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ORIGINAL ARTICLE

Variability in seed germination and seedling growth of some rice (*Oryza sativa* L.) landraces from Sikkim Himalaya: α-amylase activity and response to gibberellic acid

Poonam Chetry and Shanti S. Sharma*

Abstract

Sikkim, with a long tradition of rice cultivation, is endowed with many landraces that represent a unique genetic resource for multiple desired traits for crop improvement. Here, the seed germination and seedling growth aspects of fifteen rice landraces, including both low land and up land, from Sikkim Himalaya have been assessed. The freshly harvested seeds of eleven landraces exhibited nearly complete (96-100%) germination within 6 d with a mean germination time (MGT) ranging from 1.04 (Ruduwah and Mumpupzho) to 1.88 (Sano attey). In contrast, four landraces namely, Birinful, Doodhkatey, Dhansey and Dharmali exhibited much lower germination that ranged from 62% (Birinful) to 71% (Dharmali) after 6 d suggesting partial dormancy; the higher MGT values (2.83-4.07) imply slower rate of germination. Seed germination in these landraces further declined after 6 months of storage. The α-amylase activity, measured in germinating seeds through an agar plate assay as well as biochemically, was much lower in the landraces showing low germination (Birinful, Doodhkatey, Dhansey) than in those with high germination (Chinizho, Nepalzho, Tukmorzho). Exogenous GA_3 stimulated the enzyme activity in both the groups but the magnitude of stimulation was much stronger in the former than that in the latter. GA_3-induced stimulation of α-amylase activity in the low germinating landraces was accompanied by a correspondingly enhanced seed germination. Taken together, the germination ability of the tested landraces seems to be inter alia determined by endogenous GA_3 levels that influence the α-amylase activity. The tested rice landraces also exhibited strongly variable seedling growth parameters. For example, the root and shoot length varied from 3.52 to 8.62 cm and 5.19 to 13.62 cm respectively. The seedling growth was stimulated by exogenous GA_3 application. The findings have implications primarily for seedling establishment potential.

Keywords: α-amylase, GA_3, Rice landraces, seed germination, seedling growth, Sikkim Himalaya.

Introduction

Rice is the staple food for nearly 50% of the world population. In India, North-eastern region in particular has a strong diversity of rice cultivars (Choudhury *et al.* 2014; Singh *et al.* 2019). Sikkim, the 'valley of rice', (Denzong), is endowed with an array of indigenous rice landraces that are cultivated over small areas across different parts of the state. In recent years, different aspects of genetic diversity of rice in Sikkim have been reported. For example, Kapoor *et al.* (2017) reported the on-farm status of 52 traditional rice landraces from the state. Fifty one landraces grown at varying altitudes in different parts of the state exhibit substantial differences in agronomic traits, yield, grain quality and micronutrient (Fe, Zn) contents (Kapoor *et al.* 2019). Chettri and Sathyanarayana (2021a) documented, involving 74 landraces, the available traditional knowledge concerning diverse aspects of rice cultivation in Sikkim Himalaya. Furthermore, 53 landraces have been analysed for genetic diversity employing SSR markers with implications for breeding programme(s) aimed at improvement for desired traits (Chettri and Sathyanarayana, 2021b). In addition to the low land rice landraces that are cultivated through transplantation in irrigated fields, several rice landraces, indigenous to Lepcha community of the state, are upland and sown directly following the age-old slash and burn system. The upland

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landraces include Nepalzho, Tukmorzho, Mumpupzho, Marbonzho, Chinizho and Dharmali (Kalo), Kalo tukmar, Seto tukmar (Chettri and Sathyanarayana, 2021a). The stated rice landraces are adapted to the prevalent environmental adverse circumstances that include water deficit, low temperature, low soil nutrient contents (Kapoor *et al.* 2019). A sizable part of soils in different parts of Sikkim are acidic. Consequently, they impose plant toxicities on account of enhanced availability of aluminium (Al^{3+}), Mn and Fe. Owing to chronic exposure to these factors, it is logical to expect different landraces to have evolved varying degrees of tolerance to them. Therefore, they constitute an invaluable genetic resource inter alia for imparting tolerance to the relevant abiotic stressors.

