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Identifying Donors for Anaerobic Germination Tolerance and Direct Seeded Rice Cultivation by Exploring Seed and Seedling Traits

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ABSTRACT

Background: Anaerobic conditions in waterlogged soil affect germination rate significantly reducing rice yields. Under wet direct seeded condition, only limited availability of rice genotypes are suitable for anaerobic germination. So, there is an urgent need to identify rice genotypes for enhanced germination even under anoxic stress.

Methods: Experiment was conducted in glass house, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore. In this study, 22 rice germplams with three replications were evaluated for seed and seedling traits underlying anaerobic germination tolerance. Main aim of this study is to explore the starch and sugar relation under anoxic stress and identifying tolerant genotypes for direct seeded rice cultivation.

Result: Tolerant genotypes had early emergences, higher shoot and root length along with higher starch degradation and sugar accumulation under waterlogged condition. Cluster analysis revealed that Karuppukavuni, Kalanamak, CBMAS 14065 and Kodavilayan were identified as tolerant genotypes and suitable for wet direct seeded rice cultivation. Finally higher starch degradation coupled with higher accumulation of glucose and fructose were the key traits underlying anaerobic germination tolerance in tolerant genoytpes. Shoot length, root length and sucrose contents had higher genotypic coefficient of variance, phenotypic coefficient of variance coupled with high heritability indicating scope for enhancing anaerobic germination tolerance.

Key words: Anaerobic germination, Genotypes, Rice, Root length, Shoot length, Starch, Sugars.

INTRODUCTION

Rice is the second largest cereal crop in the world and worlds total rice production comes from Asia with a productivity of 510 million tonnes (Rauf *et al.,* 2019). Shortage of water, labour and high input makes rice production more expensive, less profitable and unsustainable (Farooq *et al.,* 2011). In India now a day's farmers are slowly adapting direct seeded rice (DSR) technology by broadcasting dry seeds (Sandhu *et al.,* 2021).

In Tamil Nadu especially Cauvery delta zones, nearly 3- 4 lakh acres of land are cultivated under DSR technology (Ganeshmoorthy *et al.,* 2014). Anaerobic germination (AG) is the major limiting factor for large scale adoption of DSR (Ghosal *et al.,* 2019). Anaerobic germination followed by shoot and root length plays a significant role in providing yield stability and adaptability under DSR (Sandhu *et al.,* 2016). AG tolerant genotypes responds in such a way that rice shoot develops quickly to reach surface of water allowing oxygen to get transported to underwater tissues. For tolerant genotypes faster and longer coleoptile growth under submergence acts as a major morphological adaptation (Hsu and Tung, 2017). Low O_2 conditions affects sugar and starch metabolism by altering glucose and fructose contents (Kogawara *et al.,* 2014).

Existing rice varieties are not speciûcally developed for direct-seeded ecosystems. Earlier rice varieties exhibited yield decline under direct seeded conditions. Varieties such as Sahbhagi dhan, DRR 42, DRR 44, CR dhan 201, CR dhan 203 and Swarna Shreya (Sandhu and Kumar, 2017) were developed by International Rice Research Institute (IRRI) and genomics-assisted derived CR dhan 801 varieties have become highly popular in India. In Tamil Nadu, landraces namely Kallurundaikar, Sivapuchithiraikar and Kuruvaikalanjiyam showed higher grain yield under dry direct seeded condition in Paramakudi (Muthuramu and Ragavan, 2022). Till date no landraces are reported to show tolerance under wet direct seeded conditions.

Under anaerobic condition, there is an urge to study the seed and seedling traits underlying anaerobic

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germination tolerance. Hence, this study was aimed with the following objectives (i) To screen the available landrace diversity of Tamil Nadu for anaerobic germination tolerance. (ii) To evaluate the seed and seedling traits in the contrasting rice genotypes to anaerobic germination tolerance. (iii) To identify the anaerobic germination tolerant rice genotypes for further field evaluation.

