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INFLUENCE OF SEED MOISTURE CONTENT AND SALICYLIC ACID TREATMENTS ON LOW AND FREEZING TEMPERATURE TOLERANCE OF FENNEL SEEDS

SUMMARY

Integrated effects of salicylic acid (SA) priming, seed moisture content (MC) and temperature (Tem) during storage were investigated on germination and seedling performance of fennel seeds. Seeds were primed in 12h at 25 °C in darkness containing one of the following solutions: 0, 100, and 200 ppm salicylic acid (SA) then the seed moisture contents were adjusted to 8, 15, 22, 29 and 36%. Following preparations, seeds were immediately stored at +10, 0 and -10 °C for 15 days then subjected to germination test at 25 °C. Results revealed that interaction of MC×SA and MC×Tem were significant for germination percentage. In comparison with control, germination percentage (GP) increased significantly at 15%MC for SA pre-treatments and after that point, GP decreased. Response of seeds was depending on SA concentrations and higher concentration had inhibitory effects on all traits. The higher the moisture content of the seeds the more they were adversely affected by temperature and when seed moisture contents were greater than 15%, seeds lost their viability at freezing temperature. Salicylic acid pre-treatments did not induce viability tolerance to subzero temperature and seed moisture contents played a more powerful role for seed viability at given conditions. It found that accompanying of high seed moisture contents with chilling temperature during seed storage not only increased the germination percentage but also improved the seedling length.

Keywords: *Foeniculum vulgare*, germination, salicylic acid, freezing stress, seed storage

INTRODUCTION

Fennel (*Foeniculum vulgare*) is a perennial medicinal herb from Apiaceae family and its leaves and seeds are used in the food and flavor industry (Khare, 2007). In many plants, the length of growing period plays an important role on yield potential because this factor causes better uses of resources, furthermore, if the canopy close earlier it will lead to better plant establishments and finally influence weed managements (Bradford, 2002). Generally, in the temperate and cold regions fennel is cultivated in spring. In this situation due to low

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temperature and continuous rain, sowing date is delayed so plant growth and development encounter with high temperature and radiation, therefore decreasing of plant performance is a usual event (Yadav & Khurana, 1999).

Response of seeds to low and freezing temperature is vary and dependent on many factors such as seed water content, seed structure, seed reserve compounds and compositions. Several studies showed that imbibed seeds were often less freezing tolerance than dry seeds (Vertucci & Farrant, 1995; Coursolle et al., 1998; Hawkins et al., 2003). Exposure of plants to sub-zero temperatures results first in extracellular ice formation, efflux of water, and cellular dehydration and the continued freezing period causes intercellular ice formation so plant cells will die (Hirt, 2004). The common results of abiotic stresses are the production of reactive oxygen species (ROS), which have a powerful damaging potential to different macromolecules in the cells, particularly to bio-membranes (Wang et al., 2009b).

In plants, complex defence systems share to alleviate stress conditions. One of the important systems is the phytohormonal involvements. Salicylic acid (SA) is one of the stress hormones that mediate activation of the defense pathways (Vankova, 2011). Lopez et al., (2008) reported that SA inhibits cell division in order to allow the reallocation of limited resources to energy costly defence responses. Salicylic acid (SA) not only is a responsive system to biotic stresses and pathogenesis but also recently studies has demonstrated that SA participates in the signalling networks (Hara et al., 2012). Salicylic acid acts primarily as a secondary signal for activation of antioxidant genes and induces stress resistance, and it might also function as an antioxidant to limit oxidative damage at the site of infection (Bai et al., 2009).

The influence of exogenous SA on plants differs by the specie types, plant stages, application modes, and the concentration of applied and endogenous SA levels (Hara et al., 2012). Several reports demonstrate that exogenous application of SA increases the cold tolerance of various species such as maize (Kang & Saltveit, 2002), potato (Mora-Herrera et al., 2005), winter wheat (Tasgin et al., 2006), grape (Zawoznik et al., 2007), rice (Wang et al. 2009a, 2009b) and cucumber (Lei et al., 2010). Senaratna et al., (2000) are shown that immersion of tomato and bean seeds in optimal concentration of SA, improved cold tolerance. Chen et al., (2011) showed that pre-treating of eggplant (Solanum melongena L.) with SA could enhance chilling tolerance by induction of gene expression involved in oxidative defence mechanisms. Application of SA enhanced expression of alternative oxidase pathway (Rhoads & McIntosh, 1992) and induces heat production (Raskin et al., 1987), these responses commonly perform under chilling stress (Moynihan et al., 1995).

