Pak. J. Bot., 42(1): 227-238, 2010.

THE ROLE OF SEED COAT PHENOLICS ON WATER UPTAKE AND EARLY PROTEIN SYNTHESIS DURING GERMINATION OF DIMORPHIC SEEDS OF *HALOPYRUM MUCRONATUM* (L.) STAPH

ZAMIN SHAHEED SIDDIQUI AND M. AJMAL KHAN*

**Institute of Sustainable Halophyte Utilization,*
Department of Botany, University of Karachi,
Karachi 75270, Pakistan.

Abstract

Role of seed coat phenolics on water uptake and early protein synthesis of *Halopyrum mucronatum* dimorphic seeds during germination were tested. Scanning electron micrographs (SEM) showed seed texture with differential deposition of secondary metabolites in both morphs. Ability of both seed morphs to retain secondary deposition was dependent on exposure to either saline or non-saline conditions. More phenols leached from the brown seed during the initial hours of soaking when compared to black seeds. Water uptake pattern was slightly different in both seed type particularly during initial hours when imbibition in black seeds showed little water uptake while in brown seeds absorption was quick in the first hour under both saline and non saline condition. Change in total protein was somewhat similar in both seeds morphs showing early increase (4 and 8 h), reaching to the maximum (12 h) and decreasing (24 and 48 h) afterward. The results are discussed in relation to seed coat phenolics, water uptake and early protein synthesis during germination.

Introduction

The seed coat is a multifunctional organ that plays a critical role in embryo nutrition during seed development and protection against detrimental agents from environment afterward (Mohamed-Yaseen *et al*., 1994; Bewley & Black, 1994; Bradford, 2000). The seed coat exerts its germination-restrictive action most of the time by being impermeable to water and oxygen or by its mechanical resistance to radicle protrusion. These properties have been positively correlated with seed coat colour due to phenolic compounds in diverse species (Debeaujon *et al*., 2000).

Water uptake is an important step towards the initiation of biochemical changes that lead to germination completion. However, rapid uptake can cause imbibition damages to the embryos of germinating seeds, particularly those that imbibe water quickly. It has been observed in several reports that seed colors are an important external factor significantly contributing in water uptake and early protein synthesis (Duran & Retamal, 1989; Wyatt, 1977; Powell., 1989; Kantar *et al*., 1996; Bewley, 1997) but the role of seed coat phenolics in water uptake and early protein synthesis in dimorphic halophytic seed are rather scarce.

Halopyrum mucronatum L. Staph., is a stoloniferous perennial halophytic grass which exhibit seed dimorphism, flowering shoots usually produces twice a year, from April to May and from September to November (Khan & Ungar, 2001). Black seeds are produced during summer and are heavier than brown seeds which are formed in winter. Both the seed morphs showed variable germination response under saline and non-saline conditions, temperature and growth regulating chemical treatments. In the present study the role of seed coat in germination of dimorphic halophytic seeds in relation to secondary metabolites deposition, water uptake pattern and their subsequent effect on early protein synthesis is presented.

Material and Methods

Scanning electron microscopy: Surface scanning of both black and brown seed coat before and after 4 h imbibition were examined. Seeds coat were scanned after (60%) ethanol washing to remove any dust or other impurities. Randomly selected samples were coated with gold and examined in JEOL scanning electron microscope Japan at different magnification power i.e., 500 µm, 1000µm and 1500µm.and 1700 µm

Seed collection: Seeds of *Halopyrum mucronatum* were collected during May to June and December to January 2006-2007 from sand dunes and flats on Hawksbay sea coast around Karachi.

Germination test: Caryopses of *H. mucronatum* collected bear two different morphology and will be referred to 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 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). Four replicates of 20 seeds each were used for each treatment and were placed at 20-25˚C ± 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 germinated after the radicle emerged. Percentage germination was recorded at 1 day interval up to 14 days. Final percentage germination was recorded at 14^{th} day of the experiment.

Imbibition: Seeds were placed in 50×9 mm tight-fitting plastic Petri plates at 25°C. Experiment was divided into 2 parts of 4 h each. First 4 h, change in weight was measured at 30 min. interval and then last 4 h at 1hrs interval. Change in weight of 25 seeds was measured in each control and treatments. Seeds were imbibed in 0, 100, 200 and 300 mM NaCl. The experiment was replicated four times.

Total phenols in leachate after soaking: Twenty five seeds of each morph were placed in 0, 100, 200 and 300 mM NaCl solution for 4, 8, and 12 h. Later the respective solution was dried in oven at 80^{o}C for 48 h. 1mL ethanol was added and total phenol estimated by Swain & Hills (1959) method.

Protein 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 for the change in total protein during germination. Approximately 100 mg healthy seeds were placed in 90 mm diam., Petri plates with 5 ml test solution (0, 100, 200, and 300 mM NaCl) enough to moist filter paper. Change in total protein was measured after 4, 8, 12, 24 and 48 h interval. Whole setup was placed at 25˚C ± 2°C in growth chamber (Hotpack programmed refrigerated incubator). The photoperiod, light intensity and relative humidity were the same as provided in germination experiment. Each treatment and control was replicated four times.

