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TESTED SEED PRIMING METHODS TO STIMULATE THE GERMINATION OF *CITRUS LIMON* L.

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Abstract

The germination of exotic plants usually encounters difficulties. The research aim was to assess the effect of different seed priming methods on lemon germination. A number of 42 test tubes were filled with 11 cm of cotton wool and 1 cm of sterilized quartz sand. Each test tube contained one lemon seed. Three seed priming methods were tested respectively hardening with hot water, osmo-hardening with polyethylene glycol and hot water, hydro-priming, and a control treatment without priming methods. The assessments were made according to the BBCH scale of growth and development. The seeds from osmo-hardening treatments needed 42 days to reach stage 10 on BBCH scale, and the control needed two extra weeks. The hardening hot treatment and hydropriming seeds need 56 days to reach BBCH 09 secondary stage. A percent of 100% germination capacity was obtained to seeds from osmo-hardening, hydropriming and control treatments after 56 days. The most successful treatment in terms of secondary stages distribution was represented by the osmo-hardening, followed by hardening, then control, and the lowest result was in the hydro-priming treatment.

Key words: *breaking dormancy, hardening, hydro-priming, osmo-hardening; seeds.*

INTRODUCTION

Seed priming methods stimulate some metabolic processes necessary for germination without germination itself occurring (Marthandan et al., 2020). The most important processes that help rapid germination and increase seed tolerance to adverse environmental conditions are due to these seed priming methods most of the time (Raj and Raj, 2019; Singh et al., 2020). Seed priming was defined as pre-sown treatment in pure water or osmotic solutions, this hydration being sufficient to allow previous germinative metabolic processes but insufficient to allow radicle emergence through the seed coat (Bojović et al., 2022; Stoian et al., 2022).

The success of seed priming is influenced by the complex interplay of factors between plant species, the water potential of the priming agent, duration of pre-treatment, temperature, vigour, seed dehydration, and storage conditions of pre-treated seeds (Arun et al., 2022; Mal et al., 2019).

In general, the seed germination process in citrus is slow (Lugassi et al., 2020) and irregular due to the presence of inhibitory factors and the physical strength of the seed coat (Javed et al., 2022). One of the best seed priming method is represented by a controlled hydration pre-treatment in which the seeds are left to soak.

This process has an important role in improving the germination rate, germination uniformity, sometimes increasing the total germination percentage and more vigorous seedling growth (Thakur et al., 2022; Chakraborty and Dwivedi, 2021).

That is why certain seed pre-treatment techniques must be tested and selected to speed up or even halve the seed germination process.

Citrus fruits are currently consumed in large quantities due to the high content of ascorbic acid with therapeutic properties (Costanzo et al., 2020; Santos et al., 2021).

The most well-known and used citrus species is *Citrus limon* L. from the *Citrus* genus, *Rutaceae* family. Lemon is used for fruit consumption, due to the aromatic oils generally extracted from the mesocarp and seeds, as well as for ornamental purposes (Khan et al., 2021; Klimek-Szczykutowicz et al., 2020).

The aim of the study was to test three seed priming methods on lemon seeds to highlight the best method with high efficiency in germination time BBCH secondary stages.

MATERIALS AND METHODS

The experiment was set up at room temperature 20±2°C to simulate representative low-cost seed priming testing methods in order to obtain healthy and resistant seedlings in the shortest time. For this purpose, 78 seeds were used for seed priming techniques, respectively hardening with hot water (HH), osmo-hardening (O) with polyethylene glycol and hot water, hydro-priming (HY), and a control treatment (C) without priming methods. For the osmo-hardening priming technique, 2 ml of Fairy (polyethylene glycol) was added to 98 ml of warm water with a temperature of 50°C, then 12 seeds were placed in equal parts in the two Petri dishes. The osmotic solution was added to the Petri dishes until the seeds were completely immersed in the priming solution.

All seeds were left to soak for 24 hours

In the end, a number of 42 test tubes were filled with 11 cm of cotton wool and 1 cm of sterilized quartz sand. In order to highlight the tested seed priming methods, the germination capacity was assessed every two weeks at three time periods A1, A2, and A3. The first assessment was done after four weeks when the seeds start to germinate (A1). A2 - was the assessment at 6 weeks from the beginning of the experiment, and A3, respectively, at 8 weeks after the experiment starts.

The formula for germination capacity (%) was calculated following Bazai and Achakzai (2006):

*Germination capacity (%) = (Number of seeds germinated/total number of tested seeds) * 100.*

The detailed evaluation comprises also the secondary stages from the BBCH scale for lemon, separately for each treatment and all three assessments (Meyer, 2018).

