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Seed vigour analysis using oxygen sensing technology in Rice (*Oryza sativa*. L) Genotypes.

P. Sadhana[1], Ch. Damodar Raju[2], Michael Kruse[3], Sebastian Bopper[3], L.V. Subba Rao[4], Aparna Kuna[5],B. Akshitha[1], S. Udaya[1]

[1]Department of Genetics and Plant Breeding, PJTSAU, College of Agriculture, Rajendranagar, Hyderabad, 500030, Telangana, India.
[2]Rice Research Centre, ARI, PJTSAU, Rajendranagar, Hyderabad, Telangana, India.
[3]Division of Seed Science and Technology, University of Hohenheim, Stuttgart, Germany.
[4]Department of Genetics and Plant Breeding, Crop Improvement section, ICAR- Indian Institute of Rice Research, Rajendranagar, Hyderabad, 500030, Telangana, India.
[5]Foods and Nutrition, Quality Control Laboratory, Rajendranagar, Hyderabad, 500030, Telangana, India.

ABSTRACT

Seed quality test is most important in agriculture to determine the planting value and to minimize the hazards of crop production. Quick and reliable vigour tests are required for supply of quality seed to the farmer. However, most commonly used method for the evaluation of vigour i.e. germination test is time-consuming and laborious. To rapidly assess the seed vigour, a Q2 scanner based on Oxygen sensing technology is used. The conventional vigour testing based on germination and seedling parameters needs a minimum of eight days for assessment. On the other hand, Q2 analysis provides a fast and accurate single-seed measurement of oxygen consumption during germination. Oxygen measurement can be used as it is directly related to seed respiration and energy production. An analysis can take 10 to 72 hours, depending on the species. The result is an easy determination of the metabolic activity of seeds. Although the Q2 analysis does not provide specific information about seedling categories in terms of the International Seed Testing Association (ISTA) (normal, abnormal etc.), it delivers fast and accurate information about homogeneity of seed vigour linked to oxygen consumption of seed lots. The Q2 analysis can be used to determine differences between seed lots in comparative trials. Oxygen-sensing technology provides a fast and automatic measurement of oxygen consumption and respiration efficiency. The present study is conducted for the evaluation of six rice genotypes for vigour using Q2 Scanner and examined the correlations between the different potential vigour indices.

Keywords: *Rice, Vigour, Q2 Scanner, Oxygen sensing technology, germination test, seed quality, International Seed Testing Association, Seed metabolic rate.*

Introduction

Rice is one of the most important crops providing staple food for half of the global population. Rice production depends on the supply of good quality seed. Seed vigour is an important characteristic of seed quality, reflecting potential seed germination, seedling growth, seed longevity, and tolerance to adversity. Strong vigour seeds can boost seed germination speed and uniformity, as well as the ultimate percentage of germination, resulting in flawless field emergence, crop performance and even high yield under various conditions [4]. Cultivars with strong seed vigour are desirable for farmers to get optimum stand establishment. The seed has to be evaluated before sowing. So, there is a need for rapid seed vigour evaluation methods. The Q2 Oxygen Sensing Technology is a new era in seed testing for basic research. It was developed by ASTEC Global. The measurements are being taken to link seed oxygen consumption over time to seed lot quality[2]. Q2 stands for Quality and Quick, as well as O2 (oxygen), the element around which the entire Q2 instrument is built.

Corresponding Author:* ***P. Sadhana*

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It allows for a quick and precise assessment of a seed lot's germination level. In addition, Q2 data are more robust and defining than traditional germination tests. It will easily determine dead, incomplete or actively germinating seeds.

Materials and Methods

Seed vigour analysis using the Q2 scanner was done at the University of Hohenheim, Germany in *Rabi*, 2019. Thirty samples (genotypes) were submitted to NBPGR, Hyderabad for testing of nematode (*Aphelenchoides besseyi*). Of these, six genotypes were free from nematode and a phytosanitary certificate was issued by Plant Quarantine Station (PQS), Hyderabad. These genotypes were taken for assessment of seed vigour using the Q2 scanner at UOH, Germany(Table .1). Experiment was done according to the procedure of [7]and[6] with some modifications as given below.