The experimental data are rather controversial, and there is no general agreement about seed moisture contents and freezing temperature tolerance, also far too little attention has been paid to role of salicylic acid pre-treatments on freezing tolerance during seed storage. The aim of this paper therefore was to examine interactions among seed moisture contents, storage temperature and salicylic acid pre-treatments on fennel seed viability and seedling growth.

MATERIAL AND METHODS

To study the effects of seed moisture contents and salicylic acid pre-treatments on low and freezing temperatures tolerance, an experimental was conducted in University of Maragheh, Iran on seeds of Fennel at 2013.

The used seeds for this purpose were collected from same age plants. Number 2 umbel was used. Seeds were screened out based on size. For gaining seeds with same moisture content, seeds were kept up at the lab temperature for 2 months. At this time, the moisture content of samples was determined by ISTA, (2005) roles and 8% seed moisture was recorded. A subsample was isolated from this sample and was maintained in a sealed container at 4 ºC for further testes and also as a control.

Before the experiment, seeds were disinfected by sodium hypochlorite (5%) for 15 minute then rinsed with tab water 3 times. To obtain water absorption equation, a sample of seed lots were immersed in the water and percentage of absorption were recorded at 5 minutes intervals. Test showed that there was no problem in water absorption and its seed coat is permeable to water and during first minutes, seed moisture content reached to 60% in wet weight bases. Maximum water absorption (about 70%) achieved after 72 hours. For preparing salicylic per-treatments, seeds were immersed in 0, 100 and 200 ppm Salicylic acid solutions for 5 min then each seed lots treatment was divided into 4 parts to make different seed moisture contents (29, 22, 15 and 8%). To decrease seeds moisture, seeds placed on lab table at 24 ºC temperatures and in order to speed up drying process, a fan with 2 ms^{-1} velocity were used. Prepared samples were divided to 3 subsamples. Each subsamples were placed in sealed plastic containers and were kept for 2 weeks in chamber with different temperatures (-10 ºC and 10 ºC) to evaluate low and freezing effects on seed viability and seedling growth.

Germination trials were conducted in roll papers and moistened with sterile distilled water to ensure adequate moisture for the seeds. Treatments were arranged in factorial spilt plot on the base of randomized completely block with 3 replications of 25 seeds for each plot. Germination percentage, root and shoot length and other traits about germination behaviour were calculated.

Germination data was analysed using SAS software (version 9.1). One-way analysis of variance (ANOVA) was conducted and Duncan's multiple range tests at 5% level of significance, was used to separate means.

RESULTS AND DISCUSSION

Seeds with different moisture content (MC) showed different germination reaction (Table). By increasing of MC from 8% to 15%, first germination percentage (GP) slightly increased, however by further increasing at seed moisture content GP was reduced significantly, depending on MC contents. The similar results also observed on radicle and shoot length, shoot dry weight and seedling dry weights (Table). The ratio of shoot length to radicle length was not changed at different MC levels. The highest and the lowest shoot dry weight was recorded from MC 15% and MC 36%, respectively that had remarkably differences with other treatments (Table 1.). Seed moisture contents have a critical role in seed longevity especially at orthodox seeds, usually in these seeds with one percentage decreasing in MC, seed longevity is increased up to twofold (Hong & Ellis, 1996). Therefore seed viability is directly affected by MC during the seed storage. When seeds deteriorate during storage, they lose their viability also any declining at seed vigor levels is probable (Rajjou & Debeaujon, 2008), therefore later seedling growth of germinated seeds is influenced by early condition. Generally if the seed moisture content increases, the storage life will decrease. If seeds are kept at high moisture contents, the viability losses could be very rapid due to mould growth. Due to extreme desiccation at very low moisture content (below 4%), seeds will deteriorate rapidly. According to the Table, SA had an inhibitory effect on germination percentage, radicle dry weight, shoot dry weight and seedling growth. The inhibitory effects for germination percentage were also severed at higher concentrations. These results indicated that SA not only affected on seed germination but also future seedling growth parameters influenced by SA. The ratio of radicle length to shoot length remained constant.