MATERIALS AND METHODS

Plant material and growth conditions

Was estimated seeds of 22 rice genotypes studied were sourced from the Tamil Nadu Agricultural University (TNAU), Coimbatore. Experiment was conducted (May to October, 2022) in glass house of Department of Crop Physiology, TNAU, Coimbatore. For both control and anaerobic germination, flooding tank (2.45 meter height, 1.57 meter width and 2.70 meter length)was used. The soil was a clay loam. Seed dormancy was broken by incubating the seeds at 50°C for one day prior to sowing. The control tank was allowed to water at regular interval to maintain thin film of water. To induce anaerobic condition, water level maintained at 15cm above the soil surface for 21 days. Seeds were allowed to germinate inside the submerged conditions. The water level in the flooding tank was observed daily morning and evening to maintain a 15 cm water level. All the genotypes were replicated thrice. Germination percentage was recorded at the end of the experiment. The number of seeds with emerged coleoptile and radicle were counted and expressed as percentage of the total number of seeds germinated to total number of seeds sown. After 6th day seeds were taken for analysis of starch content, sugar fractions and seedling traits. Moreover the germination percentage was calculated at end of the experiment (21 days after germination).

Measurements on seedling morphology

Shoot length (cm)

The length of the shoot was measured from the point of attachment on the seed to the tip in cm and the average was worked out.

Root length (cm)

The root length of the five random seedlings per replicant was measured from the point of attachment on the seed to the root tip in cm and the average was calculated.

Measurement of starch and sugar fractions

Starch determination

500 mg of rice seeds were heated with 80% ethanol for sugar removal from sample. Samples were centrifuged and 5.0 ml of water and 6.5 ml of 52% perchloric acid were added to the residues. The contents were centrifuged. 0.2 ml of the supernatant was pipetted out and made up to 1 ml with water. Add 4 ml of anthrone reagent was added and the contents were heated for 8 mins. OD measured at 630 nm (Critchley *et al.,* 2001).

Sucrose and fructose content

was estimated as per the procedure of Yaliang *et al.,* (2020). 0.1 ml sample extract was mixed with 50 µl 2 N NaOH in a test tube and the tube was placed in water bath for 5 min followed by addition of 0.7 ml 30% HCl, 0.2 ml 0.1% of resorcinol. OD value was measured at 480 nm to estimate sucrose content. To calculate. Fructose content 0.1 ml of sample was taken in a test tube and 0.2 ml of 0.1% resorcinol and 0.7 ml water was added and kept in water bath at 80°C for 10 min. OD value was measured at 480 nm. For glucose content, 4 mL enzyme mixture (10 mg *O*-dianisidine, 10 mg peroxidase and 0.1 mL glucose oxidase dissolved in 100 mL of 0.1 mol/L acetic acid buffer) was added to 2 mL supernatant and the mixture was heated at 30°C for 5 min followed by addition of and 8 mL of 10 mol/L sulphuric acid to terminate the reaction. Finally OD was taken at 505 nm (Yaliang *et al.,* 2020).

Statistical analysis

Experiment was designed as completely randomized design. Genetic parameters were performed by TNAUSTAT-statistical package. Analysis of variance (ANOVA) was used to identify significant difierences between the treatments. Statistical analysis and correlation were performed by GRAPES 1.0.0 software (General R-shiny based Analysis Platform Empowered by Statistics). Cluster analysis was performed by R studio version 4.3.0.

RESULTS AND DISCUSSION

To evaluate the seed and seedling traits in rice genotypes

Germination percentage

Early emergence is important during AG condition. Anaerobic stress decreases the germination percentage. Thavalakannan, Mapillai samba, Vellimuthu, Kothamalli samba, Karuppukavuni, Kodavilayan, CBMAS 14065 and Kalanamak showed all the seeds were germinated under stress (Table 1). Highest germination was observed in Karuppukavuni (100%) followed by CBMAS 14065 and Kalanamak (97.5%), Kodavilayan (95%) under anoxic condition. TKM13 and Anna R4 had germination percentage of 82.5 and 82.5 respectively under AG (Table 1).Tolerant genotypes had higher seed germination even under anoxic stress condition. Similar results were reported by Yang *et al.,* (2021). Germination rate was directly related to anaerobic seedling establishment under wet DSR (Yang *et al.,* 2021).