1. Initially agar solution was prepared by dissolving 3g of 0.5 % (w/v) agar in 600ml of distilled water and kept in the oven for 5mins.

2. The agar solution was placed on a vortex shaker and transferred to each well after the solution reached the temperature of 30-40º C.

3. Four replicates of 48 seeds per genotype were individually sewn on 750 µl 0.5% agar in 48-well microtiter plates (one seed per 1.5 ml well).

4. The microtiter plates were covered by lids coated with a 1.0 mm-thick membrane with fluorescence materials on the inside.
5. Two vials outside of the plates containing only air or 0% oxygen and 100% relative oxygen (actually 21% O_2 in air) were used as controls.
6. The plates were then placed in the Q2 and the instrument was programmed to scan the plates at intervals of 30mins until 140hrs at 25º C and then kept for the start of the run.
7. A robotic arm sequentially moved the light source and sensor over each well, measuring the oxygen concentration inside the well at 30mins intervals and developed oxygen consumption time courses for individual seeds.
8. The time course data from each well were collected in a database that is accessible to the Q2 analysis software.
9. The Q2 s**c**anner is well equipped with two software namely BMS (Basic Machine Software) and ANS (Analysis software). BMS is the basic software to operate the Q2 scanner. This BMS measured the data and transferred it to the PC (computer). The stored data is translated to ANS software through the ADO database engine.
10. ANS software consists of three modules (CAM, RCM, LCM) for further analysis of each replicate of a microtiter plate.
11. The curve analysis module (CAM) judged each seed oxygen consumption curve (inverse S-shaped curve) into four categories *viz.,* Germinated ok, incompletely germinated, Wrong and Dead and the data was exported to Excel.
12. After curve fitting, four replicates of each lot (genotype) were compared with the Replication comparison module (RCM) and data was exported to Excel.
13. The lot comparison module (LCM) compared six lots (genotypes) and the data was obtained in EExcel report and pdf format.
14. The Excel data was used for statistical analysis.

STANDARD Q2 VALUES

Increased Metabolism Time (IMT)

It is the time (in Hours) that the seed starts to begin increasing its metabolism. This value will be affected by the water permeability of the pericarp, the structure and composition of the coating and/or pelleting layers and the ability of the seeds to imbibe the necessary water to increase their metabolism in the germination process.

Oxygen Metabolism Rate (OMR)

It is the maximum amount of oxygen that can be consumed over time by the seed. This rate is expressed as % oxygen per hour. Because there is a direct relationship between the amount of oxygen consumed and the amount of energy used, this rate value is well related to the speed of germination. This value is one of the important parameters that can be used to describe the vigour of a seed lot. The speed of germination will also correlate to the field emergence.

Critical Oxygen Pressure (COP)

It is the lowest threshold value or amount of oxygen where seeds start to reduce respiration, and thus, metabolism as a response to the lack of oxygen. This value (expressed as oxygen %) provides an idea of how the seeds will perform under oxygen-stressed conditions.

Relative Germination Time (RGT)

It is valuable to make inferences about the germination time (or even field emergence) of a seed lot.

For seeds undergoing the germination process, the two biological factors which play major roles in the RGT are the IMT and the OMR (time to begin breaking dormancy and rate of metabolic energy use). The RGT value can be used to infer the relative germination and field emergence times. The RGT also provides an opportunity to differentiate between seed lots for their relative and critical events of crop establishment and overall yield.