Seed germination and subsequent seedling establishment are crucially important processes that eventually determine the crop productivity. These are influenced by the environmental conditions prevalent during the period of seed development. In certain rice genotypes, low germination on account of dormancy has been reported (Shiratsuchi *et al.* 2017; Zhang *et al.* 2020). The maintenance of seed dormancy and germination are tightly regulated by phytohormones particularly gibberellin (GA) and abscisic acid (ABA) that impose mutually antagonistic effects (Kucera *et al.* 2005, Finkelstein *et al.* 2008, Shu *et al.* 2016). In rice, GA_1 and GA_4 are the predominant bioactive forms so far studied (Kobayashi *et al.* 1988). During seed germination, bioactive GAs are synthesized in the embryo and transported to the aleurone layer to induce α-amylase (EC 3.2.1.1) gene expression. Thereafter, the enzyme is secreted into the endosperm to hydrolyze the stored starch into mobilizable low molecular weight sugars (Kaneko *et al.* 2002). α-amylase activity is liable to be affected by factors such as temperature, pH, enzyme concentration, substrate concentration, and also the presence of inhibitor(s)/ activator(s). In the present study, seed germination and seedling growth features of fifteen rice landraces from Sikkim Himalaya have been characterized. In addition, it was of interest to get insight into the involvement of α-amylase levels in differential germination performance of the landraces and the response to exogenous GA_3.

Materials and Methods

Source of seeds

The seeds of selected landraces of rice (*Oryza sativa* L.) were procured from the local farmers from different parts of Sikkim Himalaya during December 2018 and January 2019 and in the subsequent year. The landraces together with the respective locations of their collection (given in parenthesis) are as follows: 1. Kalo tukmar (Pentong, North Sikkim), 2. Chinizho (Lingthem, North Sikkim), 3. Dharmali (Sakyong, North Sikkim), 4. Mumpupzho (Sakyong, North Sikkim), 5. Tukmorzho (Dzongu, North Sikkim), 6. Birinful (Gerethang, West Sikkim), 7. Krishna bhog (Saramsa, East Sikkim), 8. Tulasi (Assam Lingzey, East Sikkim), 9. Ruduwah (Aho, East Sikkim), 10. Champasari (Turuk, South Sikkim), 11, Dhansey (Upper Rumtek, East Sikkim), 12. Doodhkatey (Radong, East Sikkim), 13. Lama dhan (Ranka, East Sikkim), 14. Nepalzho (Lingthem,North Sikkim), 15. Sano attey (Khamdong, East Sikkim).

Seed germination and seedling growth

The germination of seeds of all the stated landraces was assessed within two months of harvest. In selected landraces, the germination was also assessed after six months of storage. The uniform seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min followed by thorough washing with water. They were then imbibed with distilled water for 24 h at room temperature in dark. The imbibed seeds were shifted to Petri plates lined with two layers of Whatman filter paper made wet with distilled water and allowed to germinate in a seed germinator at 30°C, 65% RH and 14 h photoperiod. In case of selected landraces, the effect of GA_3 on seed germination was monitored. For this, the seeds were imbibed with GA_3 (0.1 and 1 mM) solution for 24 h prior to subjecting them to the stated germination conditions. The seeds simultaneously imbibed with water constituted the control. The seed germination was recorded every day for 6 d. Four replicates of 20 seeds each were considered for each landrace. On 7^{th} d, the seedling growth was measured in terms of root and shoot length, fresh weight and dry weight.

Determination of mean germination time (MGT)

The mean germination time was calculated according to the method described by Hartman *et al.* (1989) using the formula: $MGT = \Sigma nd/N$; n represents the number of seeds germinated after each incubation period in days (d) whereas N represents total number of seeds germinated at the end of test.

Determination of α-amylase activity

The α-amylase activity in the seeds of selected rice landraces was measured according to the method of Filner and Varner (1967). The water-imbibed or GA_3 treated seeds were homogenized with chilled Tris-HCl buffer (pH 7.2) and the homogenate was centrifuged at 10,000 g for 10 min at 4°C. The supernatant was employed as enzyme extract. The reaction mixture containing 1 ml of substrate (0.15% starch, 0.2 mM $CaCl_2$ in Tris-HCl buffer) and 1 ml of enzyme extract was incubated at 25°C for 10 min. The reaction was quenched by adding 3 ml of IKI reagent (0.6% iodine in 6% KI; 1 ml diluted to 50 ml with 0.05 N HCl) and absorbance was recorded at 620 nm. The amount of starch degraded was determined by a standard curve.

