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ORIGINAL PAPER

The role of enzyme amylase in two germinating seed morphs of *Halopyrum mucronatum* (L.) Stapf. in saline and non-saline environment

Zamin Shaheed Siddiqui · M. Ajmal Khan

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Abstract The role of enzyme amylase in two germinating seed morphs, i.e. black and brown, of *Halopyrum mucronatum* in saline and non-saline environment was examined. Both seed morphs of this halophytic grass have variations in their moisture content, total lipid, protein, sugar, phenol and tannin contents. Black seed exhibited higher activity compared to brown in saline medium. Sugar mobilization in both seed morphs was also affected due to the difference in amylase activity. However, exogenous application of GA_3 in saline medium enhanced the amylase activity and sugar mobilization. Phenolic contents were similar except for vanillic acid which was found only in brown seeds while catechol was present only in black seeds. Phenols extracted from both seed morphs were applied to determine their effects on amylase activity. Phenolic extracts obtained from brown seeds showed higher degree of inhibition of amylase activity. Results are discussed in relation to seed coat phenols, leaching, amylase activity and sugar mobilization.

Keywords Amylase · Phenols · Seed types · Leaching · Germination · Response

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M. A. Khan
Institute of Sustainable Halophyte Utilization,
University of Karachi, Karachi, Pakistan

Z. S. Siddiqui (✉)
Department of Botany,
University of Karachi,
Karachi, Pakistan
e-mail: zaminss@uok.edu.pk

Introduction

Amylases (endo-1,4-D-glucan glucohydrolase) are a class of hydrolases which are ubiquitously found in living organisms. They cleave the *ortho*-glycosidic bonds in amylose, a principal storage of polysaccharides present in seeds of various plants along with other related oligo- and polysaccharides playing key role in carbohydrate metabolism of developing and germinating seeds (Muralikrishna and Nirmala 2005).

There are few studies on the regulation of carbohydrate in seeds during germination and their early developmental stages under stress conditions (Muralikrishna and Nirmala 2005; Mei and Song 2008). However, metabolism of these compounds can be affected by a number of environmental factors such as irradiance, temperature, salinity, drought and type of ions present (Bohnert et al. 1995). Thus, the variation in carbohydrate metabolism of dimorphic seeds during early germination is poorly understood and information on the physiological events involved in this process is rather scarce.

Most of the molecular and physiological research on salinity effects is restricted to crop plants due to their importance as food for human consumption. Few studies about the effects of salinity on the physiological or molecular mechanisms of wild plants have been documented (Flowers and Flowers 2005; Munns and Tester 2008). However, abiotic stresses and increasing population have put the pressure on the crop plants leading to the investigation into the alternate means of food and shelter from plant resources. Halophytes are the most appropriate choice since research has established their nutritive and food values despite their being inhabitant of saline areas and thus having better tolerance in saline environment.

Halopyrum mucronatum (L.) Stapf. is a stoloniferous perennial coastal halophytic grass which exhibits seed dimorphism, usually shoots produce flowering twice a year, from April to May and then from September to November. Black seeds are produced during summer and are heavier than brown seeds which are formed in winter. Coastal halophytes exhibit seed polymorphism and dimorphism, and have shown great deal of variation in their ability to tolerate salt in stages throughout the life cycle (Khan and Gul 1998). Further, in several species, these polymorphism and dimorphism are reflected by a differentiation in seed size, shape and color (Maxwell et al. 1994; Imbert et al. 1997). In addition to the difference in their size and morphology, the two seed types can also differ in dispersal, dormancy and germination responses (Venable and Levin 1985; Zhang 1994; Debeaujon and Koornneef 2000; Mölken et al. 2005).

Lignin, tannin and non-tannin polyphenols have differential distribution in polymorphic and dimorphic seeds which are produced in different habitats (Khan and Ungar 1986; Bos and Jetten 1989; Carmona et al. 1991; Asiedu et al. 2000). The roles of these polyphenols are known in relation to diverse germination response (Asiedu et al. 2000), but their physiological explanation during germination especially of dimorphic halophytic seeds is rather scarce. Present study describes the difference in seed biochemical composition and the role played by these differences on the amylase activity in two germinating seed morphs under saline and non-saline medium.

Table 1 Biochemical difference in *Halopyrum mucronatum* seed morphs

Parameters	Black seeds	Brown seeds
Seed weight (g per 100 seed)	0.237 ± 0.015	0.210 ± 0.012
Seed moisture (%)	12.33 ± 0.55	9.12 ± 1.15
Total soluble protein (mg g^{-1})	179.36 ± 13.78	221.93 ± 21.55
Total sugars (mg g^{-1})	13.57 ± 0.78	17.39 ± 1.22
Total reducing sugars (mg g^{-1})	3.17 ± 0.048	5.53 ± 0.13
Total non-reducing sugars (mg g^{-1})	10.40 ± 1.22	11.86 ± 0.78
Total lipid (mg g^{-1})	208.57 ± 20.45	136.36 ± 12.55
Total TAG (g dL^{-1})	44 ± 2.55	32 ± 1.22
Total phenols (mg g^{-1})	2.15 ± 0.045	1.58 ± 0.095
Total tannin (mg g^{-1})	1.15 ± 0.033	0.65 ± 0.082

Values are in mean ± standard error

Table 2 Phenols and phenolic acids in dry *Halopyrum mucronatum* seeds

No.	Black seed	Brown seed	R_f values
1	Pyrogallol	Pyrogallol	0.08
2	2-Methyl resorcinol	2-Methyl resorcinol	0.40
3	4-Methyl resorcinol	4-Methyl resorcinol	0.25
4	*para*-Hydroxybenzoic acid	*para*-Hydroxybenzoic acid	0.54
5	Syringic acid	Syringic acid	0.79
6	Catechol	–	0.35
7	–	Vanillic acid	0.82

Materials and methods

Seeds of *H. mucronatum* were collected during May to June and December to January 2006–2007 from sand dunes and flats on Hawksbay around Karachi sea coast.

Biochemical analysis of seeds

Weights of the four lots of 100 randomly selected seeds were measured and averaged. Moisture content of both seed morphs was observed after oven drying of samples at 105 ± 2°C for 18 h. Twenty-five healthy seeds (~50 mg) were selected for each lipid, protein, and sugar and phenol examination. Total protein, sugar, reducing and non-reducing sugar, lipid, phenol and tannin were estimated using methods described by Bradford (1976), Hassid and Abraham (1957), Nelson (1944), Becker et al. (1978), Swain and Hillis (1959) and Conners (1999), respectively. Qualitative estimation of phenolic compounds was performed in TLC by the method of Harborne (1984).

