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Effects of Hydro-Priming and Hormonal Priming on Seedling Growth and Seed Germination of *Cucurbita pepo* Treated by Mercury

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Abstract

Several priming treatments have been used to increase germination, improve germination uniformity and seedling establishment under stressed conditions. A study was conducted to determine the impact of two priming techniques (hormonal priming and hydro-priming) on seedling growth and seed germination of *Cucurbita pepo* treated with different concentrations of mercury (0, 200, 500, 1000 and 2000 µM). Seeds of *Cucurbita pepo* were soaked in aerated solution of gibberellic acid, 2 mM (hormonal priming) and in distilled water (hydro-priming). The results show that the addition of Hg at 200, 500 and 1000 µM in the medium did not have a significant effect in the growth of no priming seeds of *Cucurbita pepo*. The phytotoxic effect of mercury on seedling dry weight was observed at high concentration, 2000 µM. Hydropriming and hormonal priming with gibberellic acid improved the seedling dry weight, the tolerance index and the T50 either in seeds of *Cucurbita pepo* treated or no with Hg. Similarly, results show that the hormonal priming induced by gibberellic acid enhanced the germination percentage of *Cucurbita pepo* as compared to hydro-priming seeds. It is recommended that hydropriming and hormonal priming be used as suitable priming techniques to enhance cucumber seed germination and tolerance on stress exposure.

Keywords

Hormonal Priming, GA_3, Hydro-Priming, Mercury, *Cucurbita pepo*

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1. Introduction

Mercury (Hg) is a global environmental pollutant that is present in soil, water, air and biota [1]. He is considered highly toxic to the growth of plants [2]. They produce toxic effects on the leaves where crucial functions such as photosynthesis and transpiration are carried out, cause morphological, anatomical and physiological changes, inhibit pollen germination and pollen tube formation and thus affect fruit production [3, 4]. The possible mechanisms of its phytotoxicity can be through the change of the permeability of cells membrane, affinity for reacting with phosphate groups, the replacement of essential ions and its ability to disrupt functions involving critical or non protected proteins [5, 6]. Many forms of Hg have been related to seed injuries and reducing seed viability. When Hg interacts with the SH groups to form the S–Hg–S bridge, disrupting the stability of the group, it can affect seed's germination and embryo's growth (tissues rich in SH ligands) [7].

Seeds performance of many vegetables can be improved during priming by soaking seeds in water or by inclusion of

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plant growth regulators and other pre-sowing treatments [8, 9].

Hormone pretreatment is a commonly used priming strategy to improve seed germination in stressful conditions [10, 11]. Plant growth regulators are organic compounds, which are produced in very small amount in plants and play an important role in growth, development and yield of crops and are becoming quite popular in the field of agriculture [12]. Gibberellic acid (GA_3), a phytohormone, is a tetracyclic di-terpenoid compound. GA_3 stimulate seed germination, trigger transitions from meristem to shoot growth, juvenile to adult leaf stage, vegetative to flowering, determines sex expression and grain development along with an interaction of different environmental factors [13].

The present study is conceived to evaluate the effect of hydro-priming and hormonal priming induced by GA_3 on seedling growth and seed germination of *Cucurbita pepo* treated with mercury (0, 200, 500, 1000 and 2000 µM).

2. Material and Methods

2.1. Plant Material and Priming Treatments

Cucurbita pepo L. (pumpkin) belongs to the melon family *Cucurbitaceae* which comprises approximately 95 genera and 950-980 species [14]. Seeds of *Cucurbita pepo* were soaked in distilled water (hydro-priming) and in a solution of GA_3 (2 mM) for 2h (25°C, dark). No-priming seeds (NP) were taken as a control.

After the priming treatment, the seeds were washed with distilled water and dried on filter paper at room temperature (in the shade).

2.2. Stress Induction

Seeds were allowed to germinate in plastic containers containing two layers of filter paper moistened with distilled water or a solution containing $HgCl_2$ with different concentrations (0, 200, 500, 1000 and 2000 µM).

The experiment was conducted in a growth chamber at 25°C. Throughout the tests, periodic watering by tested solution has regularly maintained the imbibition of seeds. A daily germination count (every 2 hours) was carried out for 9 days.

