Cold plasma treatment to improve wild asparagus (*Asparagus acutifolius*L.) seed germination.

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Abstract: In this work the effect of cold plasma treatment on the germination of wild asparagus was evaluated; a comparison was made with a commonly used technique to improve the germination. Cold plasma resulted more effective in increasing the germination percentage and rate and this could be due to an induced higher ability to uptake water and to a surface decontamination occurring during the plasma treatment.

Keywords: Cold plasma, seeds germination, wild asparagus, stratification, soaking.

1. Introduction

Wild asparagus (*Asparagus acutifolius*L.) is classified in the Liliaceae family that usually grows spontaneously in uncultivated areas. The species could have a high income potential due to its ability to adapt to marginal and arid lands. On the other hand, some limitations exist in the cultivation of this vegetable that contribute to its high cost on the market. The most important is related to its low and erratic seed germination that makes its cultivation economically disadvantageous. Several methods for promoting asparagus seed germination have been proposed in literature: different moist stratification methods (cold, warm, alternate temperature), soaking, hormonal, priming, etc. [1-3]. In the last decades cold plasma technology is gaining increasing interest in the agriculture scenario. [4-6] It is a fast, low-cost and pollution-free method to improve seed performance and crop yield by increasing seed germination. [7-10] This study evaluates the influence of cold plasma treatment with different gas composition and application time on germination of stratified wild asparagus seed. The results were compared to the soaking, one of the most frequently studied and/or used treatment. Before "in vitro" germination trials, the influence of stratification and of temperature on germination percentage were also evaluated.

2. Materials and Methods

The seeds used in this study were manually collected from Parco dell'Alta Murgia in Apulia (Italy).

Stratification was carried out placing the seeds in sand saturated with distilled water in plastic containers inside plastic bags to avoid dehydration of the substrate. They were, hence, stored for a period of 56 days at 25°C in the dark.

In order to study the germination process, the seeds were placed in Petri dishes with filter paper in the dark and at a fixed temperature and watered, every 2-3 days, with 3 mL of sterilized tap water. Germination percentage (G%) was calculated according to **Equation 1**.

$$G\% = \frac{n^\circ\, of germinated\ seeds}{total\ n^\circ\, of seeds} \cdot 100 \qquad (1)$$

The effect of the temperature and of the stratification step on the germination was evaluated. Both fresh (just after the collection) and 2-months stratified seeds were tested. The germination percentage at different temperature values (5, 10, 15, 20, 25, 30, 35 °C) was evaluated over 9 months (30 seeds x 3 replicates were tested for each condition).

The effect of soaking in tap water, polyethylene glycol (PEG 6000) and gibberellic acid (GA3) on G% was evaluated on 2-months stratified seeds. To this end, 90 seeds for treatment were soaked respectively in 50 mL of sterilized tap water, 0.5 M PEG 6000, and 300 ppm GA3 for 3h at 35°C. (30 seeds x 3 replicates were tested for each condition).

For plasma treatments, the gases that compose the atmosphere were chosen: oxygen (Air Liquid 99,999% purity), nitrogen (Air Liquid 99,999% purity) and a mixture of the two gases with a ratio of 20-80% to mimic the air composition. Three different treatment time were chosen for each gas feed: 1, 15and 30min. A pressure of 800mTorr and a power of 50W was used. These trials are carried out on 2-months stratified seeds (for each treatment 30 seeds x 3 replicates were considered).

To evaluate both the effect of soaking and plasma treatment, the germination percentage, the mean time germination (MTG) and the time necessary for 50% of the

seeds to germinate (T50) were monitored over 150 days. Over the whole germination time, seeds were kept at a constant temperature of 15 °C in dark conditions.

Water uptake trials were performed on 2-months stratified seeds both untreated and plasma treated with O_2-N_2 gas mixture for 1 min. Different immersion times in sterilized tap water were evaluated: 1, 3, 6, 24, 48, 72 h (20 seeds x 3 replicates were tested for each condition).

3. Results and discussion

From the study of the effect of temperature and the stratification step resulted an optimum condition that was consequently applied in the subsequent tests: 2-months stratified seeds kept at 15°C during germination measurements.

In **Figure 1** it can be seen how between the seeds soaked, only the ones soaked in water show a higher final G% value if compared to the control, lower values result from seeds soaked in PEG and GA3.

Regarding the effect of plasma treatment, the best choice for gas feed resulted to be O_2/N_2. As it can be seen in **Figure 1**, the shorter discharge duration (1 min) lead, not only to a final G% value higher than the control and the sample soaked in water, but to a lower T50 (29±3 days for the control, 35±4 days for the sample soaked in water and 24±2 for the sample plasma treated with O_2/N_2 for 1 min).Therefore, this specific plasma treatment can increase the total percentage of germinated seed and can accelerate the germination process.

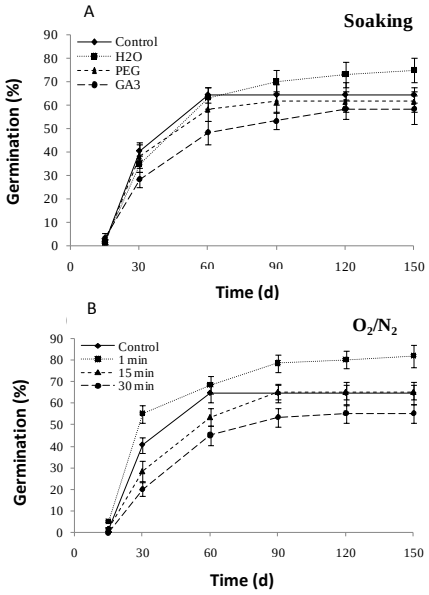

Fig. 1 Germination percentage in time for seeds treated by a) soaking in H_2O, PEG and GA3, or by b) O_2/N_2 plasma for 1min, 15min and 30min.

On this specific sample the water uptake was measured in order to understand if the faster and higher germination might depend on a better ability of the plasma treated seeds to absorb water. It resulted a slightly higher water uptake value for the plasma treated seed when compared to the control. Another factor that can contribute to the higher G% is the decontamination from moulds and fungi operated by plasma on the seed surface. This effect is vastly documented in literature and it was clearly visible on the samples during the germination. [11-13] The contamination can, in fact, decrease the number of viable seeds, therefore reducing the percentage of seeds able to germinate.

In conclusion, plasma treatment allows for a faster and improved germination of wild asparagus if compared to the untreated seeds. Moreover, plasma treatment results more efficient of a commonly used method as the soaking procedure.

4. References

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