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ANAEROBIC GERMINATION TOLERANCE IN SWARNA × *Oryza rufipogon* WILD INTROGRESSED RICE LINES: SCREENING AND MGIDI-BASED SELECTION

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ABSTRACT

In direct-seeded rice, crop establishment is greatly affected by the ability of seeds to germinate and seedlings to grow when oxygen availability is low, especially in fields that remain flooded or waterlogged after sowing. Wild rice relatives, particularly *Oryza rufipogon*, carry alleles for stress adaptation that have largely been lost during domestication and offer scope for widening the tolerance base of modern varieties. In the present study, 119 BC_2F_5 wild introgressed lines derived from the cross Swarna × *O. rufipogon*, together with three checks (Swarna, Swarna Sub1 and *O. rufipogon*) were screened for anaerobic germination tolerance under controlled anaerobic conditions at the College of Agriculture, Navile, Shivamogga, during summer 2026. Pre-soaked seeds were sown under a 10 cm standing water column for 21 days, after which seedling emergence and establishment were recorded following a one-week revival period. Based on anaerobic germination percentage 32 genotypes were classified as tolerant, 66 as moderately tolerant and 24 as susceptible, following the criteria of Manigbas *et al*. (2008). The wild parent *O. rufipogon* exhibited a tolerant reaction, whereas both checks, Swarna and Swarna Sub1, were susceptible. Anaerobic germination percentage ranged from 10.00 to 90.00 per cent and considerable variation was also evident for shoot length, root length, number of leaves, distance to the first internode, seedling length and seedling vigour index-I (SVI), with SVI exhibited the greatest variation among the traits studied. A Multi-trait Genotype Ideotype Distance Index (MGIDI) built from these seven seedling-establishment traits, applied at 15 per cent selection intensity, identified 18 superior lines, with A-219, A-87 and A-09 recording the lowest (most desirable) MGIDI values. The selected lines combined higher germination percentage with longer shoots and roots and greater seedling vigour and the selection differential was highest for SVI (41.10%), followed by root length (34.90%) and germination percentage (23.70%). These results indicate that the Swarna × *O. rufipogon* introgression population harbours useful variability for anaerobic germination tolerance and that multi-trait indices such as MGIDI provide an efficient basis for identifying donor lines combining rapid germination with vigorous early seedling growth for direct seeded rice breeding.

Key words : Rice, *Oryza rufipogon*, Wild introgression, Anaerobic germination, Multi-trait Genotype Ideotype Distance Index (MGIDI).

Introduction

Rice (*Oryza sativa* L.) is the principal staple food for more than half of the world's population and support the food security of large parts of Asia, including India. Traditional puddled transplanted culture, although reliable, is increasingly constrained by shortages of labour and irrigation water, prompting a steady shift toward direct seeded rice (DSR) in many production systems. Under DSR and particularly under wet or flush irrigated seeding,

seeds frequently germinate, while submerged or under a standing water layer, exposing the germinating embryo and emerging coleoptile to near-anoxic conditions. In general, cultivated rice varieties exhibit limited adaptation to restricted oxygen availability which suppresses starch mobilisation and coleoptile elongation, leading to weak and patchy stands and in severe cases, complete germination failure. Reliable establishment under anaerobic conditions is therefore a prerequisite for the broader and more dependable adoption of DSR technology.

Unlike vegetative-stage submergence tolerance, which is associated with the SUB1 locus, anaerobic germination tolerance is governed by distinct physiological and genetic mechanisms driven by efficient anaerobic starch-sugar metabolism, enhanced á-amylase activity and rapid coleoptile elongation that allows the shoot to reach the water surface (Ismail *et al*., 2009 and Kretzschmar *et al*., 2015). Only a limited number of genetic sources for anaerobic germination tolerance have been identified in cultivated rice, highlighting the need to explore additional genetic resources, including wild relatives, for novel tolerance alleles.

Oryza rufipogon, the wild progenitor of cultivated rice is considered as a valuable reservoir of beneficial alleles for yield, quality and stress tolerance traits that may lost during domestication and modern breeding. Backcross introgressed populations developed between elite cultivars and *O. rufipogon* allow such alleles to be incorporated into an adapted genetic background, making them directly usable in applied breeding once superior lines are identified. Because anaerobic germination tolerance is influenced by several related traits involved in seedling establishment, rather than germination percentage itself, selection based on a combination of traits may provide a more reliable approach than evaluating a single trait independently. Multivariate selection indices, such as the Multi-trait Genotype Ideotype Distance Index (MGIDI) proposed by Olivoto and Nardino (2021), combine several correlated seedling traits into combined single index and have been used more widely to identify genotypes that most closely approach a predefined ideotype.

