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RESEARCH ARTICLE :

Role of seed-Zn content on seed longevity of paddy genotypes

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SUMMARY : Ten paddy genotypes were selected to establish the role of seed-Zn content in maintaining seed longevity of paddy genotypes. The present study revealed clear genotypic variability with respect to storability among different paddy genotypes. The genotype with highest seed-Zn content (24.79 ppm) proved as good storer by recording highest seed quality parameters *viz.*, seed germination (85.70 %), seedling length (21.88 cm), seedling vigour index (1795), speed of germination (18.80), dehydrogenase enzyme activity (0.39 OD value), á-amylase activity (12.47 mm) with lowest electrical conductivity (153.40 µS/cm) and moisture content (10.62 %) at the end of twelve months of storage period. Whereas, genotypes with low seed-Zn content showed lowest seed quality parameters.

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KEY WORDS :
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BACKGROUND AND OBJECTIVES

Rice (*Oryza sativa* L.) is a "Global Grain" cultivated widely across the world and feeds millions of mankind, is the staple food for more than half of the human population. Grown in Asia for at least 10,000 years, rice (the main product of the paddy plant) has become deeply embedded in the cultural heritage of Asian societies and is the life, heart and soul of the people throughout Asia. In India, rice continues to hold the key for sustained food production by contributing 20-25 per cent to agriculture and assures food security for more than half of the total population. Out of 2234 calories per day per capita food intake, rice accounts for 30 per cent in Indian diet.

Seed being a biological or living entity, deterioration in its quality is inevitable, irreversible and inexorable. It occurs with advance in ageing, which is common for all the living organisms. In storage, number of biotic and abiotic factors *viz.*, genotypes, production location, mechanical injury to the seed, initial seed quality, seed treatment, packaging material and storage conditions influence storage potential of seeds and results in gradual seed deterioration and ultimately death of the seeds. Orthodox seeds are

characterized by their ability to tolerate desiccation and to retain their viability for a long time in the dry state. However, these seeds age during storage and eventually lose their ability to germinate. Several comprehensive reviews have identified free radical mediated lipid peroxidation (accumulation of reactive oxygen species), enzyme inactivation or protein degradation, disruption of cellular membranes, and damage to genetic (nucleic acids) integrity as major causes of seed ageing [1, 2].

Zinc plays a fundamental role in several critical cellular functions such as protein metabolism, gene expression, structural and functional integrity of biomembranes. Increasing evidence indicates that oxidative damage to critical cell compounds resulting from attack by reactive O_2 species (ROS) is the basis of disturbances in plant growth caused by Zn deficiency [3]. Zinc interferes with membrane-bound NADPH oxidase producing ROS. In Zn-deficient plants the iron concentration increases, which potentiates the production of free radicals. Zinc plays critical roles in the defense system of cells against ROS [4, 3] and thus, represents an excellent protective agent against the oxidation of several vital cell components such as membrane lipids and proteins, SH-containing enzymes and DNA. In the present study, an effort has been made to explore the role of seed Zn content in maintaining seed longevity of paddy genotypes during storage.

RESOURCES AND METHODS

Storage study was conducted at the Department of Seed Science and Technology, College of Agriculture, UAS, Raichur during 2015-16. The seeds of ten paddy genotypes *viz.*, GNV-GP-62, GNV-12-96-1, RYC 667, PAU-3105-45-3-2, GNV-MSGP-1, GNV-MSGP-10, GNV-MSGP-16, GNV-MSGP-18, GNV-MSGP-28 and GNV-MSGP-29 were stored in cloth bag for 12 months period under ambient condition. Experiment was laid out in complete randomized design in three replications. Seed samples were drawn subsequently at bimonthly intervals and tested for the following seed quality parameters.

The seed moisture content was calculated and expressed in per cent by using the standard procedure [5].

Germination per cent was determined as per ISTA rules for seed testing. The seeds were placed in rolled paper towels. Hundred seeds of four replications were tested at a constant temperature of 25°C. The number of normal seedlings were evaluated on 14th day and per cent germination was expressed on normal seedling basis [5].