Germination test

Caryopses of *H. mucronatum* collected bear two different morphologies and were referred here as summer (black) and winter seeds (brown), respectively. Hulled seeds were separated, cleaned and stored at the room temperature. Seeds were surface-sterilized with 30% NaOCl (sodium hypochlorite) for 5 min and washed several times with distilled water. Later, seeds were pre-soaked in distilled water or respective test solutions for 4 h. Germination was carried out in 90-mm diameter glass Petri plates. Seeds were placed on Whatman No. 1 filter papers moistened with 5 mL of respective test solutions (100, 200 and 300 mM NaCl) or distilled water (control) with or without 3 mM GA_3. Four replicates of 20 seeds each were used for each treatment and were placed at 25 ± 2°C in a germinator (Hotpack programmed refrigerated incubator). The photoperiod, light intensity and relative humidity were 12 h, 25 μmol m^{-2} s^{-1} and 70%, respectively. Seeds were considered to be germinated after the radicle emerged. Percentage germination was recorded at 1 day interval up

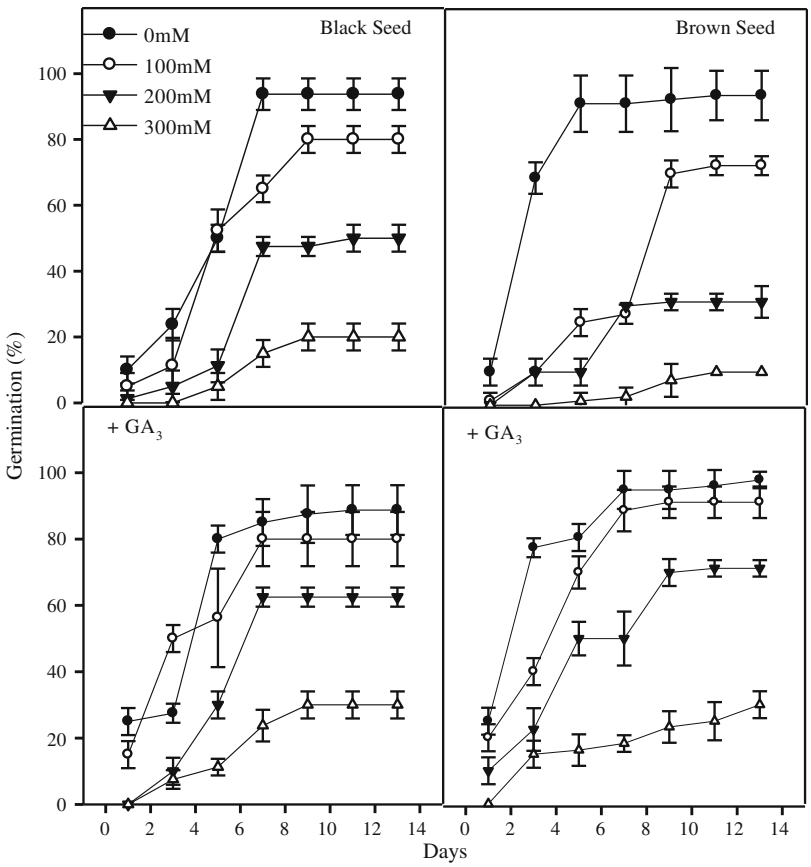

Fig. 1 Mean seed germination of *Halopyrum mucronatum* as a function of time. *Vertical lines* on graphs represent standard error. 3 mM GA_3, 0 mM distilled water, 100, 200, 300 mM NaCl concentrations

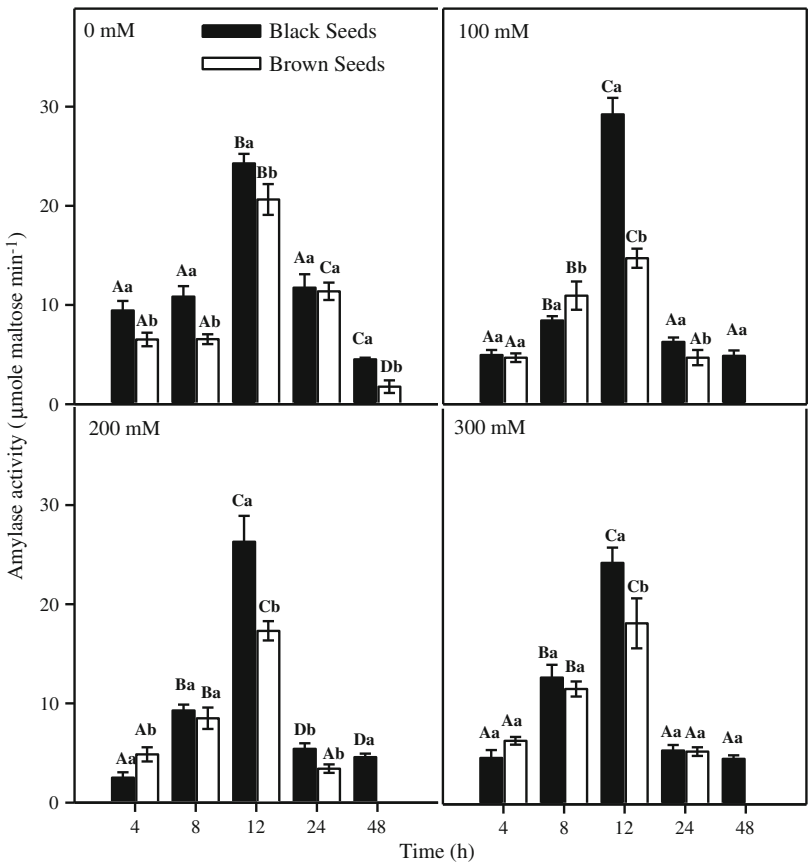

Fig. 2 Effect of salinity (NaCl) concentrations (100, 200, 300 mM) on amylase activity in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (t test)