Against this background, the present investigation was undertaken to screen a 119 BC_2F_5 wild introgressed lines derived from the cross Swarna × *O. rufipogon* for anaerobic germination tolerance, to characterise the extent of variability present for germination and early seedling establishment traits and to utilise the MGIDI approach for identifying superior introgressed lines combining rapid, uniform germination with vigorous seedling growth under oxygen deficient conditions. Such lines are expected to serve as useful donor parents for improving the performance of direct-seeded rice varieties in flood-prone ecosystems.

Materials and Methods

Experimental site and material

The screening was conducted during summer 2026 at the College of Agriculture, Navile, Shivamogga, Karnataka, India (13°58’37.19" N, 75°34’39.48" E; altitude 630 m above mean sea level). The experimental material comprised of 119 BC_2F_5 wild introgressed lines developed through backcrossing the Swarna × *Oryza rufipogon*, obtained from the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad. These lines were compared against three checks, namely Swarna (recurrent parent and susceptible check), Swarna Sub1 (submergence-tolerant check) and *O. rufipogon* (wild donor parent), comprising a total 122 genotypes evaluated for anaerobic germination response.

Screening procedure for anaerobic germination tolerance

Each genotype was evaluated in two replications, with each replication comprising a plastic cup of 15 cm height filled with 5 cm of soil. In each replication, twenty seeds per genotype, pre-soaked in water for 48 hours, were sown at a uniform depth of 1 cm. A standing water column of 10 cm was then maintained above the soil surface throughout the screening period to impose anaerobic condition and this water level was sustained for 21 days. At the end of this period the standing water was drained and the seedlings were allowed to recover for one week under normal conditions before final observations were recorded. Mean values of the two replications were used for subsequent statistical analysis.

Classification of tolerance

Anaerobic germination percentage was calculated as the number of seedlings emerged expressed as a percentage of the total number of seeds sown. Genotypes were then grouped into three tolerance classes following the criteria of Manigbas *et al*. (2008): tolerant (76-100% germination), moderately tolerant (51-75% germination) and susceptible (0-50% germination).

Observations recorded

Following the revival period, observations were recorded on all surviving seedlings for anaerobic germination percentage (AG, %), shoot length (SL, cm), root length (RL, cm), number of leaves per seedling (NL), seedling length (SNG, cm) and distance to the first

internode (DIL, cm). Seedling vigour index-I (SVI) was derived from these primary measurements using the standard formula:

SVI-I = Anaerobic germination percentage × Mean seedling length

Statistical analysis

Descriptive statistics, namely the mean, range, standard deviation and standard error were computed for anaerobic germination percentage and each of the seedling-establishment traits across the 122 genotypes evaluated.

Superior introgressed lines were subsequently identified using the Multi-trait Genotype Ideotype Distance Index (MGIDI) of Olivoto and Nardino (2021). Anaerobic germination percentage, shoot length, root length, number of leaves, seedling length and seedling vigour index-I considered traits for which higher values were desirable, while a lower value was assigned as the breeding objective for distance to the first internode, so as to favour lines that established vigorously without excessive early internodal elongation. Genotypic means for the seven traits were standardised, factor-analysed and combined into a single distance measure representing each genotype's proximity to the predefined ideotype; introgressed lines with the smallest MGIDI values were considered closest to the ideotype and at a selection intensity of 15 per cent, the eighteen lines with the lowest MGIDI values were selected. The selection differential (SD) for each trait was calculated as the difference between the mean of the selected lines and the mean of the original population and expressed both in absolute terms and as a percentage of the original population mean.

