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Non-destructive Method of Seed Vigour Testing of Traditional Rice (*Oryza sativa* L.) Varieties Conserved in Genebank under Medium-term Storage Conditions

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Plant genetic resources are commonly conserved in the form of seeds in genebanks for their present and future use in crop improvement programmes. It is commonly understood that the loss in vigour, precedes loss in viability during storage. However, vigour tests cannot be conducted routinely for a large number of varieties processed for conservation and monitoring seed quality in genebanks. Hence, rapid and non-destructive tests will be immensely useful to provide additional information during monitoring of seed samples. The seeds of 31 traditional varieties of rice, conserved in medium-term storage (MTS) module (4°C and 35% relative humidity) at ICAR-National Bureau of Plant Genetic Resources, New Delhi, for past 10 years, were used to assess the seed quality by recording the germination percentage, mean germination time, seedling length and vigour index I, electrical conductivity, potassium (K^+) and sodium (Na^+) ion concentration in leachates. Results of the study clearly confirmed the fact that K^+, Na^+ ion concentrations in leachate and K^+/Na^+ ratio can be used as reliable and non-destructive method of vigour tests for assessing the quality of rice seeds. Several varieties with similar high percentage of initial germination deteriorated drastically leading to highly variable germination percentages after 10 years in MTS, indicating genotype-specificity.

Key Words: Genotype, Germination, Leachate, Paddy, Potassium, Sodium, Vigour

Introduction

Seed vigour testing is becoming increasingly important for ranking of various kinds of seed lots in terms of physiological potential. To ascertain the quality of seeds for long-term storage in genebanks, germination tests are carried out periodically. It is commonly understood that loss in vigour precedes loss in viability during storage. Seed vigour testing is thus assigned high importance as a measure of seed quality. Being time consuming and labour intensive, vigour tests cannot be routinely conducted for the large number of accessions. Hence, rapid and non-destructive vigour tests will be immensely useful to provide additional information during monitoring of seed samples. The leakage intensity during imbibition of soybean and Chinese cabbage seeds revealed a strong relation between K^+/Na^+ ratio and germination and the evaluation of specific ion ratio K^+/Na^+ test yields more consistent results than electrical conductivity (Cheng *et al*., 2005). When the seeds are soaked for a definite period in distilled water, low vigour seeds leak out larger quantity of inorganic ions into the seed steep medium, which can be quantified using flame photometry. Potassium (K^+) ion leakage has been used as an indicator of cell membrane integrity which, in turn, is related to seed vigour (Mc Kersie and Stinson, 1980; Webes and Karseen, 1990; Dias *et al.,* 1997).

The present study was envisaged to (i) evaluate the relationship between K^+ and Na^+ in the seed leachate with the physiological parameters of seeds of thirty one traditional varieties of rice conserved for 10 years under medium-term storage (MTS) conditions in the National Genebank at ICAR-National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi and (ii) to study the correlations behaviour on seed quality traits under MTS conditions.

Material and Methods

Seed Material

Seeds of 31 traditional varieties of rice were procured from Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, in 2004 and stored in MTS module (4°C and 35% relative humidity) at ICAR-NBPGR, New Delhi. In 2014, *i.e*., after 10 years, the seeds of the 31 varieties were retrieved from MTS to monitor the seed viability, seedling vigour and to study the pattern of changes in the EC, leakage of K^+ and Na^+ ions into the seed leachate.

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Germination Test and Mean Germination Time

Fifty seeds of each variety, replicated four times, were placed evenly on top (TP method) two layers of filter papers (Axiva Sichem Biotech, India) saturated with double distilled water. Seeds were allowed to germinate in a seed germinator (Sanco, India) in dark at 30°C. The seeds with at least 2 mm long radicle were considered as germinated and final germination percentage was counted on the 7^{th} day of sowing the seeds (ISTA, 1985). Similarly, for recording mean germination time (MGT), 50 seeds of each variety, in four replications, were placed randomly and the germinated seeds were counted after every 24 h until no further germination was observed; MGT was calculated by using the formula of Ellis and Roberts (1981), as given below:

Mean germination time = $\Sigma nd/\Sigma n$

Where, n = number of seeds germinated on day d, and

d = number of days counted from the beginning of germination test.

Seedling Length and Vigour Index I

Batches of 10 seeds of each variety in four replications were sown between moist germination/towel papers and allowed to germinate at 30°C in dark for 10 days in a seed germinator (Sanco, India). The sum of mean length (cm) of root and shoot, measured on a linear scale was calculated as seedling length (Srinivasan and Saxena, 2007). The product of germination percentage and seedling vigour was tabulated for the estimation of Vigour index I (Abdul-Baki and Anderson, 1973).