Shoot and root length

Development of high yielding rice genotypes under wet DSR required certain morpho-physiological traits. Shoot length and root length have been found to relate with rice root adaptation and tolerance in AG conditions (Sandhu *et al.,* 2016). Shoot length and root length are important and basic parameters under anaerobic stress conditions. Tolerant genotypes namely Karuppukavuni, Kalanamak, CBMAS 14065, Vellimuthu and Kodavilayan had higher shoot length of 28.19 cm, 27.02 cm, 25.53 cm, 23.62 cm and 24.97 cm respectively compared to other genotypes. Moderately tolerant genotypes *viz*., TKM13, Anna R4 and Varappukudainchan recorded shoot length of 12.27 cm, 11.18 cm and 10.70 cm respectively (Table 1). The shoot length was significantly increased in tolerant genotypes. Similar findings were observed in rice by Sandhu *et al.* (2016).

Long-term anoxic stress vanquishes growth and physiological activities of rice roots causing a dramatic decrease in root activity. Root length of tolerant genotypes ranged from 13.86 cm to 9.85 cm and 12.60 cm to 7.95 cm under control and AG conditions respectively. Similar results were reported by Liu *et al.,* (2023). Moderately tolerant genotypes had root length of 10.97 cm to 4.02 cm under AG conditions (Table 1). Highest root length of 12.60 cm was observed in Kalanamak and followed by Karuppukavuni (12.51 cm) while, minimum root length was recorded in Rasagadam (4.02 cm) and CO53 (4.33 cm) under AG conditions (Table 1). Moderately tolerant genotype showed 12.94% to 49.43% reduction in root length compared to highly tolerant genotype. Cao *et al.* (2020) stated that under hypoxia stress, tolerant genotypes exhibits lower reduction of root length. Shoot and root length variables are closely related and positively correlated (Fig 4), similar to the reports of Miro *et al.,* (2017).

Starch, glucose, fructose and sucrose contents

The major source of energy for seed germination and seedling growth is degradation of stored starch in seeds (Damaris *et al.,* 2019). Starch is a key nutrient and major component that affects germination. Tolerant genotypes namely Vellimuthu, Karuppukavuni, CBMAS 14065, Kalanamak and Kodavilayan showed decreased starch contents of 8.52, 6.71, 7.01, 7.18 and 7.42 µmole/g FW respectively when measured after 6 days of anaerobic stress conditions compared to control. Starch degradation in rice seeds is a major mechanism related to submergence tolerance (Loreti *et al.,* 2016). Starch values of 7.04 and 7.67 µmole/g FW were recorded in TKM13 and Anna R4 respectively under AG environment (Fig 1a). Percentage of starch breakdown was higher in tolerant genotypes (13.12% to 37.11%) compared to moderately tolerant genotypes (5.7% to 22.92%). For survival, starch utilization tends to be significant in AG stressed rice seeds (Loreti *et al.,* 2018). All the seed quality traits were positively inter correlated and contributed towards anaerobic germination tolerance (Fig 4). Glucose and fructose content showed opposite trends as that of starch content.

Sugar availability plays a pivotal role in energy production under anoxic conditions as reported by Loreti

Table 1: Genetic variability for seed and seedling growth traits in rice seeds germinated under anaerobic conditions.

Rice genotypes	Germination percentage (%)		Shoot length (cm)		Root length (cm)	
	Control	Stress	Control	Stress	Control	Stress
Nijavara	92.5	80.0	20.06	22.56	11.65	10.28
Thavalakannan	100.0	85.0	17.74	20.98	12.11	9.00
Adukkan	95.0	95.0	22.23	20.97	11.59	10.91
Mapillai samba	100.0	85.0	17.29	14.58	13.90	11.54
Vellimuthu	100.0	90.0	22.29	23.62	12.55	11.19
Kunjoanju	95.0	95.0	17.33	20.12	10.13	8.19
Kothamalli samba	100.0	90.0	17.33	14.91	10.14	8.44
Manvilayan	92.5	65.0	18.83	14.03	11.82	7.95
Karuppukavuni	100.0	100.0	21.21	28.19	13.86	12.51
CBMAS 14065	100.0	97.5	23.35	25.53	12.99	12.00
Nootripathu	97.5	90.0	18.09	15.61	11.86	8.96
Kala namak	100.0	97.5	22.96	27.02	13.69	12.60
Kodavilayan	100.0	95.0	21.84	24.97	12.86	10.87
Kallurundai samba	95.0	90.0	18.96	15.10	9.85	8.19
TKM 13	95.0	82.5	19.83	12.27	14.45	10.06
Aanaikomban	90.0	65.0	21.22	7.68	10.71	7.96
Norungan	92.5	80.0	20.10	7.00	13.28	8.14
Varappukudainchan	92.5	70.0	18.04	10.70	13.87	9.87
CO53	97.5	85.0	18.81	3.01	9.93	4.33
Rasagadam	92.5	77.5	16.49	4.02	9.83	4.02
Anna R 4	95.0	82.5	18.22	11.18	12.05	10.97
Kullakar	95.0	87.5	17.23	4.06	9.84	4.53
SEd	4.55		0.70		0.23	
CD (p<0.05)	7.79**		1.68***		0.36***	

