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Influence of pre-harvest climatic variables and natural storage duration on seed physico-chemical profile in diverse maturity groups of rice (*Oryza sativa*) varieties

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ABSTRACT

Seed storage is a critical concern for farmers and seed banks, as it can significantly impact the quality of seeds over time. Present study was carried out during rainy (*kharif)* season of 2020–21 at ICAR-Indian Agricultural Research Institute, New Delhi to understand the physiological and biochemical factors determining seed vigour traits in relation to maturity and storage periods in rice (*Oryza sativa* L.) varieties. In the freshly harvested seeds, early and mid-maturity group varieties exhibited higher germination percentage and seed vigour indices compared to late and very late maturity varieties. However, with increasing storage duration, germination percentage and vigour gradually decreased in the early and mid-maturity groups, while late and very late groups showed an increase up to 8 months of storage, followed by a decline after 12 months. Catalase activity (CAT) and Super oxide dismutase activity (SOD) remained stable across maturity groups and storage periods, while peroxidase activity (POD) varied significantly in fresh seeds but not during storage. Hydrogen peroxide activity (H_2O_2) and α-amylase content exhibited variations among maturity groups and storage periods. The study emphasizes the crucial role of maturity group selection in seeds, both for immediate sowing and long-term storage.

Keywords: Enzyme activity, Maturity group, Rice, Seed quality trait, Storage period

Rice (*Oryza sativa* L.) is one of the important and the second largest food grain crop grown in the world both in terms of area and production. Rice cultivation in country takes place in humid tropical and sub-tropical climates characterized by high temperature and high relative humidity which leads to faster deterioration of seeds. Seed deterioration is a natural process expressed as loss of quality, viability and vigour during ageing or adverse environmental conditions. Seeds are considered to be deteriorating from the moment they reach full maturity until they are ready to germinate (Gokhale 2009) and the rate of deterioration is however influenced by seed moisture content and temperature of the storage. To ensure that seeds are suitable for planting, they need to possess good storage quality, maintaining optimal conditions until they are used for sowing. While seeds are in storage, their quality may either remain at the initial level or decline to the extent that they become unsuitable for planting (Pratt *et al.* 2009).

The storability of seeds is primarily determined by their genetic characteristics and is influenced by factors such as the seed's history before storage, maturation, and environmental conditions during both pre- and post-harvest stages. Higher grain moisture content during storage produces more heat in the grain due to high grain respiration, and this causes grain deterioration and the reduction of storage period (Barary *et al.* 2015). Seed lots with high viability are likely to have high vigour; reduced vigour is an early symptom of seed ageing. When the seed deteriorates it loses vigour (slow growth, abnormal growth, increased leachates, and susceptibility to biotic and abiotic stresses) and viability in storage. Hellevang (2009) reported that during storage, seed deteriorates rapidly and as a result substantial loss in terms of vigour and germinability occurs particularly in humid condition. Biochemical changes associated with seed deterioration are increase in membrane instability and leaching of sugars and electrolytes. Generally, early-maturing rice varieties tend to have a shorter shelf-life than late-maturing varieties but the reason behind is still unknown. Thus, the present study was undertaken to understand the physiological and biochemical dynamics with respect to the seed vigour traits in rice varieties of different maturity duration and seed storage periods.

MATERIALS AND METHODS

Seed material: A total of 20 rice varieties (5 varieties in each maturity group) were studied for the relationship

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Table 1 List of Indian rice varieties and maturity duration used for study

Early maturity varieties* (EGV)	Medium maturity varieties* (MGV)	Late maturity* varieties (LGV)	Very late maturity varieties* (VLGV)
VL Dhan-221	PNR-381	Dudheswar	Kranti
VL Dhan-81	NDR-97	Shyamala	Swarna
Jyoti	Satyabama	Mandyavijaya	Surajone
Pusa 834	Lochit	Basmati-370	
Poornima	Pant Dhan-12	Vasumati	CRD-300

*Duration of early maturity group (<110 days), medium maturity group (111–130 days), late maturity group (131–150 days), very late maturity group (>151 days)

between maturity group and storage period with respect to seed vigour traits (Table 1). Four different maturity group/ duration (from sowing to harvesting) were early (EGV), medium (MGV), late (LGV), and very late (VLGV). The experiment was conducted during rainy (*kharif)* season of 2020–21 at ICAR-Indian Agricultural Research Institute, New Delhi. The freshly harvested seeds were cleaned, dried to about 12–13% moisture content and kept under ambient conditions for storage. The observations on seed physiological and biochemical characters were recorded on fresh and stored seeds (after 1, 4, 8 and 12 months of storage).

