Effects of Desiccation, NaCl and Polyethylen Glycol Induced Water Potentials on the Sprouting of *Glycyrrhiza glabra* Rhizome Buds

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The effects of desiccation and NaCl and Polyethylen Glycol (PEG) induced water potentials on rhizome bud sprouting of the perennial weed, *Glycyrrhiza glabra* were investigated in separate laboratory experiments laid out in completely randomized designs. Each treatment was replicated four times. The water potentials of NaCl and PEG used were 0, -0.4, -0.8, -1.2 and -1.6 MPa. Desiccation experiments were conducted in factorial arrangements of treatments. Factors included two classes of rhizome diameter, thin (rhizome specific weight 0.05-0.4 g cm^{-1}) and thick (rhizome specific weight of 0.4-1.6 g cm^{-1}) and desiccation duration (exposing rhizome fragments to sunlight) of six levels (0, 6, 8, 12, 24 and 48 h). There were no significant differences between 0 and -0.4 MPa of PEG solutions. NaCl treatments from -0.4 to -1.2 and PEG treatments from -0.8 to -1.6 MPa drastically reduced rhizome bud sprouting and its rate. No bud sprouting was observed in –1.2 and –1.6 MPa of NaCl solution. For all treatments, NaCl was found to be more inhibitory than corresponding potentials of PEG solution. This

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can be attributed to the toxicity of NaCl ions. No bud survived after 48 and 36 hours desiccation in thick and thin rhizomes, respectively. Complete inhibition of sprouting occurred when rhizomes were desiccated to 42% and 70% of their original weight for thick and thin rhizomes, respectively.

Key word: Licorice, *glycyrrhiza glabra*, rhizome bud sprouting, desiccation.

در این تحقیق ، تاثیر خشک شدگی و محلول های پلی اتیلن گلایکول و سدیم کلرید هر یک با پتانسیل های ۰ ، ۰/۴-، ۰/۸-، ۱/۲- و ۱/۶- مگاپاسکال روی جوانه زنی ریزوم‌های علف‌هرز شیرین بیان (*Glycyrrhiza glabra*) در سه آزمایش جداگانه بررسی شد. در آزمایش خشک شدگی ، ریزوم ها به دو گروه نازک (وزن مخصوص ۰/۰۵ تا ۰/۴ گرم بر سانتی‌متر) و قطور (وزن مخصوص ۰/۴۱ تا ۱/۶ گرم بر سانتی متر) تقسیم شده و برای خشک شدگی برای ۰، ۶، ۸، ۱۲، ۲۴، ۴۸ ساعت در برابر هوای معمولی قرار داده شدند.

نتایج نشان داد که محلول سدیم کلرید در پتانسیل‌های ۰/۴- و ۰/۸- مگاپاسکال بطور معنی داری موجب کاهش جوانه زنی ریزوم‌ها شد و در پتانسیل‌های ۱/۲- و ۱/۶- مگاپاسکال باعث توقف آنها گردید. محلول پلی اتیلن گلایکول با پتانسیل ۰/۴- تاثیر معنی داری روی جوانه زنی ریزوم‌ها نداشت ولی در پتانسیل‌های ۰/۸- و ۱/۲- مگاپاسکال جوانه زنی راکاهش داده و در پتانسیل ۱/۶- آن را متوقف نمود. محلول سدیم کلرید ، در قیاس با محلول پلی اتیلن گلایکول با پتانسیل مشابه، تاثیر بیشتری درکاهش و یا توقف جوانه زنی ریزوم‌های شیرین بیان داشت که این ممکن است به سمیت یون‌های کلر و سدیم نسبت داده شود. درآزمایش خشک شدگی، جوانه زنی ریزوم‌های نازک و قطور به ترتیب ۳۶ و ۴۸ ساعت پس از خشک شدگی در رطوبت نسبی ۲۴٪ در دمای ۳۶-۲۹ درجه سانتی‌گراد در برابر هوای معمولی تقریبا متوقف شد. در آزمایش خشک شدگی، جوانه‌زنی ریزوم‌های نازک و قطورکه به ترتیب پس از ۳۶ و ۴۸ ساعت منجر به ۷۰ و ۴۲٪ کاهش وزن مخصوص آنها شده بود، متوقف گردید.