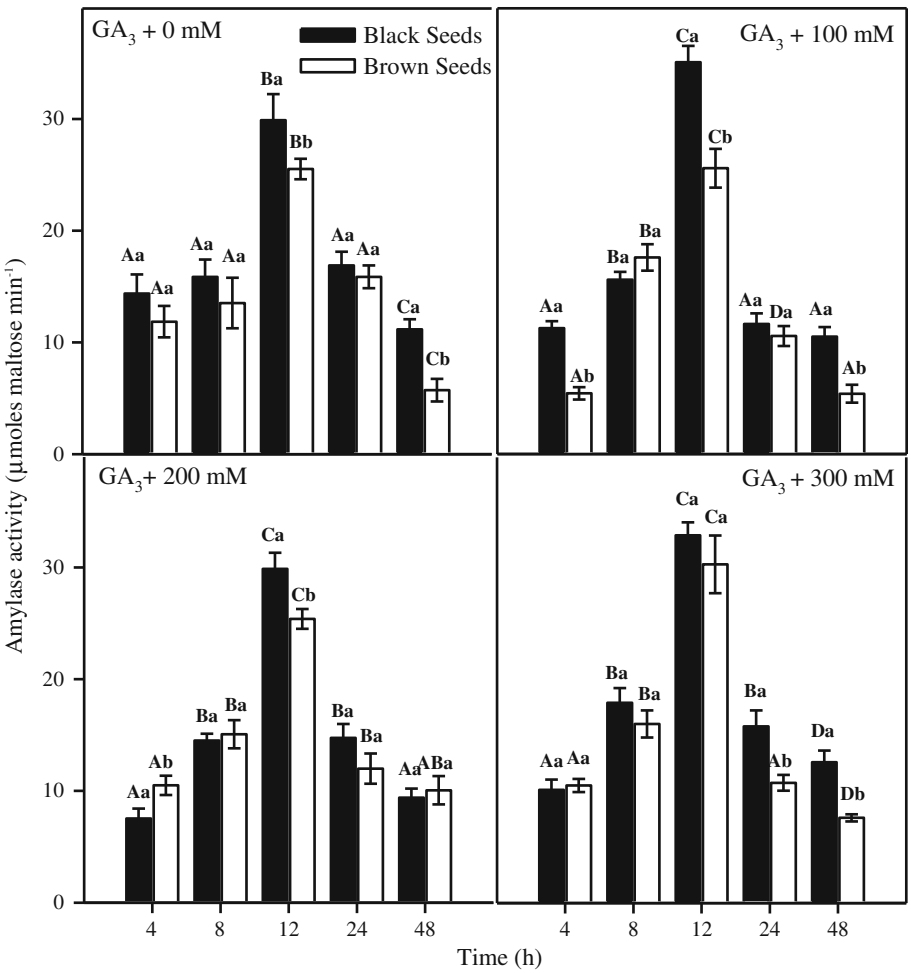

Fig. 3 Effect of exogenous application of GA_3 (3 mM) in salinity (NaCl) concentrations (100, 200, 300 mM) on amylase activity in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (*t* test)

to 13 days. Final percentage germination was recorded at 13th day of the experiment.

Biochemical changes during germination

Preliminary test was performed to examine the timing of radicle emergence under control and saline conditions to plan a schedule to test total sugars, reducing sugars and amylase activity during germination. Approximately 100 mg (~50 mg) of healthy seeds was placed in 90-mm diameter Petri plates with 5 mL test solution enough to moist filter paper, i.e. 0, 100, 200 and 300 mM NaCl with or without 3 mM GA_3 (0 served as control for salinity). GA_3 concentration was selected by a series of preliminary experiments and 3 mM was found to be the most effective concentration for *H. mucronatum* seeds germination. Petri plates were placed at 25 ± 2°C in growth chamber (Hotpack programmed refrigerated incubator). The photoperiod, light intensity and relative humidity were the same as mentioned in germination experiment. Change in total sugar (Hassid and Abraham 1957), reducing sugar (Nelson 1944) and amylase activity (Bernfeld 1955) was measured after 4, 8, 12, 24 and 48 h time interval. Amylase kinetics of black and brown seeds was calculated using substrate and pH maxima data by the method of Lineweaver and Burk (1934). The substrate saturation kinetic curve was calculated using GRAPHPAD PRISM 5.00 software. Each treatment and control was replicated four times.

Extraction procedure

Twenty-five germinating seeds of each type, i.e. black and brown, were randomly collected from each treatment and control and homogenized separately in chilled 10 mL Tris–HCl buffer pH 6.8, centrifuged at 14,000 rpm for 15 min at 4°C. Supernatant was collected, and total sugar (Hassid and Abraham 1957), reducing sugar (Nelson 1944) and amylase activity (Bernfeld 1955) were determined. Total non-reducing sugars (polysaccharides) were calculated by the following formula:

$$TNRS = TS - TRS$$

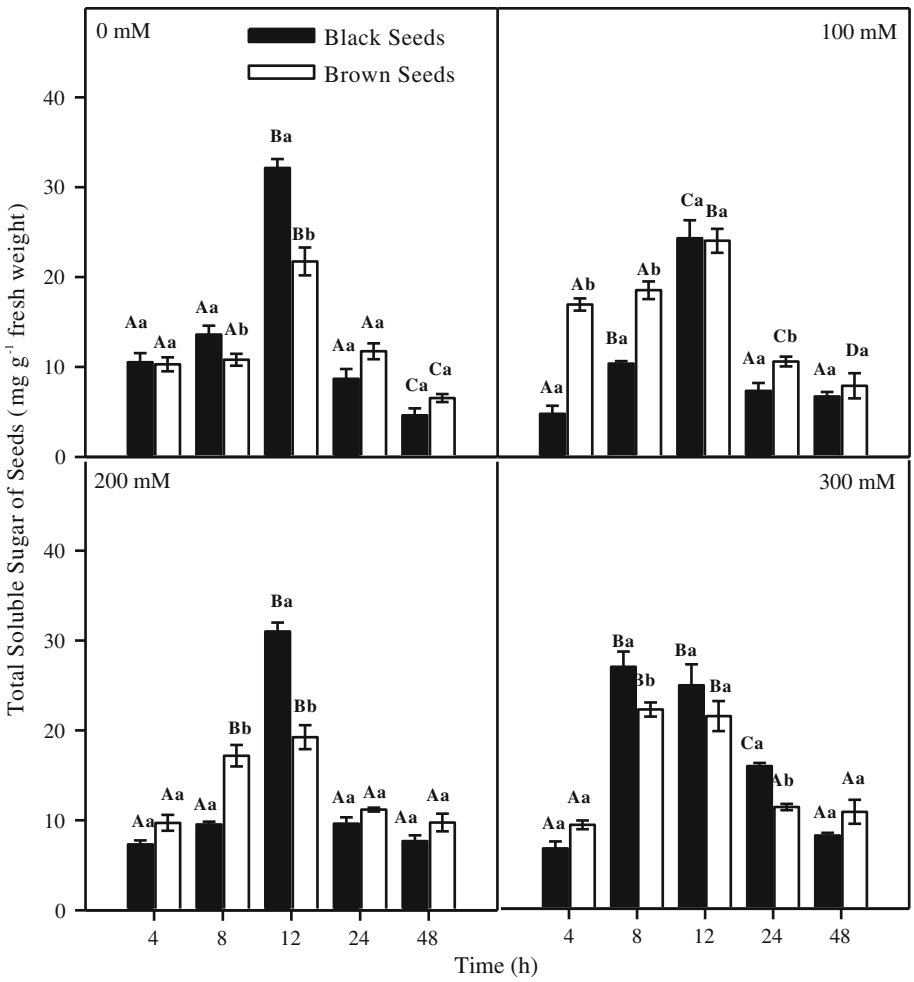

Fig. 4 Effect of salinity (NaCl) concentrations (100, 200, 300 mM) on total sugar contents in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (t test)

where TNRS = total non-reducing sugar, TS = total sugar, and TRS = total reducing sugar.

Seed phenols and amylase activity

For the analyses of seed phenols and amylase response, a partially purified *P. turgidum* amylase was mixed with 4 h imbibed seed leachates of each seed types (black and brown) in a 1:1 ratio, respectively. After 10, 20 and 30 min incubation, enzyme activity was measured by Bernfeld (1955) method.