Agar plate assay for determination of α-amylase activity

The α-amylase activity was also determined in an agar plate assay where the diffusion of α-amylase from seed tissue into

agar gel containing starch enabled assessment of enzyme activity through starch-IKI reaction (Sharma *et al.* 2010). The water-imbibed and GA_3 (0.1 mM and 1 mM) treated seeds were cut transversely and the embryo containing seed halves were placed on 2% agar plates (containing 0.2% soluble starch and 0.2 mM $CaCl_2$ at pH 7.2) and incubated at 25°C for 24 h. Thereafter, the agar plates were stained with IKI reagent as described above. The area of transparent halos formed around the half-seeds was taken as an indicator of the α-amylase activity.

Determination of protein

The protein content in the enzyme extract was determined according to the method of Bradford (1976). Following the addition of Bradford reagent to the extract, A_{595} was measured and protein contents determined using a standard curve prepared with BSA.

Statistical analysis

All experiments were performed in four replicates and repeated once. Data are represented as arithmetic means ± S.E.

Results

Seed germination

Time-course of seed germination monitored in 15 rice landraces revealed the seed germination to start as early as 24 h of incubation in all landraces except Birinful and Doodhkatey where it was delayed. With the progression of incubation period, the germination percentage gradually increased. After 6 d of incubation, 96.25 to 100% germination was evident in 11 landraces (Figure 1A). The mean germination time (MGT) in these 11 landraces ranged from 1.04 to 1.88 implying that despite the final count being comparable, the germination speed was variable to some extent. The MGT value of 1.60 and 1.88 observed in Krishna bhog and Sano attey, respectively indicates a slightly slower germination in these two landraces as compared to that in other landraces in this group (Figure 1B). In contrast, Birinful, Doodhkatey, Dhansey and Dharmali exhibited a relatively lower final germination count; a maximum of 62.5, 63.75, 70 and 71.25% germination was observed in these landraces, respectively. There was no further change beyond 6 d of incubation. The germination in this group of landraces was much slower than that in the other one. The MGT values in a decreasing order were 4.07 (Birinful), 3.33 (Doodhkatey), 2.96 (Dhansey) and 2.83 (Dharmali) implying the slowest rate of seed germination in Birinful (Figure 1B). In order to understand whether the seed germination performance was affected due to storage, the seed germination was monitored in 3 high germinating (Chinizho, Nepalzho, Tukmorzho) and 3 low germinating (Birinful, Doodhkatey, Dhansey) landraces after six month of storage at 4°C.

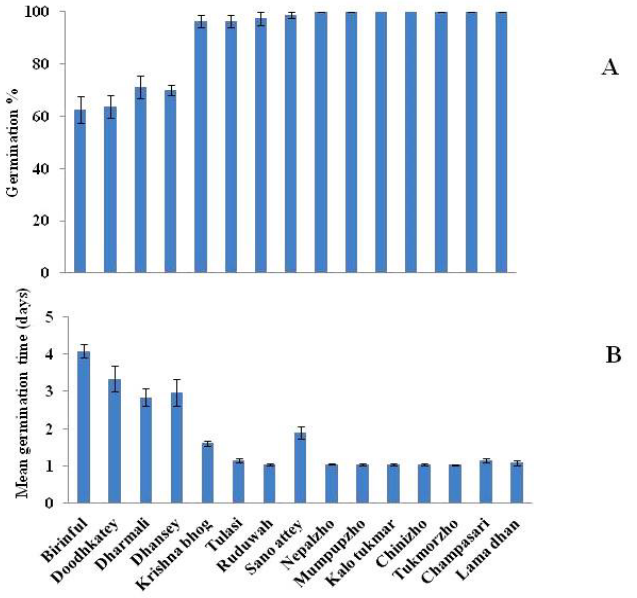

Figure 1: Seed germination percentage after 6 d (A) and mean germination time (MGT) (B) of 15 rice (*Oryza sativa* L.) landraces from Sikkim Himalaya. Values are arithmetic means of four replicates, each comprising 20 seeds ± S.E.

