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Full Length Research Paper

Sugarbeet (*Beta vulgaris* L.) seed pre-treatment with water and HCl to improve germination

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This study was conducted to evaluate the effects of pre-sowing seed treated by soaking in distilled water and two concentrates of hydrochloric Acid (0.03 N HCl and 0.3 N HCl) at three different time (2, 4 and 6 h) by factorial arrangement based on completely randomized design with three replications in the University of Mohaghegh, Ardabili (Agronomy Laboratory). For each treatment, 25 uniform seeds were washed with distilled water, dilute hydrochloric acid and concentrated hydrochloric acid at 2, 4 and 6 h. Samples were sterilized using dilute sodium hypochlorite (2%) for 5 min. The treated and untreated seeds (control) were potted on sterilized Whatman paper in Petri-dishes. At the end of experiment, the percentage of germination, rate of germination, time of germination and T_{10} were evaluated. The results indicated that percentage of germination, mean time of germination, rate of germination and T_{10}, were affected significantly in all treatments in comparison with untreated seeds as well as the type and time of treatment. Results showed that both the type and time of treatment could improve the rate of germination. Soaking six hours with diluted acid (0.03 N) or with water, improved significantly the mean time of germination, rate of germination and T10 in comparison with untreated seeds. Result also indicated that seed treatment with water or 0.03 N hydrochloric acid for 6 h improved seed germination. Ultimately, the highest and lowest level of germination rate was obtained with soaking seeds in diluted acid (0.03 N) and concentrated acid (0.3 N), respectively. Therefore seed treatment with water could be low cost and more effective and thus could be suggested.

Key words: Sugar beet, germination, HCl, water, pre-treatment.

INTRODUCTION

Emergence of sugar beet (*Beta vulgaris* L.) seedlings is a major factor limiting satisfactory stand establishment. The variability in emergence is caused in part by differences in germination and seedling vigor among seed cultivar, or between cultivars. Factors affecting sugar beet seed germination and emergence include underdevelopment and immaturity of seed, presence of chemical inhibitors in pericarp tissue, physical impairment of germination by the pericarp, seed hardness and impermeability to water and oxygen (Khazaei, 2001; Akeson and Widner, 1980). Cultural practices, soil fertility, climate and maturity at harvest also influence germination and emergence of sugar beet seeds. Commercial processing and sizing may improve germination and emergence of seed by removal of pericarp tissue containing chemical inhibitors and by eliminating empty fruits or those containing poorly developed seed. However much of the acreage is overplanted and gap filled. Effective pre-germination seed treatments increase the rate of emergence and may improve uniformity of emergence and other number of seeds resulting in plants (Taylor et al., 1998). Pre-sowing seed treatments are use to leachout germination inhibitors, soften seed coats and advance physiological processes. Various methods such as soaking the seeds in water (Jun and Ling, 2004), osmotic solutions with known matric potentials (Kaffka et al., 1997), chilling and stratification (Schutz and Rave, 1999; Jun and Ling, 2004), temperature (Demel, 1996, 1998; Demel and Mulualem, 1996), physical scarification (abrasion, removal of seed coat layers), chemical scarification (with HCl, H_2SO_4 and HNO_3), growth regulators and sound stimulation pretreatment significantly affect seed germination (Duan et al., 2004; khazaei, 2001; Bockarie and Duryea, 1993; Durrant and Mash, 1991) and have been recommended for improve seed germination in dormant seeds. Pre-sowing seed treatments have been used in a number of

Table 1. Effect of seed soaking with water and HCl on sugar beet seed germination.