Extraction procedure: Twenty five germinating seed (~50 mg) of each type i.e. black and brown were randomly collected from each treatments and control separately and homogenized in chilled 10 mL Tris-HCl buffer pH 6.8, centrifuged at 14000 rpm for 15 min at 4°C. Supernatant were collected and total protein estimated by Bradford (1976) method.

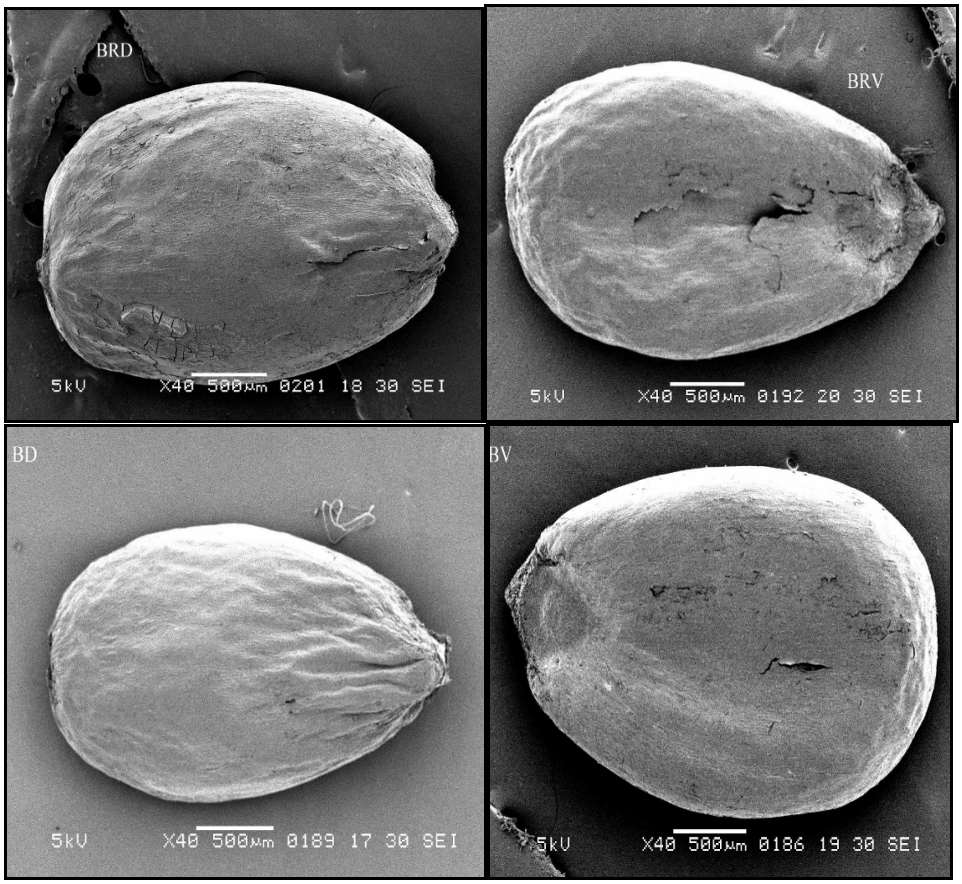

Fig. 1a. Scanning electron micrograph showing texture of both *H. mucronatum* seed morphs. (B= Black Seed BR= Brown seed V= Ventral view, D= dorsal view)

Statistical analysis: A Three-Way ANOVA analysis was used to determine significant differences among means within and among each seed morphs using time (germination duration), NaCl concentrations and seed types as factors. A Bonferroni test and paired t-test were carried out to determine if significant ($p<0.05$) difference occur in individual treatments. The significance of Bonferroni and paired t-test was represented as capital and small alphabets on the bar graphs.

Results

Secondary metabolite deposition on seed coat and their quantification: Black and brown seeds were surface scanned with JEOL electron microscope. Scanning showed secondary deposition of metabolites (Fig. 1a & 1b). Seed texture was about similar in each morph but deposition of secondary metabolites was dense and reticulated on black seeds in comparison to brown seeds where some cracks were found on dorsal surface and these were more on the ventral surface. Cracks on the black seeds appeared to be filled up due to high deposition of secondary metabolites.

