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SOME PHYSIOLOGICAL ATTRIBUTES OF DIMORPHIC SEEDS OF *HALOPYRUM MUCRONATUM* (L.) STAPF

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Abstract

Halopyrum mucronatum have dimorphic seeds and referred to as black and brown seeds. Black seeds when soaked in either non-saline or saline medium, showed lesser solute leakage in comparison to brown seeds. Amylase extracted from black seeds had optimum performance in acidic pH, high temperature and ability of early substrate utilization. Results are discussed in relation to physiological attributes of seeds produced in different seasons and their habitat in response to salinity.

Introduction

Halophytic species often show their seed dimorphism and polymorphism seed morphs produced by a single individual may differ in germination response in saline and non-saline condition (Khan & Gul, 2006). Seed dimorphism gives the species an added advantage of multiple windows of opportunity in heterogonous coastal saline habitats which show high degree of environmental fluctuations.

Since seeds are the vital component of world diet and main reproductive unit of those plant which are inhabitant of harsh environment. Most of the researches of seed physiology are restricted to crop plants (Bewley, 1997; Prado *et al.,* 2000; Potokina *et al.,* 2002; Duque & Chua, 2003; Ogawa *et al.,* 2003; Kwon *et al.,* 2008) but the reports on those plants which are adapted in harsh land and environment are rather scarce, halophytes in particular.

Halopyrum mucronatum (L.) Stapf. is perennial halophytic grass exhibit seed dimorphism. Usually plant produces flowering twice a year in two seasons i.e., summer and winter (Khan & Ungar, 2001). Black seeds are produced during summer which is heavier than brown seeds formed in winter. Both seed's showed variable germination responses under saline and non-saline condition, temperature, growth regulating chemicals treatments (Khan & Ungar, 2001; Siddiqui & Khan, 2011).

Though, some of physiological mechanism explaining diverse germination responses of both seed morphs has been reported by Siddiqui & Khan (2010) but few other mechanisms need to be studied closely. Therefore, present investigation focus on those physiological attributes like water uptake, solute leakage and amylase activity performance of two seed morphs of *Halopyrum mucronatum* in detail. Water uptake, solute leakage and amylase characteristics are supposed to be important physiological assessments during germination which not only enlighten the diverse germination response but also provide the information about how these factors affect the germination rate, tolerance and susceptibility of two seed morphs under saline and non-saline environment.

Materials and Methods

Seed collection: Seeds of *Halopyrum mucronatum* were collected during May to June and December to January 2008-2009 from the sand dunes and flat on Hawksbay sea cost around Karachi.

Seed surface area: Twenty five seeds of each morph were placed in 100 mL beaker containing 0, 100, 200 and 300 mM NaCl solution. Seeds were kept in the solutions for 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, and 8 h respectively. Later the seeds were blotted with tissue papers and subjected to seed area determination in LI COR 3100 Leaf Area Meter (±5 %). Each data is the sum of 25 seeds and expressed in bar graphs. Experiment was replicated four times.

Solute leaching: Twenty five seeds of each morph were placed in 100 mL beaker containing 50 ml of 0, 100, 200 and 300 mM NaCl solution for 1, 2, 3, 4, 5, 6, 7, 8 h respectively. Later on seed were removed from the respective beaker and conductivity of each solution was measured by conductivity meter model CMD 500 assuming increase in conductivity related to solute leakage from the seed.

Amylase characteristics: 100 mg (~50) healthy seeds were placed in 90mm diameter Petri plates with 10ml distilled water enough to moist filter paper. Amylase of both seeds morphs were extracted from 12 h samples (based on preliminary study) where optimum activity was recorded in both seed morphs. Temperature, substrate and pH maxima were examined. For temperature maxima, 1mL of both extract and substrate (1 % starch) were taken in each of 6 test tubes and subjected to 30, 40 50, 60, 70 and 80°C temperature for 15 min respectively. Similarly for substrate maxima, 1mL extract and 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% substrate concentration were added in each of 6 test tubes respectively. Further pH maxima, 1mL extract and 1mL substrate of pH 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were added in each of 6 tubes respectively. All the set up for pH and substrate maxima were incubated at 40°C for 15 min. Later enzyme activity was assayed by DNS method (Bernfeld, 1955).

Extraction: Fifty seed (~50mg) of each type black and brown were extracted after 12 h treatment in cool enzymes extraction buffers having Tris/HCl pH 6.8, 20 mM $CaCl_2$ and 0.25 % PVP. Homogenized and centrifuged the whole contents at 14000 rpm at 4°C for 15 min. One unit releases one micromole of β-maltose per min at 25°C and pH 6.8 under the specified conditions.