The germination percentage (GP) is the proportion, expressed as percentage of germinated seeds to the total number of viable seeds that were tested by following formula [15]:

$$GP = 100 \times (\text{Number of seeds germinated/total number of seeds})$$

Tolerance index (TI) was calculated using following formulae [16]:

$$TI = 100 \times (\text{Fresh weight of stress seedling/Fresh weight of control seedling})$$

The time to 50% germination (T50) was calculated according to the following formula of Coolbear et al. [17] modified by Farooq et al. [18]:

$$T50 = t_i + \{(N/2) - n_i\}\ (t_i - t_j)/n_i - n_j$$

Where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

2.3. Statistical Analysis

The data was submitted to statistical analysis by using analysis of variance one-way ANOVA Statistica 8 (Statsoft). Tukey's HSD test was performed to define which specific mean pairs were significantly different at $p < 0.05$.

3. Results

3.1. Seedling Growth

The results show that the addition of Hg at 200, 500 and 1000 µM in the medium did not have a significant effect in the growth of no priming seeds of *Cucurbita pepo*. While, the presence of 2000 µM Hg induced a decrease of seedling dry weight (Figure 1).

Generally, hydropriming and hormonal priming with GA_3 improved the seedling dry weight of *Cucurbita pepo* treated with Hg as compared to the control (no priming) (Figure 1).

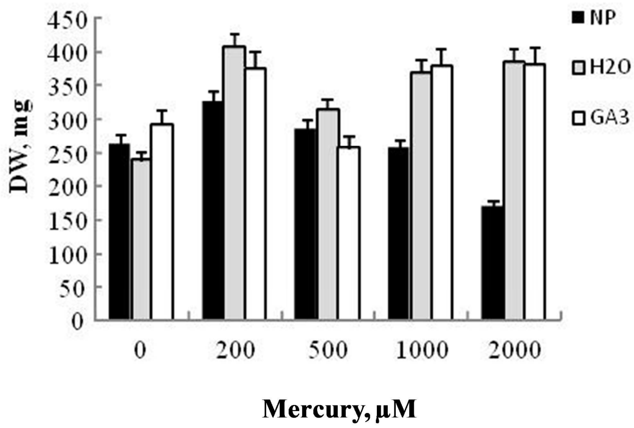

Figure 1. The dry weight of seeds per-sowed with no priming, hydro-priming and GA_3 priming in *Cucurbita pepo* treated with mercury.

3.2. Percentage of Germination

The results show that the percentage of germination in *Cucurbita pepo* was affected by elevation of Hg concentration in the medium mainly at high concentration more than 500 µM

(Figure 2). At 200 µM, we observed a stimulation of the percentage of germination in *Cucurbita pepo*.

The hydro-priming did not have a significant effect on the seed germination of *Cucurbita pepo* as compared to no priming seeds.

Interestingly, the percentage of seed germination in *Cucurbita pepo* was hardly enhanced by hormonal priming with gibberellic acid at 2 mM. In fact the GA_3 attenuated the effect of Hg mainly at 200 and 500 µM as compared to hydropriming seeds (Figure 2).