INTRODUCTION

While being a medicinal plant, Wild licorice (*Glycyrrhiza glabra* L.) is considered a noxious weed in some provinces of Iran. This plant is a rhizomatous perennial weed that spreads by seed and rhizome. Licorice competes with wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and alfalfa (*Medicago* spp.) because of its deep and extended rhizomes. Licorice rhizome bud activation and its growth rate are the two determining factors in the outcome of competition between this weed and its companion crop. Understanding the factors that influence the germination of licorice rhizome buds may help to find appropriate methods towards successful licorice management. Soil water potential can play an important role in bud germination. Barkat and Briske (1982) showed that water is the first and most important factor that affects germination. King and Oliver (1994) reported that the rate and percentage of germination increased as the availability of soil water increased. Large crabgrass (*Digitaria sanguinalis* L.) germinated at a soil water potential ranging from -0.03 to -0.1 MPa, ryegrass (*Lolium perenne* L.) germinated at a soil water potential of -1.5 MPa, but *Phalaris*

tuberosa L. did not germinate below -7.5 MPa. Koocheki and Shahroudi (1966) and Azarnivand and Jafarian Jolodar (2003) reported that the final germinating percentage and germination rate were reduced in chickpea and two species of quack grass (*Agropyron* spp.) by decreasing the water potential. Other studies show that decreasing the water potential leads to a reduction and/or delay in the germination of both halophytes and glycophytes (Katembe, 1998). NaCl, Polyethylene glycol (PEG) 6000 and mannitol can be used to create different levels of water potential. Katembe and Ungar (1998) reported that a higher concentration of NaCl (-1.5 MPa) was more inhibitory to imbibition and germination of *Atriplex* species seeds than that of iso-osmotic PEG. They also compared seed germination in *Atriplex* species at -0.25 and -1.0 MPa solutions of NaCl and PEG. The -1.0 MPa NaCl treatments resulted in significantly less germination than that of the PEG iso-osmotic solution. NaCl and PEG solutions with water potentials of -0.25 and -0.5 MPa did not significantly decrease the index germination rate (IGR) of species. At –1.5 and –1.4 MPa, both NaCl and PEG inhibited germination of *Atriplex* spp. (Katembe, 1998). Bajji and Kinet (2002) found that a water potential of -0.5 MPa caused delayed germination of *Atriplex halimus* L. seeds, and at higher water potentials, final germination percentages were drastically reduced (germination was lower than 10% in response to an external water potential of –2.1 and –2.9 MPa).

In many parts of the world hot and dry climates can be exploited to kill weed propagating organs like tubers, rhizomes, corms, etc. (Thomas, 1969). Johnson grass (*Sorghum halepense* L.) infestation may be reduced by dragging the rhizomes onto the soil surface using a sweep or a tiller during land preparation, as this facilitates desiccation by sunlight (Sullivan, 2002). Aldrich (1984) states that quack grass (*Agropyron repense* L.) is sensitive to desiccation. The rhizomes of couch grass are susceptible to desiccation and freezing. Desiccation of couch grass rhizomes can be accelerated by cutting them into fragments less than 5 cm in length. Rhizomes dried to 20% of their original weight lost their regenerative ability completely (Tu *et al*., 2001). Drying to less than 40% of the original

moisture content was lethal to all the Johnson grass rhizomes (Anderson *et al.*, 1977). Survival of Bermuda grass (*Cynodon dactylon* L.) rhizome fragments was greatly influenced by desiccation treatment, duration of desiccation treatment and the interaction of these two factors. No bud survived when rhizomes reached 50% of their original weight, indicating good tolerance to desiccation. The original dry-matter content of Bermuda grass (*Cynodon dactylon* L.) rhizomes in this experiment was 41.2%.