Results and Discussion

Classification of wild introgressed lines for anaerobic germination tolerance

Screening of the 119 BC_2F_5 wild introgressed lines together with the three checks revealed a wide variation to anaerobic conditions during germination. Of the 122 genotypes evaluated, 32 were classified as tolerant (76-100% germination), 66 as moderately tolerant (51-75% germination) and 24 as susceptible (0-50% germination) (Table 1). The wild parent, *O. rufipogon*, recorded a tolerant reaction, whereas both check varieties, Swarna and the submergence-tolerant check Swarna Sub1, fell in the susceptible class. This contrast between the checks and the tolerant introgressed lines indicates that tolerance to vegetative-stage submergence, for which Swarna Sub1 was bred, does not necessarily confer tolerance to anaerobic germination, since the two traits are associated



Plate 1 : Experimental setup of anaerobic germination screening conducted during summer 2026.



a) View of tolerant lines



b) View of moderately tolerant lines



c) View of susceptible lines

Plate 2 : Representative anaerobic germination responses of rice genotypes: (a) tolerant, (b) moderately tolerant and (c) susceptible.

with distinct physiological processes occurring at different growth stages. The tolerant reaction of *O. rufipogon*, together with a substantial group of 32 tolerant introgressed lines. Similar variation among diverse rice germplasm has been reported by Reddy *et al*. (2022), Tripathi *et al*. (2023), Mwakyusa *et al*. (2023) and Kiranmayee *et al*. (2026).

The considerable moderately tolerant class - 66 lines, representing more than half of the population indicates that a substantial proportion of the introgressed lines possessed an intermediate level of anaerobic germination tolerance. The broad distribution of lines across tolerance classes also reflects considerable variation in the expression of the trait within the introgressed population. This distribution provides scope for further selection, with the moderately tolerant group serving as a useful pool of genetic material from which lines with higher anaerobic germination tolerance can be identified and advanced for

Table 1 : Classification of BC_2F_5 wild introgressed lines and checks based on anaerobic germination tolerance (Manigbas *et al*., 2008).

S. no.	Anaerobic germination percentage	Tolerance level	Accessions
1.	76-100%	Tolerant	A-01, A-09, A-13, A-75, A-78, A-97, A-113, A-132, A-162, A-175, A-209, A-218, A-219, A-230, A-232, A-234, A-247, A-249, A-251, A-253, A-254, A-255, A-257, A-262, A-263, A-267, A-288, A-289, A-295, A-307, A-313, *Oryza rufipogon.*
2.	51-75%	Moderately tolerant	A-17, A-30, A-47, A-61, A-68, A-82, A-87, A-89, A-107, A-111, A-114, A-135, A-145, A-156, A-161, A-165, A-168, A-169, A-172, A-177, A-179, A-182, A-185, A-188, A-189, A-192, A-196, A-199, A-200, A-202, A-203, A-213, A-216, A-221, A-222, A-223, A-224, A-227, A-228, A-229, A-231, A-238, A-241, A-245, A-246, A-252, A-258, A-260, A-261, A-264, A-268, A-270, A-272, A-273, A-274, A-280, A-290, A-292, A-293, A-294, A-297, A-300, A-301, A-304, A-311, A-317.
3.	0-50%	Susceptible	A-67, A-77, A-80, A-85, A-91, A-174, A-186, A-195, A-201, A-204, A-205, A-208, A-212, A-236, A-239, A-259, A-271, A-275, A-286, A-291, A-305, A-312, Swarna, Swarna Sub-1.

Table 2 : Descriptive statistics of anaerobic germination and seedling establishment traits among BC_2F_5 wild introgressed lines.

Trait	Mean	Minimum	Maximum	Standard Deviation (SD)	Standard Error (SE)
AG	64.02	10.00	90.00	17.38	1.57
SL	22.50	11.00	34.63	4.52	0.41
RL	9.43	4.47	17.23	2.70	0.24
NL	3.05	2.00	4.00	0.59	0.05
DIL	5.42	2.35	8.27	1.07	0.10
SNG	31.93	18.45	46.33	6.01	0.54
SVI	2062.57	184.50	3414.17	698.39	63.23

AG - Anaerobic germination percentage; **SL -** Shoot length; **RL -** Root length; **NL -** Number of leaves; **DIL -** Distance to first internode; **SNG -** Seedling length; **SVI -** Seedling vigour index-I.

evaluation. Such selection may facilitate the identification of stable and promising lines for further improvement of anaerobic germination tolerance.