et al., (2016). Glucose and fructose level was higher with decreased sucrose levels in coleoptiles of all rice genotypes under AG compared to normal condition (Fig 1a and 1b). At AG conditions, Karuppukavuni, CBMAS 14065, Kalanamak and Kodavilayan had higher glucose content (45.90, 43.97, 44.46 and 44.93 μmole/g FW) and fructose content (37.58, 36.59, 38.50 and 37.15 μmole/g FW) compared to normally grown seeds (Fig 1b). During germination, mixture of glucose and fructose is formed as a result of starch degradation (Muralikrisha and Nirmala, 2005). On the other hand, TKM13, Varappukudainchan and Anna R4 recorded lower glucose content (41.99, 41.71 and 43.07 μmole/g FW) and fructose content (37.41, 36.13 and 38.40 μmole/g FW) compared to tolerant genotypes under AG conditions (Fig 2a).

Table 2: Genetic parameters of rice genotypes under anaerobic germination.

Traits	Variance			GCV	PCV	Heritability	GAM
	Vg	Vp	Ve	(%)	(%)	(%)	(5%)
Germination percentage	15.6250	23.1429	7.5179	4.4432	5.4075	67.5154	7.5208
Shoot length	8.4183	9.1717	0.7534	15.8821	16.5776	91.7855	31.3446
Root length	1.8790	1.9312	0.0522	13.7945	13.9848	97.2971	28.0300
Starch	0.0609	0.1424	0.0815	2.5785	3.9415	42.7976	3.4750
Glucose	1.6640	2.6160	4.2801	3.2029	4.0159	63.6095	5.2623
Fructose	1.3164	4.4122	3.0958	3.2303	5.9139	29.8358	3.6348
Sucrose	9.2341	11.4114	2.1773	9.9169	11.0243	80.9198	18.3769