Parameter	Untreated seed	Distilled water	HCl (0.03 N)	HCl (0.3 N)	Significant
P.G %	85 ^{c}	88.3 ^{b}	91 ^{a}	87.7 ^{b}	0.00
R.G	2.75 ^{c}	3.69 ^{b}	4.98 ^{a}	3.54 ^{bc}	0.00
M.T.G	7.76 ^{c}	5.98 ^{b}	4.57 ^{a}	6.19 ^{b}	0.00
T_{10}	4.7 ^{c}	3.9 ^{b}	2.8 ^{a}	4.3 ^{b}	0.00

Where R.G = Rate of germination (germ(s) per day); M.T.G = mean time to germination; T_{10} = time necessary (in days) to reach 10% of final germination percentage.

crops to break dormancy or leachout chemical inhibitors from seed coat to get earlier and more uniform emergence.

About *Calliganum* seeds, researchers reported that the seeds require treatment before sowing to obtain rapid, uniform and high germination (Demel, 1996, 1998; Demel and Mulualem, 1996; Schutz and Rave, 1999). Bussell and Gray (1976) used pre-sowing seed treatments in tomato to improve seed germination and emergence. Soaking and drying of immature wheat seeds resulted in breakdown of abscisic acid (ABA), improving germination (King, 1976). *Dialium guineense* seed pre-treatment with concentrated sulforic acid could effectively improved seed germination (Bockarie and Duryea, 1993). Peto (1964) found out that sugar beet seed germination can be improved with dilute hydrochloric or sulforic acid treatment. Durrant and Mash (1991) reported that sugar beet seed treatment with 0.3 M hydrochloric acid improved seed germination. Akeson et al. (1980, 1981) showed that sugar beet seed treatment with dilute hydrochloric acid improved germination and emergence in laboratory and field experiment.

The purpose of the present study was to investigate the sugar beet seed germination to different pre-sowing seed treatment and find a commercial, effective and suitable treatment for improve sugar beet seed germination.

MATERIALS AND METHODS

This experiment was conducted in the agronomy laboratory of Mohagheg Ardabili University. Sugar beet seeds were subjected to three treatments including soaking in 0.3 N concentrated hydrochloric acid and 0.03 N diluted hydrochloric acid at three different duration of 2, 4 and 6 h by factorial arrangement based on completely randomized design (CRD) with three replications.

Percentage of germination, mean time of germination, rate of germination and T_{10} were investigated. Seeds were administered treatments at certain time (2, 4 and 6 h) on the shaker set. Then, seeds were washed with distilled water and then dried.

After seeds were dried, the seeds were surface sterilized by soaking in the 2% sodium hypochlorite (NaOCl) for 5 min and subsequently rinsed thoroughly with sterilized water.

All germination experiments were conducted using three replications of 25 seeds per each treatment. Three replications of untreated seeds were studied as control condition also. Seeds were placed on Whatman filter paper moistened with 3 ml of distilled water in sterilized Petri-dishes. Then Petri dishes were transferred to germinator with continual light and darkness (14/10), constant temperature of 25°C and relative humidity between 40 - 50%. Germinated seeds were counted every 24 h for 12 days. A seed was considered to have germinated when the tip of the radicle had grown free of seed coat. Percentage of germination, rate of germination, mean time of germination and T_{10} were counted. For each trial, the final germination percentage was calculated and arcsine transformed values were subjected to analysis of variance using software of SAS Institute (1985).

Comparison of means among results were carried out through the least significant difference (LSD) at the 1% level of probability.

Mean time-to germination (MTG) was calculated using equations 1 and 2 (Duan et al., 2004; Nadjafi et al., 2006).

$$\mathrm{MTG} = \sum_{i=1}^{14} \frac{ni.di}{N} \quad (1)$$

Where, n_i = number of germinated seeds at d_i days; d_i = incubation period in days at n_i ; N = total number of seeds germinated in the treatment.

Rate of germination (in germ(s) per day) was calculated using the following equation:

$$R.G = \frac{N}{M.T.G} \quad (2)$$

Where, R.G = Rate of germination (germ(s) per day); N = total number of seeds germinated in the treatment; M.T.G = Mean time to germination; T_{10} = time necessary (in days) to reach 10% of final germination percentage (Garcia et al., 2006).