Whereas the former group exhibited 100% germination, the germination percentage declined in the latter group; 36.67, 46.67, and 46.67% germination was evident in Birinful, Doodhkatey, Dhansey, respectively (Figure 2A).

Seedling growth

The seedling growth was measured on 7 d of incubation in terms of length, fresh weight and dry weight of root and shoot. Strong gentotype-specific variations in these parameters were observed. Thus, the root length varied from 3.52 (Birinful) to 8.62 cm (Kalo tukmar). Likewise, the range of shoot length was observed to be 4.23 (Ruduwah) to 7.96 cm (Kalo tukmar). Apparently, the organ-specific differences exist that are revealed in marked variations in the ratio of root/shoot length. The latter was observed to be highest in Tukmorzho (1.48) implying longer roots than shoot and lowest in Birinful (0.63) implying the vice-versa. Similar quantitative variations were evident in case of the fresh and dry weight of root and shoot. Interestingly, all the seedling growth parameters were highest in case of Kalo tukmar (Table 1).

Seed germination and seedling growth response to GA_3

The influence of exogenous GA_3 was monitored on seed germination of three high (Chinizho, Nepalzho, Tukmorzho) and 3 low (Brinful, Doodhkatey, Dhansey) germinating landraces. GA_3 treatment led to a concentration dependent stimulation of seed germination in all the tested low germinating landraces. For example, in case of Doodhkatey,

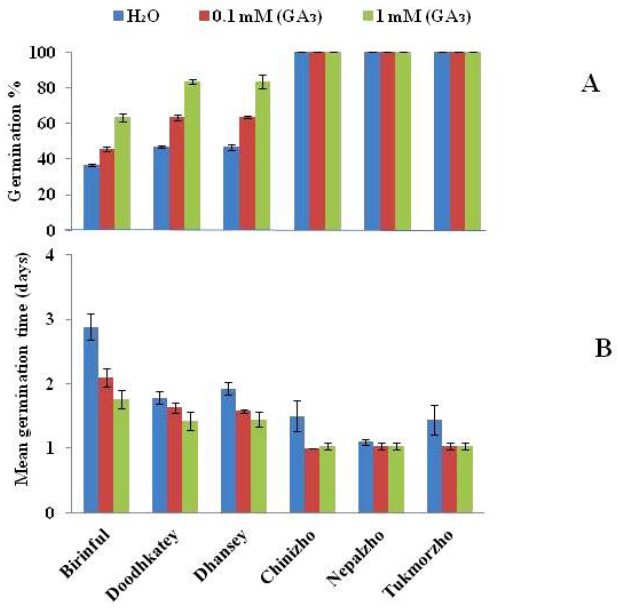

Figure 2: Germination of 6-month-stored seeds of selected rice (*Oryza sativa* L.) landraces from Sikkim Himalaya as affected by exogenous GA_3 (0.1, 1 mM). Seed germination percentage after 6 d (A) and mean germination time (B).

germination performance increased from 47% in control to 63 and 83% at 0.1 and 1 mM GA_3, respectively. Similar response to GA_3 was observed in case of Birinful and Dhansey (Figure 2A). In addition to seed germination enhancement, GA_3 induced a corresponding reduction in MGT value implying a faster rate of germination both in the low as well as high germinating landraces (Figure 2B). In response to the GA_3 treatment, the root as well as shoot length were enhanced in a concentration dependent manner in all the tested landraces. For example, in case of Birinful, an increase of 37 and 42% in root length was observed at 0.1 and 1.0 mM GA_3, respectively. These values for shoot length were 19 and 23%, respectively. Similar influence with quantitative organ-specific differences was observed in case of other landraces (Table 2). The root/shoot length ratio was affected by GA_3 to a marginal extent.