The regenerative ability of Honeyvine milkweed (*Cynanchum leave* L.) roots dried for 24 to 192 h at 5, 20, 30 ºC with relative humidity of 40, 22, and 30%, respectively, was also reduced. No shoots developed from roots dried for 24 h or longer at 20 or 30 ºC. 75% of the roots dried for 24 h at 5 ºC retained their ability to produce shoots, but this ability was lostwhen these roots were dried at 5ºC for longer periods. Root weights were also reduced by 34% following drying for 24 h at 5 ºC. Root weights were reduced more than 46% when dried for 48 h or longer at 5 ºC and 24 h or longer at 20 or 30 ºC (Soteres & Murray, 1982).

As yet no detailed studies on rhizome and drought stress tolerance have been found in the literature, the objectives of the present study were firstly to compare the effect of iso-osmotic concentrations of NaCl and PEG and desiccation on the germination of licorice rhizome buds. Secondly, to differentiate these osmotic effects from the toxic effects of Cl^- and Na^+ ions through comparison of NaCl with the metabolically inactive osmotic agent Polyethylen Glycol .

MATERIALS AND METHODS

NaCl and PEG Experiments

Wild licorice rhizomes were collected in 2003 from the Kamalshahr region, Karaj, Iran. The rhizomes were stored at +4 ºC. Before the experiment, the rhizomes were sterilized with 0.5% benzimidazole (benomyl) for 30 min, and then cut to the appropriate sizes. Buds sprouting tests were conducted using 9-cm sterile Petri dishes lined with two sheets of filter paper, with four replications. NaCl and PEG were used at five iso-osmotic concentrations corresponding to 0 (distilled water), -

0.4, -0.8, -1.2 and -1.6 MPa (Table 1). 10 rhizome buds were placed in each Petri dish, and then put in a germinator under dark conditions at 25 ºC. The number of sprouted buds was counted at 5 day intervals till the 24th day. The sprouting rate was calculated using the following equation:

$$R_S = \sum_I^N S_I / D_I$$

Where, R_s is the rate of sprouting, D_I is the day intervals and S_I is the number of sprouted rhizome buds in each interval.

Table 1**.** The amounts of NaCl and PEG used to prepare various osmotic solutions at 25 ºC.

Solution	Water potential (MPa)			
	-0.4	-0.8	-1.2	-1.6
PEG (g L^{-1})	178	262	326	381
NaCl (g L^{-1})	5.31	11.01	16.58	22.28

Both experiments (NaCl and PEG) were conducted using completely randomized design with four replicates. For statistical analysis, sprouting percentage data were transformed to arc $\sin\sqrt{x/100}$, then analyzed with SAS release 6.12 software programs.

Desiccation Experiment

This experiment was laid out in a completely randomized design with factorial arrangement of treatments and four replicates. Factors included two classes of rhizome diameter, thin (with rhizome specific weight 0.05-0.4 g cm^{-1}) and including thin (with rhizome specific weight of 0.4-1.6 g cm^{-1}), and desiccation duration (exposing rhizome fragments to sunlight) for (0, 6, 8, 12, 24 or 48 h). Rhizome fragments were weighed before and after desiccation, and their length was measured to estimate rhizome specific weight. Maximum and minimum temperatures and relative humidity during the desiccation experiments were monitored (Table 2). The rate of rhizome survival was measured by planting the treated rhizomes in sand pots. The pots were kept in the glasshouse at 25/18 ºC

(day/night) for 30 days. Then, the number of buds that had sprouted from each rhizome was counted. Analysis of variance was carried out on the percentage of buds sprouted and desiccation duration. The desiccation percentage threshold on which no rhizome bud sprouted was calculated using the polynomial equations for both thin and thick rhizomes (Figures 1 & 2).