Table 1. Means of germination percentage (GP), radicle length (RL), shoot length (SL), seedling length (SeL), ratio of shoot to radicle (S/R), radicle dry weight (RDW), shoot dry weight (SDW), seedling dry weight (SeDW)

	GP (%)	RL (cm)	SL (cm)	SeL (cm)	S/R	RDW (mg)	SDW (mg)	SeDW (mg)
MC(%)								
8	62.37a	3.76a	4.42a	8.18 a	1.006a	6.3a	19b	25b
15	66.66a	4.15a	4.48a	8.64 a	1.003a	7.4a	25a	32a
22	45.33b	2.51b	3.11b	5.63 b	1.005a	4.1a	16bc	20b
29	44.59b	2.87b	3.20b	6.08 b	1.003a	5.8a	19b	24b
36	39.25b	2.84b	3.38b	6.22 b	1.005a	7.5a	13c	21b
SA (ppm)								
0	56.17a	3.51a	3.89a	7.40a	1.0003a	8.8a	22.2a	31.0b
100	50.75b	3.03a	3.57a	6.60a	1.0005a	4.9b	17.2b	23.1a
200	48.00b	3.14a	3.71a	6.85a	1.0005a	5.0b	17.2b	23.2a
Tem (°C)								
-10	22.93c	1.53b	1.63b	3.17b	1.0009b	2.1b	7 c	2.8 b
0	68.35a	4.05a	4.92a	8.94a	1.0084a	7.9a	26a	33.9a
10	63.64b	4.09a	4.62a	8.72a	1.0052a	8.7a	23b	31.7a

Different letters in each column indicating significant difference at $p \leq 0.05$.

Germination percentage and seedling traits significantly affected by temperature. The highest values for germination percentage (68.35%) obtained from zero storage temperature that significantly was higher than that of the 10 ºC. the lowest germination percentage (22.93%) observed at -10 ºC. It showed that, fennel seeds have primary dormancy, so when seeds exposed to 0 ºC, dormancy released and germination was provoked. Baskin & Baskin, (2004) reported that seed primary dormancy is an intrinsic inhibition of early germination that can be released within a short period of moist stratification. In addition, subzero storage temperature meaningfully declined seedling growth parameters.

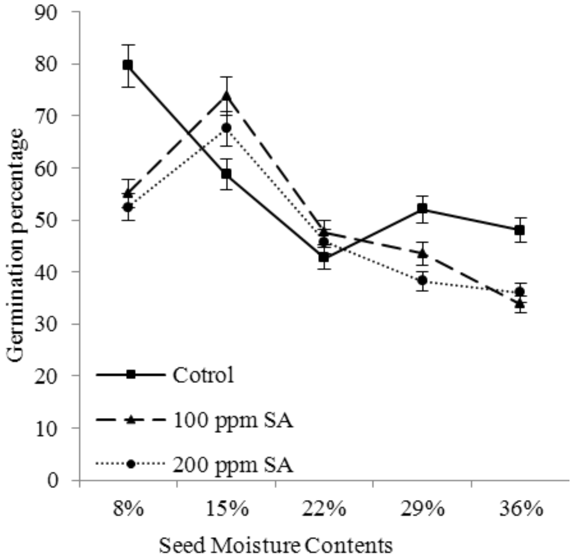

Figure 1. Interaction between moisture contents and SA pre-treatments on fennel seed germination, LSD(0.05)=5.6363

Analysis of variance revealed that interaction of MC × SA and MC × Tem for germination percentage were significant at $p \leq 0.05$. According to Figure 1, the highest value for germination percentage belongs to the control at 8% MC. However GP increased significantly at 15% MC for SA treatments and after that point, GP decreased sharply, whereas there was a gradually decreasing for control with MC increasing. Studies of Shakirova et al., (2003) determined that treatment of wheat seedling with SA causes an increasing on auxin and ABA levels but cytokinin did not change. Seed treatment with SA induced preadaptation response of barely to salt stress (El-Tayeb, 2005). Their finding illustrated that the membrane integrity and photosynthetic pigment stability improve by SA pre-treatments. Temperature plays an important role in life of seed. With regard to findings, the higher the moisture content of the seeds the more they were adversely affected by temperature. Decreasing temperature and seed moisture is an effective means of maintaining seed quality in storage.

With respect to Figure 2, the germination percentage increased to 77.33% at seeds that had 15% MC and stored at 0 ºC. The results clearly show if seed moisture content exceed from 15% and storage temperature be -10 ºC, seeds will lose their viability. But seeds with higher levels of water content could stay viable at 0 ºC and 10 ºC. The best temperature for seed storage with all seed moisture content was 0 ºC. Beside these results, a slightly declining at 36% MC was seen for seeds that stored at 10 ºC, probably at those situations, deterioration processes have begun and caused viability worsening (Figure 2). Influence of temperature on seeds with low moisture content is very little. when enzyme are dehydrated, they are much less susceptible to heat denaturation, and the dry state also prevents enzyme denaturation caused by freezing during winter in seeds, buds, and other part of perennial shrubs and trees (Salisbury & Ross, 1991). By further attention to the Figure 2, it looks that for high seed longevity, there was optimum water content for each storage temperature. This result is supported by finding of Buitink et al., (2000).