From the standard germination test, ten normal seedlings were selected at random in each replication on final count. The shoot and shoot length was measured, sum of shoot and root length constitute the seedling length and mean was calculated and expressed in centimeters. Seedling vigour index was computed by adopting the formula as suggested by [6] and expressed in whole number.

Seedling vigour index = Germination (%) × Mean seedling length (cm)

Seed germination test was conducted as described above and daily germination counts were recorded on the germinated seeds possessing radical size of 3-5 mm. The speed of germination was calculated by using formula suggested by [7].

$$\text{Speed of germination} = \frac{\text{No. of seeds germinated}}{\text{Days to first count}} + \ldots + \frac{\text{No. of seeds germinated}}{\text{Days to final count}}$$

The electrical conductivity and dehydrogenase enzyme activity were measured as per ISTA rules for seed testing [5].

Seed micronutrient content (Fe and Zn) and α-amylase activity (mm) were analyzed as per the procedure outlined by [8] and [9], respectively. The data obtained from the experiments were statistically analyzed as per [10].

OBSERVATIONS AND ANALYSIS

One of the critical factors that determine the viability of seeds in storage is seed moisture content. The moisture content of seeds recorded at bimonthly interval showed increasing trend during storage (except after second and twelve months after storage), irrespective of the genotypes. Significantly highest moisture content was observed in GNV-MSGP-18 (12.46 %) and lower in PAU-3105-45-3-2 (10.62 %) at the end of storage period (Table 1). Presence of lower moisture content during second and twelve months after storage period owing to lower atmospheric relative humidity and higher temperature causes loss of moisture content from the seeds. The increase in moisture could be due to hygroscopic nature of the seed enabling moisture absorption from the surrounding atmosphere. The

container used in the present study was cloth bag which is moisture pervious. The other probable means of seed moisture increase are metabolic release of water, insect infestation and fungal infection as reported by [11].

Irrespective of the genotypes, germination potential of seeds decreased with advancement in storage period [12, 13]. Among the genotypes, PAU-3105-45-3-2 recorded higher seed germination (97.00 %), seedling length (25.40 cm) and seedling vigour index (2413) initially as well as up to 12 months of storage (85.70 %, 21.88 cm and 1795, respectively) while, genotype GNV-MSGP-18 recorded lower quality parameters throughout the storage period. The per cent reduction was highest in GNV-MSGP-18 (11.30 %) and lowest in PAU-3105-45-3-2 (15.83 %). Even after 12 months of storage, out of ten genotypes, seven genotypes maintained germination percentage above the minimum seed certification standard of 80.00 per cent (Table 1 and 2). The decrease in quality parameters during storage is mainly due to age induced phenomenon in most kind of seeds which is inevitable and irreversible and also increases in membrane leakage as reported by [6]. Higher quality parameters observed in genotype PAU-3105-45-3-2 might be due to presence of high zinc content which reduce the lipid peroxidation by preventing the production of reactive oxygen species (ROS) during

Table 1 : Seed moisture content (%), germination (%) and seedling length (cm) as influenced by paddy genotypes during storage

Genotypes	Months of storage								
	Moisture content			Germination			Seedling length		
	0	6	12	0	6	12	0	6	12
G_1: GNV-GP-62	11.50	11.68	11.60	95.00	89.40	83.00	25.40	23.50	20.35
G_2: GNV-12-96-1	11.84	12.45	12.09	93.00	86.83	80.00	23.70	21.67	19.00
G_3: RYC 667	11.54	11.82	11.66	94.50	88.67	80.00	24.80	23.07	20.00
G_4: PAU-3105-45-3-2	10.50	10.67	10.62	97.00	91.89	85.70	25.40	23.80	21.88
G_5: GNV MSGP-1	12.15	12.48	12.32	93.00	85.60	79.00	23.20	21.37	19.21
G_6: GNV MSGP-10	11.94	12.16	12.14	93.50	87.67	80.00	24.40	21.70	19.60
G_7: GNV MSGP-16	12.34	12.56	12.46	91.00	85.20	78.50	23.10	20.70	17.79
G_8: GNV MSGP-18	12.44	12.76	12.62	92.00	85.34	76.17	21.80	20.17	17.75
G_9: GNV MSGP-28	10.50	10.85	10.63	95.00	90.60	82.00	25.37	23.20	20.00
G_{10}: GNV MSGP-29	11.95	12.32	12.15	94.00	88.50	81.50	24.50	22.10	20.00
Mean	11.67	11.97	11.82	93.8	87.97	80.58	24.16	22.12	19.55
S.E.±	0.14	0.12	0.10	0.87	0.75	1.39	0.26	0.23	0.22
C.D. (P=0.01)	0.57	0.50	0.40	3.49	3.02	5.61	1.05	0.93	0.87

