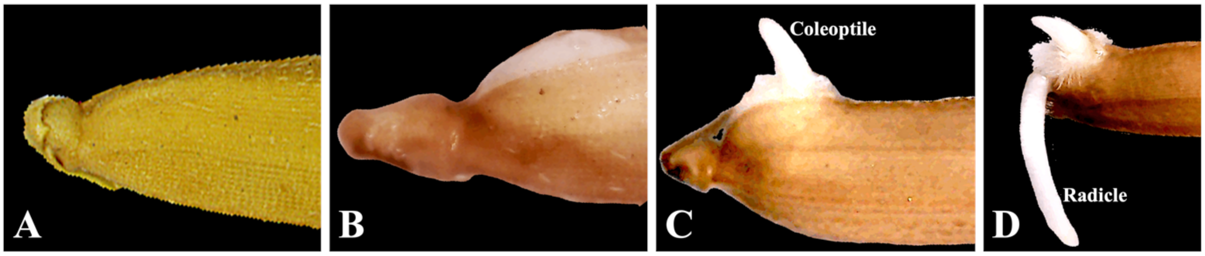

Fig. 2. Stages of rice seed germination

A: Dry Seed B: Lemma rupture stage C: Coleoptile emergence stage D: Radicle emergence stage

were then placed upright in a germination chamber.

The germination chamber was meticulously maintained at a temperature of 25 ± 2°C with a relative humidity of 80%, while diffused light was provided by a white fluorescent lamp with an intensity ranging from 750 to 1250 lx. At the conclusion of the germination test, 14 days after incubation, a count was performed to distinguish between normal seedlings, abnormal seedlings, dead seeds, and hard seeds. The germination percentage was calculated based on the number of normal seedlings.

$$\text{Germination percentage} = \frac{\text{Total number of normal seedlings}}{\text{Total number of seeds used for germination testing}} \times 100$$

Upon completion of the germination test, ten normal seedlings were randomly chosen from each replication, and the length of each seedling's root was measured using a linear scale, extending from the point where the shoot met the root to the tip of the root. Similarly, the length of each seedling shoot was measured using a linear scale, starting from the point where the shoot was attached to the root and reaching the tip of the tallest leaf. The total seedling length was calculated by adding the measurements of the shoot and root lengths. Subsequently, the analysis involved computing the average shoot length, root length, and seedling length based on these measurements from ten randomly selected seedlings in replicates for each genotype.

After measuring the individual shoot and root lengths of each seedling, the shoots and roots were carefully separated. Subsequently, the shoots from the ten selected seedlings were collected and measured using a precision four-digit analytical balance to obtain the fresh weight of the shoots. The fresh weight of the roots was recorded. Subsequently, the shoots and roots from each replication of the respective genotypes were dried overnight in an oven at 70 ± 2 °C. The resulting dry weights were measured using the same precision four-digit analytical balance and subsequently used for statistical analysis. The fresh and dry seedling weights of each replicate were computed by summing the fresh and dry weights of shoots and roots.

Seedling vigour indices, denoted as I and II, were computed for each genotype replicate. Seedling vigour index I was determined using germination percentage and seedling length following the method proposed by Abdul-Baki and Anderson (1973). Similarly, the seedling vigour index II was calculated based on the germination percentage and seedling dry weight, as recommended by Abdul-Baki and Anderson (1973).

Vigour index I = Average seedling length of 10 seedlings (cm) X germination percentage

Vigour index II = Seedling dry weight of 10 seedlings (g) X germination percentage

Correlations between early germination traits and other ESV traits: Relationships between the early germination traits (TFLR, TFCE and TFRE) and other ESV traits like shoot length (ShL), root length (RL), seedling length (SL), shoot fresh weight (ShFW), root fresh weight (RFW), shoot dry weight (ShDW), root dry weight (RDW), seedling dry weight (SdDW), germination percentage (Ger), Vigour index-I (VI.I) and Vigour index-II (VI.II) were examined through Pearson's correlation coefficient analysis (Pearson, 1932). This analysis was performed within the R statistical environment, utilizing packages such as ggplot2 and GGally, and subsequently, a correlogram was generated.

Results

To explore the relationship between early germination traits, specifically the time required for 50 percent lemma rupture (TFLR), time for 50 percent coleoptile emergence (TFCE), and time for 50 percent radicle emergence (TFLR), and other well-documented ESV traits, such as shoot length (ShL), root length (RL), seedling length (SL), shoot fresh weight (ShFW), root fresh weight (RFW), seedling fresh weight (SLFW), shoot dry weight (ShDW), root dry weight (RDW), seedling dry weight (SdDW), germination percentage (Ger), Vigour index-I (VI.I), and Vigour index-II (VI.II), an experiment was conducted using twenty randomly selected rice accessions from the 3KRGP panel. We generated respective trait values and performed a comparative analysis using Pearson correlation statistics.