Statistical analysis: A three-way ANOVA was used to determine significant differences among means within and between each seed morphs using germination time,

NaCl concentrations and seed types as factors. A bonferroni test was carried out to determine if significant ($p<0.05$) difference occur in individual treatments. The significance of test was represented as small alphabets on the bar graphs. Linear regression was fitted for amylase characteristics representation using SPSS 17.0 software.

Results

Dimorphic seeds of *H. mucronatum* respond differently when soaked in saline and non-saline medium showing increase in black seed surface area compared to brown (Fig. 1). However, substantial increase in seed area was found when black seed was soaked in the 300mM NaCl solution after 6 and 7 h treatment. At 300mM NaCl, brown seed showed substantial decrease in area which might be due to the loss of solute leakage. However, at 200mM brown seed showed slight increase over black. In general, increase in seed area of both seed morphs was noted over time duration.

Solute leakage was high in brown seed compared to black with few exceptions where black seed released more solute in the surrounding medium (Fig. 2). However, maximum leakage was observed during initial hours of the treatments. At 200mM NaCl solute leakage more or less same in both seed morphs. However, in non-saline medium more solute was leached out from the brown seed than black. In comparison with non saline medium, solute leaching was increased with soaking time in both seed morphs.

Amylase extracted from black and brown seed were used to examine the effect of various pH, temperature and substrate ranges on activity performance (Fig. 3). Amylase extracted from both seed morphs were reached to the steady state of the enzymatic reaction with different velocity. Black seed reached the steady state before 2% substrate concentration compared to brown seed which reached to the state after 2%. This showed the enzymes saturation point of black seed amylase reached earlier than brown. However, both seed morphs positively (black seed $r^2 = 0.710$, brown $r^2 = 0.868$) correlated with the substrate concentration at certain levels.

Effect of temperature on the amylase activity of both seed morphs were noted showing different optimum ranges (Fig. 3). Amylase extracted from black showed optimum activity at 50ºC compared to brown seed where maximum activity was found at 40ºC. However, some activity was measured at 60 and 70ºC in black seed amylase where in brown seed amylase which was completed inhibited after 60ºC.

Amylase extracted from both seed morphs showed almost similar activity pattern in various pH (Fig. 3). Optimum activity in both black and brown seed morphs were at the pH range 6.5 to 7.0. Brown seed amylase showed some activity at pH 8.0 compared black showing more stability in alkaline environment.

