



The impact of hydro-priming duration on seed invigoration and field emergence of milk thistle

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Abstract

Laboratory tests and a field experiment were conducted in 2015, to evaluate the effects of hydro-priming duration (0, 8 and 16 hours) on seed invigoration and seedling emergence of milk thistle. All laboratory tests were arranged as completely randomized design with four replications and the field experiment was carried out as randomized complete block design in six replications. Hydro-priming significantly increased seedling dry weight and seed vigor index, but reduced electrical conductivity of seed leachates and mean germination time. Seed priming had no significant effect on germination percentage. Invigoration of milk thistle seeds by hydro-priming resulted in significantly higher seedling emergence percentage and lower emergence time in the field, compared with unprimed seeds. The highest improvement in seed invigoration and seedling emergence was achieved by 16 hours hydro-priming. Thus, this hydro-priming duration could be used as a simple method for enhancing seed vigor and field emergence of milk thistle.

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Introduction

In recent decades, extensive research has been conducted on medicinal plants, and medicines that contain natural active ingredients have opened new horizons for pharmacists and researchers. Therefore, the pharmaceutical industry and research groups of many countries have turned their attention to the cultivation and production of medicinal plants. Milk thistle (*Silybum marianum* L.) is an annual or biennial plant of the *Asteraceae* family. This plant is known commercially as medicinal plant in Europe, China, Argentina and Iran, but it has been reported as a deleterious weed in some countries (Khan *et al.* 2009; Ghavami and Ramin, 2007; Zehtab-Salmasi & Moghbeli, 2008). Milk thistle is used for treatment of some disorders such as liver and gastrointestinal diseases and poisoning caused by several funguses (Dvorak *et al.* 2003).

Various seed priming techniques have been developed, including hydro-priming (soaking in water), halo-priming (soaking in inorganic salt solutions), osmo-priming (soaking in solutions of different organic osmotica), thermo-priming (treatment of seeds with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and bio-priming (hydration with biological compounds) (Ashraf & Foolad 2005). The beneficial effects of priming have been reported for many field crops such as mungbean (Ghassemi-Golezani *et al.* 2014), pinto bean (Ghassemi-Golezani *et al.*, 2010b), rapeseed cultivars (Ghassemi-Golezani *et al.*, 2013b), borage (Ghassemi-Golezani *et al.*, 2013a), maize (Parera & Cantliffe, 1994), sugar beet (Sadeghian & Yavari, 2004), and chickpea (Ghassemi-Golezani and Hosseinzadeh-Mahootchi, 2013). Hydro - priming markedly improved seedling establishment and early vigor of mungbean, resulting in faster development, and higher yields (Ghassemi-Golezani *et al.*, 2014).

The positive effects of hydro-priming on seed germination and establishment have widely been studied in different plant species (Ghassemi-Golezani *et al.* 2008a, b, 2010b). However, the response of milk thistle seeds to priming duration is not well understood.

Since germination percentage and seedling field emergence of milk thistle is poor (Sedghi *et al.*, 2010; Mokhtari-Karchegani *et al.*, 2014; Nasiri *et al.*, 2014), this research was carried out to evaluate the effects of different hydro-priming durations on seed invigoration and seedling emergence of milk thistle.

Material and methods

Seed preparation

Seeds of milk thistle (*Silybum marianum* L) were obtained from Pakan bazr, Esfahan, Iran. Experiments were conducted at the Seed Technology Laboratory and Research Farm of the University of Tabriz. Moisture content of the seed lot was determined as 5.4% by grinding the seeds and then drying at $103\pm 2^{\circ}\text{C}$ for 17 hours (ISTA 2010). Seeds were pre-treated with a mixture of Benomyl and Thiram fungicides at a rate of 3.3g/kg, in order to control possible fungal contamination during priming. Seed sample was divided into three sub-samples. One of the sub-samples was considered as control (unprimed) and the other two sub-samples were prepared for hydro-priming.