Table 2 provides descriptive statistics for various parameters related to early germination and ESV traits, demonstrating significant variation among the 20 genotypes. The germination percentage trait exhibited the highest coefficient of variation (CV), followed by seedling length and vigour index I.

Pearson correlation test revealed significant correlations between the early germination traits and other ESV traits (Figure 2). Positive correlations were observed between TFLR and TFCE (0.859), TFLR and TFRE (0.840), and TFCE and TFRE (0.853). Conversely, significant negative correlations were found between early germination traits (TFLR, TFCE, TFRE) and traits associated with seedling length (ShL, RL, SL), with correlation coefficients ranging from -0.317 ($1.37x10^{-02}$) to -0.780($2.11x10^{-13}$) at respective p-values. Similarly, significant negative correlations were detected between early germination traits and traits related to the fresh weight of 14-day-old seedlings (ShFW and SLFW), with correlation values ranging from -0.523 ($1.85x10^{-05}$) to -0.672 ($4.25x10^{-09}$). Notably, no significant associations were observed between early germination traits and RFW.Similarly, no significant associations were found between.

Furthermore, significant negative correlations were identified between early germination traits and seed germination percentage, ranging from -0.319 ($1.31x10^{-02}$) for TFLR and Ger to -0.481 ($9.92x10^{-05}$) for TFCE and Ger. Comparable results were observed for vigour index-II and early germination traits, with high correlation coefficient values of -0.878 between TFLR and VI.I and the lowest correlation coefficient values of -0.744 between TFCE and VI.I, all at a P value of 1.01×10^{-11}. No associations were found between early germination traits and VI.II. The effectiveness of DSR cultivation depends early seedling emergence and uniform establishment. Seedling emergence is influenced by environmental conditions as well as favourable ESV traits of rice genotypes, bred to suit the challenges and opportunities of DSR farming. Rapid seed germination and post-germination heterotrophic seed-

Table 2. Descriptive statistics table of early germination and ESV traits

Trait	N	Mean	Minimum	Maximum	Standard Error	Standard Deviation	CV(%)
TFLR (hrs)	20	30.16	21.86	44.47	1.71	7.65	3.94
TFCE (hrs)	20	40.61	28.18	76.30	2.59	11.59	3.50
TFRE (hrs)	20	54.32	37.01	82.12	3.15	14.11	3.85
ShL (cm)	20	14.41	8.76	17.94	0.56	2.50	5.77
RoL (cm)	20	18.27	13.94	22.94	0.55	2.46	7.42
SdL (cm)	20	31.97	22.01	40.33	1.06	4.75	6.72
ShoFW (g)	20	0.4761	0.2154	0.9786	0.0455	0.2034	2.34
RoFW (g)	20	0.0846	0.0391	0.1254	0.0063	0.0284	0.98
SdLFW (g)	20	0.5607	0.2626	1.0455	0.0484	0.2166	2.59
ShoDW (g)	20	0.0802	0.0424	0.1324	0.0048	0.0215	3.73
SdLDW (g)	20	0.1344	0.0892	0.2060	0.0074	0.0331	4.06
Ger (%)	20	94.69	89.36	97.27	0.50	2.24	42.34
VI-I	20	3098.62	2023.64	3862.77	110.23	492.98	6.29
VI-II	20	12.73	8.58	19.32	0.68	3.06	4.16

ShL: Shoot length RL: Root length SL: Seedling length
ShFW: Shoot fresh weight RFW: Root fresh weight SLFW: Seedling fresh weight
ShDW: Shoot dry weight RDW: Root dry weight SdDW: Seedling dry weight
Ger: Germination percentage VI.I: Vigour index-I VI.II: Vigour index-II
TFLR: Time to 50 percent lemma rupture
TFCE: Time to 50 percent coleoptile emergence
TFRE: Time to 50 percent radicle emergence
TFLR, TFRE, and dry weight-related traits of 14-day-old seedlings (ShDW and SLDW).

ling growth are crucial factors that determine seedling emergence in the field (Finch-Savage *et al.,* 2010).

Discussion

Under field conditions, delay in seedling emergence is predominantly due to late germination and failure or non-uniformity in seedling emergenceis due toa slow heterotrophic seedling growth rate, as reported in many horticultural crops (Hegarty and Royle, 1978; Durrant, 1984; Finch-Savage and Phelps, 1993; Finch-Savage *et al.,* 1998; Whalley *et al.,* 1999; Rowse and Finch Savage, 2003; Finch-Savage, 2004; Finch-Savage *et al.,* 2010). Early germination traits, such as time to 50 per cent radicle emergence, have been reported to have a positive influence on other ESV traits, such as seedling emergence potential, percentage normal seedlings, seedling vigour, and seedling growth rate in many horticultural crops (Matthews and Khajeh-Hosseini, 2007; Dutt and Geneve, 2007; Matthews and Powell, 2012; Mavi *et al.,* 2016; Demir *et al.,* 2019; Demir *et al.,* 2020).