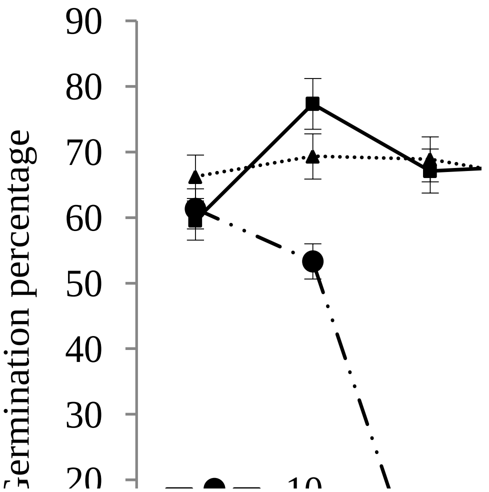

Figure 2. Interaction between moisture contents and different storage temperatures (ºC) on fennel seed germination, LSD (0.05)=5.6363

Similar results were also recorded for interaction between seed moisture contents and seedling morphology properties (Figure 3, 4 and 5). The superior values for radicle length, shoot length and seedling length were produced by seeds that had kept at 0 ºC with greater seed moisture contents. An increasing trend was visibly seen from 8% to 36% MC for those seeds. Whereas, we did not see a tangible trend for 10 ºC, however, a decreasing for values started at 36% MC. Due to killing effects of subzero temperatures at high MC, we had not data for those levels. These results in agree with finding of Ladror et al., (1986) that found, seeds of snap bean with high initial moisture (12%) perform better than the seeds with low moisture content (8%) at low temperature. They also reported that high moisture content is essential for maintaining the functional integrity of membranes that lead to chilling injury tolerance. McKersie & Leshem, (1994) reported that in seeds with low moisture content, water is either tightly bound to proteins and sugars or is able to form a glass phase, and thus does not freeze and damage cells. El-Maarouf-Bouteau & Bailly, (2008) reported that, ROS accumulation can be beneficial for seed germination and seedling growth, as a signal. In imbibed seeds, presence of free water and low cytoplasmic viscosity, would allow ROS to travel within the cell but in dry quiescent seeds, the absence of free water and an elevated cytoplasm viscosity probably limit the diffusion of ROS and reduce function of ROS in germination.

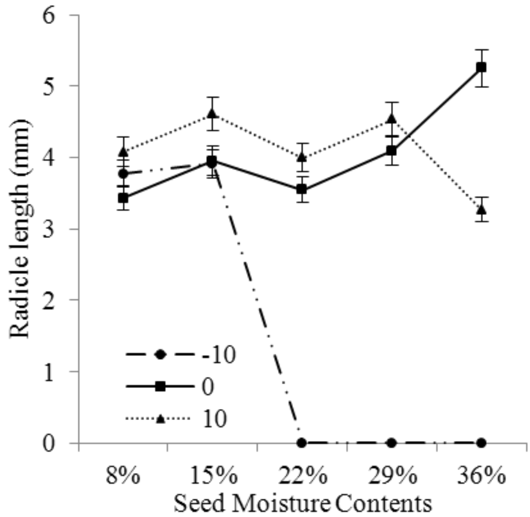

Figure 3. Interaction between moisture contents and different storage temperatures (ºC) on radicle length, LSD (0.05)= 0.6315

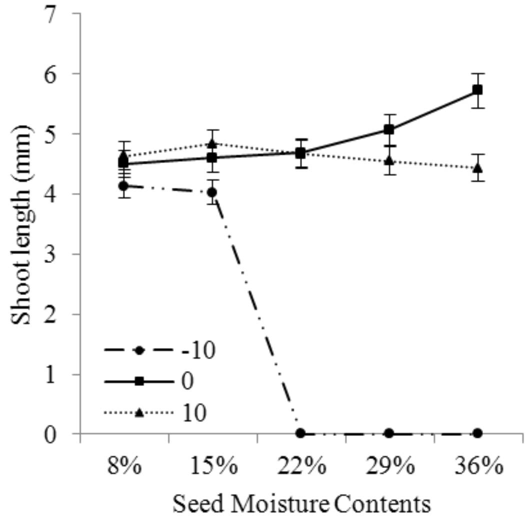

Figure 4. Interaction between moisture contents and different storage temperatures (ºC) on shoot length, LSD (0.05) = 0.5531