Variability for anaerobic germination and seedling-establishment traits

Anaerobic germination percentage varied widely among the introgressed lines, ranging from 10.00 to 90.00 *per cent* with a mean of 64.02 per cent (SD = 17.38, SE = 1.57), reflecting the broad genetic base created by introgression from *O. rufipogon* (Table 2). Shoot length ranged from 11.00 to 34.63 cm with mean 22.50 cm, while root length varied between 4.47 and 17.23 cm with mean 9.43 cm, indicating that lines differed not only in the proportion of seeds that emerged but also in how vigorously the emerging seedlings elongated under continued anaerobic conditions. The number of leaves per seedling was comparatively uniform, ranging from 2.00 to 4.00 with mean 3.05, whereas the distance to the first internode, spanning 2.35 to 8.27 cm with mean 5.42 cm, showed moderate variation. Seedling length, an integrated measure of root and shoot growth, ranged from 18.45 to 46.33 cm with mean 31.93 cm.

Seedling vigour index-I, being a composite trait derived from germination percentage and seedling length, showed the widest relative spread of all traits examined, ranging from 184.50 to 3414.17 with mean 2062.57. This magnitude of variation indicates that introgressed lines differing only relatively in germination percentage could still differ substantially in overall vigour once differences in seedling length were compounded into the index, making SVI-I a sensitive discriminator among introgressed lines. Comparable ranges of variability for anaerobic germination and associated seedling traits among rice germplasm have been reported by Sudeepthi *et al*. (2019), Arulmozhi *et al*. (2020), Reddy *et al*. (2022), Tripathi *et al*. (2023), Gilbert *et al*. (2024), Neelima *et al*. (2025) and Kiranmayee *et al*. (2026), all of whom emphasised that such variability offers valuable scope for identifying superior donors for anaerobic germination tolerance. The wide variation recorded across the seven traits in the present population confirms that the Swarna × *O. rufipogon* introgressed lines constitute a useful genetic resource for improving early seedling establishment of rice under direct-seeded, flood-prone conditions.

Multi-trait selection of superior introgressed lines using MGIDI

Selection for anaerobic germination tolerance based on germination percentage alone can underestimate introgressed lines that germinate adequately but establish poorly or *vice versa*, since successful seedling

Table 3 : Selection differential for anaerobic germination and seedling-growth traits based on MGIDI.

Trait	Factor	MGIDI Original Mean (Xo)	Selected Mean (Xs)	Selection Differential (SD)	SD (%)	Selection Direction	Goal (%)
AG	FA1	64.00	79.20	15.20	23.70	Increase	100
RL	FA1	9.43	12.72	3.29	34.90	Increase	100
NL	FA1	3.05	3.50	0.45	14.60	Increase	100
SVI-I	FA1	2062.57	2910.57	848	41.10	Increase	100
SL	FA2	22.50	24.21	1.71	7.61	Increase	100
DIL	FA2	5.42	4.98	-0.44	-8.10	Decrease	100
SNG	FA2	31.93	36.93	5.00	15.70	Increase	100

AG - Anaerobic germination percentage; **SL -** Shoot length; **RL -** Root length; **NL -** Number of leaves; **DIL -** Distance to first internode; **SNG -** Seedling length; **SVI -** Seedling vigour index-I.

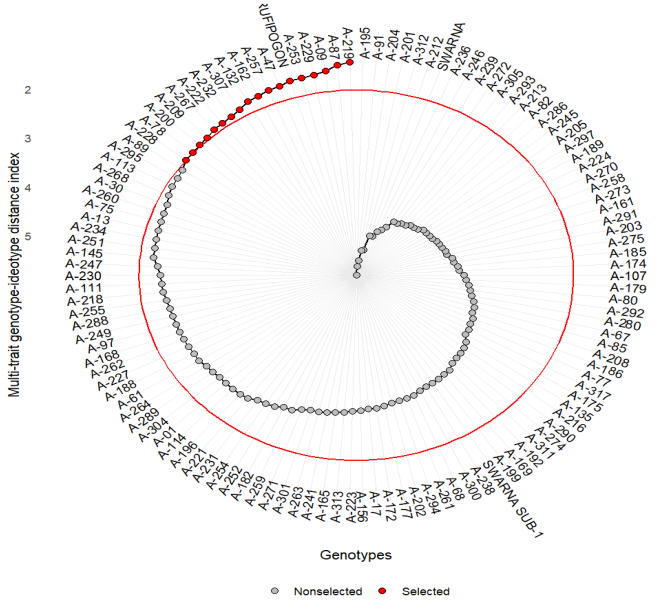


Fig. 1 : Radar plot showing MGIDI-based ranking and selection of wild introgressed rice lines.

establishment under oxygen-deficient conditions depends on the combined expression of germination, elongation and overall vigour. The MGIDI framework was therefore used to combine anaerobic germination percentage, shoot length, root length, number of leaves, seedling length and seedling vigour index-I for which higher values were preferred, with a lower value for distance to the first internode was considered, so that lines were preferred for vigorous establishment without excessive early internode elongation.