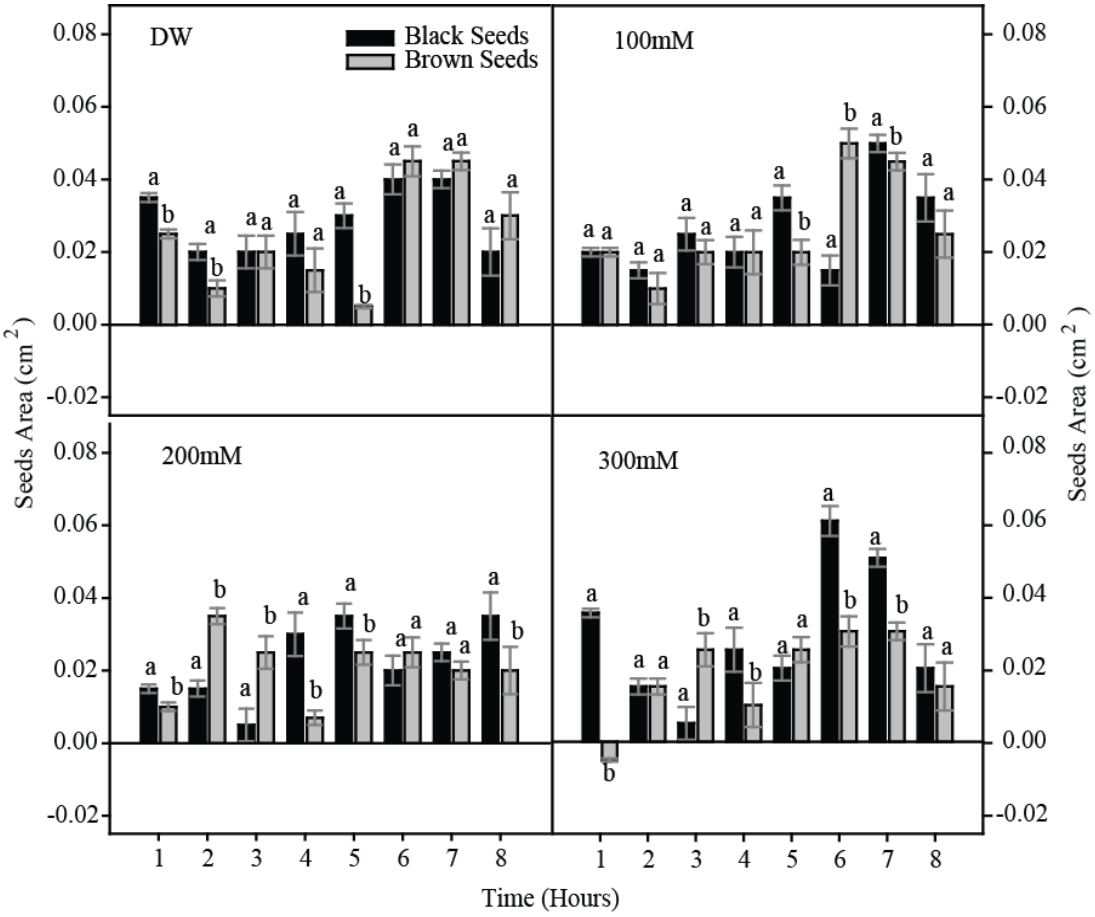

Fig. 1. Effect of water uptake on the area of both seed morphs in saline and non-saline medium. Vertical line on the bar graphs means ± standard error. Similar alphabets on the bar graph stand for non-significant values (Bonferroni test *p*-0.05).

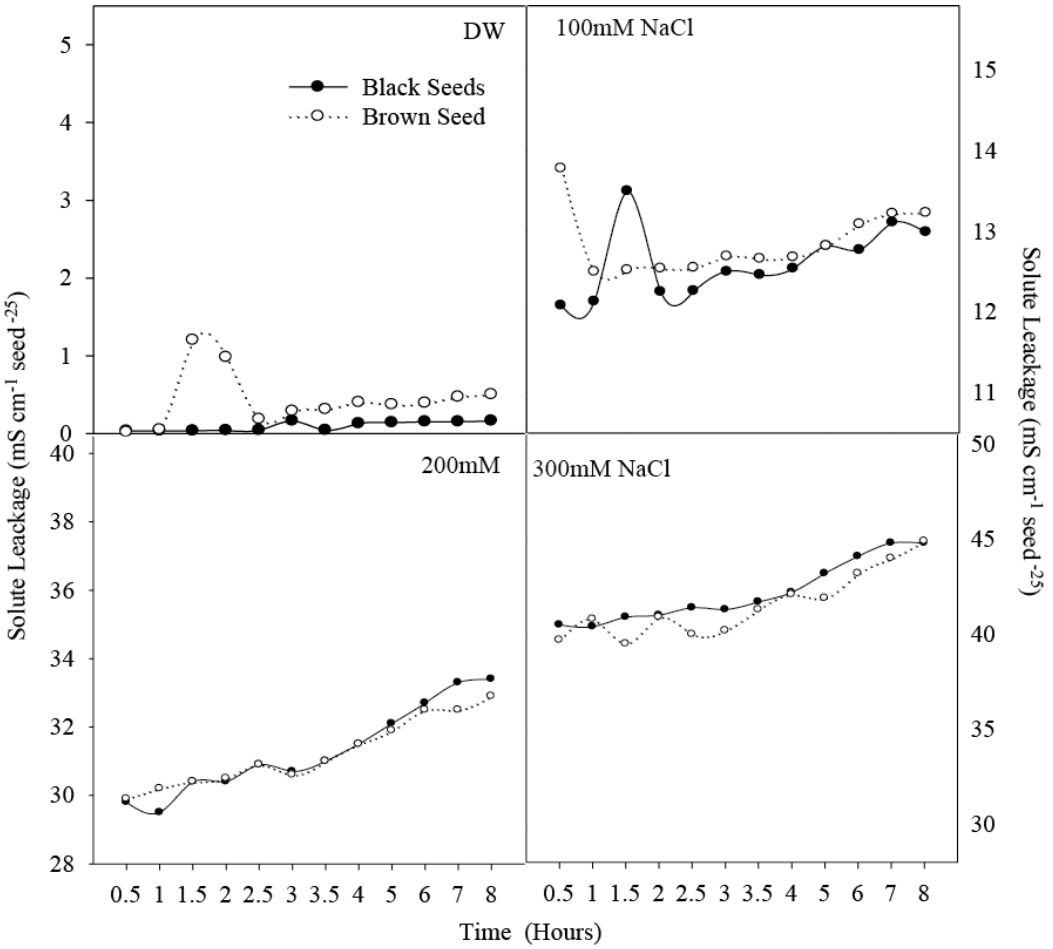

Fig. 2. Solute leakage from black and brown seeds of *Halopyrum mucronatum* in saline and non-saline medium.

