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Variation in seed dormancy and α-amylase activity in Indian rice (*Oryza sativa*) accessions

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ABSTRACT

Lack of adequate seed dormancy is the major reason for pre-harvest sprouting in field under wet weather conditions. This results in significant economic loss for grain industry around the world. Objective of this study was to evaluate rice seed dormancy and the use of α-amylase enzyme activity as an indicator of dormancy level. There was, significant variation for degree of dormancy among 100 accessions, procured from the Regional Agricultural Research Station (RARS), Titabar, India. Cheniahu with highest duration of dormancy (25 days) can be a potential donor for dormancy, which can be exploited for development of rice variety with desired period of dormancy to resist the problem of pre- and post-harvest sprouting. Rice seeds were germinated and α-amylase activity was measured 0, 3, 5, 7, 10 and 12 days of germination. Significant steady increases in á-amylase activity were observed from third day of germination but decreased after 10 days of germination. The α-amylase activity was low in dormant genotypes and high in non-dormant genotypes and proved to be an efficient marker of the seeds dormancy level.

Key words: α-amylase activity, Pre-harvest sprouting, Seed dormancy

Rice (*Oryza sativa* L.), the major cereal crop playing significant role in shaping the diet, culture and economy of millions of people across the globe, is the leading food source for mankind and it feeds more than half of the world's population. Northeast India including Assam is considered as one of the primary centers of origin of rice representing a rich source of genetic diversity and reservoir of valuable gene system. In Asom, geoclimatic variations and agriculture's dependence on rainfall have resulted in three distinct rice (*indica* type) growing seasons: summer rice which extends from March-April to June-July, winter rice which extends from June-July to November-December and autumn rice which extends from November-December to May-June.

Summer rice is sown in March/April and harvested in June/July and its cultivation is considered as playing gamble in the context of uncertainties in return. This crop is grown with minimum inputs with deficiency of water during vegetative phase of growth and plenty of water at maturity. Thus, summer rice encounters plenty of rainfall at maturity stage along with rise in temperature and humidity. This results in sprouting of rice grain within the panicle in the standing crop. Sprouted grains are imperfect in appearance, generally not accepted for milling and not suitable for human consumption, because of presence of α-amylase (Bewley and Black 1985). These grains also cannot be used for seeding. A major factor leading to preharvest sprouting (PHS) is a lack of seed dormancy, the failure of viable seeds to germinate under favourable conditions (Lin *et al*. 2009, Ullrich *et al*. 2009). In summer rice, the main effects of pre-harvest sprouting are a lower yield due to harvest losses and more importantly, a reduction in end-product quality. Grain α-amylase activity is used as the major indicator of pre-harvest sprouting, because of the effect it has on flour properties and because of its abundance in germinating grains. So, development of high-yielding varieties of rice with a dormancy period of 3-4 weeks is of utmost importance to improve the productivity and quality of rice. Knowledge on nature and amount of genetic variability present in a gene pool and the heritability of the traits are important for achieving genetic improvement of quantitative traits. Thus, information of genetic parameters such as, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) is useful to ascertain the extent of genetic variation among the traits measured. Moreover, the environmental influence on the trait can be understood by the estimation of heritability, and this also reflects the component of variation that is inherited to the subsequent generation and is exploited for crop improvement. Genotypic

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coefficient of variation does not provide a clear picture of the extent of genetic gain to be achieved from selection unless heritable fraction of variation is known, indicating the importance of heritability estimation. Improvement in the mean genotypic value of the selected families over base population is the genetic advance, which depends on genetic variability in the base population, heritability of character under selection and selection intensity.

Till date, not a single modern high-yielding rice variety with resistance to pre- and post-harvest sprouting has been developed and only limited attempt for development of such a variety has been reported so far utilizing indigenous rice materials. Considering these, there is an urgent need for proper characterization of traditional summer rice of Asom based on dormancy traits.