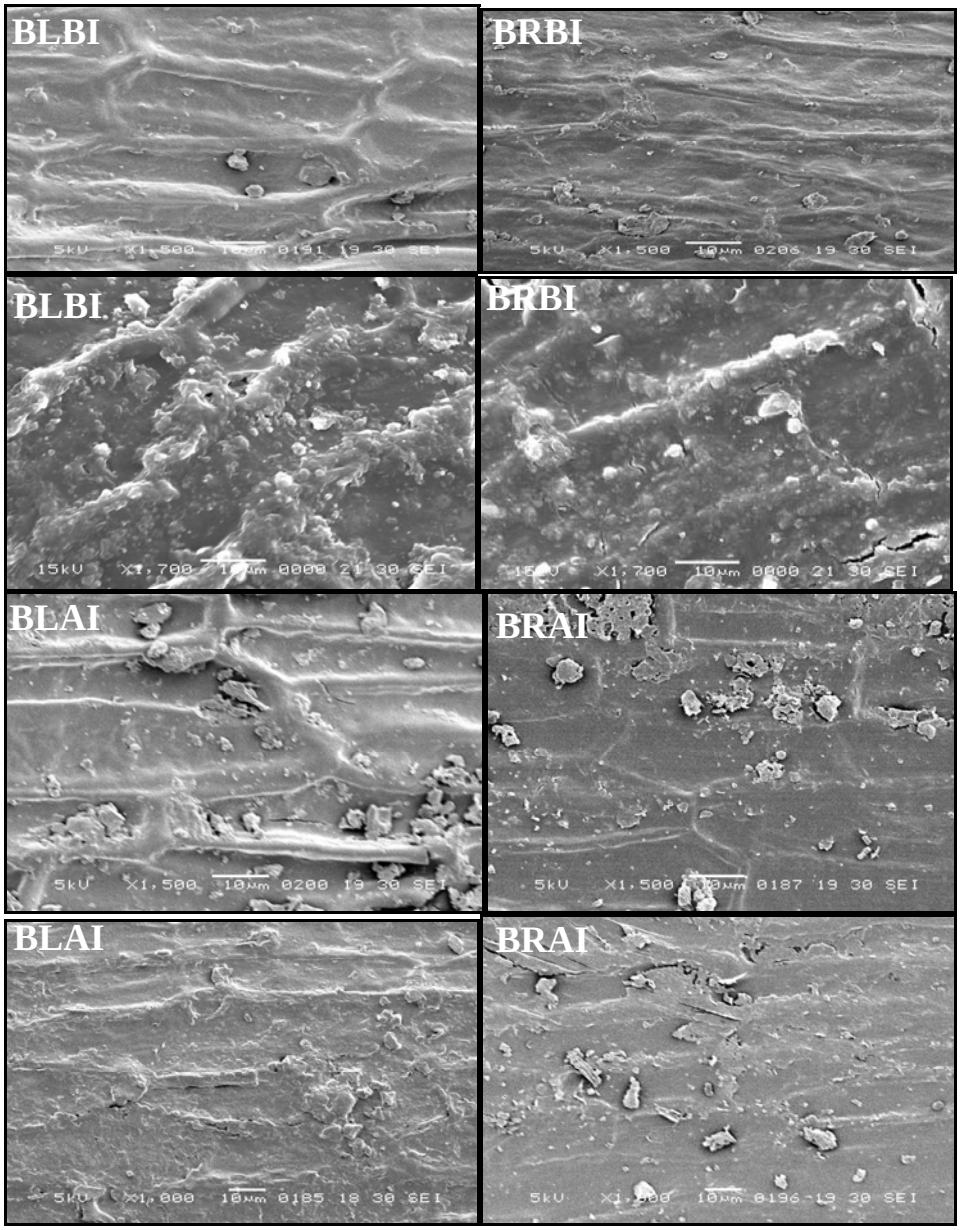

Fig. 1b. Scanning electron micrograph showing secondary deposition on seed surface. (B= Black Seed BR= Brown seed BI= before imbibition, AI= 4hrs after imbibition).

When seeds are imbibed in water, they not only absorb water but some metabolites from the seed were also leached out into the surrounding medium. For examining this phenomenon, two experiments were conducted. In the first, seeds were placed in water to examine the effects of soaking on the secondary metabolites deposition solubility in

surrounding medium (water). For this purpose, seeds were placed in water for 4 h and the observations are shown in micrographs in Figure 1b which shows more deposition on the black seeds than on brown seeds. However cracks widening was much pronounced in both seed morphs due to seed soaking. Further, to verify the observations of the first experiment, seeds were placed in distilled water and NaCl Solution (100, 200, 300 mM) in the second experiment. The total phenol was quantified in respective solutions and is illustrated in Fig. 2, showing quick and high phenols leaching in respective solution from brown seeds compared to black. Leaching of total phenols in black seeds was lesser and late compared to brown seeds.

Seed germination: Complete germination was recorded at the end of 2nd week. Final percent germination varied significantly with the salinity (Fig. 3, $F = 2330.69$, $p<0.001$). In saline medium, final percent germination was higher in black seeds compared to brown. However, final percent germination was similar to control for both seed morphs. Contrary to control, final percent germination was reduced drastically with increase in salinity. However, maximum inhibition was recorded in brown seeds when seeds were treated with 300 mM NaCl.

Water uptake pattern: Water uptake by both seed morphs of *H. mucronatum* were examined for 8 h. Change in seed weight was the criterion to assess the imbibition pattern. For that, experiment was conducted into two phases of 4 h each. In first 4 h, seed weight was measured at 30 minute interval, while in second phase; measurement was taken at 1 h interval (Fig. 4). In non saline medium (distilled water), black seed did not start water uptake earlier than brown seeds, 30min and 1hrs in particular. As the imbibition proceeded, brown seeds showed considerable decrease in weight at 1.5 and 2 h interval indicating early metabolites leaching from the seeds. In black seeds decrease in weight was however, recorded as late as 3 h after imbibition. After 4 h, water uptake patterns were identical in both seed morphs.