Table 2**.** Temperature and relative humidity during desiccation experiment of *Glycyrrhiza glabra* L. rhizome buds

Date	Temperature (ºC)		Relative humidity (%)	
	max.	min.	max.	min.
6th August	39.2	26.8	34	16
7th August	39.0	27.6	38	10
8th August	39.4	25.8	22	13

RESULTS AND DISCUSSION

The effect of water potential concentration on bud sprouting was significantly different ($p< 0.01$) for NaCl and PEG. The final bud sprouting percentage and the rate of bud sprouting reduced as the PEG water potential decreased from 0 to -1.2 MPa (Figures 3 & 4). There were no significant differences between 0 and -0.4 MPa of PEG-induced water potential. There were significant differences among NaCl treatments, which is in concord with the results of Katemb *et al.* (1998). NaCl treatments from -0.4 to -1.2 MPa, drastically reduced the number of rhizome bud sprouting and their rate of sprouting (Figures 3 & 4). Bajji *et al*. (2002) reported the same result for *Atriplex spp* seeds. Maximum rate of sprouting occurred 10 to15 days after treatments (Figures 2). No bud sprouting was observed in –1.2 and –1.6 MPa of NaCl solution. For all treatments, NaCl was found to be more inhibitorier to water uptake than corresponding potentials of PEG solution, as was reported by Rahimian *et al.* (1989) and Katembe *et al*. (1998). However, our results do not agree with findings of Kafi and Goldani (2000) and Aparecida *et al.* (2003). The higher inhibitory effect of NaCl is believed to be related to the characteristics of NaCl, which can readily penetrate and move through the cell

membrane and enter the cytoplasm where the Na^+ or Cl^- can be toxic to cell organelles. In contrast, PEG cannot penetrate the cell wall and cell membrane.

Desiccation Experiment:

The duration of desiccation significantly reduced rhizome bud sprouting (Figures 1). However, rhizome specific weight did not have significant effects on rhizome bud sprouting. Sprouting decreased about 40% and 50% after 24 hours desiccation and no bud survived after 48 and 36 hours desiccation in thick and thin rhizomes respectively.

Complete inhibition of bud sprouting occurred when rhizomes were desiccated 42% and 70% of their original weight. Water losses were more rapid for thin rhizomes compared with thick ones. The water loss was rapid and then became gradual (Figures 2).

This experiment suggests that exposed rhizomes left on the soil surface for a relatively short period of time would essentially lose their ability to produce shoots and develop into new plants.

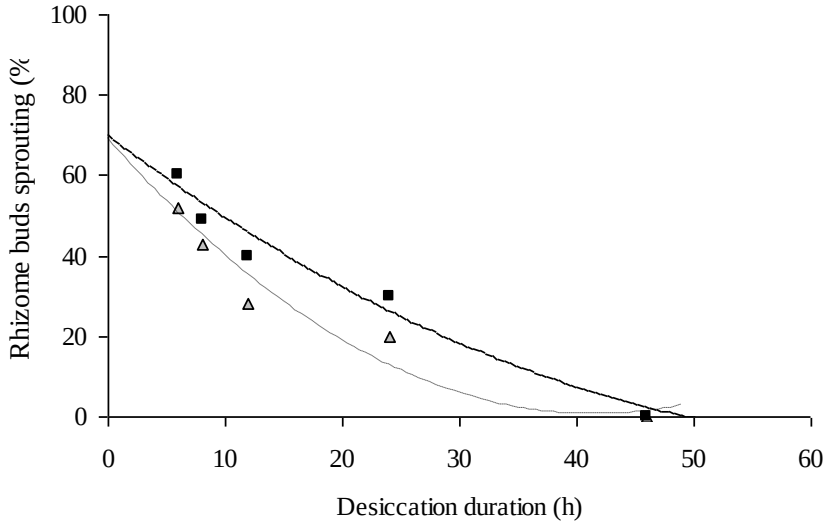

Figure 1. The effect of desiccation durations on bud sprouting of thin (Δ) and thick (■) rhizomes of *Glycyrrhiza glabra* L. (-----) represent fitted line for thin rhizomes where $y = 0.04x^2-3.3x+69$, $R^2 = 0.97$ and (—) represent fitted line for thick rhizomes where $y = 0.016x^2-2.2x+70$, $R^2 = 0.98$.