MATERIALS AND METHODS

Grains of hundred rice genotypes were obtained from the Regional Agricultural Research Station (RARS), Titabar during the harvesting season in June-July 2008 (Table 1). Panicles from each plant were collected on the 35th day after heading when the grains were fully filled and were immediately stored at 4°C for maximum one month to maintain dormancy. The grains were oven dried at 100°C (± 2°C) for 6 hr and converted into fine powder and kept in a desiccator for analytical works. The dormancy was assessed following Wan *et al.* (1997). For evaluation of dormancy, 100 seeds from each plant were placed on doubled sheets of filter paper moistened with distilled water in a dish of 6 cm diameter, and maintained at 30°C and 100% relative humidity for 7 days in germinator. Each genotype was tested with three repeats. Germination was judged when the radicle or coleoptile expanded by >3 mm. The degree of seed dormancy was scored as the mean percentage of germinated seeds. Those genotypes with seed germination =25% were considered to be strongly dormant, whereas those with seed germination =50% were considered to be weakly dormant (Das 1989). Duration of dormancy was calculated for each of the genotypes when 10% of the germination was achieved in each genotype. The α-amylase activity at different days of germination was estimated by the method as described by Sadasivam and Manickam (1996).

Table 1 Rice genotypes used in the present investigation

Genotype	Genotypes	Genotypes	Genotypes
Cheniahu	Ch 63	Betguti Ahu	As 305/2
Kolong	Nilaji II	IET 6148	As 180/2
Kola Meghi Ahu	Rongadoria (Local)	IET 10898	As 317
ARC 10372	Koijanori III	Banglami	As 323
DKH 36	Koijanori II	Boga Ahu	As 324
H 1120	Koijanori I	Changa Ahu	As 27
Sonamukhi	Sarimohia Ahu	Kajoli Ahu	As 327
Gareem	Tinimohia Ahu	As 55	As 159/2
Betguli Ahu	Bahmori	Koimurali	As 314
Ahu Joha 2	Borkola Ahu	Moren Chenkol	As 312
Guni Ahu	Daukola Maghi	Chidon	As 209
Rikhoijoi	Boga Bengena Gutia	Bizor 3	As 292
Bongal Ahu	Kola Bengena Gutia	Bizor 1	As 1224
New Sali	Kolamaghi	Bairing	As 204
Rasi	Sohalia Ahu	Ahu I	As 178/3
IET 6223	Kola Ahu	Basanta Bahar	As 206
Govind	Panja Sali	Dubori Cinga	As 36/20
Jai Bangla 1	Rongadoria 3	Garem I	As 75
Kanua	Bongal doria	As 156	As 313
Kosamani	Iharsal	As 330	As 310
Baibi 2	Begon Bisi	As 229	As 105
Bongaloni	Goal Bhog	As 195	As 47
Jai Bangla 2	Poromi Ahu	As 39/13	As 39
IET 10896	Naga Ahu	As 177/2	As 34
Ronga Gutia	Shesamthat	As 38/2	As 38/2

Extraction of α-amylase: 1 g of rice sample was extracted in 10 ml of ice cold 10 mM calcium chloride solution overnight at 4°C. The extract was centrifuged at 12 000 g at 4°C for 30 min. The supernatant was used as enzyme source.

Estimation of amylase activity: 1 ml of 1% starch solution and 1 ml of enzyme extract was pipetted in a test tube and was incubated at 27°C for 15 min. Then the reaction was stopped by adding 2 ml of DNS reagent and the solution was heated in a boiling water bath for 5 min. While the tubes were warm, 1 ml 40% sodium potassium tartarate solution was added and then cooled it in running tap water. The volume was made up to 10 ml by the addition of distilled water and the absorbance was read at 560 nm. The reaction was terminated at zero time in the control tube. A standard graph was prepared with 0-100 µg maltose. A unit of α-amylase was expressed as mg of maltose produced during 5 min incubation with 1% starch.