Working principle

The working chemical in the Q2 scanner is "yellow" dye-coated transparent caps. It is a fluorescent chemical which responds to the amount of oxygen. Its fluorescent lifetime changes and gives off different values at different oxygen amounts which are measured by the sensor and translated into oxygen curves. The sensor is designed with a photodiode which emits two different lights based on the amount of oxygen present in the well. The detection of oxygen with a fluorescent coating (yellow dye) is based on the phenomenon that oxygen can quench the fluorescence. This means that both the fluorescent intensity as well as the fluorescence lifetime decrease as a function of a rising oxygen partial pressure. Initially, a blue light is emitted when the oxygen per cent in the well is high. At the end of the run when oxygen per cent decreases the sensor emits red light which indicates the lack of oxygen in the well Fig 1(a). Therefore, the oxygen curves were produced for the amount of oxygen present in the well. As the seed in the well starts increasing its metabolic rate, it starts using oxygen proportionately, hence depleting oxygen in the well. This causes the oxygen curve to start dropping over time. At a certain point in time, the oxygen in the well inevitably is used up. This causes the curve to flatten indicating that there is no more oxygen for the seed to use at that point in time, which is the end of the test. A typical inverse S (sigmoid) shaped curve is produced by the germinating seeds.

Oxygen consumption by rice seeds during germination mainly displayed an inverse S-shaped (Sigmoid) curve. Each well was scanned automatically by the Q2 machine at 30-minute time intervals. The oxygen level measurements were collected and stored in Excel data format and converted to data curves showing the oxygen consumption pattern of individual seeds. Through the Curve Analysis Module (CAM) individual seeds are categorized into dead, incompletely germinated, germinated ok and wrong based on the pattern of the curve. The seeds that showed constant oxygen consumption were categorized as linear or incompletely germinated (Fig 2 a). The seeds that showed straight lines or no oxygen consumption were categorized as dead seeds (Fig 2 b). The seeds that showed negative values for the parameters were categorized as wrong (Fig 2 c). The seeds that showed curves other than sigmoid were neglected (Fig 2 d). The seeds that showed an inverse sigmoid curve of oxygen consumption pattern were categorized as Germinated Ok (Fig 2 e). After categorizing individual seeds through CAM analysis, two replications of each lot (genotype) were compared through the Replication Comparison Module (RCM) (Fig 3 a).

Through the Lot Comparison Module (LCM) different lots are compared and the overall graph is displayed (Fig 3 b). However, through LCM analysis only six lots can be compared per run. Through the Q2 scanner, four oxygen metabolism parameters were determined for each inverse S-shaped curve *viz*., IMT (Increased Metabolism Time), OMR (Oxygen Metabolism Rate), RGT (Relative Germination Time) and COP (Critical Oxygen Pressure) (Fig 1 b).

Among the 6 genotypes taken to UOH, Germany, genotype RNR-29183 did not germinate in the Q2 scanner. The mean performance of six genotypes for all the four Q2 parameters and other seedling vigour traits are presented in Table 2.

Results and Discussions

Correlations between oxygen metabolism parameters and seedling vigour traits

In this study, five traditional indices including four laboratory parameters (Germination %, Seedling length, Dry weight and Seedling vigour index) and one field parameter i.e. Field emergeence (FE) were used as control indices for seedling vigour assessment. Significant correlations were observed between oxygen metabolism parameters (Q2 ASTEC VALUES) and seedling vigour parameters. Of the four Q2 parameters, OMR showed a significantly positive correlation with COP (0.972**) and a significantly negative correlation with IMT (-0.902*). Similarly rapid methods for evaluating the vigor of sweet corn results showed that oxygen metabolism rate (OMR) and critical oxygen pressure (COP) were suitable to evaluate seed vigor of sweet corn[9]. COP showed a significant negative correlation with IMT (-0.916*). Similarly IMT (Increased metabolism time) was significantly correlated with radicle emergence in malting barley through Q2 scanner[3]. RGT showed a significant negative correlation with germinated Ok (-0.939*). Seedling length (cm) showed a significant negative correlation with germination (%) (-0.927*). The seedling vigour index showed a significant positive correlation with seedling dry weight (0.940*). Field emergence showed no significant correlation with any of the Q2 parameters.

Of all the Q2 parameters none of the parameters showed a positive significant correlation with seedling vigour traits, but RGT was considered as the optimum oxygen metabolism parameter to evaluate seedling vigour as it showed a positive non-significant correlation with the seedling vigour parameters when compared to other Q2 parameters. Similarly, the results suggested IMT (Increased metabolism time) does not seem to correlate with germination, but the RGT again correlated well with the emergence speed and germination[1].