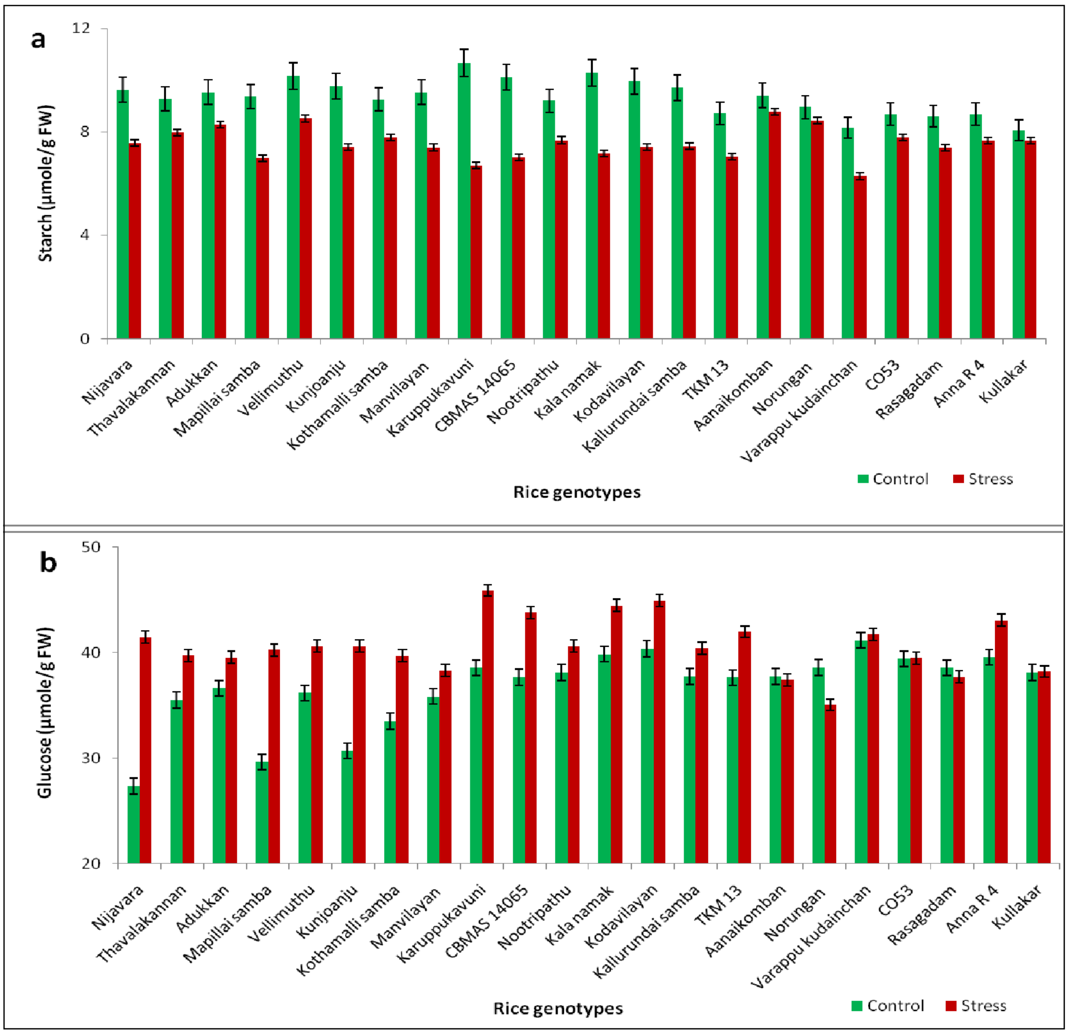

Fig 1: Genetic variability for starch (a) and glucose (b) contents in rice seeds subjected to anaerobic stress.

Rough and brown rice showed increment in reducing sugars during germination (Moongngarm and Saetung 2010). Enhancement in reducing sugar plays a significant role in AG tolerance.Thus, AG tolerant genotypes degrade starch contents into soluble sugars at a faster rate.

Anaerobic stress generally decreased the sucrose contents. Sucrose degradation was faster in tolerant genotypes compared to moderately tolerant genotypes. Tolerant genotypes recorded lower sucrose levels of 15.22 µmole/g FW in Karuppukavuni while, higher level of 33.32 µmole/g FW in Vellimuthu under AG. In moderately tolerant genotypes, less sucrose content of 19.37 µmole/g FW was observed in TKM13 while, Norungan recorded 36.19 µmole/g FW of sucrose under AG environment (Fig 2b). Similar results were found on the report of Magneschi and Perata (2009) who stated that sucrose catabolism is an adaptive way to channeling sucrose into AG metabolism in rice and maize crops under anoxia conditions. During seed germination sucrose is the major source for embryo development that is consumed in large scale by tolerant lines compared to moderately tolerant genotypes. Due to these reasons, tolerant landraces under AG stress catabolise sucrose into glucose and fructose contents.

Correlation and cluster analysis of physiological traits under AG conditions

Based on variations, 22 rice genotypes were clubbed into three clusters using R studio. Under AG conditions cluster technique clearly defines the clusters on basis of seed and seedling parameters (Fig 3). The results of cluster analysis showed that, cluster 1 (Highly tolerant genotypes) included Karuppukavuni (4), Kalanamak (12), Kodavilayan (13) and CBMAS 14065 (10) genotypes. TKM 13 (15), Anna R4 (21), Manvilayan (8), Kallurundai samba (14), Nootripathu (11), Mapillai samba (4), Kothamalli samba (7), Nijavara (1), Adukkan (3), Vellimuthu (5), Thavalakannan (2), Kunjoanju (6) were grouped in cluster 2 (Moderately tolerant genotypes). Six genotypes namely Kullakar (22), CO53 (19), Rasagadam (20), Varappu kudainchan (18), Aanaikomban (16) and Norungan (17) were grouped in cluster 3 (Moderately sensitive genotypes) (Fig 3). Inter traits correlation was also confirmed by correlation graph. Highly