The data generated from three replications were analyzed using ANOVA and the genetic parameters such as phenotypic and genotypic coefficient of variation (PCV and GCV), heritability in broad-sense (h^2 in %), and genetic advance as percent of mean (genetic gain) were worked out as suggested by Johnson *et al.* (1955). Correlation coefficients were calculated according to Al-Jibouri *et al*. (1958). The significance of a given variance was determined by calculating the respective 'F' values (Panse and Sukhatme 1978).

RESULTS AND DISCUSSION

Seed/grain dormancy

Seed dormancy, a condition that temporarily suspends visible growth of meristems, is one of the factors that hamper germination. In nature, dormancy can be an advantage for some species because it renders resistance to pre-harvest

sprouting and prevents germination until favourable conditions for plant development prevail. The germination test is preferred because it does not require expensive equipment and is rapid in obtaining results (Lin *et al*. 2009).

There was a significant variation in grain dormancy among the different genotypes (Table 2). The average germination percentage was found to be 16.77% with highest in Kajoli Ahu (58.21%) and lowest (0.57%) in Guni Ahu, Nilaji II, IET 6148, Banglami and Basanta Bahar. From the results obtained, summer genotypes can be classified as: 5 genotypes (Guni Ahu, Nilaji II, IET 6148, Banglami, Basanta Bahar) are with germination less than 1%, 24 genotypes with less than 5%, 19 genotypes with less than 10%, 13 genotypes with less than 15%, 10 genotypes with less than 20%, 9 genotypes with less than 30%, 4 genotypes with less than 40%, 13 genotypes with less than 50% and 7 genotypes (Chidon, As 159/2, Bairing, As 314, As 312, Bizor 3, Kajoli Ahu) with less than 60% germination. Those genotypes with seed germination 25% (Guni Ahu, Nilaji II, IET 6148, Banglami, Basanta Bahar, Cheniahu, Rongadoria etc) were considered to be strongly dormant, whereas those with seed germination 50% (Chidon, As 159/2, Bairing, As 314, As 312, Bizor 3, Kajoli Ahu, Kolong etc) were considered to be weakly dormant (Das 1989).

Duration of dormancy was calculated as days for each of the genotypes when 10% of the germination was observed. The rice genotypes differed significantly from one another. The average duration of dormancy was 6.64 days. The highest duration of dormancy was recorded for Cheniahu (25 days) and lowest (1 day) for Kolong, IET 6223, Jai Bangla 2, Ch 63, Kajoli Ahu, Chidon, Bizor 3, Bairing and Garem I.

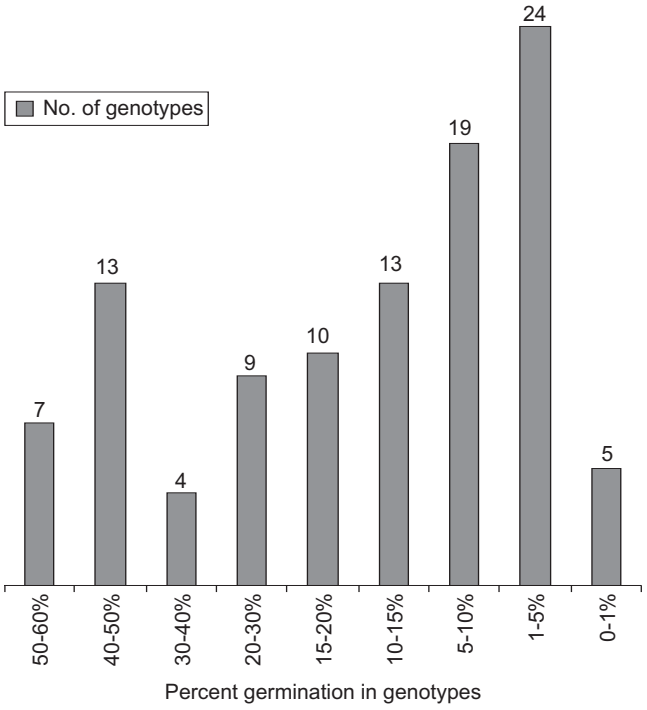
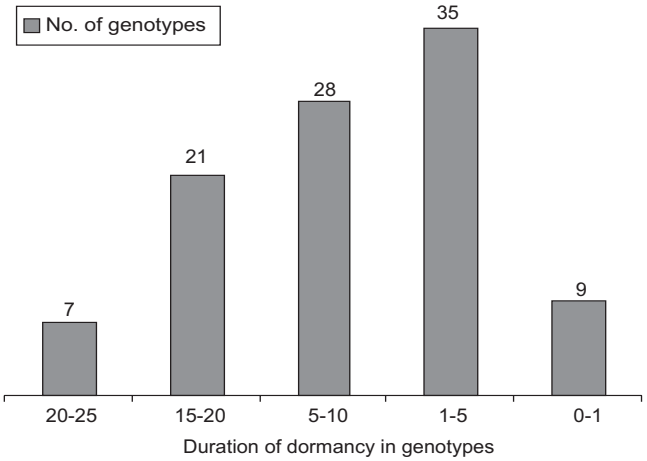