RESULTS AND DISCUSSION

Germination percentage mean and other studied objects are given in Tables 1, 2 and 3. Comparison of means showed that, 0.03 N hydrochloric acid treatment signifycantly increased seed germination and rate of germination. Mean time for germination (days) improved in all treatments when compared to the untreated seed (Figures 1 and 2). Ultimately, the high level rate of germination was obtained by soaking seed with 0.03 N hydrochloric acid for 6 h (6.39 germs per day) in comparison with untreated seeds (2.73 germs per day), (Figure 3).

Seed treatment with dilute acid (0.03 N hydrochloric acid) for 6 h significantly improved seed germination whereas concentrated acid (0.3 N HCl) probably had side effect on seed embryo.

Presowing seed treatment in all time, enhanced germi-

Table 2. Effect of seed soaking time on sugar beet seed germination.

Parameter	Untreated seed	2 h	4 h	6 h	Significant
P.G %	85 ^{c}	88 ^{b}	89 ^{ab}	90 ^{a}	0.02
R.G	12.90 ^{c}	13.98 ^{b}	15.66 ^{b}	21.14 ^{a}	0.00
M.T.G	7.76 ^{c}	6.34 ^{c}	5.97 ^{b}	4.43 ^{a}	0.00
T_{10}	4.70 ^{b}	4.11 ^{b}	3.70 ^{b}	3.20 ^{a}	0.00

Where R.G = Rate of germination (germ(s) per day); M.T.G = mean time to germination; T_{10} = time necessary (in days) to reach 10% of final germination percentage

Table 3. Interaction effect of type and time of treatment on sugar beet seed germination.

Parameter	P.G %	R.G	M.T.G	T10
a1b1	86 ^{bc}	3.03 ^{e}	7.10 ^{e}	4.67 ^{d}
a1b2	87 ^{bc}	3.22 ^{e}	6.74 ^{e}	4.00 ^{cd}
a1b3	92 ^{a}	5.61 ^{b}	4.10 ^{b}	3.00 ^{abc}
a2b1	89 ^{ab}	3.71 ^{d}	6.00 ^{d}	3.67 ^{bcd}
a2b2	92 ^{a}	5.58 ^{b}	4.12 ^{b}	2.67 ^{ab}
a2b3	92 ^{a}	6.39 ^{a}	3.60 ^{a}	2.00 ^{a}
a3b1	89 ^{ab}	4.12 ^{cd}	5.39 ^{cd}	4.00 ^{cd}
a3b2	88 ^{b}	3.12 ^{e}	7.05 ^{e}	4.33 ^{d}
a3b3	86 ^{bc}	3.85 ^{c}	5.58 ^{c}	4.67 ^{d}
Untreated seed	85 ^{c}	2.73 ^{f}	7.76 ^{f}	4.67 ^{d}
Significant	0.02	0.00	0.00	0.03
CV %	9.8	7.29	4.11	17.41

Where R.G = Rate of germination (germ(s) per day); M.T.G = mean time to germination; T_{10} = time necessary (in days) to reach 10% of final germination percentage