In saline medium (100 mM NaCl) water uptake was identical in both seed morphs, showing increase in seed weight with time. At 200 mM, concentration black seeds showed no imbibition initially, but slight imbibition after 1.5 h. However, in brown seed, imbibition increased and after 1 h, weight was reduced. Water uptake at all phases was similar in both morphs after initial period. At 300 mM NaCl, brown seeds showed early uptake with no decrease in weight compared to black seeds where no change in weight was recorded until 1.0 h. However, the rest of the uptake pattern was similar (Fig. 4).

Change in total protein: Change in total protein content was significant in response to NaCl concentration (Fig. 5, $F = 3.96$, $p<0.05$), the duration of seed's exposure to NaCl ($F = 17.31$ $p<0.001$) and seed type ($F = 65.85$, $p<0.001$). However, interaction of all three factors was highly significant ($F = 7.80$, $p<0.001$).

Change in protein pattern was somewhat similar in both seeds morphs showing early increase, reaching to the maximum (12 h) and decreasing afterward. Maximum increase was recorded in both seed types when treated with 100 mM NaCl, but black seeds exhibited more protein content compared to brown. Apparently, it was observed that protein content was high in black seeds during early hour's treatment with 100 mM and 300 mM NaCl compared to brown seeds. In late hours, protein content reached to minimum in both seed types.

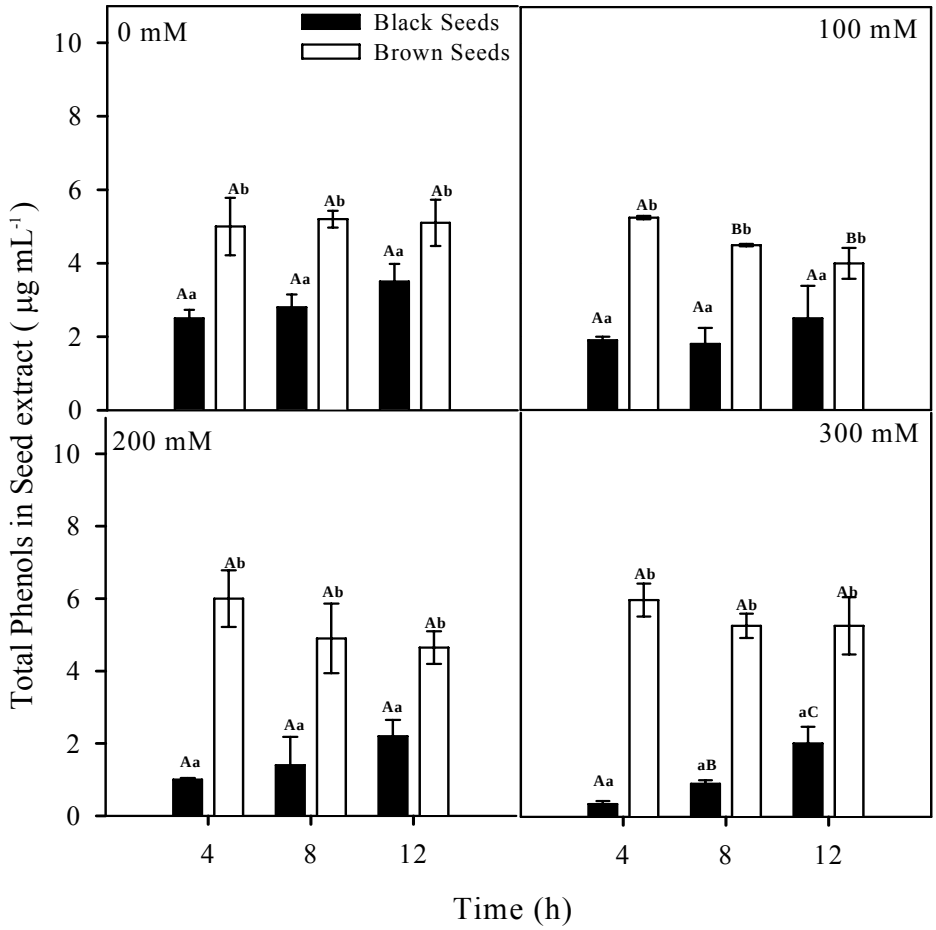

Fig. 2. Total phenols in seed morphs extract due to soaking. Vertical lines on the bar represent means ± standard error. Values on the bars having similar capital letters between time interval within seed morphs are not significantly different at p=0.05 (Bonferroni test). Similar small letters between the seed morphs in each time interval are not significantly different at p=0.05 (t-test).

Discussion

Black seeds germinated more in comparison to brown seeds under saline conditions indicating physiological differences between two morphs. Further, secondary metabolites deposition was higher in black seeds in comparison to brown seed. After 4 h soaking in water, the deposition on the black seeds was still higher than brown seeds which became almost smooth due to the rapid leaching in the surrounding medium. Quantification of total phenols in the medium indicated more leaching from brown seed compared to black (Fig. 2). Furthermore, water uptake patterns showed, absorption in brown seeds was quick in the initial hours than black (Fig. 4).