Discussion

Some physiological attribute of *H. mucronatum* seed morphs in saline and non-saline medium were investigated. Observation showed substantial increase in black seed area when seed were soaked in saline and non saline solution compared to brown seeds. Present study elucidate water uptake in terms of seed area measurement rather than weight of the seeds. Solute leakage was high in brown seed compared to black with few exceptions where black seed released more solute in the surrounding medium. However, maximum leakage was observed during initial hours of the treatments.

Seed morphology played significant role in water uptake initiating early metabolism which are pre-requisite of germination completion (Bewley, 1997; Siddiqui & Khan, 2010). Further, seed texture, morphology, size and color have greatly influenced the seeds germination in two ways. First by being impermeable to water and oxygen, the second is that seed coat mechanical resistance to radicle emergence (Serrato-Valenti, 1993; Tyler, 1997; Debeaujon *et al.,* 2000; Fengshan, 2004; Mie & Quan, 2008). Reports suggest that dark colored seeds germinate slowly compared to light colored but showed higher final percent germination (Wyatt, 1977; Powell, 1989; Kantar *et al*., 1996). Probably, light colored seed uptake water more rapidly and therefore suffer greater solute leakage compared to dark seeds. For example, red seed of *Sinapis arvensis* L., uptake water more rapidly compared to black ones (Duran & Retamal, 1989). White colored seed in legumes imbibe quickly, suffer greater solute leakage than colored seeds but germinate earlier with low final germination (Kantar *et al.,* 1996).

Present study suggested that increase in black seed area might be due to water uptake and least solute leakage thus improved black seed area compared brown. It might be concluded that black seed has more water and solute retain ability in saline and non saline medium compared to brown seed causing increase seed area compared to black thus enhance the final percent germination of the black seed (Siddiqui & Khan, 2010).

Amylase extracted from both black and brown seed morphs reaching to the steady state of the enzymatic reaction with slight variation. Black seed reached the steady state before 2% substrate concentration compared to brown seed which reached the state more than 2% indicating the amylase saturation point in black reached earlier than brown seed. Amylase extracted from black showed optimum activity at 50ºC compared to brown seed. Optimum activity in both black and brown seed morphs were at the pH range 6.5 to 7.0. Brown seed amylase showed some activity at pH 8.0 compared to black indicating some brown seed stability in alkaline environment.