Vigour Index I = Seedling length (cm) × Germination percentage

Electrical Conductivity Test and Assay of Ion Concentration in Leachate

A total of 25 seeds of each variety, replicated four times, were soaked in 25 ml double distilled water for 24 h at 30°C in dark. The seed leachate conductivity (µsi/cm/gfw of seed) was estimated using a digital conductivity meter (Control Dynamics) after calibration with a solution of 0.01 M KCl (Mathews and Bradnock, 1968).

K^+ and Na^+ ion concentrations in the seed leachate were measured using a flame-photometer (Jenway, U.K.) after calibration with 10 ppm solution of KCl and NaCl, respectively. The ratio of K^+ and Na^+ was calculated on the basis of the concentration of K^+ and Na^+ (µg/g seeds) in the seed leachate.

Data Analyses

Collected data were statistically analysed by using SPSS software (version 16.0) and treatment means were compared by applying Duncan's multiple range test (DMRT) at 5% probability.

The data recorded as percentage were transformed to the respective angular (arcsine) values before subjecting them to statistical analyses.

Results and Discussion

The initial germination (before storage in October, 2004) ranged from 85 to 99%, whereas final germination (after 10 years of storage in September, 2014) in MTS was recorded between 5.33 to 98%. For all the parameters tested, wide ranges of values were observed in 10-year-old conserved rice varieties. The range of mean germination time (1.44-5.66 days), seedling length (7.68-47.84 cm), vigour index I(53.26-4304.10), electrical conductivity (49.48-112.82 µsi/cm/gfw of seed)), K^+ in the seed leachate (55.68-129.18 µg/g seeds), Na^+ (2.45-11.62 µg/g seeds) and the ratio of K^+/Na^+ (5.70-30.72) were recorded in 31 traditional varieties of rice germplasm (Table 1).

The details of data on seed vigour parameters, EC, K^+, Na^+ and K^+/Na^+ ratio are presented in Table 2. Genotypic effects were recorded on almost all the parameters tested. Out of 31 rice varieties tested, 12 varieties showed ≥ 85% germination after 10 years of storage in MTS; 5 varieties showed ≥ 50% and remaining showed < 50% germination. In general, the varieties showing higher seed germination

Table 1. Range of seed quality parameters of traditional varieties of rice conserved for 10 years under medium-term storage conditions

Seed quality parameter	Minimum (Mean)	Maximum (Mean)
Initial germination (%) - 2004	85.00 (67.19)	99.00 (85.34)
Final germination (%) - 2014	5.33 (13.16)	98.00 (83.41)
Mean germination time (days)	1.44	5.66
Seedling length (cm)	0.68	47.84
Vigour index-I	53.26	4304.10
Electrical conductivity (µsi/cm/gfw)	49.48	112.82
Potassium ion (µg/g seeds)	55.68	129.18
Sodium ion (µg/g seeds)	2.45	11.62
Ratio (K^+/Na^+)	5.70	30.72

Values in parentheses are arc sine transformed values for germination percentage maintained higher vigour and vigour index also. Seed longevity is a quantitative trait controlled by several genes scattered throughout the genome and influenced largely by environmental conditions (Kochanek *et al.,* 2010). Niedzielski *et al.* (2009) also observed that wheat, secale and triticale seed lots with high initial germination percentages revealed widely varying longevities. In the present study, several varieties where the initial germination was very high (96-99%), final viability declined to various levels indicating that genotype plays a very important role in controlling viability and also that vigour assessment is essential to differentiate the performance potential of various genotypes. Lower EC values of electrical conductivity were recorded in seeds with high germination percentage; however, no consistent linear trend was observed in relation to linear decrease in seed germination values. Most of the varieties that showed higher germination (≥ 85%) exhibited relatively low ratio of K^+/Na^+ ions, except Bhudkud, Khuddi and Ratna.

It is also evident from Table 3 that germination exhibited a significantly positive correlation with seedling length (r =0.860), Vigour index I(r = 0.934) and Na^+ ion (r = 0.681), but significantly negative correlation with mean germination time (r = -0.724 and the ratio of K^+/Na^+(r = -0.763). Seedling length was recorded to be significantly and positively correlated with Vigour index I (r = 0.957) and Na^+ ion (r = 0.656), but negatively correlated with the K^+ ion (r = -0.367) and the ratio of K^+/Na^+ (r = -0.791).