α-amylase activity in germinating seeds

It was of interest to monitor whether the observed variations in seed germination performance of the rice landraces are associated with the level of seed α-amylase activity that is responsible for hydrolysis of starch and in turn availability of low molecular weight sugars to support the initiation and progression of seed germination process. The activity of α-amylase was determined in the seeds of selected low as well as high germinating landraces through agar plate zymographic as well as biochemical assay. In the agar plate assay, the water-imbibed (control) seeds of low germinating landraces namely, Birinful, Doodhkatey and Dhansey exhibited a very low level of enzyme activity as is evident from the small halo areas around the seed halves. Lowest halo was observed in case of Doodhkatey. In contrast, the α-amylase activity levels were much higher, as indicated by greater halo areas, in case of the seeds of high germinating landraces (Figure 3A). The GA_3 treatment of seeds led to enhanced α-amylase activity both in case of low and high germinating landraces. In case of the former, the magnitude of GA_3-induced stimulation showed concentration as well as genotype specificity. Thus, in case of Birinful and Dhansey, stimulation of enzyme activity was observed only at higher (1 mM) GA_3 concentration whereas in Doodhkatey, both

Table 1: Seedling growth parameters of 15 rice **(***Oryza sativa* L.) landraces from Sikkim Himalaya measured on 7 d in terms of root length, fresh weight, dry weight (RL, RFW, RDW), shoot length, fresh weight, dry weight (SL, SFW, SDW), and root/shoot length ratio (R:S). Values are arithmetic means; n= 40-80 ± S.E.

Landraces	RL (cm)	RFW (mg)	RDW (mg)	SL (cm)	SFW (mg)	SDW (mg)	R:S
Birinful	3.52 ± 0.06	5.19 ± 0.06	1.28 ± 0.07	5.6 ± 0.16	4.27 ± 0.11	1.06 ± 0.11	0.63
Doodhkatey	4.63 ± 0.21	6.31 ± 0.09	1.25 ± 0.21	6.49 ± 0.12	10.37 ± 0.11	1.04 ± 0.07	0.71
Dharmali	3.62 ± 0.13	7.15 ± 0.12	1.23 ± 0.28	5.42 ± 0.13	12.15 ± 0.08	1.57 ± 0.09	0.67
Dhansey	4.94 ± 0.12	6.44 ± 0.13	1.12 ± 0.42	5.99 ± 0.12	8.18 ± 0.07	1.05 ± 0.05	0.82
Krishna bhog	4.54 ± 0.17	7.13 ± 0.11	1.95 ± 0.12	5.93 ± 0.09	6.46 ± 0.08	2.02 ± 0.12	0.77
Tulasi	4.97 ± 0.22	8.29 ± 0.09	1.54 ± 0.13	5.72 ± 0.15	5.12 ± 0.09	1.28 ± 0.05	0.87
Ruduwah	5.47 ± 0.23	7.13 ± 0.11	1.25 ± 0.09	4.23 ± 0.16	8.13 ± 0.09	2.02 ± 0.07	1.29
Sano attey	4.37 ± 0.26	6.54 ± 0.07	1.64 ± 0.08	5.05 ± 0.21	5.35 ± 0.11	1.51 ± 0.05	0.87
Nepal zho	6.62 ± 0.12	11.14 ± 0.13	3.03 ± 0.11	7.32 ± 0.17	12.54 ± 0.04	2.58 ± 0.07	0.90
Mumpupzho	7.84 ± 0.13	10.29 ± 0.06	2.51 ± 0.06	6.92 ± 0.12	9.25 ± 0.06	2.68 ± 0.12	1.13
Kalo tukmar	8.62 ± 0.11	13.62 ± 0.07	3.22 ± 0.11	7.96 ± 0.08	14.38 ± 0.15	2.53 ± 0.07	1.08
Chinizho	7.43 ± 0.21	10.37 ± 0.08	2.91 ± 0.09	5.67 ± 0.18	12.37 ± 0.09	1.98 ± 0.12	1.13
Tukmorzho	7.94 ± 0.19	9.72 ± 0.11	2.67 ± 0.15	5.37 ± 0.12	7.42 ± 0.14	2.46 ± 0.06	1.48
Champasari	7.43 ± 0.21	12.89 ± 0.09	2.80 ± 0.05	6.87 ± 0.11	13.57 ± 0.12	2.72 ± 0.06	1.08
Lama dhan	4.72 ± 0.21	8.37 ± 0.12	1.84 ± 0.07	5.06 ± 0.11	7.56 ± 0.09	1.45 ± 0.09	0.93