Table 2 Analysis of variance for dormancy properties in summer rice

Sources of variation	df	Mean sum of squares											
		Grain dormancy (%) (Days)	Duration of dormancy	Germination at 3 DAH (%) DAG	Amylase activity at 3	Germination at 5 DAH 5% DAG	Amylase activity at 5 (%)	Germination at 7 DAH (%) DAG	Amylase activity at 10 DAG	Germination at 10 DAH (%)	Amylase activity at 12 DAG	Germination at 12 12 DAH (%)	Amylase activity at 12 DAG
Replications	2	33.79266	44.18	21.624	0.019	0.128	0.004	33.793	0.002	3.007	0.013	129.62	0.008
Genotypes	99	81 278.31**	82 71.12**	38 571.89**	3.838**	51 707.24**	7.568**	81 278.31**	21.268**	107 977.48**	59.967**	243 728.88**	30.390**
Error	198	46.096869	117.82	32.635	0.075	56.075	0.049	46.097	0.738	306.965	0.241	68.38	0.244

df, degrees of freedom; **Significant at 1% level; DAH, days after harvest; DAG, days after germination



Cheniahu (25 days) with maximum dormancy was followed by Nilaji II (21 days), Rongadoria (Local) (21days), Guni Ahu (20 days), IET 6148 (20 days), Banglami (20 days), Basanta Bahar (20 days), Kola Meghi Ahu (15 days) and Koijanori III (15 days). Nine genotypes had duration of dormancy equal to 1 day with germination above 35.0% and can be considered as non-dormant. Thirty five genotypes had duration of dormancy more than 1 day and less than 5 days, 28 genotypes with less than 10 days, and 21 genotypes with less than 20 days. Seven genotypes had duration of dormancy ranging from 20-25 days with germination less than 3% can be considered as highly dormant.

Kalita *et al*. (1994) studied seed dormancy in summer rice germplasm which were insensitive to photoperiod and reported that Cheniahu showed the highest dormancy period which supports the present findings. Haloi (1998) reported that there is a wide variation in grain dormancy in summer rice with degree of dormancy varying from 5 to 42 days. Cheniahu, Nilaji II, Rongadoria (Local), Guni Ahu, IET 6148, Banglami, Basanta Bahar were identified as potential donors for dormancy and can be used in breeding programmes. This is only if the dormancy induced genes are totally/ majorly transferred in the new variety. Recently our group has detected two QTLs for seed dormancy (qSD5, qSD11) and one for duration of dormancy (qSDD5) in cross involving Cheniahu, thereby indicating presence of genes in traditional rice germplasm (Rathi *et al*. 2011). This indicates that there is sufficient genetic variability for seed dormancy in the genetic resources, which can be exploited for development of rice variety with desired period of dormancy to resist the problem of pre- and post-harvest sprouting.