RESULTS AND DISCUSSIONS

The lemon seeds' germination capacity was the highest at the third assessment A3 in the treatments with osmo-hardening, hydropriming, and control (Figure 1). After 4 weeks, at the first assessment (A1), the seeds from the osmo-hardening treatment presented a germination rate with 8% lower compared to the control. Then, this percentage increased after 6 weeks (A2) at 25% compared to the control. At the last assessment (A3) all the seeds from the osmo-hardening treatment succeed to complete the germination stage, at the same value recorded in control. The hardening with hot water was not so beneficial for lemon seeds. At the first assessment (A1), the difference compared to the control was by 50%. At the following evaluations the seeds started to germinate and the difference was 8% lower compared with the control treatment. The seeds from the hydropriming treatment were with 25% lower than the control in A1. After 6 weeks, the difference was with 8% (A2) and in the subsequent evaluation (A3), all the seeds succeeded to germinate (Figure 1).

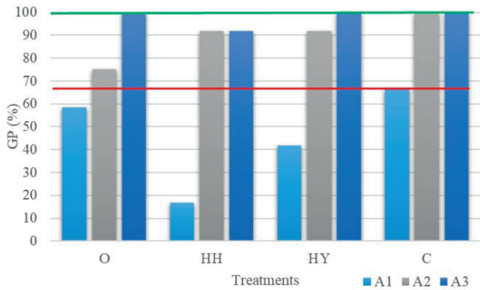

Figure 1. Germination capacity at all three assessments A1 after 4 weeks, A2 after 6 weeks, and A3 after 8 weeks for all treatments O-osmo-hardening, HH-hardening with hot water, HY-hydropring and C-control

All the seed priming methods gave positive results in terms of seed germination, except hydropriming (Figures 2, 3). At the first assessment, the osmo-hardening stimulated 41.67% of the seed to reach BBCH 03 stage. This number was lower with 25% compared with the control treatment, however, the control treatment seeds were not in other BBCH development stages. In the O treatment, 8.33% of the seeds evolved into both BBCH 05 and 06. Compared with the control, the O treatment produced seeds in 2 more developmental stages. At the second assessment, the same treatment (O), had 50% of the seeds in BBCH 03 and 8.33% in BBCH 05, BBCH 10, and BBCH 11.

The BBCH 03 was higher with 16.67 compared to control, while the BBCH 05 was lower with 25% compared with the control. Also, even the number of secondary stages present in the seed development of O and C was the same, the O jumped two secondary stages, so therefore the seeds were more evolved (Prasad et al., 2022). At the last assessment, 33.33% of the seeds were in BBCH 03 for O treatment. This percent was doubled for the same secondary stage from the control treatment. The secondary stages BBCH 05, BBCH 06 and BBCH 19 were each represented with 16.67% from the total share of the seeds. In the control treatment only BBCH 19 was present with 33.33% higher percent compared to O treatment. Only 8.33% of the seeds from O treatment were in BBCH 09 and the same percentage in BBCH 11. Hereby, 6 secondary stages were present, double the number present in the control treatment. This could be explained by the continuous beneficial effect of osmotic stress upon the seed and the stimulation in time of the embryo (Bafoil et al., 2019; Golmohammadzadeh et al., 2020).

The increased water temperature used for seed priming with (HH-hardening with hot water) did not had the same effect like the combination of osmotic solution with hot water (Figures 2, 3). The seeds at the first assessment (A1) of the HH treatment comprised 8.33% of the seeds in BBCH 03 and the same share reached BBCH 06. Only these two secondary stages were therefore present, with one extra compared with the control treatment. The second assessment (A2) highlighted 41.67% seeds in BBCH 05 with 8.34% higher than in control. The secondary stage BBCH 06 was represented by 33.33% of the seeds with 16.67% higher than the control. Also, the seeds in BBCH 03 were represented in a share of 16.67%, this time lower with 16.67% compared to control. It could be observed that even if the total germination capacity was higher at the second assessment in the control treatment, the HH seed priming method gave a fast development of the embryo (Raj and Raj, 2019; Dinesha et al., 2022; Arun et al., 2022). At the end of the experiment, the seeds from HH treatment were in BBCH 05 and BBCH 19 (33.33%), in BBCH 06 (16.67%) and in BBCH 09 (8.33%). Compared with the control, only BBCH 19 was with 16.67% lower in HH. The other two secondary stages present in the control treatment were BBCH 03 (66.67%) and BBCH 10 (16.67%). Therefore, the majority of the seeds could be considered over-evolved compared with the control treatment.

The hydropriming treatment (HY) had the closest results with the control treatment, being less efficient for lemon seeds (Figures 2, 3). At the first assessment (A1), the seeds were in BBCH 03 in a share of 41.67%, with 25% lower compared to control. At the second assessment (A2) in HY treatment the seeds were in 3 secondary stages BBCH 03 (41.67%), BBCH 05 (16.67%) and BBCH 06 (33.33%), missing BBCH 09, compared to control. These seed percentages share in HY were with 8.34% higher compared to control for BBCH 03, with 16.66% lower for BBCH 05 and with 16.66 higher for BBCH 06. At the last assessment (A3), the treatments shift trends compared with the previous one. From the 4 secondary stages present in HY treatments, a share of 33.33% of the seeds were in BBCH 03 and BBCH 19. The differences from the control were twice lower for BBCH 03 and with 16.67% lower for BBCH 19. Then, 25% of the seed from HY were in BBCH 05, and for the control treatment only 16.67% were in BBCH 10.