At a selection intensity of 15 *per cent*, the eighteen introgressed lines with the lowest MGIDI values and therefore closest to the predefined ideotype were identified. Line A-219 recorded the lowest MGIDI value (1.45), followed by A-87 (1.50) and A-09 (1.59). These top ranked lines combined high germination percentage (75-85%) with above average shoot and root length and correspondingly high seedling vigour index values, illustrating that the leading introgressed lines performed well across the traits rather than only in single component trait. The wild parent *O. rufipogon* itself ranked sixth (MGIDI = 1.67), reaffirming its role as a source of favourable alleles for anaerobic germination tolerance underlying the performance of the selected introgressed lines.

The selection differential calculated for each trait among the eighteen selected lines confirmed that the MGIDI procedure met the intended shift toward the predefined ideotype. Positive selection differentials were recorded for all traits favoured for increase, the largest was obtained for seedling vigour index-I (41.10%), followed by root length (34.90%), anaerobic germination percentage (23.70%), seedling length (15.70%), number of leaves (14.60%) and shoot length (7.61%). Distance to the first internode showed a negative differential (-8.10%) (Table 3). Consistent with the breeding objective of restricting excessive internode elongation while the seedling was still under water. The magnitude of the differential for SVI, considerably larger than that for germination percentage alone, this highlights the usefulness of a composite, multi-trait selection approach, as SVI-I integrates germination success with seedling length and provides a broader measure of seedling vigour than germination percentage alone.

These results are in agreement with the general observation that multi-trait indices such as MGIDI outperform single character selection when the target trait is physiologically complex, since they allow breeders to capture genotypes that are consistently favourable across a set of correlated traits rather than exceptional in only one (Olivoto and Nardino, 2021). In the present study, the strengths of the top ranked lines, particularly A-219, A-87 and A-09 in combining above-average



Fig. 2 : Strengths-weaknesses analysis of selected introgressed lines under anaerobic germination conditions.

germination with vigorous shoot and root growth and their relatively low distance to the first internode, indicate that these introgressed lines offer a favourable combination of traits for practical use as donor parents. Together with the wild parent *O. rufipogon*, which ranked among the top performers, these lines represent promising genetic resources that can be advanced directly in breeding programmes aimed at developing rice varieties with improved anaerobic germination tolerance for direct-seeded cultivation.

Conclusion

The present investigation demonstrated substantial genetic variability for anaerobic germination tolerance and associated seedling establishment traits among BC_2F_5 wild introgressed lines derived from the cross Swarna × *Oryza rufipogon*. Of the 122 genotypes screened, 32 introgressed lines were classified as tolerant, 66 as moderately tolerant and 24 as susceptible, with the wild parent *O. rufipogon* exhibiting a tolerant reaction and Swarna and Swarna Sub1 were susceptible. Considerable variation was also observed for shoot length, root length, number of leaves, distance to the first internode, seedling length and seedling vigour index-I, with SVI showing the widest range among the traits studied. Multi-trait selection using MGIDI, applied at 15 per cent selection intensity, identified eighteen superior introgressed lines that combined higher germination percentage with vigorous shoot and root growth and superior seedling vigour, with A-219, A-87 and A-09 emerging as the most promising genotypes. The results indicate that the Swarna × *O. rufipogon* introgressed population contains substantial variability for anaerobic germination and associated seedling-establishment traits, providing useful scope for selection. The MGIDI-selected lines combined higher germination with vigorous seedling growth, indicating their potential as donor material for improving crop establishment under direct-seeded and flood-prone conditions. The selected lines showed favourable performance across multiple traits rather than for germination percentage alone, supporting the usefulness of a multi-trait selection approach. The greater selection differential observed for seedling vigour index-I further indicates the importance of early seedling growth under anaerobic conditions. These lines can therefore be considered promising genetic resources for further breeding and evaluation. Molecular characterisation of the *O. rufipogon*-derived genomic regions in these lines would further help clarify the genetic basis of anaerobic germination tolerance and support their effective utilisation in breeding programmes for developing rice varieties better suited to direct-seeded and flood-prone production systems.

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