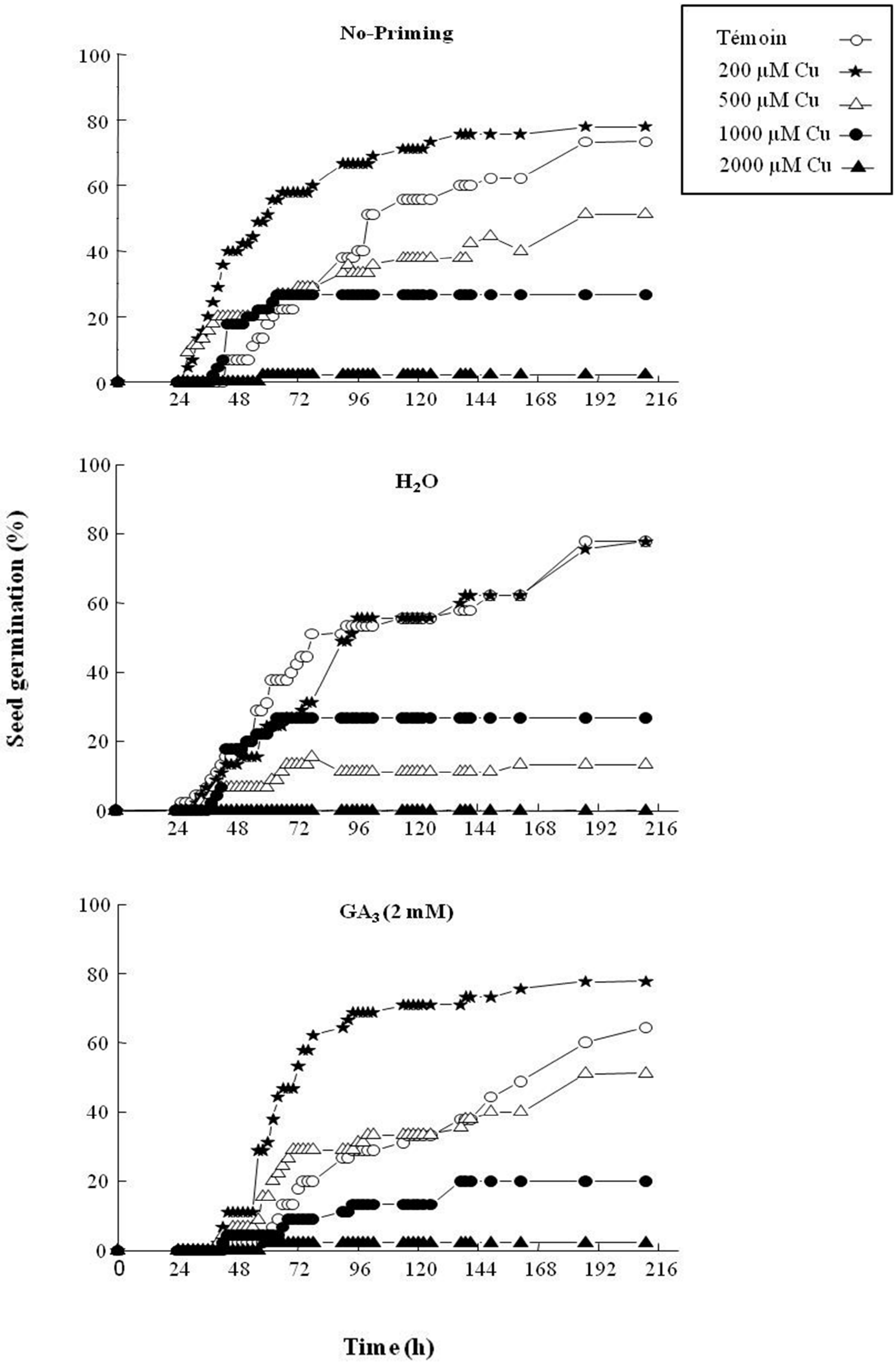

Figure 2. The percentage of germination in seeds per-sowed with no priming, hydro-priming and GA_3 priming in *Cucurbita pepo* treated with mercury.

3.3. Tolerance Index

The elevation of the Hg concentration in the medium induced a significant decrease of the tolerance index in *Cucurbita pepo* as compared to no priming seeds. Results show that the tolerance index was slightly improved by hydro priming and hormonal priming essentially in seeds treated with 200 and 500 µM of Hg (Figure 3).

3.4. T50

The presence of Hg at different concentrations (200, 500, 1000 and 2000 µM) in the medium significantly decreased the T50 in *Cucurbita pepo.*

The hydropriming improved the T50 only in *Cucurbita pepo* seeds no treated with Hg and in seeds treated with low concentration, 200 µM. Similarly, the hormonal priming with GA_3 enhanced the T50 in the no treated seeds and in seeds treated with low concentration of Hg (Figure 3).