Weather parameters: Prevailing weather parameters such as maximum temperature (T_{max}), minimum temperature (T_{min}), average temperature (T_{mean}), morning relative humidity (RH-M), evening relative humidity (RH-E), average relative humidity (RHavg), and cumulative rainfall (Fig. 1) were measured.

Estimation of seed physiological parameters

Seed moisture content (%): Moisture content of seed was determined on dry weight basis as per ISTA rules (ISTA 2021):

$$\text{Moisture content (\%)} = \frac{W2 - W3}{W2 - W1} \times 100$$

where W_1, Weight of empty container with its cover (g); W_2, Weight of container with its cover and ground seeds before drying (g); W_3, Weight of container with its cover and ground seeds after drying (g).

Germination test: Germination test was conducted on pure seed fraction of freshly harvested seeds (after about 1 month of harvest) using 100 seeds in three replications of each of the varieties. The test was conducted following the between paper (BP) method and keeping at 25±1°C temperature and 93±2% relative humidity following ISTA protocol (ISTA 2021).

Seed vigour index: Seed vigour indices were estimated following the procedure suggested by the Abdul-Baki and Anderson (1972) as:

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

Seed vigour index II = Germination (%) × Mean seedling dry weight (mg)

Seed biochemical traits

Observations were recorded on seed biochemical traits,

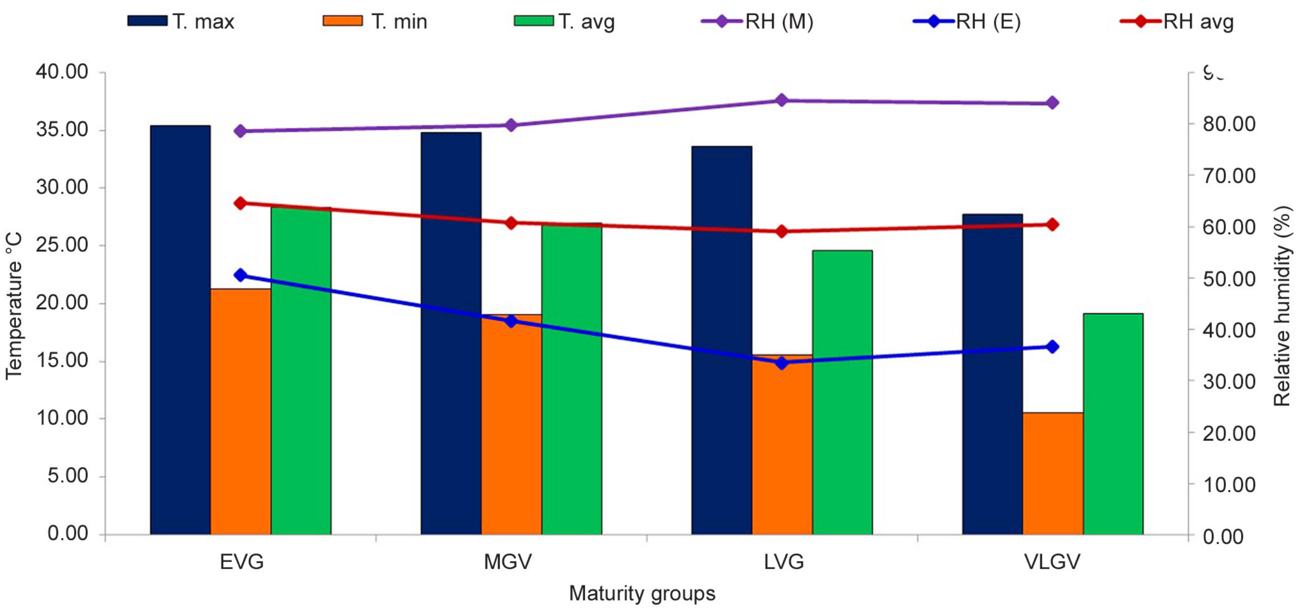

Fig. 1 Climatic variables during seed development period for various maturity group of varieties.
EGV, Early group varieties; MGV, Mid group varieties; LGV, Late group varieties; VLGV, Very late group varieties.