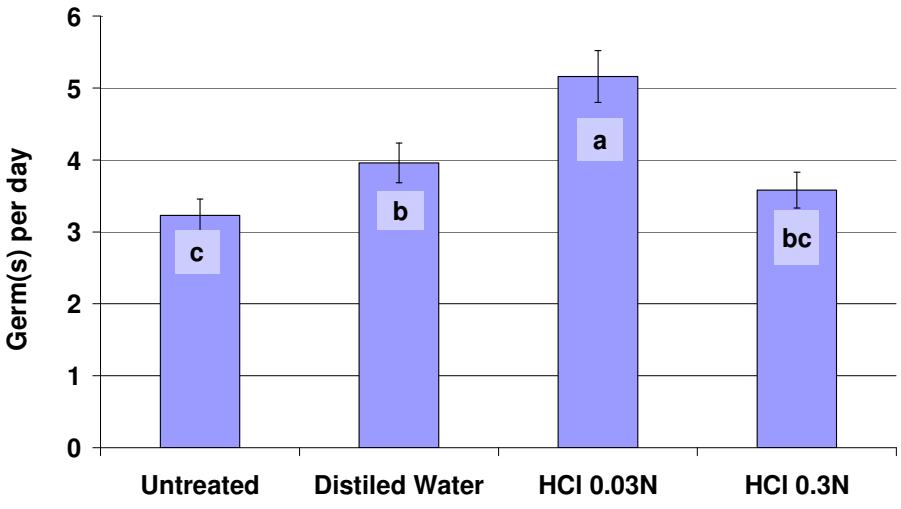

Figure 1. Effect of seed soaking with water and HCl on rate of germination (germ(s) per day).

nation. Result showed that seed treated with water or 0.03 N hydrochloric acid for 6 h improved seed germination (Figure 4). In this study, the cost of each treatment was important. Therefore seed treatment with water was found

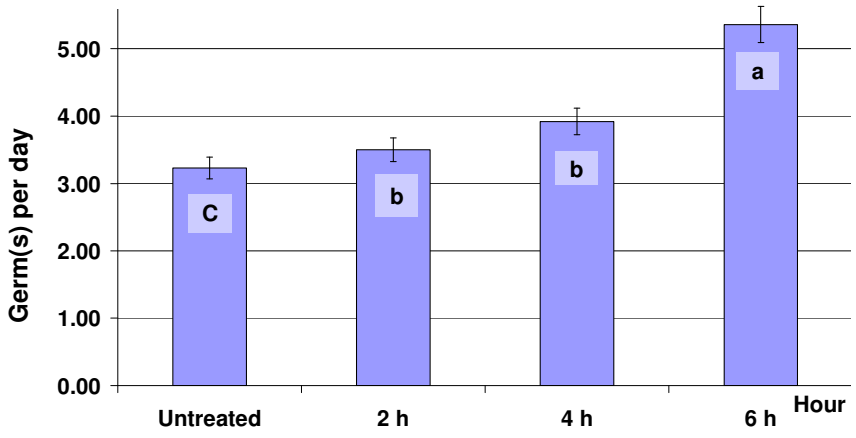

Figure 2. Effect of seed soaking time on rate of germination (germ(s) per day).

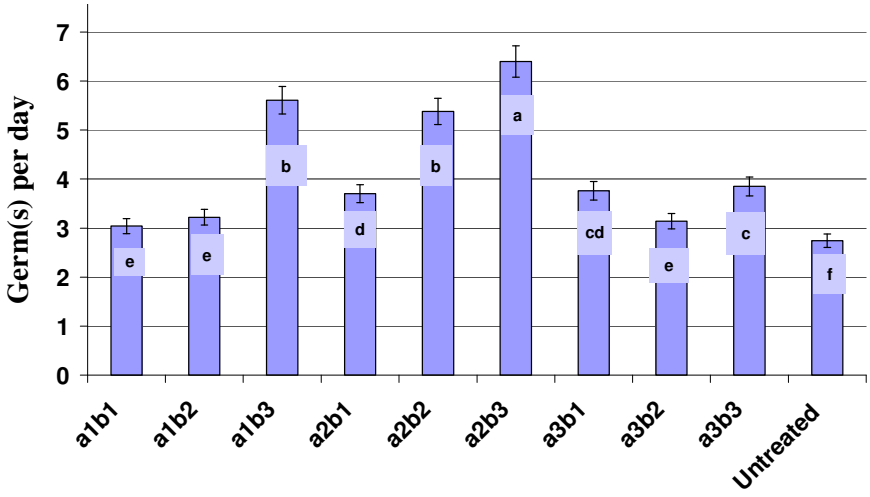

Figure 3. Interaction effect of type and time of treatment on rate of germination (germ(s) per day).

to be low cost, more effective and thus was suggested.

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