Statistical analysis

A three-way ANOVA was used to determine significant differences among means within and among each seed morphs using germination time, NaCl concentration and seed types as factors. A Bonferroni test and paired t test were carried out to determine if significant ($P < 0.05$) difference occurs in individual treatments. The significance of Bonferroni test and paired t test was represented as small and capital alphabets on the bar graphs.

Results

Biochemical analysis in dry seeds

Biochemical composition of two seed morphs was examined and illustrated in Table 1. Moisture content, total lipid, phenols and tannin were higher in black seeds compared to brown. High protein, sugar and reducing sugar were observed in brown seeds. Pyrogallol, 2-methyl resorcinol, 4-methyl resorcinol, *p*-hydroxybenzoic acid, syringic acid and catechol were observed in black seed. However, brown seed exhibited the same phenol compositions except catechol which was replaced by vanillic acid (Table 2).

Germination

Germination percent as time function showed significant variation ($F = 14.06$, $P < 0.001$) between both seed types showing higher percent germination in black seeds compared to brown throughout the experiment (Fig. 1). However, salinity reduced the percent germination more in

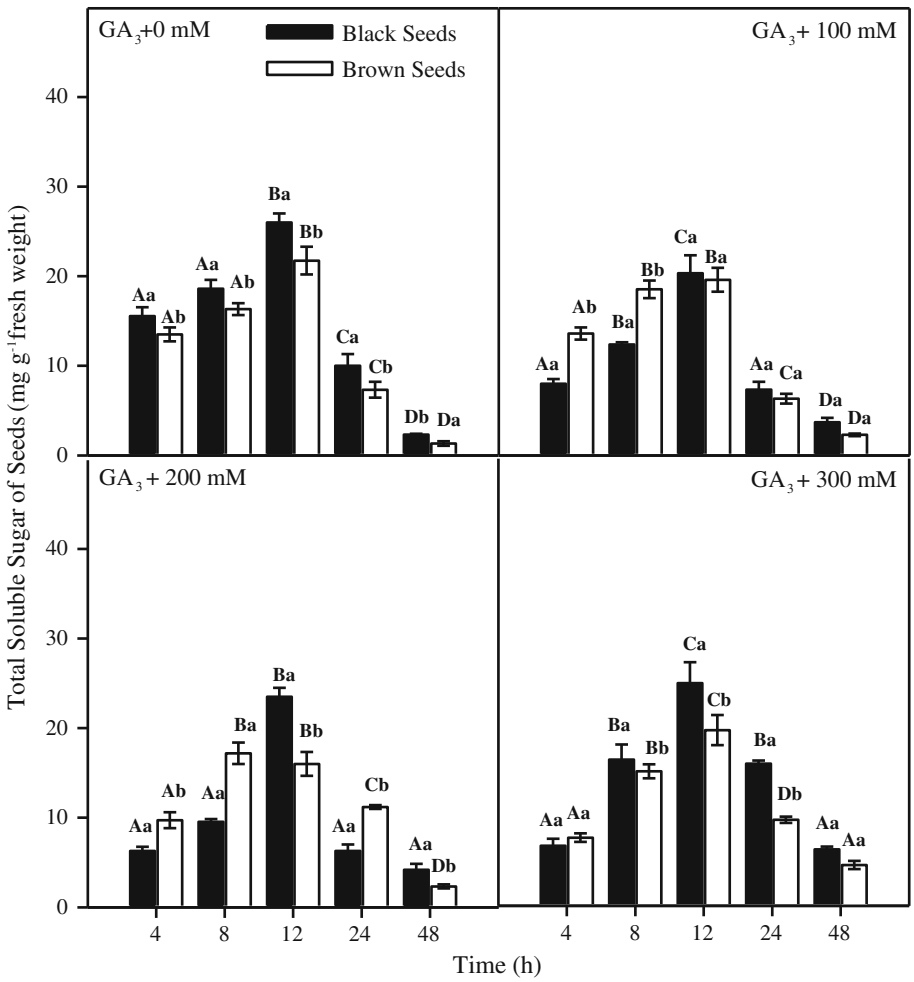

Fig. 5 Effect of exogenous application of GA_3 (3 mM) in salinity (NaCl) concentrations (100, 200, 300 mM) on total sugar in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (*t* test)

brown seeds compared to black ($F = 233.09$, $P < 0.001$). Percent germination over time in saline medium was rapid in brown seed compared to black. At 100 and 200 mM NaCl, more black seeds germinated in comparison to brown. Exogenous application of GA_3 in saline medium significantly enhanced the percent germination of both seed morphs ($F = 1318.48$, $P < 0.001$). However, brown seeds showed more pronounced effect of GA_3 compared to black especially in 100 and 200 mM NaCl. Slight increase in germination percent was recorded at 300 mM NaCl in both seed morphs.

Amylase response

Amylase activity of both seed morphs was different in response to salinity ($F = 185.53$, $P < 0.001$, Fig. 2). However, maximum activity of both seed morphs was achieved after 12 h in all treatments including control. Black seeds show maximum activity when treated with 100 mM NaCl compared to brown seeds ($P < 0.001$). In general, amylase activity in brown seeds was less than black seeds throughout the experiment and no enzyme activity was observed in brown seeds after 48 h in all treatments.

Exogenous application of GA_3 significantly enhanced the amylase activity in both seed morphs (Fig. 3, $F = 14.22$, $P < 0.001$). Likewise, optimal amylase activity was recorded after 12 h in all treatments including control. Compared to saline medium, GA_3 showed greater increase in the amylase activity after 48 h particularly in brown seeds ($F = 15.93$, $P < 0.001$). Exogenous GA_3 application recorded a substantial in amylase activity in brown seeds under saline conditions compared to those not treated with GA_3.

Total soluble sugar

Total sugar was higher in germinating black seeds at 12 h in all treatments compared to brown (Fig. 4). While at 100, 200 and 300 mM NaCl, total sugar was higher in brown than in black seeds after 4 and 48 h. However, exogenous GA_3 application showed increase in total sugar contents under saline conditions (Fig. 5). GA_3 alone significantly reduces the total sugar content in both seed morphs