Table 2 Effect of maturity groups, storage duration and their interaction on seed physiological and biochemical parameters

Factor	Moisture content	GP	SV-I	SV-II	EC	WSS	CAT	POD	SOD	H_2O_2	Alpha amylase
Maturity group	**	**	**	**	**	**	**	**	**	**	**
Storage period	**	**	**	**	**	**	**	**	**	**	**
Maturity duration × Storage period	*	**	**	**	**	**	**	**	**	**	**

*, $P<0.05$; **, $P<0.01$. GP, Germination percentage; SV-I, Seed vigour index I; SV-II, Seed vigour index II; EC, Electrical conductivity; WSS, Water soluble sugars; CAT, Catalase; POD, Peroxidase; SOD, Superoxide dismutase.

viz. ROS scavenging enzymes like catalase (Aebi 1984), peroxidase (Castillo *et al.* 1984), superoxide dismutase (µM/min/gFW) (Dhindsa *et al.* 1981), hydrogen peroxide (Rao *et al.* 1996), and α-amylase (Jones and Varner 1966).

Statistical analysis: The data recorded in the varieties were analyzed for seed vigour traits following the statistical methods as described by Panse and Sukhateme (1985).

RESULTS AND DISCUSSION

Climatic conditions during seed maturity: The EGV and MGV group varieties experienced a higher atmospheric temperature at the time of seed maturity whereas in case of LGV and VLGV varieties there was a gradual lowering of temperature during seed maturity (Fig. 1).

Seed physiological traits: Maturity groups, Storage duration and their interaction effect significantly ($P<0.01$) influenced all the seed physiological and biochemical parameters of rice varieties (Table 2). In freshly harvested seeds, EGV and MGV varieties showed elevated germination percentages and seed vigour indices when contrasted with LGV and VLGV groups. Nonetheless, as the duration of storage increased, there was a gradual decline in both germination percentage and vigour within the EGV and MGV groups. In contrast, the LGV and VLGV groups exhibited an initial upswing in these parameters up to 8 months of storage, followed by a subsequent decline after 12 months.

Moisture content: Among the maturity groups, EGV consistently has the lowest moisture content throughout all the storage periods. LGV and VLGV showed higher moisture content compared to EGV but lower than MGV. MGV consistently has higher moisture content compared to all other maturity groups. For all maturity groups, there is a noticeable decrease in moisture content as the storage period increases. Freshly harvested seeds of different maturity groups varieties had moisture content ranging from 19.24 (EGV) to 21.77% (VLGV). Over the storage period (1, 4, 8, 12 months), there was a consistent decrease in moisture content. Similar to EGV, MGV seeds showed a consistent decrease in moisture content during storage. LGV seeds had the highest initial moisture content at 0 month (Fig. 2A). The moisture content of seeds during storage is the most convincing factor affecting the longevity.