In the present study, we established the relation between the early seed germination traits and the other conventional ESV traits, which will facilitate in better understanding of the ESV trait and for betterment of the DSR system.

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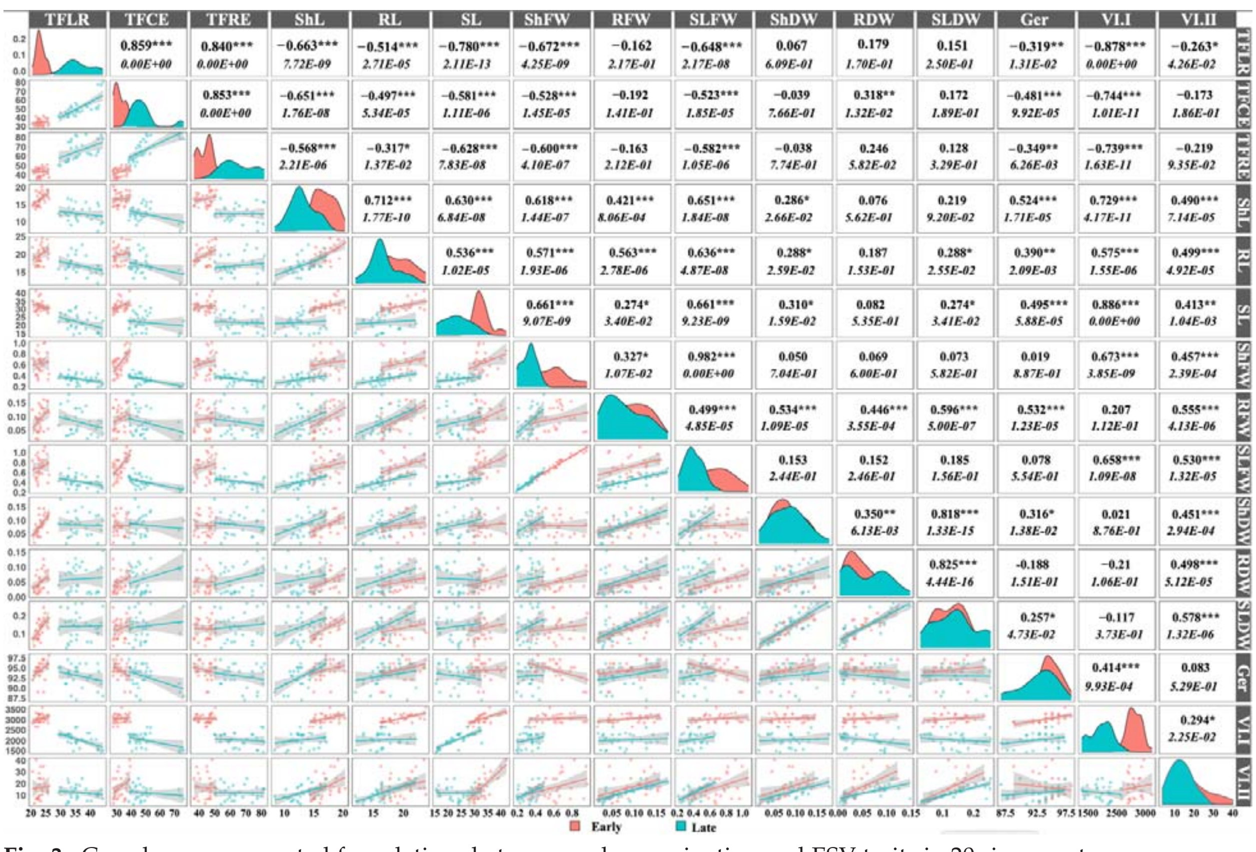

Fig. 3. Correlogram generated for relations between early germination and ESV traits in 20 rice genotypes

ShL: Shoot length (cm) RL: Root length (cm) SL: Seedling length (cm) ShFW: Shoot fresh weight (g)
RFW: Root fresh weight (g) SLFW: Seedling fresh weight (g) ShDW: Shoot dry weight (g) RDW: Root dry weight (g)
SdDW: Seedling dry weight (g) Ger: Germination percentage (g) VI.I: Vigour index-I VI.II: Vigour index-II
TFLR: Time to 50 percent lemma rupture TFCE: Time to 50 percent coleoptile emergence
TFRE: Time to 50 percent radicle emergence Values given in italics are the P values

availability of essential resources.

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