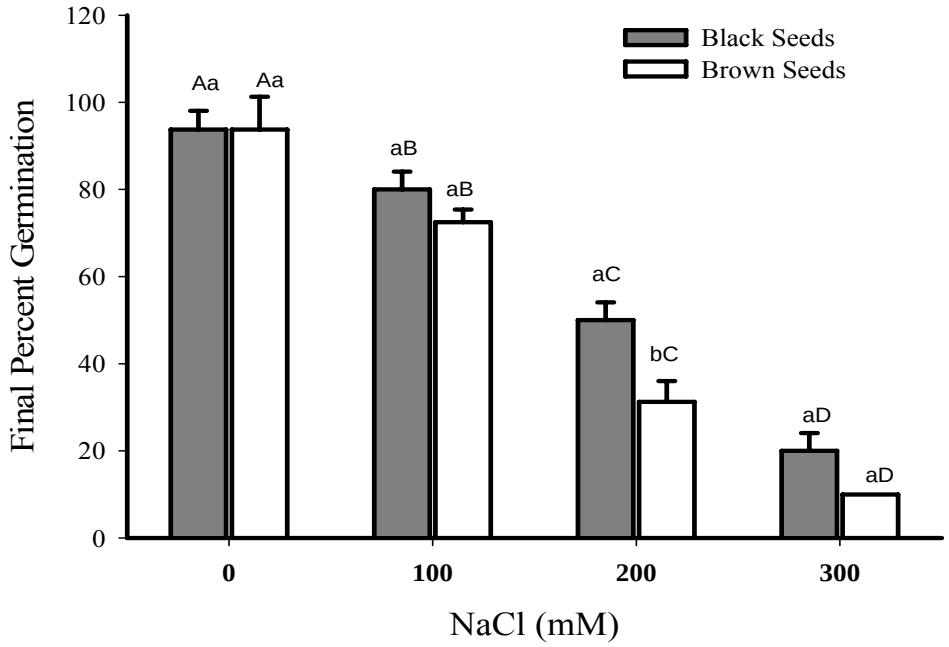

Fig. 3. Mean final percent germination of *Halopyrum mucronatum* seed. Vertical lines on graphs represent standard error. 0 mM = Distilled water, 100, 200, 300 NaCl concentrations. Values on the bars having similar capital letter between concentration within each of the two seed morphs are not significantly different at p=0.05 (Bonferroni test). Similar small letter between the seed morphs within each concentration are not significantly different at p=0.05 (t-test).

Seed coat texture, morphology and size have great influence on the ability of seeds by two ways 1) impermeable to water and oxygen and 2) seed coat mechanical resistance to radicle emergence (Wolf & Fiske, 1981; Serrato-Valenti *et al.*, 1993; Tyler, 1997; Debeaujon *et al*., 2000; Fengshan *et al*., 2004). These properties have been positively correlated with seed coat color perhaps due to the presence of phenolic compounds in species diverse seed germination responses (Asiedu *et al*., 2000). There are several reports which demonstrated that dark colored seeds germinate slowly compared to light colored seed but their final percent germination was higher (Wyatt, 1977; Powell., 1989; Kantar *et al*., 1996). Further, water uptakes of light colored seeds occur more rapidly and therefore suffer greater imbibition damage compared to dark seeds. For instance, 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 imbibition damages than colored seeds but germinate earlier with lesser final percent germination (Kantar *et al*., 1996). Like wise, dark seeds of *Panicum miliaceum* L. have heavier seed coats, thus imbibe and germinate more slowly than light colored seed (Kahn *et al*., 1996). Hence light color could be attributed for the rapid water uptake and more imbibition damage in brown seed under saline and non-saline condition than black seeds. Therefore final percent germination of brown seeds was reduced but they reached to optimum level earlier than black ones.

