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ORIGINAL ARTICLES

The use of SAS software for analysis of seedling growth in salvia (*Salvia officinalis* L.)

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ABSTRACT

Hydro-priming is a very important seed treatment technique for rapid germination. In order to the hydropriming influence on seedling growth in salvia (*Salvia officinalis* L.), this experiment was conducted in 2011 by a completely randomized design with four replications. The factor was including hydropriming (0(H1), 8(H2), 16(H3) and 24(H4) h). The results showed that the effect of hydropriming was significant on germination percentage, seedling length, seedling dry weight and seedling vigour in *Salvia officinalis*. Mean comparison showed that the highest germination percentage, seedling length, seedling dry weight and seedling vigour were achieved by H4 and lowest germination percentage, seedling length, seedling dry weight and seedling vigour were achieved by H1. The results of this experiment showed that the use of hydropriming can increase the seedling vigour in salvia while the lowest of seed characteristics were achieved under no-priming.

Key words: Hydropriming, seedling growth, salvia (*Salvia officinalis* L.).

Introduction

Cactus seeds on the soil surface in the desert are subjected to periods of drought that last for up to a few months, and thus they are typically under discontinuous hydration (or discontinuous dehydration) Apparently, they can tolerate long periods of dehydration after single or multiple hydration events and subsequently germinate in accordance with the previous hydration experience. This was verified in three cactus species from the Sonoran Desert Seeds of *Stenocereus thurberi* hydrated for 72 or 80 h followed by a dehydration period lasting for 4, 14, 70, 120 or 181 d germinated 2-3 d earlier and had 1 4-2 times shorter mean germination time (MGT) than untreated seeds. Seeds given shorter hydration periods also began to germinate sooner than the controls MGT was shorter only when the hydration period was 48 h or longer. Final germination percentages were not affected by these treatments, only the MGT Except for differences in germination percentages, similar results were found for *Pachycereus pecten-aboriginum* and *Ferocactus peninsulae*. When the cycle of 24 h hydration followed by 4 d dehydration was repeated one or two times, the effect was cumulative: MGT was equal to 48 and 72 h hydration, respectively These results suggest a phenomenon of "seed hydration memory," the ability of seeds to retain during dehydration periods those physiological changes that result from seed hydration Thus, treated seeds subsequently germinated earlier then untreated seeds, regardless of the duration of dehydration period This led to a greater biomass accumulation and thus to higher survival in seedlings from treated than from untreated seeds (Dubrovsky, 1996). To better understand seed germination, a complex developmental process, developed a proteome analysis of the model plant Arabidopsis for which complete genome sequence is now available. Among about 1,300 total seed proteins resolved in two-dimensional gels, changes in the abundance (up- and down-regulation) of 74 proteins were observed during germination sensu stricto (i.e. prior to radicle emergence) and the radicle protrusion step. This approach was also used to analyze protein changes occurring during industrial seed pretreatments such as priming that accelerate seed germination and improve seedling uniformity. Several proteins were identified by matrix-assisted laser-desorption ionization time of flight mass spectrometry. Some of them had previously been shown to play a role during germination and/or priming in several plant species, a finding that underlines the usefulness of using Arabidopsis as a model system for molecular analysis of seed quality. Furthermore, the present study, carried out at the protein level, validates previous results obtained at the level of gene expression (e.g. from quantitation of differentially expressed mRNAs or analyses of promoter/reporter constructs). Finally, this approach revealed new proteins associated with the different phases of seed germination and priming. Some of them are involved either in the imbibition process of the seeds (such as an actin isoform or a WD-40 repeat protein) or in the seed dehydration process (e.g. cytosolic glyceraldehyde-3-phosphate dehydrogenase). These facts highlight the power of proteomics to unravel specific features of complex developmental processes such as germination and to detect protein markers that can be used to characterize seed vigor of commercial seed lots and to develop and monitor