Seed priming

Two sub-samples were primed by soaking the seeds in distilled water for 8 and 16 hours. All priming treatments were performed in an incubator adjusted on $20\pm 1^{\circ}\text{C}$ under dark conditions. After hydro-priming, Seed samples were dried at $20-25^{\circ}\text{C}$ for 24 hours.

Laboratory tests

All laboratory tests were carried out as completely randomized design with four replications.

Conductivity test

Four replications of 50 pre-weighed seeds of each seed lot were soaked in 250 ml distilled water in plastic containers covered with caps to prevent evaporation loss and entry of foreign material. All the containers were incubated at 20°C for 24 hours. Conductivity was measured with an electrical conductivity meter (WTW 3110). The results were expressed on initial seed weight basis ($\mu\text{S}/\text{cm}/\text{g}$).

Germination and seedling growth

Four replicates of 25 seeds were germinated between moist double layered rolled filter papers. The rolled papers with seeds were put into plastic bags to avoid moisture loss. Seeds were allowed to germinate at $20\pm 1^\circ\text{C}$ in the dark for 21 days. Germination was considered to have occurred when the radicles were 2 mm long. Germinated seeds were recorded in daily intervals. Mean germination time (MGT) was calculated according to Ellis and Roberts (1980) as:

$$\text{MGT} = \frac{\sum(\text{D} \times \text{n})}{\sum \text{n}}$$

Where n is the number of seeds germinated on day D, D is the number of days counted from the beginning of the test. Seedlings with short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated (ISTA 2010). At the end of germination test (21 days), radicles and shoots were cut from the cotyledons and then dried in an oven at $75\pm 2^\circ\text{C}$ for 24 hours.

The dried radicles and shoots were weighed to the nearest milligram and the mean radicle and shoot dry weights and consequently mean seedling dry weight were determined. Seed vigor index (SVI) was estimated as:

$$\text{SVI} = \text{SDW} / \text{MGT}$$

Where MGT is mean germination time and SDW is seedling dry weight.

Field emergence

The field experiment was conducted as randomized complete block (RCB) design with six replicates. Each plot consisted of six rows, with 3 m length and 25 cm apart. Seeds were treated with benomyl at a rate of 3 g/kg before sowing. The seeds were sown at early May in a sandy-loam soil at a depth of about 3 with a density of 100 seeds/m². Number of emerged seedlings in each plot was counted in daily intervals until seedling establishment became stable. Seedling emergence time was calculated in accordance with the equation introduced by Ellis & Roberts (1980).

Statistical analyses

Analysis of variance (ANOVA) of the laboratory and field emergence data were carried out, using Gen Stat 12 software. Excel software was used to draw Fig.s. Means were compared by applying duncan test at 5% probability.

Results and discussion

Seed invigoration

Electrical conductivity of seed leachates was significantly ($P\leq 0.01$) influenced by seed hydro-priming time. Mean electrical conductivity of seed leachates significantly decreased as hydro-priming duration increased (Table 1). This result is in agreement with that reported for maize (Mir-Mahmoodi *et al.* 2011) and chickpea (Ghasemi-Golezani *et al.* 2012).

The effects of hydro-priming on germination time, seedling dry weight and vigor index of milk thistle were also significant ($P\leq 0.01$), but germination percentage was not significantly affected by seed priming ($P> 0.05$). Seedling dry weight and seed vigor index increased, but mean germination time decreased with increasing hydro-priming duration. In this study, 16 h of hydro-priming resulted in the lowest mean germination time and the highest seedling dry weight and vigor index (Table 1). These results are in agreement with the findings of Mir-Mahmoodi *et al.* (2011) in maize. Ghasemi-Golezani *et al.* (2008a) and Ghasemi-Golezani & Hosseinzadeh -Mahootchi (2013) also reported that hydropriming increased seedling dry weight of lentil and Chickpea, respectively. The positive effects of hydro-priming on seed invigoration are probably due to the stimulatory effects on the early events of germination process. When a dry seed is soaked in water, the uptake of water occurs in three stages: I) a rapid initial water uptake due to the seed low water potential, II) a less increase in water uptake, but physiological activities related with germination are initiated, and III) a rapid uptake of water as a result of radicle emergence. In hydro-priming, seeds are held at a water potential that allows stages I and II of imbibition to occur, but prevents radicle extension, and then seeds are dried back to the original moisture level.