Seed germination and seed vigour indices: Germination percentages (GP) of EGV (70%) and MGV (54%) were significantly higher than those of late (7%) and VLGV (5%) maturity groups in freshly harvested seeds. After one month of storage, germination percentage was significantly increased across the maturity groups. In case of EGV and MGV, germination (%) increased from freshly harvested seeds (70% and 54%) to 1 month of stored seeds and decreased during storage periods i.e. after 4 months, 8 months and 12 months after storage. In case of LGV and VLGV, germination percentage increased from freshly harvested seed to 1 month, 4 months, 8 months, and significantly decreased in 12 months of storage (Fig. 2B). A significant difference between the maturity group and storage periods for seed vigour index-I (SVI) was observed which varied from 150.60 (LGV in freshly harvested seeds) to 3790.39 (MGV after one month of storage). SVI-I in freshly harvested seeds in the EGV had the highest value (2122.64) than that in the MGV (1619.08), LGV (150.60), and VLGV (177.76). A significantly higher value was found for SVI-I for freshly harvested seeds and 1 month stored seeds in EGV and MGV. Later it decreased significantly started from 4, 8 and 12 months after storage in the EGV and MGV varieties. In case of the LGV and VLGV seed vigour index-I was increased from freshly harvested seed to 1 month, 4 months and 8 months after storage and decreased at 12 months after storage (Fig. 2C). The EGV and LGV showed significant differences in seed vigour index-II (SVI-II) from freshly harvested seeds to 1, 4, 8, and 12 months after storage. In EGV and MGV, the SVI-II significantly increased from freshly harvested seeds to 1 month of stored seeds. From 4 months of stored seeds to 8 months and 12 months of stored seeds, it significantly decreased in EGVs. In LGV, the SVI-II had significantly increased from freshly harvested seeds to 1, 4, and 8 months of stored seeds but significantly decreased after 12 months of storage (Fig. 2D). Krishnaswamy and Sheshu (1990) reported varietal differences in the longevity of rice seeds stored under ambient conditions. In the early and mid-maturity groups, the GP, SV-I, and S V-II values were highest initially and declined gradually during the storage periods. As seeds age, their physiological and biochemical processes slow down, leading to a decrease in their overall vigour and viability (Groot *et al.* 2012). On the other hand, the LGV and VLGVs exhibited a different pattern which showed GP, SV-I, and SV-II values increased during the initial storage period up to 8 months. This increase in seed vigour might be attributed to processes such as after-ripening or dormancy release. Seeds of certain species or varieties may