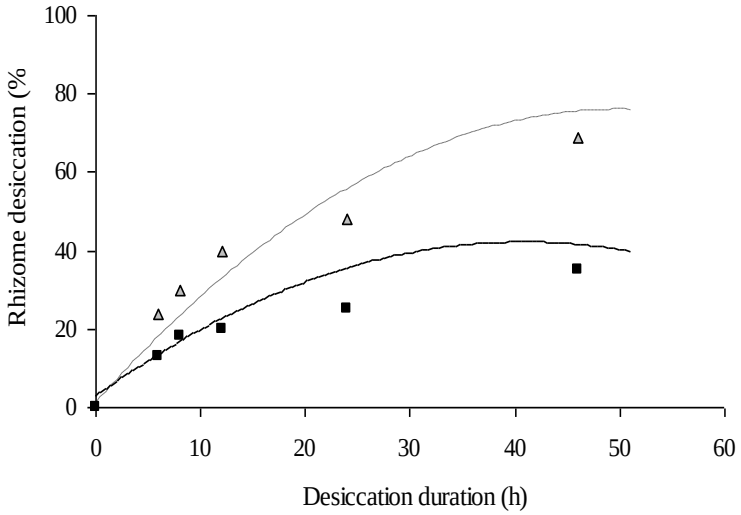

Figure 2. The relationship between rhizome specific weight of thin rhizome (Δ): 0.05-0.4 g/cm & thick rhizome (■): 0.4-1.6 g/cm of *Glycyrrhiza glabra* L. and desiccation percentage under different desiccation durations. (-----) represent fitted line for thin rhizomes where $y = -0.03x^2-3x+1$, $R^2 = 0.94$ and (—) represent fitted line for thick rhizomes where $y = -0.023x^2-1.89x+3.4$, $R^2=0.96$.

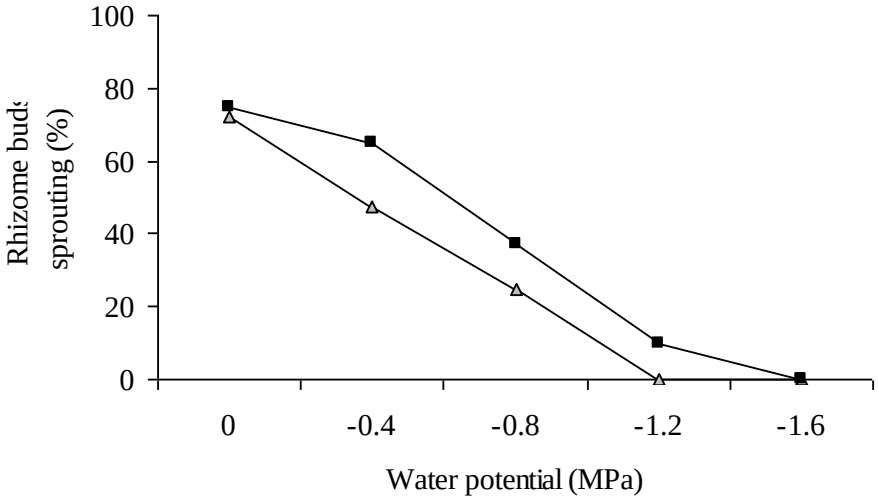

Figure 3. Comparison of rhizome buds sprouting of *Glycyrrhiza glabra* L. in different NaCl (Δ) and PEG (■) induced water potentials.