The electrical conductivity recorded highly positive correlation with K^+ ion (r = 0.841) and Na^+ ion (r = 0.296). The K^+ ion content in the leachate registered a significant positive correlation with K^+/Na^+ ratio (r =0.460), while Na^+ ion showed a highly significant negative correlation with K^+/Na^+ ratio (r = -0.829). Loss of cell membrane integrity is considered to be one of the primary and early events of the ageing process in seed (Mc Donald, 1999; Mathew and Powell, 2006). Consequently, seeds with low vigour exhibit higher degree of loss of their cellular constituents such as inorganic ions. Present results, however, show that K^+ ions are leached maximum by imbibing seeds. Such cation leakage has been used as an indicator of cell membrane integrity in cotton (Woods Stock *et al.,* 1985), radish, cabbage and broccoli (Min, 1995), maize (Miguel and Marcos Filho, 2002) and soybean (Cheng *et al.,* 2005). Also, it has been observed that the leakage of individual cation was significantly correlated (K^+ ion negatively and Na^+ ion positively) with seedling length along with K^+/Na^+ ratio. Cheng *et al.* (2005) demonstrated a strong relation between K^+/Na^+ ratio in the imbibition medium and germinability of seeds of soybean and Chinese cabbage. Since these ions are physiologically important, K^+/Na^+ ratio in soaking water could be more consistent and considered as a better indicator of vigour level than the EC of the seeds (Woodstock, 1985; Dias *et al*., 1997; Cheng *et al*., 2005). Highly vigorous seed lots often leak more charged particles into the imbibing medium initially, but the ions are later absorbed back leading to higher EC initially, followed by a gradual decrease, however, when the integrity of the membrane decreases due to disorganization and disintegration process during ageing, the ability of the membrane to reabsorb the ions also consequently decreases. Similarly, it has been postulated by Cheng *et al*. (2005) that poor membrane integrity in partially or completely aged seeds disables ion pumping mechanism, thus preventing the Na^+ ion from being pumped out into the soaking medium, thereby resulting in low amount of Na^+ in the soaking medium. Thus, when cells leak out more K^+ and less Na^+ through the cell membrane, a higher K^+/Na^+ ratio is obtained in seeds with lower membrane integrity.

Our results corroborate the findings of Cheng *et al.* (2005), who reported inconsistency between electrolyte leachate conductivity and vigour levels in Chinese cabbage seeds while observing highly consistent relationship between K^+/Na^+ ratio and vigour levels. Wang *et al.* (2003) also reported that the higher seed vigour was correlated with lower concentration of K^+ and Ca^{2+} in leachate from *Ulmus pumila* and *Brassica perkensis* seeds.

It is a general opinion that the seeds with low initial germination undergo accelerated deterioration (Mead and Gray, 1999). Poor correlation between initial germination and longevity of a seed lot is a well acknowledged fact. In the present study also, several varieties with similar high percentage of initial germination have undergone deterioration at differential rates, leading to highly variable final germination percentages.

On the basis of above results, it can be inferred that K^+ ion leakage and K^+/Na^+ ratio are more sensitive measures for assessing the physiological potential, rather than EC measurement in rice seeds. It can be used as a non-destructive method for monitoring the seed quality of rice germplasm conserved in genebanks. As many

Table 2. Seed quality of traditional varieties of rice stored for 10 years under medium-term storage conditions