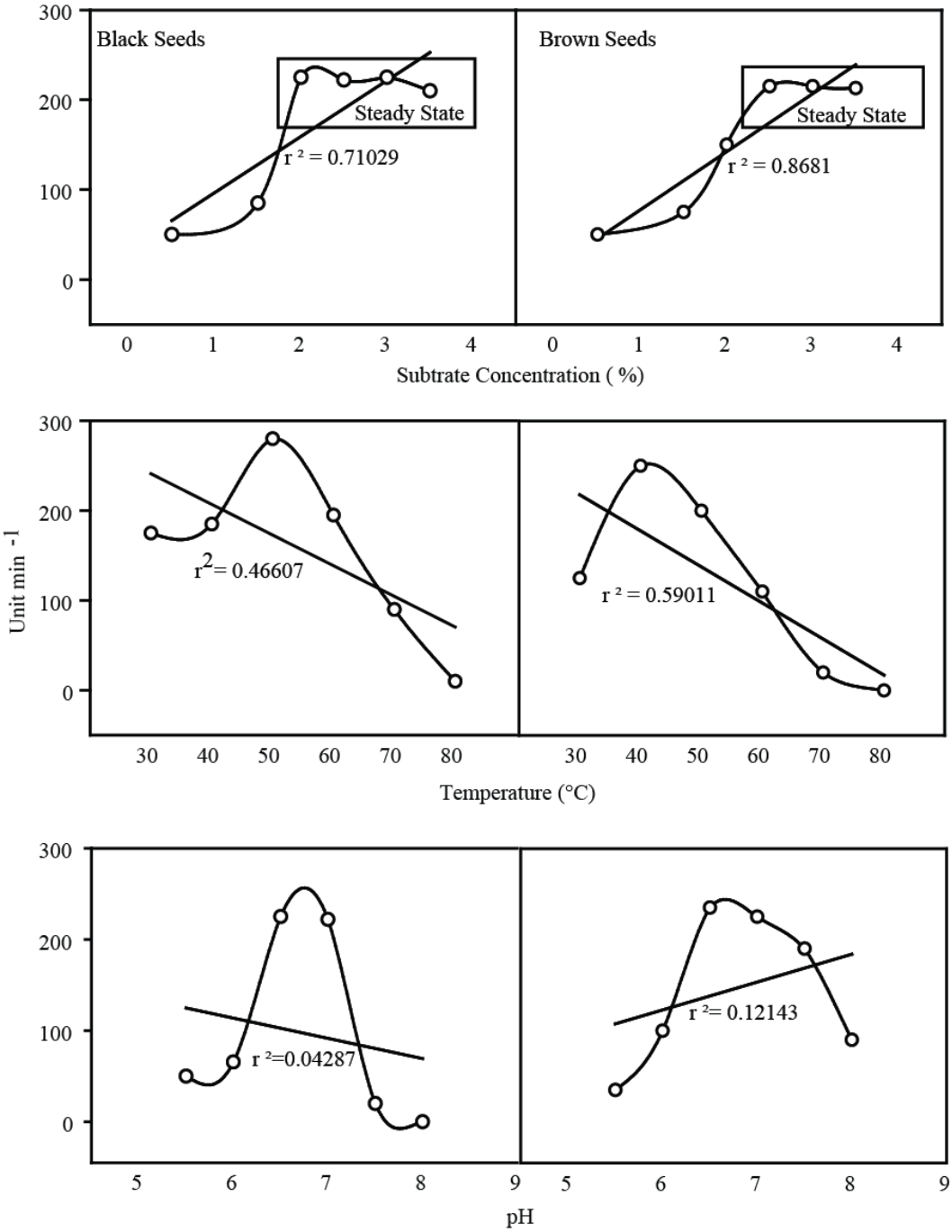

Fig. 3. Enzymes amylase characteristics of both (black and brown) *Halopyrum mucronatum* seed morphs. Symbol on graph r^2 = linear regression value.

Data suggests that black seed amylase seems to be more physiologically efficient to utilize the substrate in acidic environment with some stability in high temperature regime. It is presumed that black seed which are produced in summer might have different protein nature and have adaptability against extreme environment to cope with it. These physiological attribute reflect the variation in the amylase proteins of both seasonally produced seed of this halophytic grass which could be studied closely.

Among hydrolytic enzymes, amylase is the most abundant, playing key role in starch metabolism of germinating seed (MacGregor *et al.,* 1988; Sugimoto *et al.,* 1998; Sultana *et al.,* 2000; Siddiqui & Khan, 2011). Changes in the activity pattern of amylase in germinating plant seeds have been well documented (MacGregor *et al.,* 1988; Sun & Henson, 1991; Rehman *et al.,* 2011; Murtaza & Asghar, 2012). The observations so far obtained have indicated that the amylase activity is under significant influence of variegated environmental conditions such as temperature

(Sultana *et al.,* 2000), stress (Jazayeri *et al.,* 2007) or pH (Tripathi *et al.,* 2007). However, information on amylase characteristic particularly halophytic grass is lacking. Present study showed that the characteristics of amylase extracted from two different seasonally produced seed of *H. mucronatum* are varied showing better ability of black seed amylase to utilize substrate in extreme temperature and acidic pH environment. This could not be a reason of black seed better germination in saline and non saline environment but also suggest the tolerance ability against high temperature and saline habitat.

Black seed exhibits more water retain ability compared to brown which not only improve seed surface area but also release less solute from the black seed into the surrounding saline and non-saline medium avoiding imbibition damage. Less solute leakage, better water uptake and extracted amylase characteristic of black seeds may be the reason for better germination performance in saline and non saline environment (Khan & Ungar, 2001; Siddiqui & Khan 2010). Investigation on amylase proteomic of the two seed morphs of *Halopyrum mucronatum* could uncover many questions like amino acid sequence, nature of proteins and their stability and tolerance of this plant in extreme habitat of sea coast.

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