Table 2 : Seedling dry weight (mg), speed of germination and seedling vigour index as influenced by paddy genotypes during storage

Genotypes	Months of storage								
	Seedling dry weight (mg)			Speed of germination			Seedling vigour index		
	0	6	12	0	6	12	0	6	12
GNV-GP-62	8.44	8.43	7.67	22.50	20.54	18.19	2319	2030	1628
GNV-12-96-1	7.72	7.34	6.90	20.00	19.56	17.72	2252	1900	1521
RYC 667	8.39	7.67	7.18	21.37	19.96	18.12	2309	2014	1659
PAU-3105-45-3-2	9.5	9.53	8.59	23.70	21.12	18.8	2413	2127	1795
GNV MSGP-1	7.78	7.21	6.44	20.53	19.63	17.42	2251	1836	1538
GNV MSGP-10	8.17	7.37	6.79	21.10	19.66	17.82	2255	1966	1541
GNV MSGP-16	7.47	7.30	6.56	19.68	19.31	17.32	2149	1821	1421
GNV MSGP-18	7.35	6.27	6.06	19.00	17.78	17.05	2050	1727	1406
GNV MSGP-28	9.43	7.88	8.39	22.75	20.39	18.45	2363	2079	1715
GNV MSGP-29	8.37	7.60	6.99	21.25	19.81	18.07	2306	1969	1571
Mean	8.26	7.66	7.15	21.19	19.78	17.90	2267	1947	1580
S.E.±	0.17	0.21	0.18	0.22	0.28	0.23	44.97	21.97	45.74
C.D. (P=0.01)	0.66	0.83	0.71	0.88	1.16	0.90	180.97	88.39	184.07

storage [3].

Similar trend was also noticed in case of speed of germination. Rate of germination declined with the progress in the storage in all the genotypes but extent of reduction varied among the genotypes. The genotype PAU-3105-45-3-2 recorded highest speed of germination in all the months of storage (23.70 at initial and 18.80 at the end of storage) over rest of the genotypes, whereas lowest was observed in GNV-MSGP-18 (Table 2). Highest speed of germination might be due to higher seed index hence, seed with higher initial capital food reserve always showed rapid and fast germination [14].

Deterioration alters the semi-permeable property of the membrane and the membrane integrity. The conductivity of the seed leachate was found to be good index of seed viability [15], vigour [16] and deterioration [17]. In the present study, electrical conductivity of the seed leachate increased with increase in period of storage [18, 19]. Among the genotypes, PAU-3105-45-3-2 released lower electrolytes to seed leachate and genotype GNV-MSGP-18 recorded more EC by releasing more electrolytes to seed leachates (Table 3). The significant variation in EC may be due to the anatomical structure, membrane permeability and composition of seed coat.