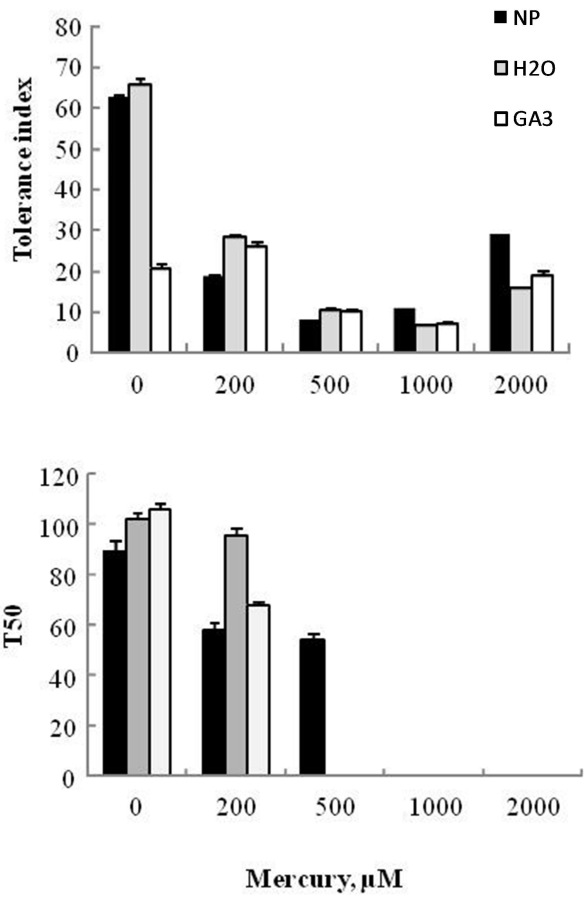

Figure 3. Tolerance index and T50 in seeds per-sowed with no priming, hydro-priming and GA_3 priming in treated with mercury.

4. Discussion

High concentration of trace metal elements produced toxic effects on seedling growth and can severely limit the yield [2]. In the present study, the seedling growth performance of *Cucurbita pepo* and the seed germination were tested in different concentrations of mercury (0, 200, 500, 1000 and 2000 µM). Our results indicated that the addition of mercury at 200, 500 and 1000 µM in the medium did not have a significant effect in seedling dry weight of no priming seeds in *Cucurbita pepo*. While, at high dose of Hg, 2000 µM, a decrease of seedling dry weight was signaled. Additionally, the results show that the percentage of germination in *Cucurbita pepo* was affected by elevation of Hg concentration in the medium. Similarly to our results, the seedling growth of *Albizia lebbeck* showed high percentage of tolerance to mercury at 1 mM concentration. A concentration of 7 mM of mercury produced a significant inhibition to seedling length as compared to control [2]. Mercury at high concentration decreased the seed germination of *Albizia lebbeck* [2]. The morphological parameters such as germination percentage, root length, shoot length; fresh weight and dry weight of seedlings were decreased with increasing dose of $HgCl_2$ concentrations in soybean [19].

Mercury treatment (1, 3, 5 and 7 mM) decreased seed germination, shoot, and root length and seedling dry weight in *Vigna radiate* [1]. According to Iqbal et al. [2], the inhibition due to the presence of mercury in the substrate provided evidence that the element in medium if present in excess may be inhibitory to plant growth and development.

Seed priming is a technique that can be applied to improve germination and growth in heavy metal-contaminated areas [20]. In this work, hydropriming and hormonal priming with GA_3 improved the seedling dry weight, the tolerance index and the T50 either in seeds of *Cucurbita pepo* treated or no with Hg. Similarly, Results show that the hormonal priming induced by GA_3 enhanced the germination percentage of *Cucurbita pepo*. This process may enhance the enzymes that are responsible for germination [21].

Primed seeds tend to show better germination and growth even when imposed to stressful conditions [20]. Although the mechanisms on how priming improves these parameters are still unclear, it has been suggested that the strategy activates a series of physiological processes that improve plant growth under stressful conditions [22], including the induction of antioxidant systems [23].

5. Conclusion

It is concluded that mercury treatment at 200, 500 and 1000 µM did not have a significant effect in the growth of no priming seeds of *Cucurbita pepo*. The phytotoxic effect of mercury on seedling dry weight was observed at high concentration, 2000 µM. Hydropriming and hormonal priming with GA_3 improved the seedling dry weight, the tolerance index and the T50 either in seeds of *Cucurbita pepo* treated or no with Hg. Similarly, the hormonal priming induced by GA_3 enhanced the germination percentage of *Cucurbita pepo* as compared to hydropriming seeds.

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