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priming treatments (Gallardo *et al*., 2001). Common waterhemp is an obligate outcrosser that has high genetic variability. However, under selection pressure, this weed shows population differentiation for adaptive traits. Intraspecific variation for herbicide resistance has been studied, but no studies have been conducted to determine the existence of variation for other adaptive traits that could influence weed management. The objective of this study was to examine the existence of different seed dormancy regulatory mechanisms in common waterhemp. Seed dormancy regulation, in response to different temperature and moisture regimes, was studied through germination experiments and proteome analysis using two common waterhemp biotypes (Ames and Everly) collected from agricultural fields in Iowa, and one biotype (Ohio) collected from a pristine area in Ohio. Without stratification, germination percentage among the different biotypes was 9, 29 and 88% for Ames, Everly, and Ohio respectively. The germination rate of seeds from Ames was dramatically increased after incubation at either 4 or 25 C under wet conditions, whereas germination of seeds from Everly was only increased at 25 C under wet conditions. The Ohio biotype showed no change in germination response to any of the incubation treatments. Germination studies indicated that the rate of seed dormancy alleviation differed between biotypes. Seed protein profiles obtained from the three biotypes differed in protein abundance, number, and type. A putative small heat-shock protein (sHSP) of 17.6 kDa and isoelectric point (pI) 6.1 increased whereas a putative glyceraldehyde-3-phosphate dehydrogenase (G3PDH) of 30.9 kDa and pI 6.4 decreased in abundance in the Ames biotype as seed dormancy was reduced in response to incubation at 4 C and wet conditions. These two proteins did not change in the Everly and Ohio biotypes, suggesting that these proteins changed their abundance in response to seed dormancy alleviation. The results of this study suggest that differences in seed dormancy levels between the biotypes were due to different physiological regulatory mechanisms (Leon *et al*., 2006). Poor germination and seedling establishment are major problems in arid and semi-arid environments, and these characteristics are considered to be important factors in later plant growth and yield. Laboratory experiments were conducted on freshly harvested pyrethrum (*Tanacetum cinerariifolium*) seeds to investigate the effects of light (influenced by the seeding method) and seed hydropriming on germination, and shoot and root growth at 25 °C. Exposure to light could reduce germination from 52% to 22% and increase the mean germination time (MGT) from 7 to 12 days. The responses of hydroprimed and unprimed seeds to salt and drought stress were determined at osmotic potentials of 0 (distilled water), −0.3, −0.6, −0.9, −1.2 MPa in NaCl and PEG6000. Seed germination and seedling growth were inhibited by increasing salt and drought stress. The germination percentage of unprimed seeds was reduced from 52% to 16% in −1.2 MPa NaCl, and no seeds germinated at osmotic potentials ≤−0.9 MPa PEG. Both shoot and root growth were inhibited at osmotic potentials ≤−0.9 MPa NaCl and ≤−0.6 MPa PEG. Hydropriming shortened the delay of MGT at all osmotic potentials, and improved the germination percentage in distilled water (from 52% to 59%) and resistance to salt stress with nearly double germination (from 16% to 29%) at the highest salt concentration. When non-germinated seeds were transferred to distilled water after 20 days of incubation in total up to 12–15% of NaCl and 25–27% of PEG stressed seeds did not recover. These results show that the inhibition of the germination and seedling growth at the same osmotic potential of NaCl and PEG resulted from drought stress rather than salt toxicity, and that hydropriming is an effective tool to improve the quality of pyrethrum seeds (Li *et al*., 2011). Therefore, the objective of this study was to evaluate the hydropriming influence on seedling growth in salvia (*Salvia officinalis* L.).

Materials and Methods

In order to the hydropriming influence on seedling growth in salvia (*Salvia officinalis* L.), this experiment was conducted in 2011 by a completely randomized design with four replications. The factor was including hydropriming (0(H1), 8(H2), 16(H3) and 24(H4) h) and then in the laboratory at each Petri dish 100 seeds were placed between two layers of paper culture and Petri dishes were placed in Germinator for 13 days at 20 to 22C. After 13 days, 10 seedlings were selected and was determined seedling length and then placed on electrical Owen for 48h at 75ºC and determined seedling weight by electrical scale. Finally, germination percentage determined for salvia by following formula:

(Number of Seeds Germinated / Total Number of Seeds on Petri Dish) * 100

Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System [SAS, 1988] and followed by Duncan's multiple range tests. Terms were considered significant at $P < 0.05$.