Germination and α-amylase activity

Amylase is an enzyme that breaks starch down into sugar. Amylases are of three types: α-amylase, β-amylase and γ-amylase. The discussion of the biochemistry of pre-harvest sprouting would not be complete without looking at the enzymes that effect the mobilization of the reserves stored in the starchy endosperm. Generally, the activity of α -amylase does not exist in dry rice seeds, but is rapidly induced and increased as the process of germination takes place (Charoenthaikij *et al.* 2009). The α-amylase activity was measured on 0, 3, 5, 7, 10 and 12 days after germination of different rice genotypes. The α-amylase activity in the resting grain i e on 0 day of germination could not be detected at all. However, there was a steady increase in the α-amylase activity from 3rd day of germination. But the α-amylase activity level decreased after 10 days of germination. On third day Kajoli Ahu, Ch 63, Chidon recorded the same highest percentage of germination with Kajoli Ahu having highest α-amylase activity while IET 6148 recorded lowest germination per cent with lowest α-amylase activity. On fifth day, Kajoli Ahu recorded the highest percentage of germination with highest α-amylase activity while IET 6148 recorded lowest germination per cent with lowest α-amylase activity. On seventh day, 'Kajoli Ahu' recorded the highest percentage of germination with highest α-amylase activity while Nilaji II, Banglami, Guni Ahu and IET 6148 recorded same lowest germination per cent with Nilaji II having the lowest α-amylase activity. On tenth day, As 177/2 recorded the highest percentage of germination with highest α-amylase activity while Guni Ahu, Nilaji II, IET 6148 and Basanta Bahar recorded same lowest germination per cent with Guni Ahu having lowest α-amylase activity. On twelfth day Bairing recorded the highest percentage of germination with highest α-amylase activity while Cheniahu recorded lowest germination per cent with lowest α-amylase activity. Kajoli Ahu recorded the highest level of α-amylase activity at 3, 5 and 7 days after germination. As 177/2 and Bairing recorded highest level of α-amylase activity at 10 and 12 days after germination respectively. IET 6148 recorded the lowest level of α-amylase activity at 3 and 5 days after germination. Nilaji II, Guni Ahu and Cheniahu recorded the lowest level of α-amylase activity at 7, 10 and 12 days after germination respectively. Ayrenor and Ocloo (2007) germinated paddy rice for 0, 5, 7 and 9 days and observed significant increase in amylase activity during rice germination up to nine days. In the present study, highest α-amylase activity was recorded for the maximum germination per cent while lowest for minimum germination per cent. Krishnasamy and Seshu (1990) reported that the rice cultivars with high α-amylase activity showed higher germination rate and faster seedling growth at the early stage which supports the present findings. Statistical analysis revealed that there was a positive association between α-amylase activity and germination. The present finding corroborates the results of Williams and Peterson (1973) who observed a strong relationship between α-amylase activity and seedling growth. Ching *et al*. (1977) reported that the rate of emergence was significantly correlated with α-amylase activity in endosperm of five days and three days old seedlings of barley (*Hordeum vulgare* L.). Kajoli Ahu recorded highest mean α-amylase activity and germination which can be classified as non-dormant while

IET-6148, recorded lowest mean α-amylase activity and germination which can be classified as dormant. This finding reveals that α-amylase activity is low in dormant genotypes and high in non-dormant genotypes and is supported by the findings of Vieira *et al*. (2002) who reported that the α-amylase activity is an efficient indicator of the degree of dormancy in the rice seeds. Similarly, Ullrich *et al.* (2009) also considered α-amylase, a reliable indicator of dormancy as it is synthesized *de novo* during the germination process.