However, oxygen-sensing technology is not able to predict reliably the field emergence of rice seeds because of the relatively low correlation coefficient. Similar observations were made and they indicated that RGT and OMR should be indicated as the optimal oxygen sensing indices to rapidly and automatically evaluate seedling vigour of Chinese fir and Masson pine, respectively[7]. Relative germination rate and oxygen metabolism rate were the optimal indices to evaluate the seedling vigour of conventional indica rice and conventional japonica rice, respectively[6]. However, these oxygen metabolism indices would not give reliable predictions of field emergence due to relatively low correlation coefficients. Sometimes a combination of tests is used on a seed lot to more accurately estimate the planting potential.

Future Scope of study

The present study helps in assessing the seed vigour and metabolic activity of seed rapidly in 10 to 72 hrs using oxygen sensing technology over conventional vigour tests.

Summary

Oxygen consumption through the Q2 scanner displayed inverse S-shaped (sigmoid) curves. The oxygen consumption pattern of individual seeds was categorized into dead, incompletely germinated, germinated ok and wrong based on the pattern of the curve through the Curve analysis module (CAM). Replications of each seed lot were compared through the Repetition comparison module (RCM) and the seed lots were compared in the Lot comparison module (LCM).

Of the four Q2 parameters, none of the parameters showed a positive significant correlation with seedling vigour traits, but RGT showed a positive non-significant correlation with germination (%), seedling vigour index and field emergence. Of all the Q2 parameters, RGT was considered the optimum oxygen metabolism parameter to evaluate seedling vigour as it showed a positive correlation with the seedling vigour parameters. Considering all other traits, JGL -11470 has low RGT indicating that the seed lot has faster germination.

Table 1. The details of the genotypes used for the Q2 experiment

S.No.	Genotype	Parentage
Lot 1	JGL-3828	Sambamahsuri / Agani
Lot 2	RNR-29090	Tellahamsa X (BPT 5204/NLR145)/BLNR6
Lot 3	RNR-29092	BPT 5204 X NBR-16
Lot 4	JGL-3844	Sambamahsuri/ARC 5984//Kavya
Lot 5	JGL-11470	JGL 418/Gedongbetan
	RNR-29183	CN 1757-5-3-7 MLD-18 X RNR15048

Table 2. Mean performance of six genotypes for Q2 Astec values and Seed vigour traits at UOH, Germany

	Genotypes	Q2 Graph Data					Q2 Astec values				Seed vigour lab data				
S.No	Entry	Dead	Incomplete	Wrong	Neglect	Germ ok	IMT (hrs)	COP (%)	RGT (hrs)	OMR (%/hr)	G.P (%)	SL (cm)	Dry wt. (g)	SVI	F.E (%)
Lot 1	JGL-3828	5	0	0	7	88	45.4	6.91	99.3	1.83	90.5	25.7	0.08	7.25	75
Lot 2	RNR-29090	21	1	0	23	55	42.7	6.7	104.8	1.73	93.5	25.23	0.09	8.38	100
Lot 3	RNR-29092	8	1	0	40	51	43.9	6.87	108.9	1.75	96.5	22.7	0.09	8.59	100
Lot 4	JGL-3844	1	0	0	13	86	30.1	7.42	95.2	2.1	90	27.63	0.09	7.85	95
Lot 5	JGL-11470	0	1	0	11	88	33.8	7.22	95.2	1.93	94	23.7	0.1	9.36	80

Table 3. Correlation coefficients between oxygen metabolism indices and the seed vigour traits of the traditional method