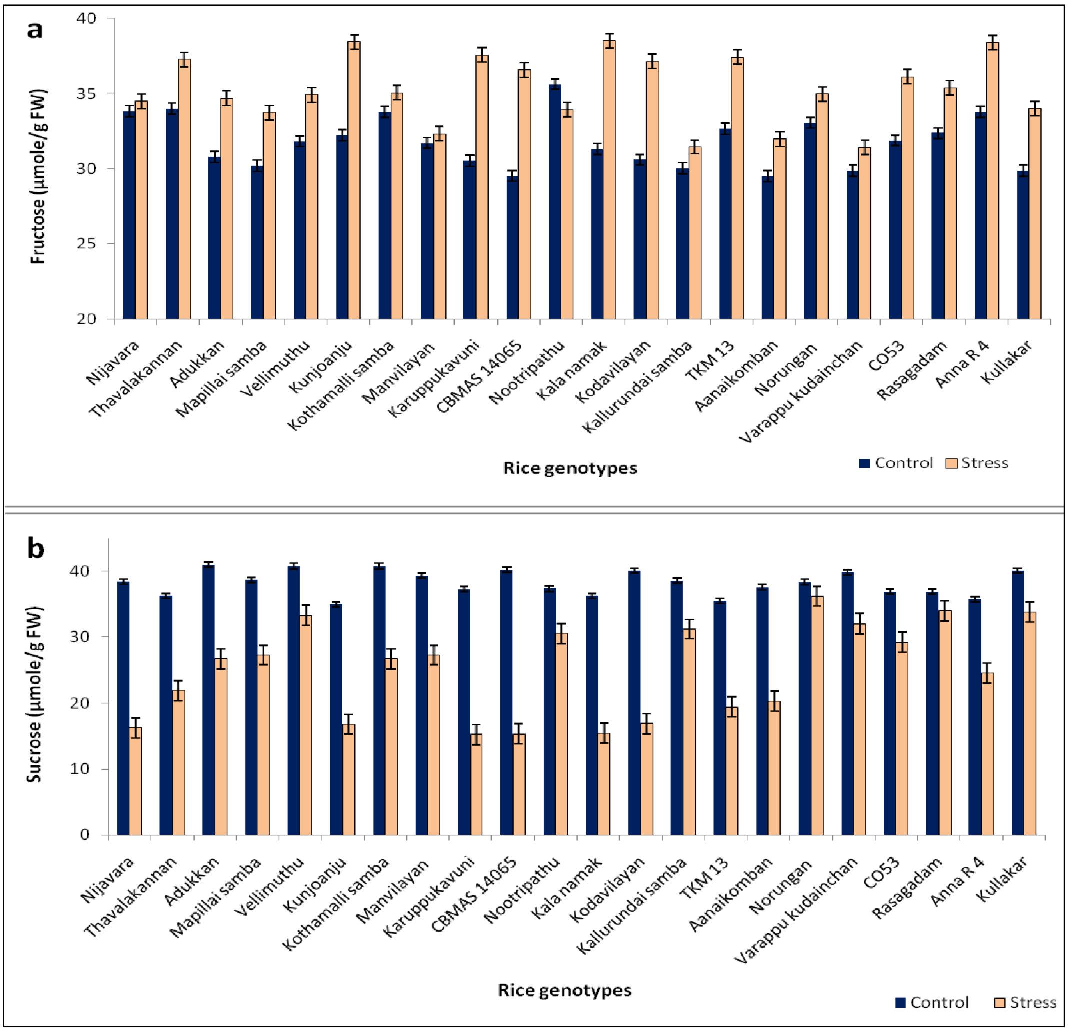

Fig 2: Genetic variability for fructose (a) and sucrose (b) contents in rice seeds subjected to anaerobic stress.

positive correlation was showed between shoot length, root length, glucose and fructose content with germination percentage (Fig 4). Starch and sucrose showed negative correlation with germination percentage under AG stress (Fig 4). All the physiological traits have contributed to tolerance in AG conditions. Similar results were supported by Miro *et al.,* (2017).