Variability parameters for dormancy related parameters are represented in Table 3 and Table 4. The difference between GCV and PCV was very narrow for all the characters analyzed. The maximum GCV value was 124.08% for germination at three days after harvest and minimum was 30.85% for amylase activity at seven days after germination while the maximum PCV value was 124.16% for germination at three days after harvest and minimum was 31.40% for amylase activity at five days after germination. For amylase activity low estimates of genotypic and phenotypic variation were observed in the present study as revealed from the estimates of GCV and PCV.

The heritability in broad sense ranged from 94.97% in amylase activity at seven days after germination to 99.96% in germination at twelve days after harvest. Genetic advance at 5% mean values ranged from 61.92 in amylase activity at seven days after germination to 255.45 in germination at three days after harvest. The high estimates (>80%) of heritability for dormancy characters indicated considerable genetic variation and lower influence of environment in the expression of these characters. High heritability with high genetic advance was recorded for grain dormancy, duration of dormancy and germination indicating the involvement of increased additive gene action and therefore, selection will be effective to a great extent. High heritability with low genetic advance was found for amylase activity indicating that these characters might be under the control of non-additive (dominance and epistasis) gene action. Amongst all the dormancy attributes, grain dormancy trait deserves top most priority in selection in developing dormant high-yielding varieties because this character was attributed with high variability.

In the present study, correlation coefficients at genotypic and phenotypic levels for all the traits were computed. In general, phenotypic correlation coefficients were marginally lower than the genotypic correlation coefficients which might be due to masked or modifying effect of environments (Johnson *et al*. 1955). Grain dormancy and duration of dormancy are negatively correlated for both phenotypic and genotypic correlation (Table 5). Negative correlation of pre-harvest sprouting with dormancy duration was also reported by Seshu and Sorrells (1986). They also reported that preharvest sprouting was positively correlated with germination which is consistent with the reports of the present findings. Duration of dormancy is negatively correlated with germination and amylase activity for both phenotypic and genotypic correlation. Germination rate at 3, 5, 7, 10, and 12 days showed positive correlation with amylase activity at 3, 5, 7, 10 and 12 days after germination for both phenotypic and genotypic correlation respectively. Seshu and Krishnasamy (1987) reported significant positive correlation between rate of germination and α-amylase activity in rice

Table 3 Estimates of variability parameters for dormancy and germination properties in summer rice

Character	Range		Mean	PCV	GCV	Heritability in	Genetic advance
	Maximum	Minimum	± SEm	(%)	(%)	broad sense (%)	(5%)
Grain Dormancy (%)	58.22	0.57	16.77±0.28	98.69	98.64	99.91	203.12
Duration of Dormancy (Days)	25.00	1.00	6.64±0.44	80.04	79.19	97.89	161.41
Germination at 3 DAH (%)	44.43	0.57	9.18±0.23	124.16	124.08	99.87	255.45
Germination at 5 DAH (%)	57.15	0.57	11.16±0.31	118.33	118.23	99.84	243.35
Germination at 7 DAH (%)	58.22	0.57	16.77±0.28	98.69	98.64	99.91	203.12
Germination at 10 DAH (%)	66.96	0.57	20.44±0.72	93.42	93.23	99.57	191.64
Germination at 12 DAH (%)	96.00	1.00	42.48±0.34	67.45	67.43	99.96	138.88

PCV, Phenotypic coefficient of variation; GCV, genotypic coefficient of variation; DAH, days after harvest, DAG, days after germination

Table 4 Estimates of variability parameters for amylase properties in summer rice

Character	Range		Mean	PCV	GCV	Heritability in	Genetic advance
	Maximum	Minimum	± SEm	(%)	(%)	broad sense (%)	(5%)
Amylase activity at 3 DAG	0.58	0.03	0.27±0.01	41.73	41.13	97.14	83.49
Amylase activity at 5 DAG	0.90	0.19	0.51±0.01	31.40	31.24	99.03	64.05
Amylase activity at 7 DAG	1.83	0.43	0.86±0.03	31.65	30.85	94.97	61.92
Amylase activity at 10 DAG	2.63	0.73	1.30±0.02	34.63	34.53	99.40	70.91
Amylase activity at 12 DAG	2.10	0.46	1.01±0.02	31.83	31.64	98.80	64.79