Table 3 : Electrical conductivity (µS/cm), dehydrogenase enzyme activity and α-amylase activity (mm) as influenced by paddy genotypes during storage

Genotypes	Months of storage								
	Electrical conductivity			Dehydrogenase enzyme activity			α-amylase activity		
	0	6	12	0	6	12	0	6	12
GNV-GP-62	109.87	132.00	194.13	0.73	0.58	0.36	18.67	15.56	11.43
GNV-12-96-1	126.40	142.40	174.00	0.58	0.5	0.29	16.78	14.10	10.53
RYC 667	123.17	137.95	184.73	0.7	0.52	0.35	18.44	15.54	11.43
PAU-3105-45-3-2	99.35	115.00	153.40	0.83	0.6	0.39	20.38	16.85	12.47
GNV MSGP-1	133.65	147.95	171.78	0.56	0.48	0.29	16.67	13.67	10.47
GNV MSGP-10	124.25	140.65	182.43	0.59	0.50	0.30	17.32	14.84	10.67
GNV MSGP-16	137.65	153.00	171.18	0.48	0.37	0.29	16.50	13.45	10.1
GNV MSGP-18	142.40	159.90	160.35	0.41	0.33	0.21	15.98	12.64	9.84
GNV MSGP-28	113.00	128.80	198.65	0.71	0.56	0.37	19.67	15.76	12.29
GNV MSGP-29	124.05	140.00	186.88	0.68	0.51	0.32	17.56	14.98	11.23
Mean	123.38	139.77	182.75	0.63	0.50	0.32	17.79	14.73	11.04
S.E.±	0.87	0.97	0.74	0.11	0.02	0.02	0.60	0.38	0.42
C.D. (P=0.01)	3.49	3.91	2.97	NS	0.09	0.08	2.39	1.51	1.70

NS=Non-significant

Table 4 : Protein content (%) and seed zinc content (ppm) as influenced by paddy genotypes during storage

Genotypes	Months of storage			
	Protein content		Zinc content	
	0	12	0	12
GNV-GP-62	9.60	9.60	20.00	19.80
GNV-12-96-1	9.90	9.90	17.49	17.49
RYC 667	9.90	9.90	13.00	12.98
PAU-3105-45-3-2	9.50	9.50	25.00	24.79
GNV MSGP-1	8.90	8.90	13.01	12.78
GNV MSGP-10	9.10	9.10	12.61	10.57
GNV MSGP-16	9.70	9.70	12.34	9.71
GNV MSGP-18	9.10	9.10	12.07	9.77
GNV MSGP-28	9.40	9.40	26.84	25.84
GNV MSGP-29	9.30	9.30	17.49	17.46
Mean	9.44	9.44	17.95	17.09
S.E.±	0.38	0.38	0.63	0.56
C.D. (P=0.01)	NS	NS	2.55	2.27

NS=Non-significant

Zinc plays a fundamental role in maintaining structural and functional integrity of biomembranes. As seeds of GNV-MSGP-18 and GNV-MSGP-16 contained less seed-Zn content it might have lead to more cellular membrane deterioration causing more leakage of solutes from the membrane [6].

The dehydrogenase enzyme activity and á-amylase is a good stable metabolic marker to estimate the degree of vigour in seeds [20] and have positive association with vigour and viability of seeds [21, 22]. The dehydrogenase and á-amylase enzyme activity decreased with the advancement in storage period. At the end of storage PAU-3105-45-3-2 recorded higher dehydrogenase and á-amylase enzyme activity (0.39 OD value and 12.47 mm), whereas, GNV-MSGP-18 recorded lower (0.21 OD value and 9.84 mm) (Table 3). Decrease in enzyme activity may be related to age induced deterioration which is a common phenomenon in any living entity and difference in genotypic response may be due to variation in inherent genotypic composition to withstand the impact of ageing [23].

Irrespective of the genotypes, seed protein content decreased during storage. Similar results with low protein content in aged seeds were documented by [24, 25]. Protein content did not differ significantly among the genotypes during storage period. However, numerically higher protein content was observed in GNV-12-96-1 (Table 4). Reduction in protein could be related to increase in moisture content that might have activated the proterolytic enzymes [11].