Table 1. Means of seed quality parameters of milk thistle affected by hydro-priming duration in the laboratory.

Hydro-priming duration (h)	Electrical conductivity ($\mu\text{S}/\text{cm}/\text{g}$)	Germination (%)	Germination time (days)	Seedling dry weight (mg)	Seed vigor Index
0	55.52 ^a	76 ^a	5.038 ^a	9.58 ^b	1.902 ^c
8	21.24 ^b	80 ^a	4.036 ^b	10.83 ^b	2.704 ^b
16	12.06 ^c	83 ^a	3.226 ^c	14.66 ^a	4.542 ^a

Different letters at each column indicate significant difference at $P \leq 0.05$.

Field emergence

Hydro-priming had significant effects on seedling emergence percentage and time ($P \leq 0.01$). Seedling emergence percentage linearly and significantly increased with increasing hydro-priming duration (Fig 1a), but emergence time exponentially decreased, with no significant difference between 8 and 16 hours priming (Fig 1b). The highest emergence percentage and the lowest emergence time of hydro-primed seeds for 16 hours are clear indications of a positive effect of seed priming on improving and synchronizing seedling emergence of milk thistle in the field (Fig 1).

Kibite & Harker (1991) reported that seed hydration of wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and oat (*Avena sativa*) seeds improved the uniformity of seedling emergence. Harris *et al.* (1999) also found that hydro-priming enhances seedling emergence and early vigor of upland rice, maize and chickpea, resulting in faster development, earlier flowering and maturity and higher yields. These results suggest that hydro-priming is a useful technique for improving seedling vigor and establishment of milk thistle in the field.

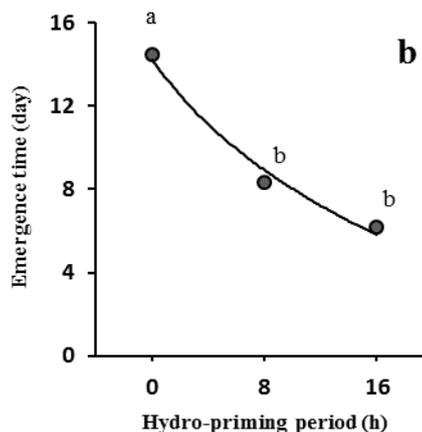
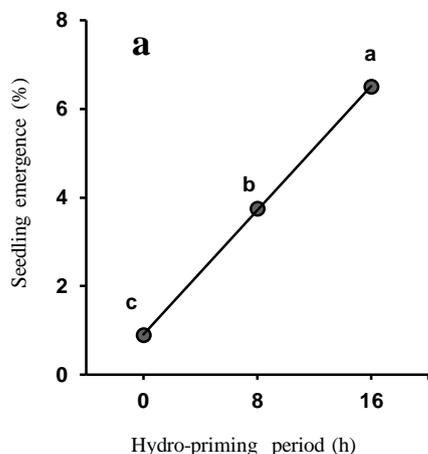


Fig. 1. Field emergence percentage (a) and time (b) of milk thistle in response to hydro-priming duration. Different letters indicate significant difference at $P \leq 0.05$.

Conclusion

Hydro-priming is helpful in reducing the risk of poor stand establishment under a wide range of environmental conditions. Our findings revealed that hydro-priming is a simple and very useful technique for improving seed invigoration and seedling emergence of milk thistle. These effects most likely continue to enhance field performance and yield of this important medicinal plant.

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