S. No.	Traditional varieties	Accession No.	Initial germination (%) in 2004	Final germination (%) in 2014	MGT (days)	Seedling length (cm)	Vigour index I (Seedling length × germination percentage)	Electrical conductivity (µsi/cm/gfw)	K^+ (µg/g seeds)	Na^+ (µg/g seeds)	Ratio (K^+/ Na^+)
1	Bawara Haruna	IC-133156	99.00 (85.34^{a})	98.00(83.41)^{a}	1.44^{n}	40.28^{bc}	3940.6^{abc}	63.83^{efgh}	76.23^{hijkl}	9.20^{abcd}	9.00^{ghi}
2	Nokhi	IC-135521	99.00 (85.34^{a})	98.00(83.41)^{a}	1.75^{lmn}	41.82^{abc}	4095.2^{ab}	61.63^{efgh}	61.47^{mn}	7.15^{cdefg}	8.71^{ghi}
3	Kera Khan	IC-134580	93.00 (74.65^{bc})	94.00(75.92)^{ab}	1.91^{klmn}	36.98^{bcd}	3485.8^{de}	61.15^{efgh}	60.88^{mn}	6.90^{cdefgh}	9.22^{ghi}
4	Khuraban	IC- 134752	99.00 (85.34^{a})	94.66(76.80)^{ab}	1.68^{lmn}	37.53^{bc}	3553.7^{cde}	69.15^{cdefg}	72.31^{ijklm}	9.54^{abc}	7.58^{hi}
5	Kansari	IC-134447	96.00 (78.49^{b})	93.33(75.17)^{ab}	1.83^{lmn}	35.69^{cd}	3323.6^{e}	67.85^{cdefg}	63.76^{lmn}	11.62^{a}	5.70^{i}
6	Khuddi	IC-134642	99.00 (85.34^{a})	93.00(74.82)^{ab}	1.55^{mn}	35.23^{cd}	3274.4^{e}	76.01^{bcde}	94.27^{cde}	6.50^{defghi}	14.88^{fgh}
7	Chipda	IC-133610	99.00 (85.34^{a})	90.00(72.04)^{ab}	2.59^{hijklm}	47.84^{a}	4304.1^{a}	49.48^{h}	55.68^{n}	5.79^{efghij}	9.60^{ghi}
8	Pandari Luchai	IC- 135117	92.00 (73.56^{cd})	89.33(70.98)^{ab}	1.96^{klmn}	43.15^{ab}	3854.7^{bcd}	63.63^{efgh}	76.57^{ghijkl}	8.50^{bcde}	9.06^{ghi}
9	Bhata Khuji	IC- 134669	99.00 (85.34^{a})	88.00(69.88)^{b}	1.92^{klmn}	41.40^{bc}	3649.1^{cde}	75.17^{bcde}	80.72^{fghij}	10.16^{ab}	7.93^{hi}
10	Ratna	IC- 135624	96.00 (78.49^{b})	88.00(69.88)^{b}	2.67^{ghijkl}	28.85^{ef}	2531.0^{f}	85.47^{b}	114.81^{b}	4.46^{ghijk}	25.88^{abcd}
11	Bhudkud	IC-133267	99.00 (85.34^{a})	86.00(68.03)^{bc}	2.08^{jklmn}	26.75^{efg}	2298.3^{f}	112.82^{a}	129.18^{b}	11.56^{a}	14.93^{fgh}
12	Gurmatiya	IC-134030	99.00 (85.34^{a})	85.66(68.26)^{bc}	1.76^{lmn}	40.46^{bc}	3477.6^{de}	57.06^{gh}	60.29^{mn}	7.31^{cdefg}	8.27^{ghi}
13	Mararin Buta	IC-133353	99.00 (85.34^{a})	78.66(62.48)^{c}	1.91^{klmn}	30.80^{de}	2421.1^{f}	65.08^{defg}	81.31^{efghij}	5.13^{fghijk}	15.89^{efg}
14	Kosam Khuta	IC-134713	92.00 (73.56^{cd})	66.66(54.83)^{d}	2.91^{fghijk}	26.50^{efg}	1760.0^{g}	64.40^{efg}	80.13^{fghij}	7.53^{bcdef}	10.67^{ghi}
15	Lako Kunwar	IC- 134891	96.00 (78.49^{b})	62.66(52.32)^{de}	2.39^{ijklmn}	27.50^{ef}	1717.2^{g}	83.71^{b}	89.45^{cdefgh}	4.61^{ghijk}	19.34^{def}
16	Lal Koram	IC- 134805	96.00(78.49^{b})	57.33(49.20)^{ef}	3.73^{def}	12.34^{jkl}	707.20^{hij}	56.76^{gh}	68.91^{jklm}	3.24^{jk}	22.08^{bcdef}
17	Newari	IC-135447	85.00 (67.19^{ef})	50.66(45.36)^{fg}	3.52^{efgh}	15.68^{ijk}	792.97^{hij}	59.68^{fgh}	77.81^{fghijk}	3.05^{jk}	26.03^{abcde}
18	Ramker	IC- 135869	96.00 (78.49^{b})	50.66(45.36)^{fg}	3.77^{def}	17.71^{hij}	896.46^{hi}	81.26^{bc}	113.87^{b}	3.72^{ijk}	30.65^{a}
19	Londi	IC- 134882	99.00 (85.34^{a})	45.33(42.29)^{gh}	4.80^{abc}	22.25^{fghi}	990.20^{h}	61.14^{efgh}	82.61^{efghi}	3.36^{jk}	24.58^{abcd}
20	Chhoti Lal	IC-134913	92.00 (73.56^{cd})	42.66(40.76)^{ghi}	3.32^{efghi}	17.91^{hij}	762.78^{hij}	65.91^{defg}	85.74^{defgh}	3.09^{jk}	27.71^{abc}
21	Koliyari	IC- 134780	99.00 (85.34^{a})	37.33(37.64)^{hij}	4.91^{abc}	20.30^{ghi}	765.26^{hij}	54.54^{gh}	68.21^{jklmn}	3.27^{jk}	21.00^{cdef}
22	Luchai	IC- 135027	96.00 (78.49^{b})	37.33(37.56)^{hij}	2.89^{fghijk}	23.30^{fgh}	874.89^{hi}	55.22^{gh}	66.18^{klmn}	2.85^{jk}	23.19^{abcde}
23	Karhani	IC-134516	96.00(78.49^{b})	34.66(36.00)^{ij}	3.98^{cde}	9.21^{kl}	322.00^{jk}	65.45^{defg}	77.09^{ghijk}	3.13^{jk}	25.89^{abcd}
24	Longa	IC-134983	96.00(78.49^{b})	33.33(35.13)^{j}	4.54^{bcd}	13.17^{jkl}	449.53^{ijk}	67.61^{cdefg}	84.76^{efghi}	3.54^{jk}	23.95^{abcd}
25	No:22	IC- 135543	88.33 (70.02^{de})	30.66(33.21)^{j}	3.10^{efghij}	23.29^{fgh}	682.60^{hij}	61.11^{efgh}	87.22^{cdefgh}	3.04^{jk}	29.17^{ab}
26	Lodiyar	IC-134966	99.00 (85.34^{a})	18.66(25.37)^{k}	3.70^{defg}	19.88^{hi}	350.00^{jk}	79.28^{bcd}	98.54^{cd}	3.61^{jk}	27.47^{abc}
27	Safed Ludka	IC-135170	91.00 (72.53^{cd})	16.00(23.46)^{k}	3.53^{efgh}	9.30^{kl}	149.73^{k}	68.48^{cdefg}	90.62^{cdef}	4.23^{hijk}	21.72^{bcdef}
28	Nirguni	IC- 135507	99.00 (85.34^{a})	12.00(20.08)^{kl}	2.20^{jklmn}	11.03^{kl}	152.40^{k}	59.08^{fgh}	68.92^{jklm}	2.45^{k}	30.72^{a}
29	Lodiyari	IC- 134967	99.00 (85.34^{a})	10.66(18.98)^{kl}	3.34^{efghi}	7.68^{kl}	82.20^{k}	83.95^{b}	112.27^{b}	4.49^{ghijk}	25.20^{abcd}
30	Lohandi	IC-134975	96.00 (78.49^{b})	10.66(18.45)^{kl}	5.17^{ab}	10.26^{kl}	113.22^{k}	73.75^{bcdef}	99.77c	3.58^{jk}	27.91^{abc}
31	Nunga	IC- 135550	99.00 (85.34^{a})	5.33(13.16)^{l}	5.66^{a}	10.05^{kl}	53.26^{k}	67.64^{cdefg}	89.77^{cdefg}	3.35^{jk}	27.30^{abc}