Character	Germinated Ok (Q2)	IMT (hrs)	COP (%)	RGT (hrs)	OMR (%/hr)	G.P (%)	Seedling length (cm)	Seedling dry weight (g)	Seedling Vigour Index	Field Emergence (%)
Germinated Ok (Q2)	1.000	-0.531	0.712	-0.939*	0.738	-0.728	0.500	-0.000	-0.225	-0.802
IMT (hrs)		1.000	-0.916*	0.753	-0.902*	0.338	-0.437	-0.603	-0.301	-0.008
COP (%)			1.000	-0.810	0.972**	-0.453	0.447	0.378	0.085	-0.224
RGT (hrs)				1.000	-0.843	0.698	-0.570	-0.240	0.043	0.604
OMR (%/hr)					1.000	-0.628	0.644	0.233	-0.091	-0.204
G.P (%)						1.000	-0.927*	0.462	0.716	0.418
Seedling length (cm)							1.000	-0.372	-0.654	-0.057
Seedling dry weight (g)								1.000	0.940*	0.151
Seedling vigour index									1.000	0.177
Field Emergence (%)										1.000

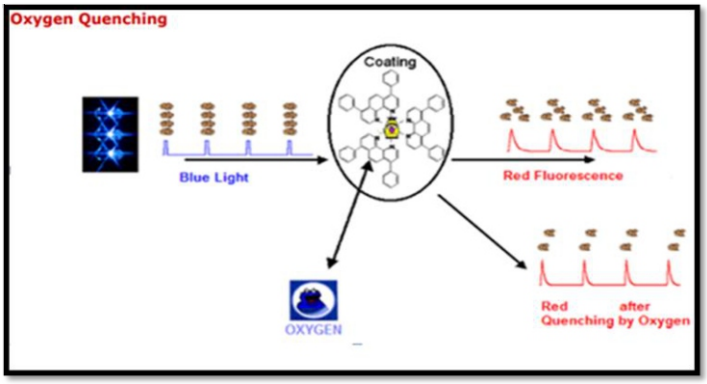


Fig 1 (a) Working principle of Q2 scanner – Oxygen Quenching

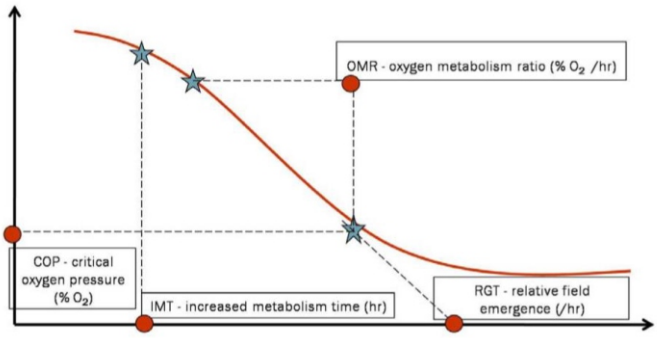
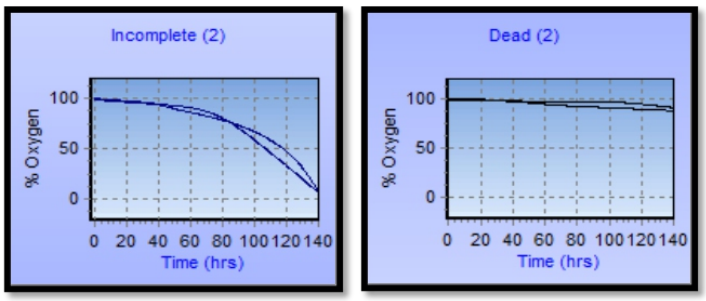
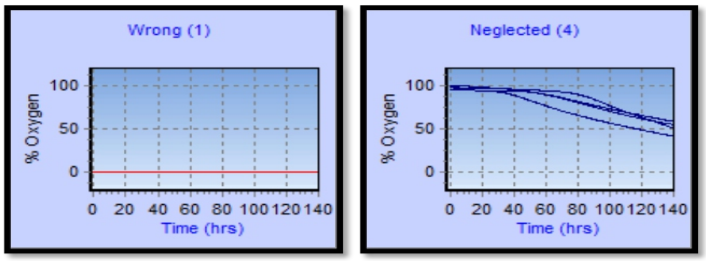