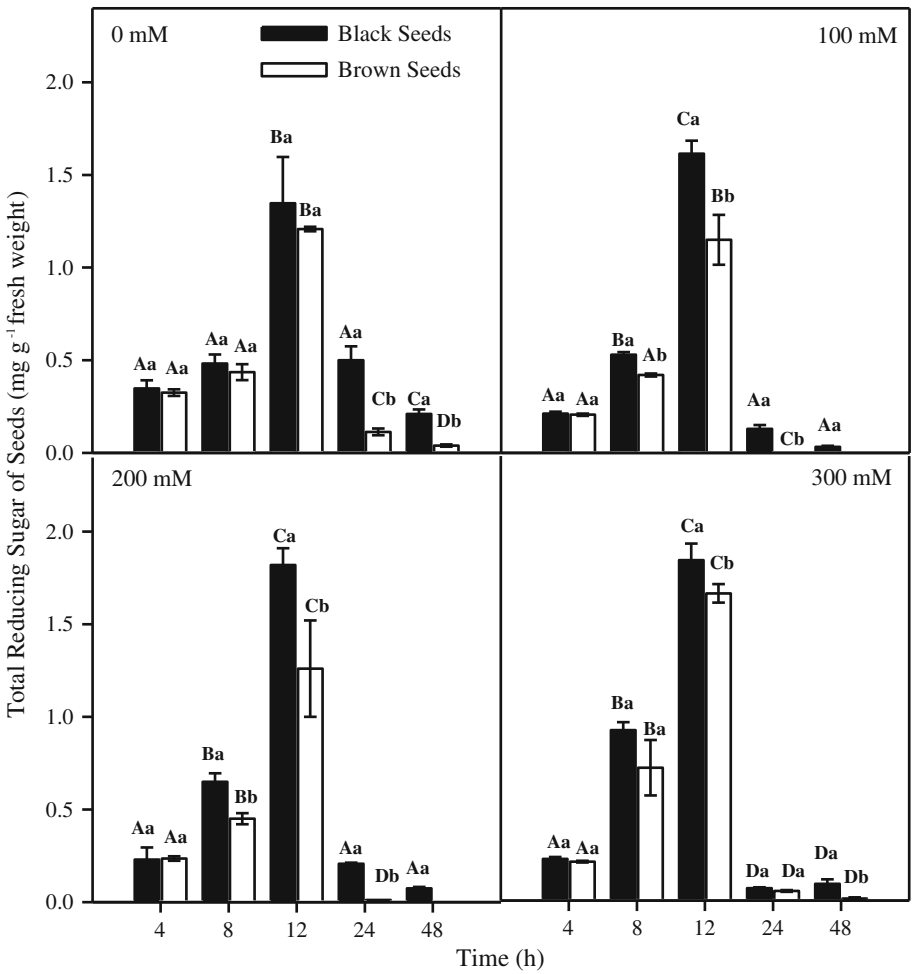

Fig. 6 Effect of salinity (NaCl) concentrations (100, 200, 300 mM) on total reducing sugar contents in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$

($F = 8.99$, $P < 0.01$) compared to the NaCl. Substantial decrease in total sugar was recorded in brown seed especially after 24 and 48 h of the germination compared to black.

Total reducing sugar

Total reducing sugar was higher in black seeds after 12 h when treated with 100 and 200 mM NaCl (Fig. 6). In general, total reducing sugar followed the similar trend in both seed morphs showing gradual increase with time reaching optimum level after 12 h and then decreased with a further increase in time. Exogenous GA_3 application in NaCl treatment showed significant effect on total reducing sugar in both seed morphs (Fig. 7, $F = 257.68$, $P < 0.001$). GA_3 significantly increases initially the total reducing sugar (4 and 8 h of the experiment) and gradually decreases after 12 h in both seed morphs ($P < 0.001$). However, maximum increase was recorded in black seed when treated with GA_3 + 100 mM NaCl. Reducing sugar was higher in black seeds compared to brown particularly during early hours (4, 8, and 12 h) treatment. Likewise, compared to NaCl treatment only, exogenous GA_3 application in NaCl solution improved the total reducing sugar content in brown seeds. More pronounced effect was found particularly at late hours in brown seeds treated with GA_3 together with 100 and 200 mM NaCl compared to black.

Change in total non-reducing sugar

Total non-reducing sugar was estimated as difference in the values between total sugar and total reducing sugar showing a significant effect during germination ($F = 38.96$, $P < 0.001$, Fig. 8). Compared to reducing sugar, total non-reducing sugars were significantly higher in brown seeds compared to black with few exceptions. However, total non-reducing sugars were non-significantly higher in brown than black seeds after 4 and 48 h. Higher amounts of total and non-reducing sugars in brown seeds are an indication that these sugars were unable to be mobilized, and thus take part in germination. Exceptionally, maximum non-reducing sugars were found in black

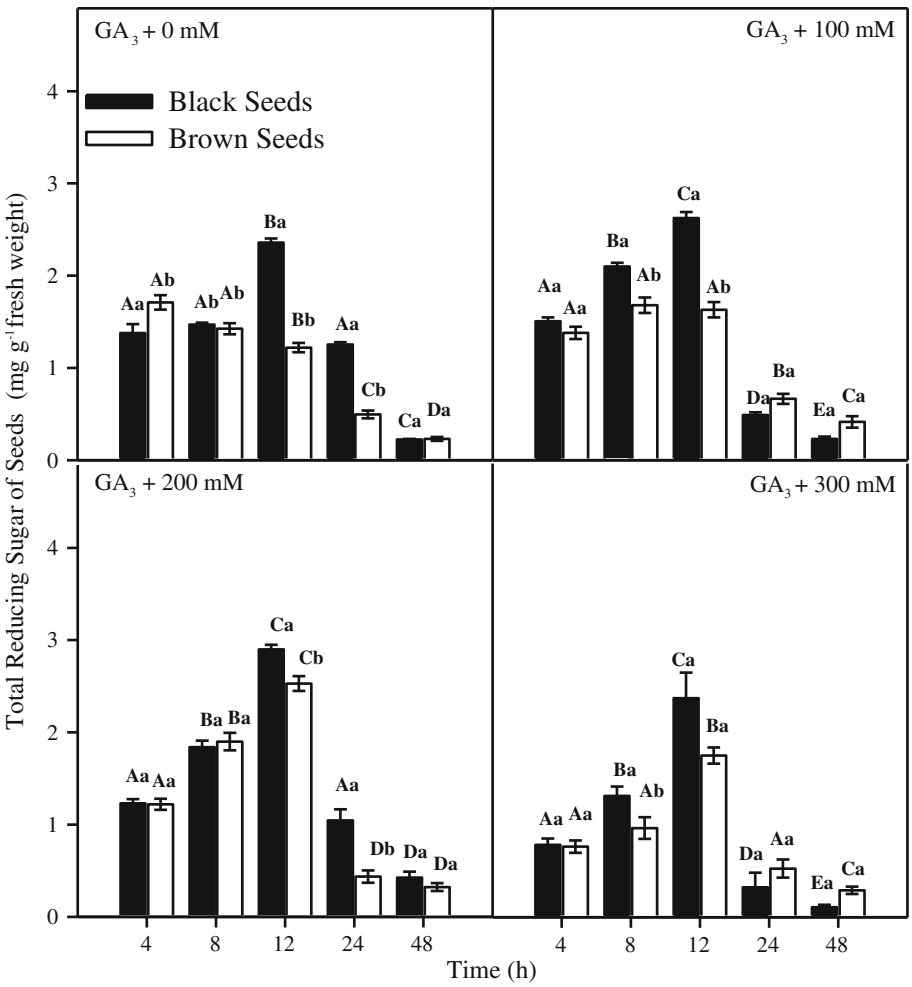

Fig. 7 Effect of exogenous application of GA_3 (3 mM) in salinity (NaCl) concentrations (100, 200, 300 mM) on total reducing sugar in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letter* between each time interval within each of the two seed morphs are not significantly different at $P = 0.05$ (Bonferroni test), *similar small letter* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (*t* test)

seeds after 12 h of control and 200 mM NaCl treatment samples.