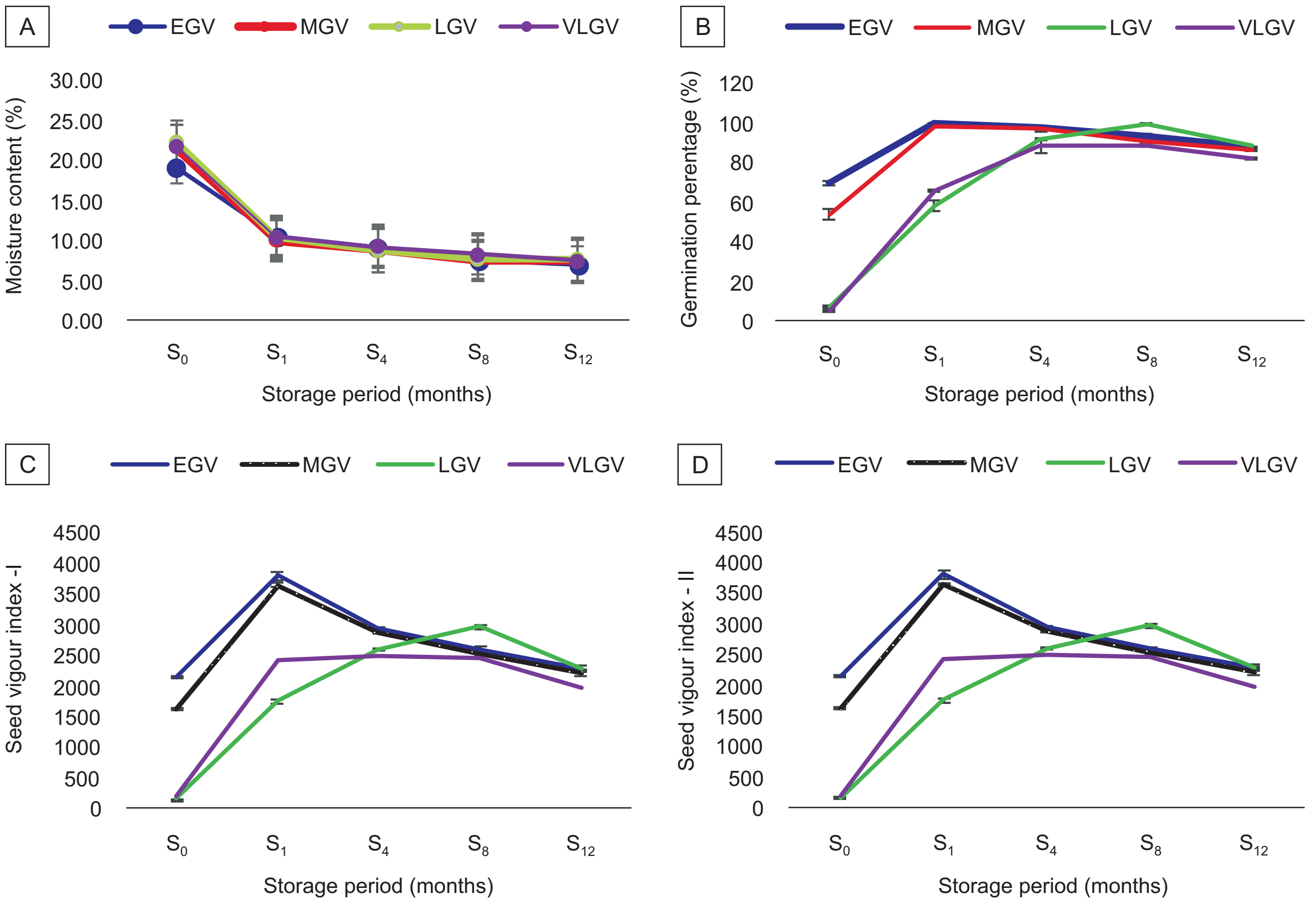

Fig. 2 Effect of seed maturity group and storage duration on (A) Moisture percentage; (B) Germination percentage; (C) Seed vigour index-I; and (D) Seed vigour index-II.
S_0, Freshly harvested seeds; S_1, 1 month stored seeds; S_4, 4 months stored seeds; S_8, 8 months stored seeds; S_{12}, 12 months stored seeds; EGV, Early group varieties; MGV, Mid group varieties; LGV, Late group varieties; VLGV, Very late group varieties.

require a period of storage to undergo physiological changes that promote germination and vigour (Bewley and Black 1994). The LGV and VLGV varieties experienced a lower atmospheric temperature and relative humidity (RH_{min}) during the time of seed maturity compared to EGV and MGV which may lead to induction of seed dormancy and may result in maintaining high vigour even after 8 months of storage (Fig. 1). Paul *et al.* (2019) also reported presence of physical seed dormancy in greengram seeds when there was lower atmospheric temperature during seed maturity. Beyond 8 months of storage, the GP, SV-I and SV-II values started to decline, indicating that extended storage durations negatively affected seed vigour even in these groups. This decline could be associated with factors such as increased oxidative stress, lipid peroxidation, or accumulation of reactive oxygen species during prolonged storage (Pukacka and Ratajczak 2007).

Seed biochemical traits

Antioxidants

Catalase (CAT), Peroxidase (POD) and Superoxide dismutase activity (SOD; µM/min/g FW): As the fresh seeds were stored for 4 months, 8 months and 12 months, the CAT activity was decreasing in EGV and MGV. In the LGV and VLGV the activity increases significantly from freshly harvested seeds to 4 and 8 months (0.0267 and 0.0298), and decreases at 12 months of storage (Fig. 3A). On the other hand, peroxidise activity (POD) decreased significantly from fresh seeds to 4, 8 and 12 months of stored seeds in EGV and MGV. In the seeds of the LGV and VLGV, the activity was increased but not significantly from fresh seeds to 4 months (0.0239 and 0.0219) stored seeds to 8 months of storage. Later the activity significantly decreases from 8 months of stored seeds to 12 months of stored seeds in the LGV and VLGV (Fig. 3B). SOD activity decreased significantly from 4 months of storage, to 8 months and 12 months of storage in EGV and MGV. There was a significant decrease in SOD activity from 8 months of storage to 12 months of storage in the EGV and MGV. There was a significant increase in the SOD activity in the LGV and VLGV in fresh seeds and 4 months of stored seeds. In LGV, the SOD activity was not significantly increased from 4 months to 8 months (181.23) but significantly decreased to 12 months (70.15) (Fig. 3C). We found that in the early and medium maturity group varieties, the ROS scavenging enzymes like CAT, POD, and SOD activity decreases from 1 month of stored seeds to 4, 8 and 12 months of stored seeds. This decline in enzyme activity indicates a reduction in the capacity of