Results and Discussion

The results showed that the effect of hydropriming was significant on germination percentage, seedling length, seedling dry weight and seedling vigour in *Salvia officinalis*. Mean comparison showed that the highest

germination percentage, seedling length, seedling dry weight and seedling vigour were achieved by H4 and lowest germination percentage, seedling length, seedling dry weight and seedling vigour were achieved by H1.

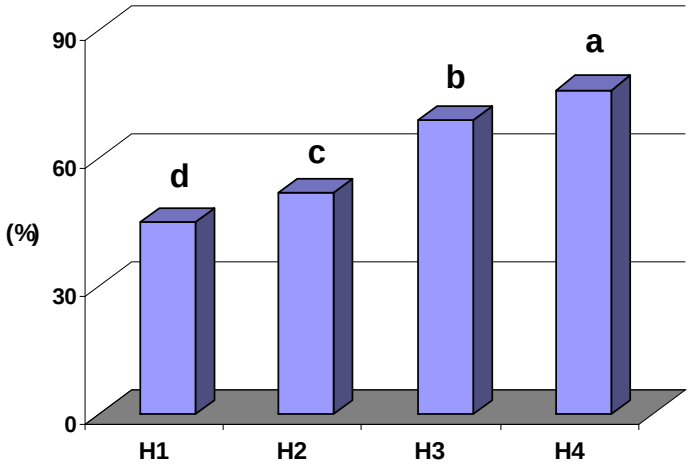

Fig. 1: Germination percentage under different levels of hydropriming.

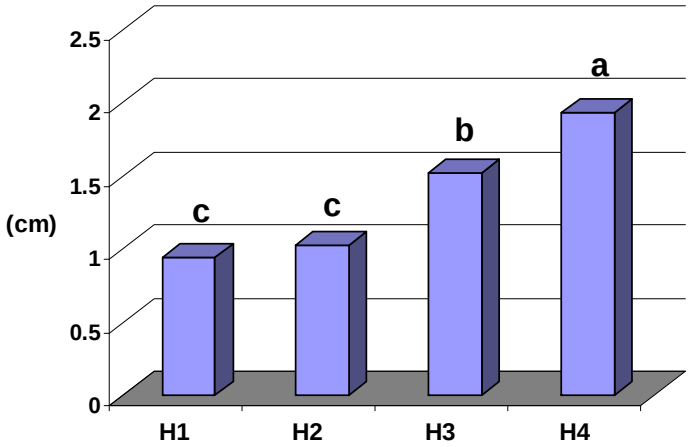

Fig. 2: Seedling length under different levels of hydropriming.

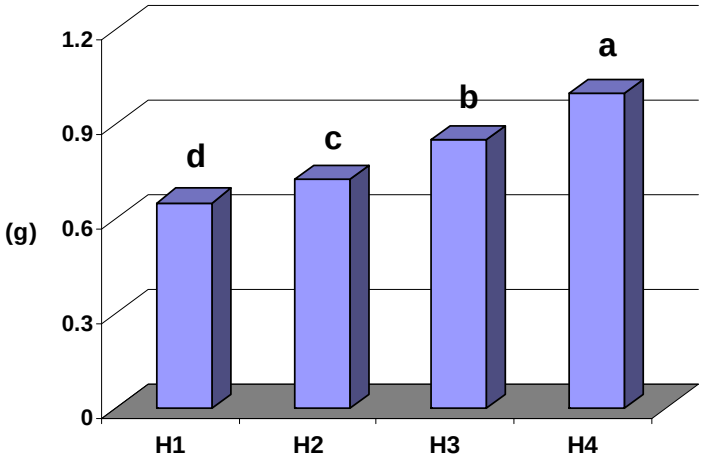

Fig. 3: Seedling weight under different levels of hydropriming.