Variability, heritability and genetic advance

Always genotypic coefficient of variance (GCV) was found lower than phenotypic coefficient of variance (PCV) for seed and seedling traits (Table 2). Due to less influence of environmental characters, difference between PCV and GCV was less for all the traits. Minimum difference of GCV and PCV were observed in germination percentage, shoot length, root length, starch and glucose contents (Table 2). Thus, the selection on these traits might be effective in bringing significant genetic enhancement. Highest heritability was observed in shoot length (91.785%), root length (97.297%) and sucrose content (80.919%) under AG stress. Genetic advance per cent as mean were recorded higher in shoot length (31.344%), root length (28.030%) and sucrose content (18.376%) under anoxic condition. Similar results were reported by Beena *et al.* (2021) in rice genotypes subjected to heat stress. Results revealed that, higher GCV, PCV coupled with high heritability was noticed for the traits

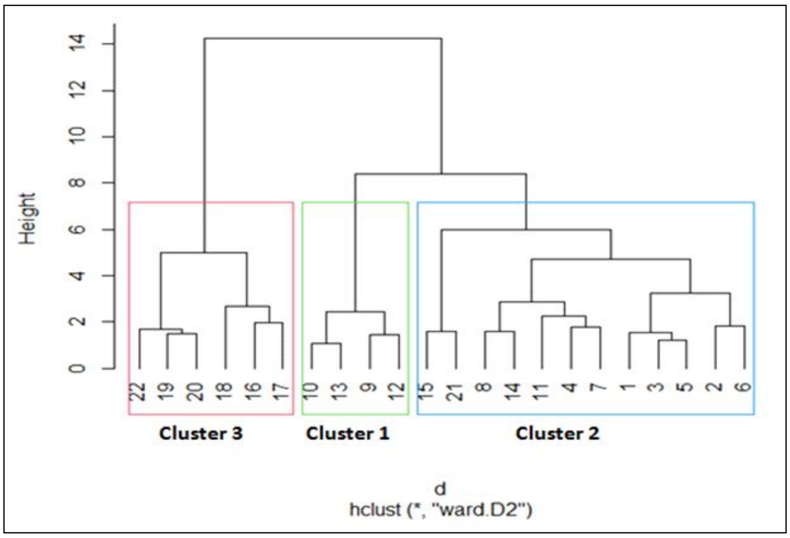

Fig 3: Hierarchical cluster analysis using seed and seedling traits under anaerobic condition.

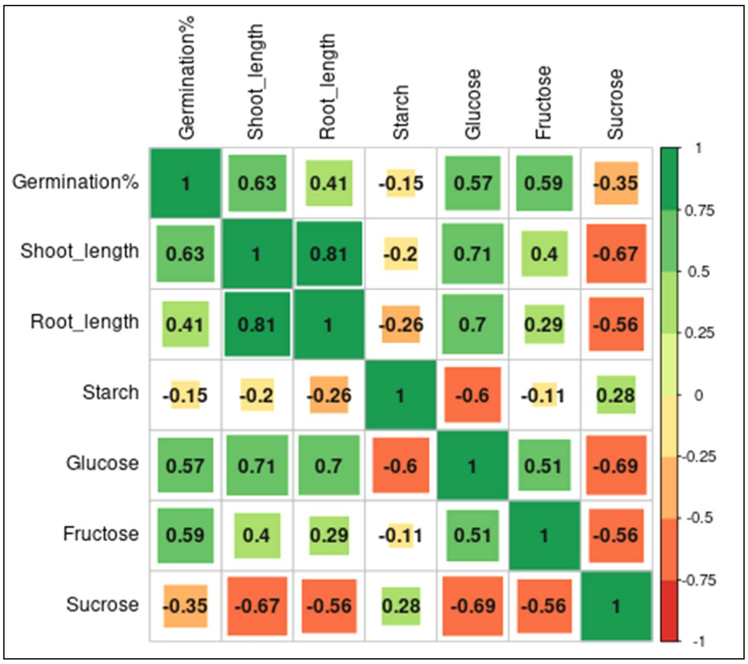

Fig 4: Correlation of seed and seedling traits associated with anaerobic germination tolerance.

viz., shoot length, root length and sucrose concentrations. Thus, these traits might be useful as selection criterion for screening anaerobic germination tolerance.

CONCLUSION

Rice cultivation under direct seeded condition is economical and eco-friendly. Under AG growth traits like shoot length and root length swap away in opposite direction showing significant relation with seedling establishment. Tolerant genotypes namely Karuppukavuni, Kalanamak, CBMAS 14065 and Kodavilayan had greater starch degradation and sucrose catabolism accompanied with higher glucose and fructose compared to moderately tolerant genotypes under submerged condition. Hence, these seed traits can be used as an index to screen rice genotypes for anaerobic germination tolerance.

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Conflict of interest

The authors declare that there is no conflict of interest.

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