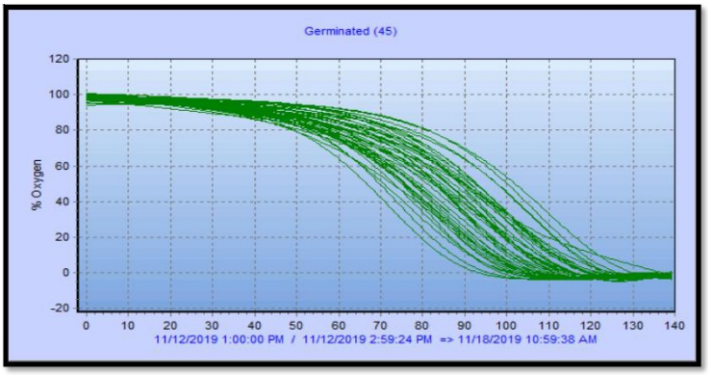
Fig 1 (b) Inverse S (sigmoid) shaped curve showing the ASTEC values.



a) Incomplete b) Dead



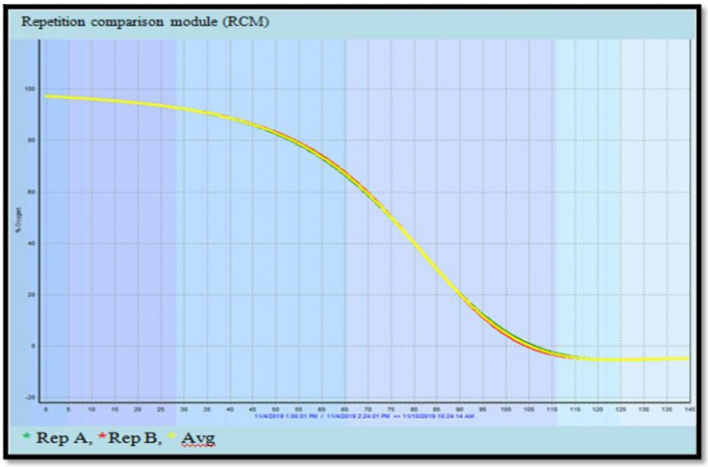
c) Wrong d) Neglected



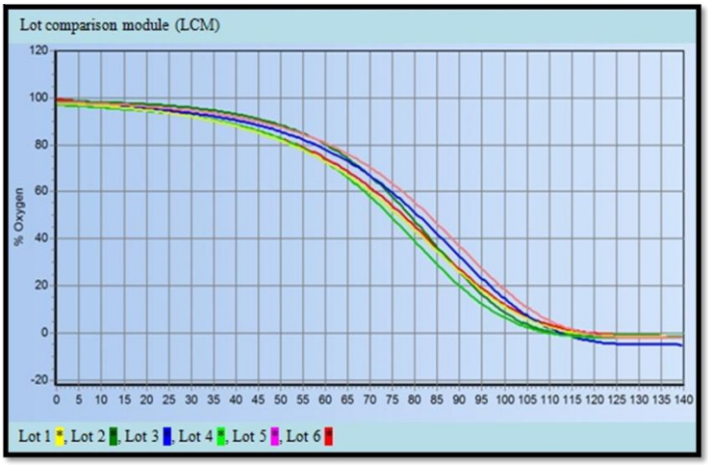
(e) Germinated ok

Fig 2. Curve analysis module graph showing individual seed curve patterns.

(a) Incomplete (b) Dead (c) Wrong (d) Neglect (e) Germinated ok



(a) Repetition comparison module (RCM)



b) Lot comparison module (LCM)

Fig 3. Graphs showing (a) Repetition comparison module (RCM)

(b) Lot comparison module (LCM)

Author's contribution

Conceptualization of research (CDR, MK, SB, LVSR, AK); Designing of experiments (CDR, MK, SB); Execution of field and lab experiments and data collection (PS, BA, SU, MK, SB); analysis of data and interpretation (PS, SB, CDR); Preparation of manuscript (PS, BA, US, CDR).

Conflict of interest

All the authors have declared and confirmed that they have no conflict of interest..

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