All tested treatments reached BBCH 19 after 8 weeks from the experiment start (Figures 2, 3). This aspect sustains the potential development of lemon seeds to succeed the same BBCH stage, independent to treatment. The differences between treatments are visible in the share of seeds that reach this final development stage, with more than 50% in control treatment. Both HH and HY treatments show a similar trend in BBCH stages at the end of the experiment, with BBCH 09 and 19 present. Compared to control, these two treatments present a shift only in BBCH 09 to BBCH 10 (control).

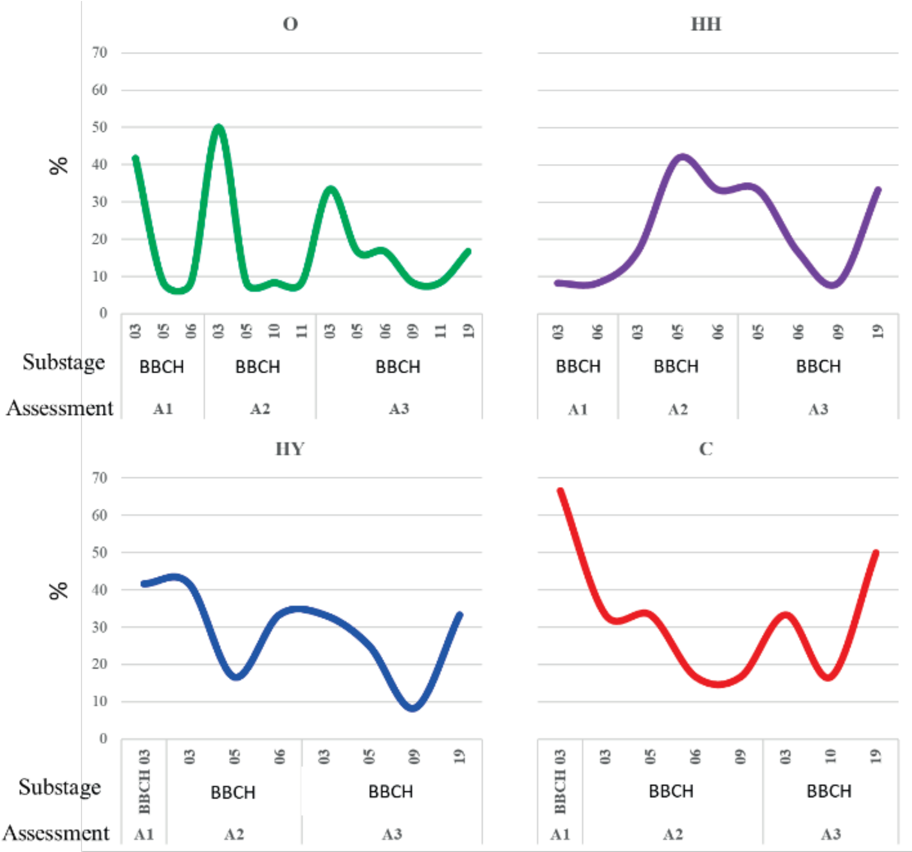

Figure 2. The percentages of germinated seeds for each BBCH sub-stages at all treatments from all three assessments

Figure 3. Phenological growth stages and BBCH assessed stages for *Citrus* spp. L. BBCH 03 - end of bud swelling: green scales slightly separated; BBCH 05 – radicle emerged from seed; BBCH 06 - elongation of the radicle; BBCH 09 - green leaf tips visible; BBCH 10 - first leaves separating: green scales slightly open leaves emerging; BBCH 11 - first leaves visible; BBCH 19 - first leaves fully expanded; classification used after Meier (2018)

The presence of BBCH 05, 06, 09, and 19 in HH indicates a gradual development evolution stimulated by this treatment, while the presence of 03 in HY-treated seeds indicates a slower development induced by this treatment (Figures 2, 3). An interesting case is visible in the O treatment, with BBCH 03, 05, 06, 09, 11, and 19 present at the end of the experiment. This indicates a step-by-step evolution of the germination process, with seeds gradually reaching each BBCH stage. The presence of numerous BBCH stages in this treatment sustains it as a solution to forecast the germination process, with the gradual development of embryos and plants.

CONCLUSIONS

The germination capacity was the highest in the control treatment starting with the day 42 of the experiment. This assessed parameter classify the priming methods on the second place the osmo-hardening, then hydropriming and at the end hardening with hot water.

All the tested seed priming methods highlight different embryo developmental strategies regarding the secondary stages present. The seeds from osmo-hardening treatments had at all the assessments the highest number of BBCH secondary stages present. Then, the following treatments in terms of seed stimulation were hardening with hot water, hydropriming and at the end the seeds from the control treatment with the lowest secondary stages present at the last assessment after 56 days.

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