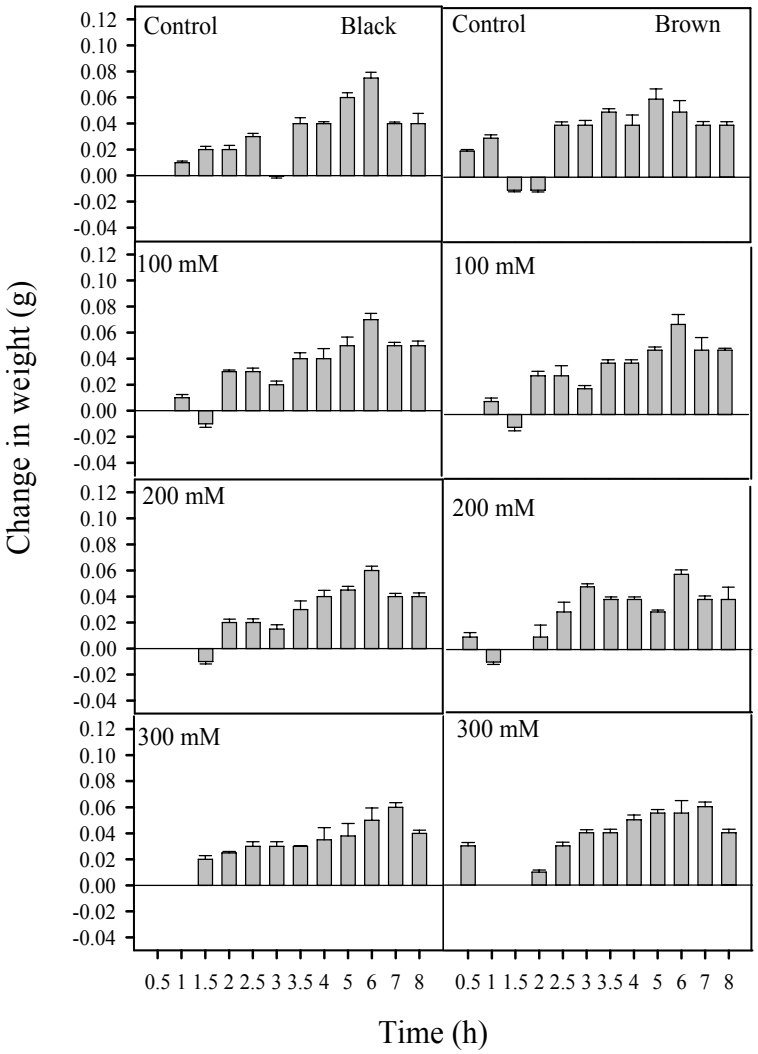

Fig. 4. Water uptake pattern of *Halopyrum mucronatum* seed morphs in saline (100, 200, 300 mM NaCl) and non-saline (0 mM NaCl) medium. Vertical lines on the graphs represent mean ± standard errors.

Early water uptake was an important event during germination to commence the metabolic machinery (Bewley, 1997). Apparently, in present study imbibition response of black seed in treatment solution was slower due to high polyphenols deposition which is observed by SEM photographs compared to brown ones. Therefore, it is presumed that black seed which exhibit high concentrations of total phenols and tannin depositions during development have slow water uptake and less phenol leaching which might be a reason of slow germination as time function but high final percent germination. It has been reported that germination rate and percent increases linearly with water accessibility and quality (Gummerson, 1986; Bradford, 1990; Dahal & Bradford, 1990). Likewise

germination percentages are reduced at lower water potential (Grundy *et al*., 2000; Kebreab & Murdoch, 2000). Further when water potential is lower than -0.5 MPa, seed physiological adjustment is needed (Ni & Bradford, 1992; Bradford, 1995). When water potential is below the threshold for radicle emergence, a metabolic advancement takes place (Kaur *et al*., 2000; 2005). The minimal water potential for seed germination shifts with seed physiological status (Ni & Bradford, 1992), dormancy (Meyer & Pendleton, 2000), and imbibition conditions (Dahal & Bradford, 1994; Alvarado & Bradford, 2002; Rowse & Finch-Savage, 2003). The ability of seed to take up water can change during the whole germination process. The initiation of radicle emergence is often more sensitive to water than subsequent seedling growth (Allen *et al*., 2000). This sensitivity or ability of seed to take up water may be under physiological control (Dahal & Bradford, 1990; Welbaum & Bradford, 1991), and seeds remained desiccation tolerant only before embryo growth is initiated.

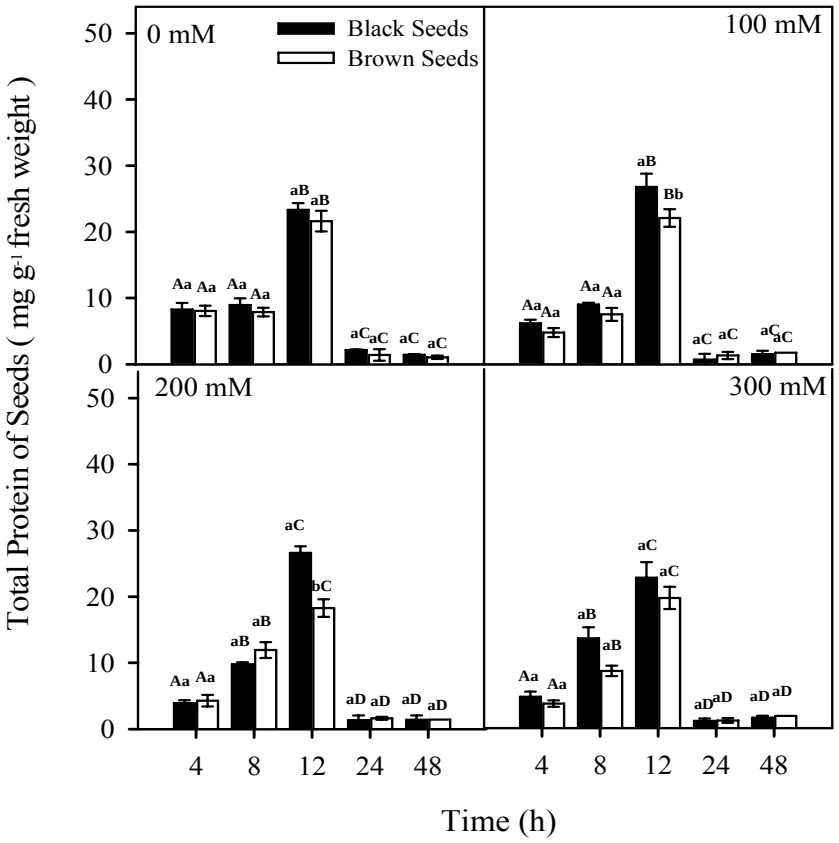

Fig. 5. Effect of salinity concentrations (100, 200, 300 mM NaCl) on total protein content in germinating seed morphs (black and brown) of *Halopyrum mucranatum*. Vertical lines on the bar represent means ± standard error. Values on the bars having different at p=0.05 (Bonferroni test). Similar small letter between the seed morphs in each time interval are not significantly different at p = 0.05 (t-test).