Table 2: Effect of GA_3 on seedling growth of selected rice (*Oryza sativa* L.) landraces from Sikkim Himalaya differing in germination performance. The 6-month-stored seeds were imbibed with water (control) or treated with GA_3 (0.1, 1 mM) for 24 h prior to incubation for germination. Seedling growth was measured on 7 d in terms of root length, fresh weight, dry weight (RL, RFW, RDW), shoot length, fresh weight, dry weight (SL, SFW, SDW), and root/shoot length ratio (R:S). Values are arithmetic means; n= 40-80 ± S.E.

Landraces	*RL(cm)*	*RFW (mg)*	*RDW (mg)*	*SL (cm)*	*SFW (mg)*	*SFW (mg)*	*R:S*
Birinful (H_2O)	3.02 ± 0.05	5.21 ± 0.05	1.31 ± 0.12	3.26 ± 0.11	4.28 ± 0.09	1.02 ± 0.12	0.92
0.1 mM (GA_3)	4.14 ± .07	6.25 ± 0.08	2.05 ± 0.09	3.89 ± 0.08	6.06 ± 0.08	1.63 ± 0.05	1.06
1 mM (GA_3)	4.29 ± .06	6.53 ± 0.11	2.34 ± 0.11	4.01 ± 0.09	7.07 ± 0.12	2.17 ± 0.08	1.07
Doodhkatey (H_2O)	4.19 ± 0.11	6.29 ± 0.10	2.28 ± 0.08	5.73 ± 0.09	9.22 ± 0.03	2.48 ± 0.07	0.73
0.1 mM (GA_3)	6.27 ± 0.09	7.23 ± 0.05	2.48 ± 0.10	7.24 ± 0.07	6.97 ± 0.09	2.33 ± 0.09	0.87
1 mM (GA_3)	7.43 ± 0.07	7.51 ± 0.08	2.84 ± 0.11	7.80 ± 0.11	7.44 ± 0.08	2.77 ± 0.05	0.95
Dhansey (H_2O)	5.26 ± 0.09	6.12 ± 0.13	1.57 ± 0.09	5.42 ± 0.07	9.88 ± 0.11	1.25 ± 0.09	0.97
0.1 mM (GA_3)	6.24 ± 0.08	6.69 ± 0.09	2.54 ± 0.07	5.84 ± 0.16	10.03 ± 0.08	2.29 ± 0.13	1.07
1 mM (GA_3)	6.47 ± 0.11	6.92 ± 0.11	2.62 ± 0.12	5.72 ± 0.10	10.29 ± 0.14	2.41 ± 0.12	1.13
Chinizho(H_2O)	6.52 ± 0.11	9.42 ± 0.11	2.23 ± 0.13	4.93 ± 0.06	10.21 ± 0.08	2.17 ± 0.08	1.32
0.1 mM (GA_3)	8.27 ± 0.08	10.57 ± 0.03	3.06 ± 0.04	6.42 ± 0.09	11.59 ± 0.06	3.22 ± 0.06	1.29
1 mM (GA_3)	9.43 ± 0.03	9.23 ± 0.15	3.44 ± 0.06	7.04 ± 0.07	7.93 ± 0.07	3.52 ± 0.05	1.34
Nepalzho (H_2O)	6.89 ± 0.03	9.27 ± 0.03	2.64 ± 0.10	6.98 ± 0.07	11.08 ± 0.06	2.58 ± 0.15	0.99
0.1 mM (GA_3)	7.28 ± 0.09	7.59 ± 0.09	3.34 ± 0.12	5.64 ± 0.08	7.44 ± 0.09	2.92 ± 0.11	1.29
1 mM (GA_3)	8.37 ± 0.05	8.24 ± 0.07	3.67 ± 0.13	6.24 ± 0.12	7.96 ± 0.03	3.42 ± 0.09	1.34
Tukmorzho (H_2O)	7.21 ± 0.04	6.27 ± 0.06	2.45 ± 0.13	5.35 ± 0.03	6.24 ± 0.05	2.21 ± 0.05	1.18
0.1 mM (GA_3)	7.94 ± 0.06	7.24 ± 0.10	3.11 ± 0.07	6.23 ± 0.09	7.32 ± 0.13	2.87 ± 0.07	1.27
1 mM (GA_3)	8.26 ± 0.08	8.39 ± 0.08	3.36 ± 0.09	6.42 ± 0.08	8.21 ± 0.11	3.24 ± 0.11	1.29