Application of GA_3 significantly increased the total non-reducing sugar initially, but after 12 h gradually decreased in both seed morphs (Fig. 9). Total non-reducing sugars were high in black seeds compared to brown in control and GA_3 + 300 mM NaCl. However, in brown seeds, it was high at 100 and 200 mM NaCl throughout the germination periods. Comparing salinity treatments only, exogenous application of GA_3 decreased total non-reducing sugar contents in all treatment and control except for the treatments 4 and 8 h. Compared to black seeds, more decreases were recorded in brown seeds in the later hours (24 and 48 h) of the treatments and control.

Effect of seed phenols on amylase activity

Phenols that leached from brown seeds caused more ($P < 0.01$) inhibition in amylase activity compared to black seeds (Table 3). However, complete inhibition was recorded from phenols leached from brown seed while 83.2% inhibition was recorded phenols leached from in black seeds. Qualitative analysis of phenols from both seed types showed that pyrogallol, syringic and vanillic acid were main phenolic compounds which were leached out from brown seed while pyrogallol and syringic were from black seeds (Table 4).

Substrate saturation kinetics

Substrate saturation kinetics (V_{max} and K_m values) of seed morphs amylase was examined and illustrated in Fig. 10. Black seed amylase showed maximum reaction velocity ($V_{max} = 285.6$) with high affinity (less $K_m = 74.03$ value) to substrate compared to brown ($K_m = 272.3$, $K_m = 80.7$).

Discussion

Final germination percent of both seed types varied over time considerably showing higher percent germination in

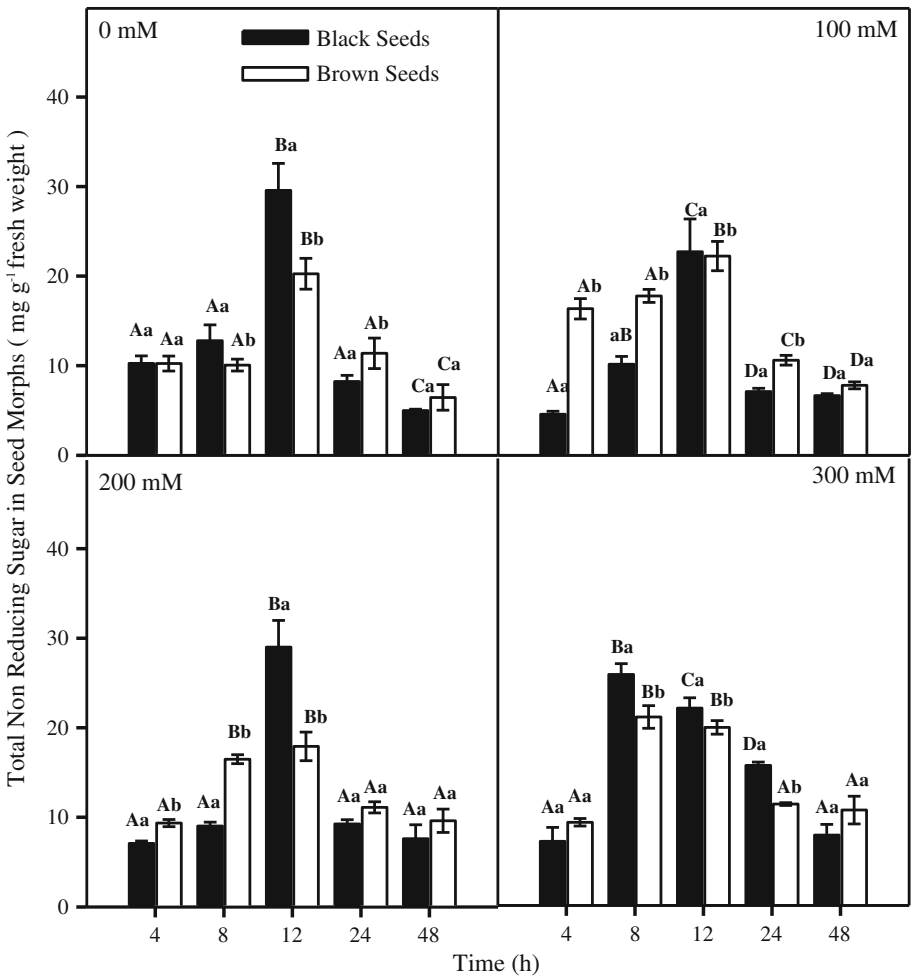

Fig. 8 Effect of salinity concentrations (100, 200, 300 mM NaCl) on total non-reducing sugar content in germinating seed morphs (black and brown) of *Halopyrum mucronatum*. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letters* between time interval within seed morphs are not significantly different at $P = 0.05$ (Bonferroni test). *Similar small letters* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (t test)

black seeds compared to brown. However, final percent germination was improved by exogenous application of GA_3 in saline medium (Fig. 1).

Seed coat morphology influenced the seed germination process in two ways. First by making seed coat impermeable to water and oxygen, the second is that seed coat exerts mechanical resistance to radicle emergence reducing growth potential of the growing embryo (Serrato-Valenti et al. 1993; Tyler 1997; Debeaujon et al. 2000; Fengshan et al. 2004; Mei and Song 2008). It has been reported that dark colored seeds germinate slowly compared to light colored but showed higher final percent germination (Wyatt 1977; Powell 1989; Kantar et al. 1996). Furthermore, light colored seed uptakes more water rapidly and therefore suffers greater imbibition damage compared to dark seeds. For instance, red seed of *Sinapis arvensis* L. uptakes water more rapidly compared to black ones (Duran and Retamal 1989). White colored seed in legumes imbibes quickly, suffers greater imbibition damages than colored seeds but germinates earlier with low final germination (Kantar et al. 1996). In the present study, therefore, rapid water uptake could be attributed to the light color of brown seeds perhaps due to lesser secondary metabolites deposited on the seed coat and consequent imbibition damage under saline condition. Therefore, it is presumed that final percent germination of brown seeds may be reduced but may reach to their optimum level earlier than black seeds.

Black seeds exhibited higher amylase activity compared to brown although exogenous GA_3 application enhanced the amylase activity in brown seeds. Compared to reducing sugar, total sugar including non-reducing sugar was considerably higher in brown seeds than black. More total sugar including non-reducing sugar and less reducing sugar in brown seeds might be an indication that these sugars are unable to mobilize and thus take part in germination.