Total protein content in both seed types varied significantly with increase in NaCl concentration. Black seeds showed increase in protein contents compared to brown. Protein synthesis is an important event which occurs early during germination and is often regulated by several abiotic factors including temperature, salinity etc. (Bewley & Black, 1994; Reuzeau & Cavalie, 1997). The results showed that black seed protein synthesis was less affected by NaCl stress compared to brown ones. Perhaps it is due to high polyphenols deposition which might optimize the imbibition process and thus may allow the seed coat to absorb selective and appropriate amount of water during the phase I of the water uptake (Bewley & Black, 1994). It has been observed that the early phase of imbibition is an important event during which the triggering of germination process occurs. Under optimal conditions, early seed imbibition and metabolic reactivation lead to the synthesis of new mRNA and protein (Bewley, 1997; Reuzeau & Cavalie 1997; Bradford *et al*., 2002). Change in the protein content and types of protein have been documented during germination process (Reuzeau & Cavalie, 1997, Bradford, 2002, Bano & Aziz, 2003). Further, It has been reported that morphological and physiological status of seed during development affect the early protein synthesis during germination (Koornneef *et al*., 2002; Potokina *et al*., 2002; van der Geest, 2002; Duque & Chua, 2003; Jabrin *et al*., 2003; Ogawa *et al*., 2003; Rajou *et al*., 2004; Bove *et al*., 2005).

It may therefore be concluded that seed coat morphology like secondary metabolites deposition on the seed coat of both seeds morphs and their ability to retain metabolites during soaking are responsible for diverse germination response. These phenolics not only affected the early water uptake of the seeds but may also have greater influence in triggering the early protein synthesis. Further, it is also presumed that in black seed coat more polyphenols which retained for longer periods in saline medium might be a reason for better tolerance and more final percent germination compared to brown seeds.

References

Allen, G.J., S.P. Chu and K. Schumacher. 2000. Alteration of stimulus-specific guard cell calcium oscillations and stomatal closing in *Arabidopsis* det3 mutant. *Science*, 289:

Alvarado, V. and K.J. Bradford. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell & Environ*., 25: 1061-1069.

Asiedu, E.A., A.A. Powell and T. Stuchbury. 2000. Cowpea seed coat chemical analysis in relation to storage seed quality. *African Crop Sci. J*., 8: 283-294.

Bano, A and N. Aziz. 2003. Salt and drought stress in wheat and the role of abscisic acid. *Pak. J. Bot*., 871-884.

Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell*, 9: 1055-1066.

Bewley, J.D. and M. Black 1994. *Seeds Physiology of Development and Germination*. Plenum Press, New York.

Bove, J., P. Lucas, B. Godin, L. Oge, M. Jullien and P. Grappin. 2005. Gene expression analysis by cDNA-AFLP highlights a set of new signaling networks and translational control during seed dormancy breaking in *Nicotiana plumbaginifolia*. *Plant Mol. Biol*., 57: 593-612.

Bradford, K.J. 1990. A water relations analysis of seed germination rates. *Plant Physiol.,* 94: 840-849.

Bradford, K.J. 1995. Water relations in seed germination. In *Seed Development and Germination* (Eds.) J. Kigel and G. Galili. Marcel Dekker, New York, USA. p. 351-396.

Bradford, K.J. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Sci*., 50: 248-260.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Ann. Biochem.*, 72: 248-254.

Dahal, P. and K.J. Bradford. 1990. Effects of priming and endosperm integrity on seed germination rates of tomato genotypes. II. Germination at reduced water potential. *J. Exp. Bot*., 41: 1441-1453.

Dahal, P. and K.J. Bradford. 1994. Hydrothermal time analysis of tomato seed germination at suboptimal temperature and reduced water potential. *Seed Sci. Res*., 4: 71-80.

Debeaujon, I., M. Karen, Léon-Kloosterziel and M. Koornneef. 2000. Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol.,* 122: 403-414.

Duque, P. and N.H. Chua. 2003. IMB1, a bromodomain protein induced during seed imbibition, regulates ABA- and phyA-mediated responses of germination in *Arabidopsis*. *Plant J*., 35: 787-799.

Duran, J.M. and N. Retamal. 1989. Coat structure and regulation of dormancy in *Sinapis arvensis* L. seeds. *J. Plant Physiol.,* 135: 218-222.

Fengshan, M.A., E.W.A. Cholewa, T. Mohamed, C.A. Peterson and M. Gijzen. 2004. Cracks in the palisade cuticle of soybean seed coat with their permeability to water. *Ann. Bot.,* 94: 213-228.

Grundy, A.C., K. Phelps, R.J. Reader and S. Burston. 2000. Modeling the germination of *Stellaria media* using the concept of hydrothermal time. *New Phytol*., 148: 433-444.