In the present study, Zn content of seeds were estimated before keeping the seeds for storage and at end of storage period to know the association with seed quality parameters. Irrespective of the genotypes, slight

Table 5 : Correlation between micronutrient (Fe and Zn) content and seed quality parameters at initial month of storage

Parameters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
X_1	1									
X_2	.854**	1								
X_3	.914**	.871**	1							
X_4	.946**	.926**	.954**	1						
X_5	.894**	.939**	.889**	.925**	1					
X_6	-.948**	-.918**	-.908**	-.968**	-.910**	1				
X_7	.950**	.946**	.898**	.953**	.966**	-.952**	1			
X_8	.932**	.893**	.973**	.969**	.885**	-.945**	.928**	1		
X_9	-.099	.297	-.087	-.037	.164	-.059	.123	.010	1	
X_{10}	.814**	.719*	.875**	.842**	.769**	-.874**	.785**	.863**	-.138	1

**. Correlation is significant at 0.01 level (2-tailed). *. Correlation is significant at 0.05 level (2-tailed).

X_1 : Germination (%) X_2: Seedling length (cm) X_3: Seedling dry weight (mg) X_4: Speed of germination

X_5: Seedling vigour index X_6: Electrical conductivity(µS/cm) X_7: Total Dehydrogenase activity X_8: α- Amylase activity (mm)

X_9: Protein content (%) X_{10}: Zinc content (ppm)

Table 6 : Correlation between micronutrient (Fe and Zn) content and seed quality parameters after 12 months of storage

Parameters	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}
X_1	1									
X_2	.937**	1								
X_3	.907**	.840**	1							
X_4	.942**	.941**	.949**	1						
X_5	.888**	.941**	.930**	.963**	1					
X_6	.945**	.912**	.951**	.979**	.932**	1				
X_7	.908**	.876**	.914**	.944**	.924**	.956**	1			
X_8	.894**	.901**	.965**	.977**	.977**	.964**	.933**	1		
X_9	-.521	-.596	-.538	-.578	-.596	-.624	-.488	-.654*	1	
X_{10}	.872**	.786**	.927**	.868**	.847**	-.854**	.788**	.890**	-.505	1

**. Correlation is significant at 0.01 level (2-tailed). *. Correlation is significant at 0.05 level (2-tailed).

X_1 : Germination (%) X_2: Seedling length (cm) X_3: Seedling dry weight (mg) X_4: Speed of germination

X_5: Seedling vigour index X_6: Electrical conductivity(µS/cm) X_7: Total Dehydrogenase activity X_8: α- Amylase activity (mm)

X_9: Protein content (%) X_{10}: Zinc content (ppm)

decline in Zn content were noticed during storage. Among the genotypes, GNV MSGP 28 maintained higher Zn content (26.84 ppm at initial and 25.84 ppm at the end of storage period) throughout the storage period (Table 4). These type of results have not been reported earlier so far and hence there is a scope for indepth study on this area in order to correlate the seed quality traits with that of micronutrient in seeds during storage there by, selection of genotypes during breeding programme can be thought off for transferring genes responsible for maintenance of micro element in seeds during storage to the promising genotypes having short seed viability.

Correlation between seed quality parameters and seed-Zn content :

There is an evidence in the literature demonstrating that role and association of Zn in enhancing the seed germination and vigour [26, 27]. Zinc is involved in biosynthesis of plant hormone, indole acetic acid (IAA), auxin metabolism and is a component of variety of enzymes like, carbonic anhydrase, alcohol dehydrogenase, glutamic dehydrogenase *etc*. plays an important role in enhancing the seed germination. In the present study, seed zinc content exhibited positive significant association with all the seed quality parameters except electrical conductivity and protein content at initial and also at the end of storage period (Table 5 and 6). Significant positive association between seed-Zn content and quality parameters during the storage might be due to the defence mechanism of seeds/ seedlings against to the production of reactive oxygen species (ROS) which is unavoidable. One of the defense enzymes against ROS is superoxide dismutase which is Zn dependent [3, 28].

Conclusion :

A comprehensive assessment of the quality parameters revealed the critical role of seed-Zn content in regulating the metabolic processes associated with seed storability. Therefore, it is necessary that while breeding paddy varieties for better seed storability, screening for high Zn content would serve as more effective criteria, as compared to low Zn content.

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