Values in parentheses are arc sine transformed values for germination percentage given in column 4 and 5

Table 3. Coefficient of correlation (r) between physiological and biochemical parameters of traditional varieties of rice

	Germination (%)	Mean germination time (days)	Seedling length (cm)	Vigour index I	Electrical conductivity (µsi/cm/gfw)	K^+ ion (µg/g seeds)	Na^+ ion (µg/g seeds)	Ratio (K^+/Na^+)
Germination (%)	1	–0.724**	0.860**	0.934**	0.029	–0.250*	0.681**	–0.763**
Mean germination time (days)		1	–0.684**	–0.728**	–0.061	0.191	–0.538**	0.566**
Seedling vigour (cm)			1	0.957**	–0.089	–0.367**	0.656**	–0.791**
Vigour index				1	–0.044	–0.343**	0.713**	–0.822**
Electrical conductivity (µsi/cm/gfw)					1	0.841**	0.296**	0.115
Potassium ion (µg/g seeds)						1	–0.088	0.460**
Sodium ion (µg/g seeds)							1	–0.829**
Ratio (K^+/Na^+)								1

** Correlation is significant at the 0.01 level
* Correlation is significant at the 0.05 level

as 12 varieties were found to be good storers for up to 10 years, maintaining full complement of seed viability and vigour as per international genebank standards.

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