GA_3 concentrations were effective to comparable extents. In contrast, in case of the high seed germinating landraces, the both the GA_3 concentrations substantially stimulated the enzyme activity (Figure 3A). Similar findings were observed in biochemical assay of α-amylase in the water imbibed and GA_3 (1 mM) treated seeds of both the groups of landraces (Figure 3B). As is clear from Figure 3B, in control (H_2O-imbibed) seeds, the level of α-amylase activity was much lower in case of the low seed germinating landraces (Birinful, Doodhkatey, Dhansey) than that in the high seed germinating landraces (Chinizho, Nepalzho, Tukmorzho). Furthermore, the GA_3 treatment induced an enhancement of α-amylase activity in the seeds of both groups of landraces; however, the magnitude of enhancement was greater in case of the low seed germinating landraces (Figure 3B). For example, the GA_3-induced enhancement of enzyme activity was 73 and 32 % in Birinful and Chinizho, respectively.

Discussion

This study aimed at determining the seed germination and seedling growth characteristics of certain rice landraces cultivated in specific parts of Sikkim Himalayas. The genetic aspects of diversity of rice landraces from the region have been reported (Chettri and Sathyanarayana, 2021b). These unique genotypes are apparently a great genetic resource as they likely possess multiple useful traits including tolerance to diverse adverse growth conditions which they experience chronically. As such, the information about early seedling establishment potential has implications for overall productivity and yield of the concerned genotypes. This is also desirable for the purpose of evaluation of the stated landraces for relative sensitivity/tolerance to abiotic stress factors such as those associated with acid soils of sizable parts of the state.

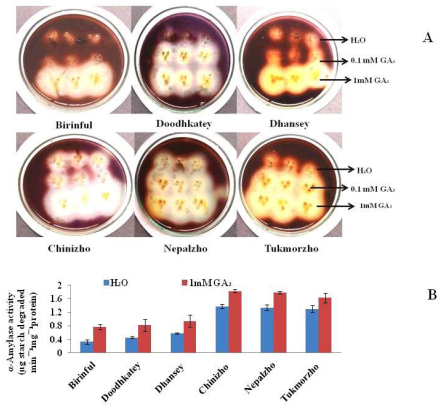

Figure 3: Effects of exogenous GA_3 on α-amylase activity in germinating seeds of selected rice (*Oryza sativa* L) landraces from Sikkim Himalaya differing in germination performance: α-amylase activity was determined in agar plate assay (A) and biochemical assay (B). Seeds were imbibed either in water (control) or treated with the stated concentration(s) of GA_3 for 24 h prior to the stated assays. For B, n=3 ± S.E.

In a time-course analysis, the freshly harvested seeds of eleven landraces exhibited nearly complete (96.25-100%) germination within 6 d although the germination speed varied to some extent as evident from the mean germination time (MGT) range of 1.04 (Ruduwah, Mumpupzho) to 1.88 (Sano attey). The MGT value is inversely related with the speed of germination. In contrast, four landraces namely, Birinful, Doodhkatey, Dhansey and Dharmali exhibited substantially lower germination; maximum germination ranged from 62% (Birinful) to 71% (Dharmali) after 6 d with no change thereafter; high values of their MGT (2.83-4.07) indicate slower germination rates. The findings point to heterogeneity within the seed lot of this group of landraces with lower germination performance where a subset was likely dormant. Incidentally, all these four landraces are low land. Seed dormancy of varying types and magnitudes is commonly encountered in many a plant species; more frequently in wild plants but also in crop species (Baskin and Baskin 2004). Seed dormancy in certain rice varieties has been reported (Zhang *et al.* 2020) that is a matter of concern in view of the significance of synchronous germination for crop species. The process of seed germination and dormancy is strongly influenced by the environmental conditions prevalent during the period of seed development. The seeds of the stated four landraces likely experienced the factors responsible for induction of partial dormancy reflected in heterogeneity of seed germination. The dormant seeds in some cases require after-ripening and thus germinate following a certain period of storage. However, in the present study, 6-month-stored seeds of the low germinating landraces namely, Birinful, Doodhkatey and Dhansey showed a further decline in germination percentage as compared to that in the freshly harvested seeds pointing to the possibility of a low level of longevity. The seed longevity could often be enhanced through manipulation of storage conditions particularly the temperature and relative humidity regime. Such interventions need to be assessed.