Gummerson, R.J. 1986. The effect of constant temperatures and osmotic potential on the germination of sugar beet. *J. Exp. Bot*., 41: 1431-1439.

Jabrin, S., S. Ravanel, B. Gambonnet, R. Douce and F. Rebeille. 2003. One-carbon metabolism in plants. Regulation of tetrahydrofolate synthesis during germination and seedling development. *Plant Physiol*., 131: 1431-1439.

Kahn, M., P.B. Cavers, M. Kane and K. Thompson. 1996. Role of the pigmented seed coat of proso millet (*Panicum miliaceum* L.) in imbibition, germination and seed persistence. *Seed Sci. & Res*., 7: 21-25.

Kantar, F., C.J. Pilbeam and P.D. Hebblethwaite. 1996. Effect of tannin content of faba bean (*Vicia faba*) seed on seed vigour, germination and field emergence. *Ann. App. Biol.,* 128: 85-93.

Kaur, P., A.K. Gupta and N. Kaur. 2005 Embryo is not required for initiation of *a*-amylase activity in germinating cowpea seeds. *Ind. J. Biochem. Biophy*., 42: 161-165.

Kaur, S., A.K. Gupta and N. Kaur. 2000. Effect of GA3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. *Plant Gr. Reg.*, 30: 61-70.

Kebreab, E. and A.J. Murdoch. 2000. The effect of water stress on the temperature range for germination of *Orobanche aegyptiaca* seeds. *Seed Sci. Res*., 10: 127-133.

Khajeh-Hosseini, M., A.A. Powell and I.J. Bingham. 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. Tech*., 31: 715-725.

Khan, M.A. and I.A. Ungar 2001. Alleviation of salinity stress and the response to temperature in two seed morphs of *Halopyrum mucronatum* (Poaceae). *Aust. J. Bot.,* 49: 777-783.

Koornneef, M., L. Bentsink and H. Hilhorst. 2002. Seed dormancy and germination. *Cur. Opin. Plant Biol*., 5: 33-36.

Meyer, S.E. and R.L. Pendleton. 2000. Genetic regulation of seed dormancy in *Purshia tridentata* (Rosaceae). *Ann. Bot*. 85: 521-529.

Mohamed-Yaseen, Y., S.A. Barringer, W.E. Splittstoesser and S. Costanza. 1994. The role of seed coats in seed viability. *The Bot. Rev*., 60: 246-260.

Ni, B.R. and K.J. Bradford. 1992. Quantitative models characterizing seed germination responses to abscisic acid and osmoticum. *Plant Physiol*., 98: 1057-1068.

Ogawa, M., A. Hanada, Y. Yamauchi, A. Kuwahara, Y. Kumiya and S. Yamaguchi. 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. *Plant Cell*, 15: 1591-1604.

Potokina, E., N. Srevinasulu, L. Altschmied, W. Michalek and A. Graner. 2002. Differential gene expression during seed germination in barley (*Hordeum vulgare* L.). *Func. Integ. Gen*., 2: 28-39.

Powell, A.A. 1989. The importance of genetically determined seed coat characteristics to seed quality in grain legumes. *Ann. Bot*., 63: 169-195.

Rajjou, L., K. Gallardo, I. Debeaujon, J. Vandekerckhove, C. Job and D. Job. 2004. The Effect of *α*-Amanitin on the *Arabidopsis* seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. *Plant Physiol*., 134: 1598-1613.

Reuzeau C. and G. Cavalie.1997. Changes in RNA and protein metabolism associated with alterations in the germination efficiency of sunflower seeds. *Ann. Bot*., 80: 131-137.

Rowse, H.R. and W.E. Finch-Savage. 2003. Hydrothermal threshold models can describe the germination response of carrot (*Daucus carota*) and onion (*Allium cepa*) seed populations across both sub- and supra-optimal temperatures. *New Phytol*., 158: 101-108.

Serrato-Valenti, G., L. Cornara, M. Ferrando and P. Modenesi. 1993. Structural and histochemical features of *Stylosanthes scaba* (Leguminoseae; Papilionoideae) seed coat as elated to water entry. *Can. J. Bot*., 71: 834-840.

Swain, T. and W.E. Hillis. 1959. The phenolic constituent of *Prunus domestica. J. Sci. Food & Agric*., 10: 63-68.

Tyler, J.M. 1997. Effect of impermeable seed coat on germination of seed for early maturing soybean. *Seed Tech*., 19: 45-50.

Van der Geest, A.H.M. 2002. Seed genomics: germinating opportunities. *Seed Sci. Res*., 12: 145-153.

Welbaum, G.E. and K.J. Bradford. 1991. Water relations of seed development and germination in muskmelon (*Cucumis melo* L.): VI. Influence of priming on germination responses to temperature and water potential during seed development. *J. Exp. Bot*., 42: 393-399.

Wolf, D.D. and D.A. Fiske. 1995. Planting and managing switch grass for forage, wildlife, and conservation.Virginia Cooperative Extension publication no. 418-013. Virginia Tech, Blacksburg, VA.

Wyatt, J.E. 1977. Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat color. *J. Am. Soc. Hort. Sci*., 102: 478-480.

(Received for publication 15 June 2009)