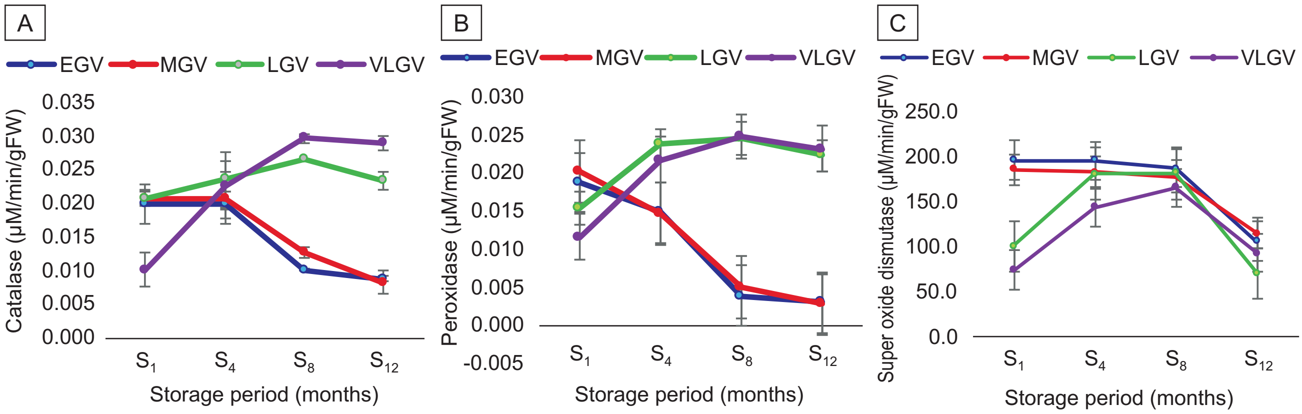

Fig. 3 Effect of seed maturity group and storage duration on (A) Catalase activity; (B) Peroxidase activity; and (C) Superoxide dismutase activity.

S_0, Freshly harvested seeds; S_1, 1 month stored seeds; S_4, 4 months stored seeds; S_8, 8 months stored seeds; S_{12}, 12 months stored seeds; EGV, Early group varieties; MGV, Mid group varieties; LGV, Late group varieties; VLGV, Very late group varieties.

the seeds to scavenge ROS, such as hydrogen peroxide (H_2O_2), superoxide radicals (O^{2-}), and hydroxyl radicals (•OH) (Bailly *et al.* 1998). But on the other hand, these enzymes increased in 1 month stored seeds to 8 months and significantly decreased from 8 months to 12 months of storage in LGV and VLGV. The initial increase in enzyme activity may indicate an up-regulation of the antioxidant defence system in response to seed aging and oxidative stress (Gill and Tuteja 2010). This decline of ROS could be attributed to factors such as enzyme degradation, inhibition by ROS-induced damage, or alterations in gene expression related to antioxidant defence pathways (Noctor *et al.* 2018).

Hydrogen peroxide activity (H_2O_2; µm/g): There was a significantly high activity of H_2O_2 in fresh seeds to 4 months stored seeds in EVG, MGV, LGV and VLGV groups (Table 3). There was a significant increase in H_2O_2 content in the LGV and VLGV from 4 months of stored seeds to 8 month of storage. A significant decrease was recorded in the activity of H_2O_2 from 4 to 8 and 12 months seeds after storage in EGV and MGV (Table 3). As a signaling molecule, H_2O_2 is involved in various physiological processes, including seed dormancy release, germination and defense responses (Bailly *et al.* 2008). This reduction from 4 to 12 months in EGV and MGV may be due to the consumption of H_2O_2 by various antioxidant systems present in the seeds, including enzymatic antioxidants such as catalase, peroxidases, and superoxide dismutase, as well as non-enzymatic antioxidants like ascorbate and glutathione (Bailly *et al.* 2008, El-Maarouf-Bouteau *et al.* 2013). But in case of LGV and VLGV the level of H_2O_2 increases up to 8 months of storage and decreases from 8 to 12 months of storage. However, the subsequent decrease in H_2O_2 levels from 8 to 12 months of storage suggests a potential decline in ROS production or an enhancement of antioxidant defense mechanisms (Bailly *et al.* 2008).