Seed germination and dormancy, comprising complex physiological as well as biochemical changes, which are strictly regulated by specific hormonal interactions (Kucera *et al.* 2005). A predominant involvement of ABA/GA ratio in seed dormancy and germination of rice has been reported (Liu *et al.* 2014 Liao *et al.* 2018). The role of GA_3 in controlling and promoting seed germination is evident from the fact that the GA deficient mutants failed to germinate in the absence of exogenous GA application. The bioactive GA content of the rice seeds have direct correlation with the expression pattern of the genes associated with GA metabolism. The germination performance of *germination-defective1* (*gd1*) rice mutant was poor because of the increased expression of *OsGA2ox3* and reduced expression of *OsGA20ox1*, *OsGA200x2*, and *OSGA3ox2* genes (Guo *et al.* 2013). A major function of GA_3 is to coordinate the induction of α-amylase activity in germinating seeds (Appleford and Lenton, 1997) necessary for hydrolysis of starch to mobilize low molecular weight sugars that support the germination process. In order to get insight into whether differential germination behaviour of rice landraces was associated with seed α-amylase activity, we determined the levels of α-amylase activity in germinating seeds of three low (Birinful, Doodhkatey, Dhansey) and three high (Chinizho, Nepalzho, Tukmorzho) germinating rice landraces. The observations both from agar plate and biochemical enzyme assay revealed the lower magnitude of seed germination in the former group of landraces to be a consequence inter alia of low level of α-amylase activity (Figure. 3). Furthermore, the α-amylase activity could be enhanced by exogenous GA_3 in both the groups of landraces with a much greater magnitude in case of the landraces with low germinating seeds than in the counterpart with high germinating seeds. The GA_3-induced enhancement of α-amylase activity in the low germinating landraces coincided with the enhanced seed germination as well as reduced MGT. Taken together, the data suggest the low seed germination ability of the landraces with low seed germination to be largely due to the low endogenous GA_3 levels. Apparently, the tested rice landraces differ quantitatively in GA biosynthetic abilities that are largely determined genetically. Indeed, the *GA-deficient-1* (*gib-1*) mutant of tomato and *Arabidopsis ga1–3* mutant in Arabidopsis showed no germination unless exogenous GA_3 were given; however, the germination was possible when endosperm caps were removed (Ni and Bradford 1993 Lee *et al.* 2002). Besides the induction of hydrolytic enzymes, GA also induces the synthesis of cell wall remodeling proteins, which results in embryo growth and endosperm weakening during seed germination (Linkies and Leubner-Metzger, 2012). Therefore, appropriate endogenous GA_3 levels and its ratio to seed germination inhibitors (e.g., ABA) are essential for realization of optimum germination potential of the seeds.

The tested rice landraces exhibited a strong variation concerning the seedling growth measured in terms of length, fresh weight, dry weight of root and shoot as well as the ratio of root to shoot length. The root and shoot length were the highest in Kalo tukmar that is an upland landrace. The seedling growth was stimulated by GA_3 application in a genotype specific manner. Our findings are in conformity with several other reports involving diverse plant species. For example, exogenous GA_3 enhanced the germination rate and seedling growth in the perennial grass *Leymus chinensis* (Ma *et al.* 2018) and *Zea mays* L. (Ghodrat and Rousta 2012).

Conclusion

In brief, certain rice landraces from Sikkim Himalaya were characterized by low germination performance as well as

α-amylase activity as compared to the others. Exogenous GA_3 stimulated both implying a role of GA_3 in differential germination behaviour of the tested landraces. The findings have implications for their seedling establishment potential.

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