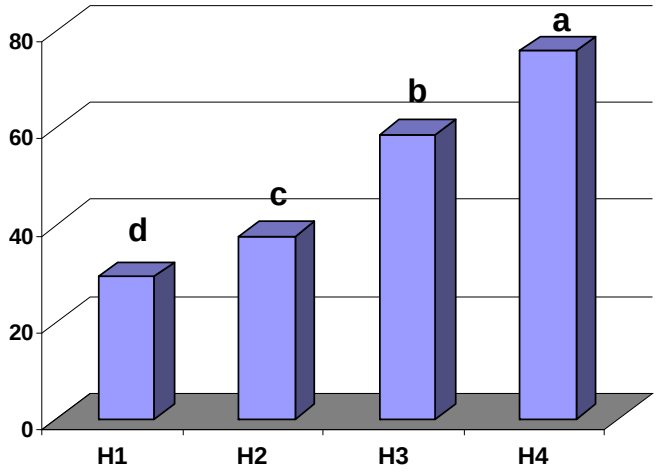

Fig. 4: Seedling vigour under different levels of hydropriming.

The results of this experiment showed that the use of hydropriming can increase the seedling vigour in salvia while the lowest of seed characteristics were achieved under no-priming. Knowledge and prediction of seasonal weed seedling emergence patterns is useful in weed management programs. Seed dormancy is a major factor influencing the timing of seedling emergence, and once dormancy is broken, environmental conditions determine the rate of germination and seedling emergence. Seed dormancy is a population-based phenomenon, because individual seeds are independently sensing their environment and responding physiologically to the signals they perceive. Mathematical models based on characterizing the variation that occurs in germination times among individual seeds in a population can describe and quantity environmental and after-ripening effects on seed dormancy. In particular, the hydrothermal time model can describe and quantify the effects of temperature and water potential on seed germination. This model states that the time to germination of a given seed fraction is inversely proportional to the amount by which a given germination factor (e.g., temperature or water potential) exceeds a threshold level for that factor. The hydrothermal time model provides a robust method for understanding how environmental factors interact to result in the germination phenotype (i.e., germination pattern over time) of a seed population. In addition, other factors that influence seed dormancy and germination act by causing the water potential thresholds of the seed population to shift to higher or lower values. This relatively simple model can describe and quantify the germination behavior of seeds across a wide array of environmental conditions and dormancy states, and can be used as an input to more general models of seed germination and seedling emergence in the field (Bradford, 2002). A laboratory experiment was conducted to evaluate the effects of hydropriming (3 and 6 h) and osmopriming (3 and 6 h KNO_3; and 1 h polyethylene glycol [PEG] 6000 at −0.2 MPa) on the germination of seeds from the safflower cultivar Kuseh. Hydropriming significantly improved the germination, germination rate, germination uniformity and shoot/root ratio, and decreased the time to 50% germination, compared to the control. Hydropriming for 3h and 6 h KNO_3 also significantly improved most of the germination parameters, but overall, the 6 h hydropriming treatment achieved the best results and therefore, this treatment was used to evaluate the effect of priming in a field experiment with a single seed lot from each of three cultivars (Kuseh, PI and ILIII). Hydropriming resulted in higher seedling emergence and an increase in seedling emergence rate for all three cultivars, although there was a significant priming × cultivar interaction. The greatest improvement in field emergence after priming was seen in PI, which also had the lowest initial germination. Hydropriming for 6 h may be used to improve field establishment of safflower. In addition, this treatment is simple, cheap and does not need expensive chemicals and sophisticated equipment (Ashrafi and Razmjoo, 2010). The objectives of a study were to evaluate the effect of hydro-priming and hydration media on the germination of dry bean cultivars. Seed performance was evaluated based on germination test**,** seed moisture content**,** electrical conductivity and water uptake. The study constituted two set of experiments. The result of the first experiment revealed that seed priming, cultivars, and their interaction were significant for percent germination at the 2nd, 4th and 8th day and normal seedlings percentage at the 8th day. Seeds failed to produce normal seedlings for both 4 and 8 h seed priming treatments, while the control (no priming treatment) produced large percentage of normal seedlings. The second experiment was designed to examine the cause for the failure of germination in the primed seed in the first experiment. There was significant difference between cultivars for germination percent at the 2nd day, while only the media of hydration was significant at the 8th day count. None of the factors were significant at the 4th day. There was significant difference among hydration media and cultivars for normal seedlings at the 8th day, while the interaction was non-significant. Completely immersing the seed in water-filled flask did not cause the failure of

germination in the first experiment; rather hydration followed by dehydration treatment was the possible cause. The better performance of the control in both experiments indicated that hydro-priming seems unnecessary in dry bean (Abebe and Modi, 2009).

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