Alpha amylase activity (mg/ml/g): Higher α-amylase content in the EGV and MGV as compared to the LGV and VLGV across the storage periods was observed (Table 3). In the EGV and MGV seeds, α-amylase content decreased from fresh seeds to 4 months of stored seeds and decreased gradually from 4 months to 8 and 12 months. In the LGV, significant increase was recorded in α-amylase from fresh seeds to 4 months stored seeds. There was a significant increase in α-amylase content from fresh seeds to 4 months stored seeds and 8 months of stored seeds in VLGVs (Table 3). Alpha amylase activity decreases during

Table 3 Effect of seed maturity group and storage duration on hydrogen peroxide activity and α-amylase activity

	Hydrogen peroxide activity					Alpha amylase activity				
Maturity group \ Storage period	S_1	S_4	S_8	S_{12}	Mean	S_1	S_4	S_8	S_{12}	Mean
Early group varieties (EGV)	1.222	1.374	0.994	0.514	1.026	0.901	0.701	0.672	0.646	0.730
Mid group varieties (MGV)	1.281	1.419	0.999	0.412	1.028	0.918	0.676	0.643	0.635	0.718
Late group varieties (LGV)	0.400	1.73	1.810	0.520	1.116	0.616	0.645	0.657	0.622	0.635
Very late group varieties (VLGV)	0.501	1.322	1.427	0.365	0.902	0.645	0.666	0.675	0.621	0.652
Mean	0.849	1.462	1.307	0.453	CD (G)	0.770	0.672	0.662	0.631	CD (G)
CD (S)		0.104			0.104		0.011			0.011
SE(m)		0.036			0.036		0.004			0.004
CD (G × S)			0.207					0.021		

S_0, Freshly harvested seeds; S_1, 1 month stored seeds; S_4, 4 months stored seeds; S_8, 8 months stored seeds; S_{12}, 12 months stored seeds.

the storage periods in EGV and MGV. These varieties are harvested when the seeds have higher moisture content which provides favourable conditions for enzymatic activities, including α-amylase. Afterwards, the decrease in moisture content during drying resulted a decline in α-amylase activity over time (Devi *et al.* 2016). Moreover, during the initial storage period, seeds may experience metabolic changes that lead to the depletion of substrates required for α-amylase activity, further contributing to the decrease in enzymatic activity. The seeds of LGV and VLGV may have lower moisture content at harvest compared to early and mid-maturity group varieties. As a result, the initial α-amylase activity is relatively low in these seeds. The increased moisture availability enhances enzymatic activity, leading to an increase in α-amylase activity up to 8 months of storage (Devi *et al.* 2016). However, beyond 8 months of storage, the activity of microorganisms, particularly fungi, can result in the degradation and inactivation of α-amylase, leading to a subsequent decrease in α-amylase activity from 8 to 12 months of natural storage (Yang *et al.* 2016).

These results emphasise the importance of considering maturity group of a variety for selecting seeds during sowing since early and mid-maturity group variety produce seed with higher initial germination percentage and vigour indices, making those suitable for immediate sowing. For LGV and VLGV seed production at *kharif* season and sowing at next *kharif* season where as in case of EGV and MGV seed production at winter (*rabi*/*boro*) season and sowing in ensuing *kharif* season may result in better seed quality and stand establishment as EGV and MGV maintain high vigour up to 4 months of natural ambient storage whereas LGV and VLGV maintain vigour even after 8 months. Farmers may be benefited by this decision in maintaining optimum crop stand with high vigour and ultimate productivity.

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