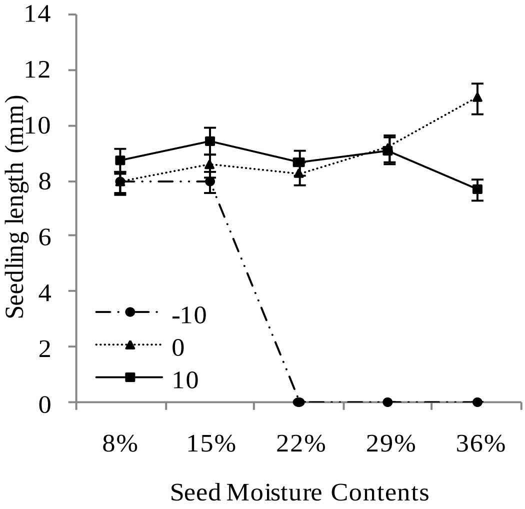

Figure 5. Interaction between moisture contents and different storage temperatures (ºC) on seedling length, LSD (0.05) = 1.0465

In general, in seeds with high moisture content, there is free and freezable water that cause ice generation in cytoplasm that could potentially destroy the biomembranes. It must be noted that with increasing the moisture content, seedling growth was promoted at 0 ºC. With regard to these results, the best condition for conservation in all moistures was 0 ºC, it seems that synchrony of water uptake and lack of deterioration factors (for example high temperatures) causes seed pre-germination processes completes in normal conditions therefore such seeds when puts in germination standard tests or seeding tests show a high performance.

CONCLUSIONS

In summary, our results revealed that SA could be effective in specific range of seed moisture contents. This range possibly is near to first stage of water imbibition (about 15%). Bio-membranes is one of the important site for SA effects. Therefore, at low seed moisture contents due to disrupting of membrane integrity not only an improvement on germination did not occur but also inhibition effects might be accelerated. Although at higher seeds moisture contents, the speed of seed deterioration was enhanced when moisture contents reach to 30%. It seems that application of SA is useful at a narrow range of moisture contents. Fortunately, at this range seed viabilities are not susceptible to low and freezing temperatures.

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UTICAJ SADRŽAJA VLAGE U SJEMENU I TRETMANA SALICILNOM KISELINOM NA TOLERANCIJU NA NISKE TEMPERATURE I TEMPERATURE ISPOD NULE KOD SJEMENA KOMORAČA

SAŽETAK

Ispitivani su integrisani efekti preparata salicilne kiseline (SK), sadržaja vlage u sjemenu (SV) i temperature (Tem) tokom skladištenja na klijanje i osobine klijanaca sjemena komorača. Sjemena su tretirana 12 h na 25 °C u mraku sa sadržajem jednog od sljedećih rastvora: 0, 100 i 200 ppm salicilne kiseline (SK), a zatim je sadržaj vlage u sjemenu podešen na 8, 15, 22, 29 i 36%. Nakon pripreme, sjemena su odmah skladištena na +10, 0 i -10 °C na period od 15 dana, a zatim su podvrgnuta testu klijanja na 25 °C. Rezultati su pokazali da je interakcija između SV×SK i SV×Tem bila značajna za procenat klijanja (PK). U poređenju sa kontrolom, procenat klijanja (PK) je značajno porastao kod 15% SV za prethodne tretmane SK-om, a nakon toga je PK opao. Reakcija sjemena je zavisila od koncentracije SK-a, a veća koncentracija je imala inhibicijski efekat na sve osobine. Što je veći sadržaj vlage u sjemenu veći je bio negativni uticaj temperature, a kada je sadržaj vlage u sjemenu bio veći od 15% sjemena su izgubile svoje životne aktivnosti na temperaturama ispod nule. Prethodni tretmani salicilnom kiselelinom nisu podstakli toleranciju životnih aktivnosti na temperature ispod nule, te je sadržaj vlage u sjemenu imao snažniju ulogu kod životnih aktivnosti sjemena u datim uslovima. Utemeljeno je da visok sadržaj vlage u sjemenu, praćen hladnoćom tokom skladištenja sjemena ne samo da je povećala procenat klijanja, već je takođe i poboljšala dužinu klijanaca.

Ključne riječi: *Foeniculum vulgare*, klijanje, salicilna kiselina, stres od temperature ispod nule, skladištenje sjemena