It is presumed that greater amylase activity and subsequent more reducing sugar in germinating black seed might be the main biochemical cause for their better final percent germination compared to brown seeds under saline medium. Reports have suggested that mobilized

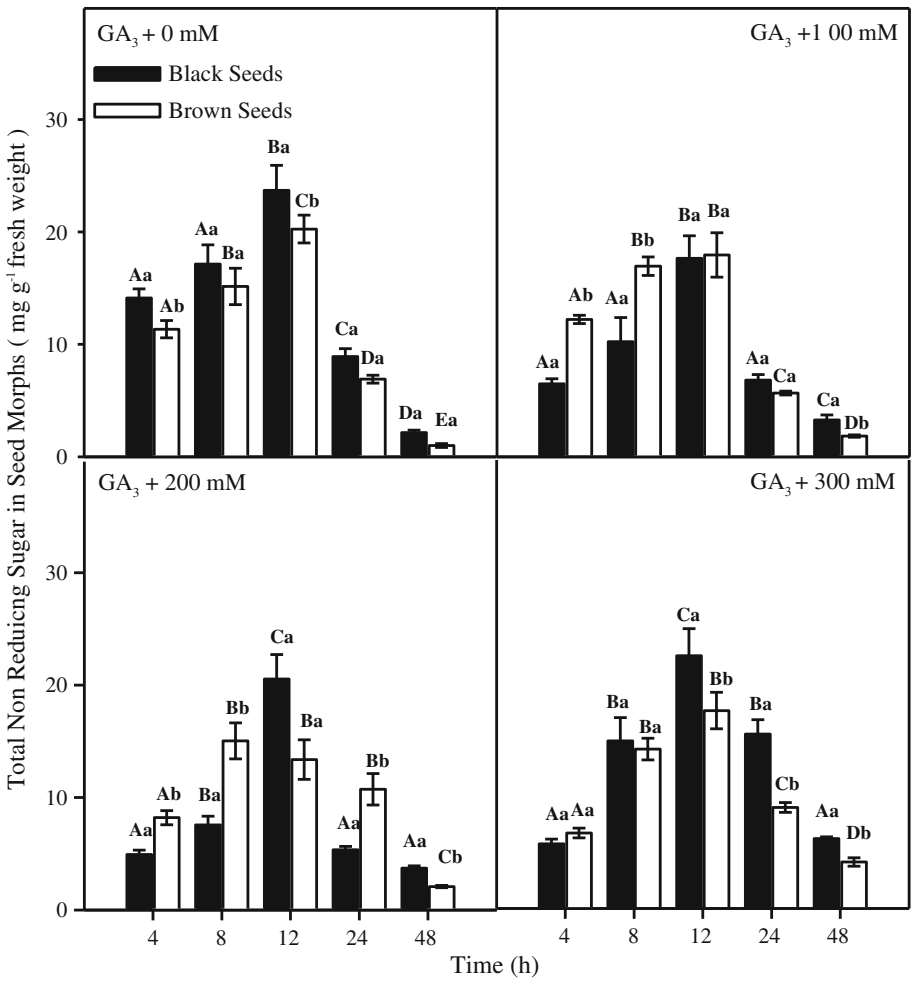

Fig. 9 Effect of exogenous application of GA_3 (3 mM) on total non-reducing sugar in germinating seed morphs (black and brown) of *Halopyrum mucronatum* in saline (100, 200, 300 mM NaCl) and non-saline (0 mM) medium. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letters* between time interval within seed morphs are not significantly different at $P = 0.05$ (Bonferroni test). *Similar small letters* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (*t* test)

Table 3 Inhibition of *Panicum* alpha-amylase by *Halopyrum* seeds extract

Sample code	Incubation time (min)	Enzyme activity (U mL^{-1} min^{-1})	Relative activity (%)	Inhibition (%)
Control	–	720 ± 25.3	100 ± 15.6	–
Black seed	10	495 ± 1.99	68.7 ± 2.55	31.3 ± 2.33
	20	420 ± 19.55	58.3 ± 1.25	41.7 ± 0.88
	30	121 ± 11.25	16.8 ± 1.33	83.2 ± 1.44
Brown seed	10	Nil	Nil	100
	20	Nil	Nil	100
	30	Nil	Nil	100

Values are in mean ± standard error

Table 4 Phenols and phenolic acids in *Halopyrum mucronatum* seed morphs after soaking

No.	Black seed	Brown seed	R_f values
1	Pyrogallol	Pyrogallol	0.08
2	*para*-Hydroxybenzoic acid	*para*-Hydroxybenzoic acid	0.54
3	Syringic acid	Syringic acid	0.79
4		Vanillic acid	0.82

carbohydrate in germinating seeds is the main source of energy and proper substrate for other pathways which are requisite for completion of germination (Mayer and Pojakoff-Mayber 1975; Lin and Kao 1995; Mei and Song 2008). This carbohydrate mobilization is inhibited by NaCl (Prakash and Prathapasenan 1988; Prado et al. 2000).

It has already been observed that more phenols were leached out from brown seed of *H. mucronatum* in saline medium during germination compared to black (Siddiqui

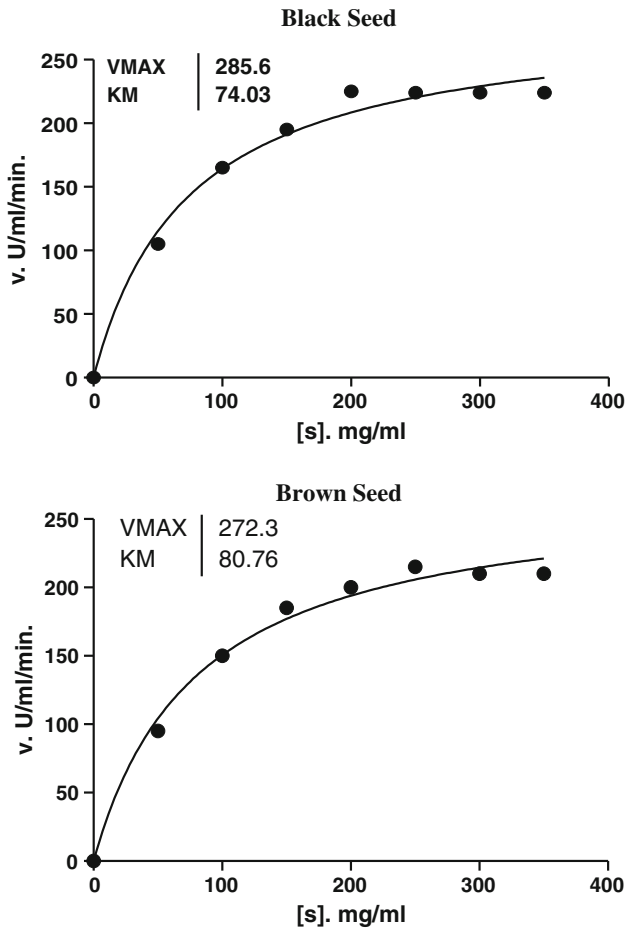

Fig. 10 Substrate saturation kinetics (V_{max} and K_m values) of amylase extracted from both seed morphs of *Halopyrum mucronatum*

and Khan 2010, Fig. 11). Hence, it is presumed that more phenols leaching from brown seed during germination in saline medium might have produced co-solute effect (NaCl + brown seed phenols) on amylase activity which results in lower amylase activity and subsequent lower mobilization of storage sugar.