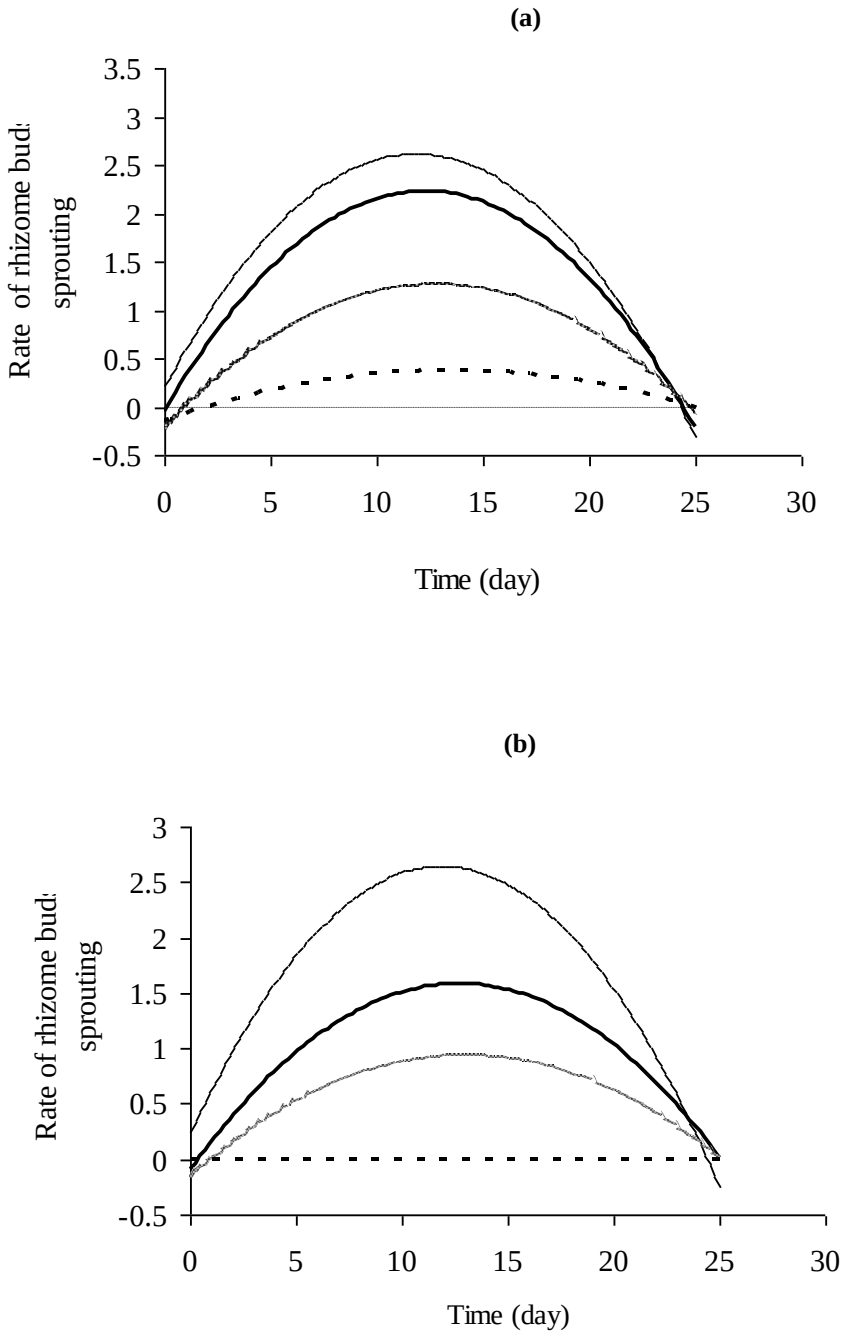

Figure 4. Comparison of rhizome buds sprouting rate of *Glycyrrhiza glabra* L. under influence of different water potentials induced by PEG (a) and NaCl (b) solutions. (_____), (▬▬▬), (.....), (– – –), (-------) represent fitted lines for 0, -0.4, -0.8, -1.2, -1.6 MPa.

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