PCV, Phenotypic coefficient of variation; GCV, genotypic coefficient of variation; DAH, days after harvest, DAG, days after germination

Table 5 Correlation data genotypic (Above diagonal) and phenotypic (Below diagonal) between dormancy properties in summer rice

Character	Grain dormancy (%)	Duration of dormancy (Days)	Germination at 3 DA (%)	Amylase H at 3 DAG	Germination activity at 5 DAH (%)	Amylase activity at 5 DAG	Germination at 7 DAH (%)	Amylase activity at 7 DAG	Germination at 10 DAH (%)	Amylase activity at 10 DAG	Germination at 12 DAH (%)	Amylase activity at 12 DAG
Grain Dormancy (%)	1.000	–0.672**	0.9162**	0.3155**	0.930**	0.1865	1.000	0.335**	0.985**	0.2967**	0.9456**	0.378**
Duration of dormancy (Days)	–0.6647**	1.000	–0.5733**	–0.2046*	–0.6071**	–0.1361	–0.672**	–0.3707**	–0.683**	–0.293**	–0.7705**	–0.2923**
Germination at 3 DAH (%)	0.9153**	–0.5678**	1.000	0.3937**	0.9951**	0.1981*	0.9162**	0.3721**	0.8749**	0.3057**	0.8317**	0.3903**
Amylase activity at 3 DAG	0.3109**	–0.1988*	0.3877**	1.000	0.4025**	0.6503**	0.3155**	0.5552**	0.2855**	0.3717**	0.2931**	0.4547**
Germination at 5 DAH (%)	0.9291**	–0.5998**	0.9935**	0.3974**	1.000	0.2237*	0.93**	0.3907**	0.8911**	0.3223**	0.8525**	0.3961**
Amylase activity at 5 DAG	0.1861	–0.1321	0.1975*	0.6388**	0.2226*	1.000	0.1865	0.6029**	0.1816	0.4096**	0.1842	0.2795**
Germination at 7 DAH (%)	1.000	–0.6647**	0.9153**	0.3109**	0.9291**	0.1861	1.000	0.335**	0.985**	0.2967**	0.9456**	0.378**
Amylase activity at 7 DAG	0.3267**	–0.3559**	0.3624**	0.5319**	0.3816**	0.5861**	0.3267**	1.000	0.3067**	0.6195**	0.3251**	0.4697**
Germination at 10 DAH (%)	0.9826**	–0.6744**	0.873**	0.2812**	0.8884**	0.1799	0.9826**	0.2974**	1.000	0.317**	0.965**	0.353**
Amylase activity at 10 DAG	0.2955**	–0.2889**	0.3041**	0.3638**	0.3209**	0.4067**	0.2955**	0.6013**	0.3152**	1.000	0.3258**	0.7227**
Germination at 12 DAH (%)	0.9449**	–0.7625**	0.8311**	0.2887**	0.8516**	0.1833	0.9449**	0.3162**	0.9628**	0.3246**	1.000	0.3721**
Amylase activity at 12 DAG	0.3756**	–0.2885**	0.3873**	0.4432**	0.3939**	0.2768**	0.3756**	0.4553**	0.35**	0.7152**	0.3699**	1.000

*Significant at 5% probability level, **Significant at 1% probability level; DAH, days after harvest; DAG, days after germination

which is consistent with the present findings.

Conclusion

There exists wide variation in grain dormancy among the genotypes studies. There was a steady increase in the α-amylase activity from 3rd day of germination, but the α-amylase activity level decreased after 10 days of germination. The α-amylase activity is an efficient indicator of the degree of dormancy in the rice seeds.

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