It has been suggested that solubility of different phenols in a medium is varied due to the presence of salts such as potassium, sodium nitrate and chloride (Noubigh et al. 2008). Furthermore, it was observed that in black seeds, amylase activity was rapid and stable throughout the germination period particularly in saline medium. There might be several possible reasons for this. First, black seeds contain more tannins and phenols compared to brown seed (Table 1). These phenols and tannin are retained in saline medium during germination. This ability to retain secondary metabolites of black seed is important in regulating water uptake particularly saline water. More leaching of phenols with vanillic acid as its main component from brown seeds caused reduction in amylase activity. Reports suggest that amylase activity is inhibited by the salt (NaCl) and phenol (vanillic acid) (Rohn et al. 2002; Singh et al. 2004; EL-Barghathi and Asoyri 2007; Noubigh et al. 2008; Chetan et al. 2008). Contrarily, there are several reports showing stress tolerance properties of polyphenols (Heimler et al. 2005; Díaz-Batalla et al. 2006).

A complete inhibition in amylase activity from phenols leached from brown seeds was observed compared to those leached from black seed. This shows that phenols are retained in black seed for a longer period and may improve tolerance to salinity which contributes enhanced amylase activity and subsequent sugar mobilization leading to higher germination. Phenols in brown seed do not produce co-solute effect (NaCl + phenols) on amylase activity leading to lesser sugar mobilization and to lower seed germination. It is well known that vanillic acid is potential inhibitor of germination and amylase activity (EL-Barghathi et al. 1989; Asoyri 2003). In present study, exogenous GA_3 application enhanced the germination and amylase activity in both seed morphs confirming the antagonistic response of GA_3 against salt and vanillic acid stress (Plyler and Proseus 1996; Debeaujon et al. 2000; EL-Barghathi and Asoyri 2007). Like sesquiterpene, facnesol, sesquiterpene lactone, argrophylline A and B, vanillic acid has more or less similar inhibitory effect on GA_3 biosynthesis (Dvorakova et al. 2008).

Enzyme kinetics also showed that black seed amylase has better reaction velocity and more affinity (V_{max} = 285.6, K_m = 74.03) to sugars compared to brown (V_{max} = 272.3, K_m = 80.76) showing rapid utilization of sugar reserves leading to completion of germination. Successful utilization of sugar reserves is an important step toward initiation of germination process as it is an early source of energy and substrate (Mayer and Pojakoff-Mayber 1975; Lin and Kao 1995; Bewley 1997; Zhou et al. 1998; Deckers et al. 2004; Muralikrishna and Nirmala 2005; Mei and Song 2008).

H. mucronatum usually spreads through stolons but seed production is also carried out as an alternate source of reproductive strategy and to maintain genetic variation so that new genetic combination can arise which is often regulated by ecological factors (Khan and Ungar 1986; Baskin and Baskin 2000). High phenols in summer seeds are perhaps the consequence of temperature, humidity, and evaporation rate which are high in local coastal sites during summer thus causing high salt concentration in the sand dunes of coastal areas. Phenol regulation and deposition in seed might be the strategy to survive and reproduce under stress environment. Further, the black seed of *H. mucronatum* is perhaps due to catechol which not only imparts its black appearance due to oxidation but also exhibits better tolerance in saline environments compared to brown which lack catechol but possess vanillic acid.

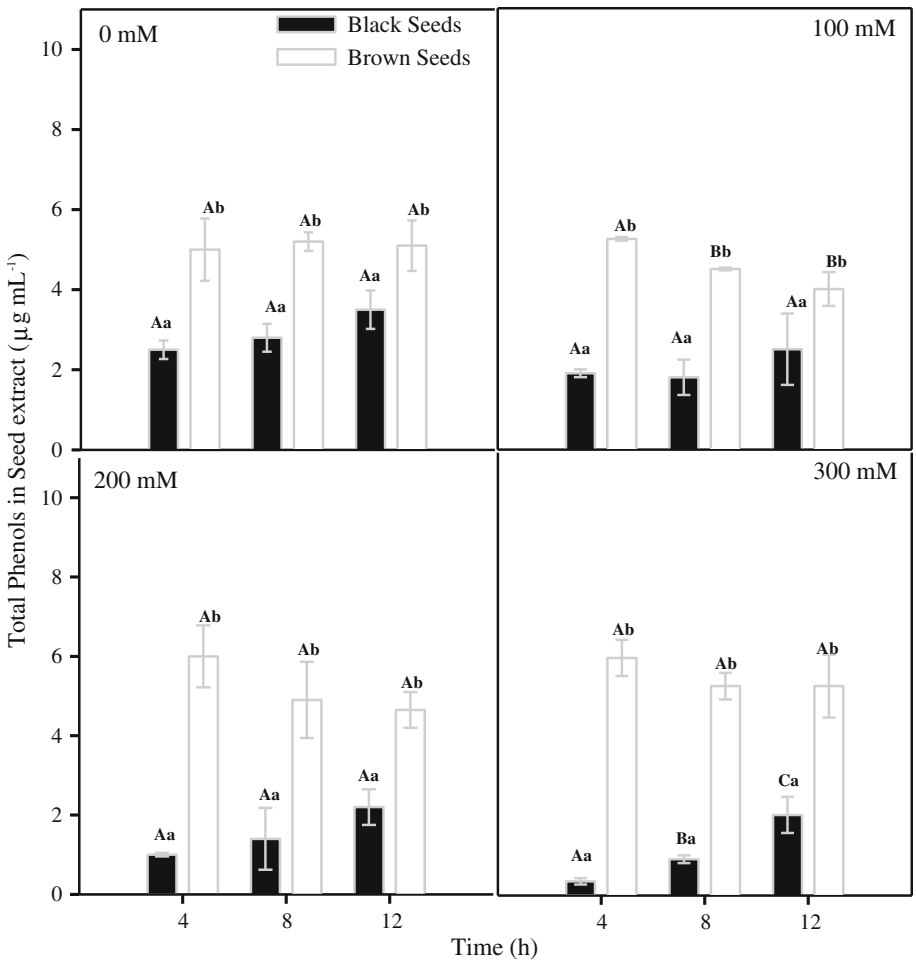

Fig. 11 Total phenols in seed morphs extract due to soaking. *Vertical lines* on the bar represent mean ± standard error. Values on the bars having *similar capital letters* between time interval within seed morphs are not significantly different at $P = 0.05$ (Bonferroni test). *Similar small letters* between the seed morphs in each time interval are not significantly different at $P = 0.05$ (t test) (data published in PJB by Siddiqui and Khan 2010)

Conclusion

Variable response of *H. mucronatum* seeds during germination could be due to the variation of phenolic contents of seed types. These endogenous phenols have differential effects on amylase activity during germination. Due to hard seed coat, most of the phenols are retained within the black seeds for relatively longer period in comparison to brown seeds.

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