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# Effect of Different Priming Treatments on Quality of Long-Term Stored Seeds of *Apium graveolens* var. *rapaceum* DC

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## Abstract

In order to improve germplasm preservation of local cultivar "Friulano", belonging to *Apium graveolens* var. *rapaceum* (Miller) Gaudin) seed germination of a seed lot, stored for prolonged time at -20°C, was assessed in comparison to that of a commercial cultivar. In order to increase the seed performance during germination, three different priming pre-treatments (hydropriming for 24 h, osmopriming for 24 h, and osmopriming for 7 days) were performed. Additionally, seed sowing was undertaken on both potting soil and Petri dish plate condition. Analysis of seed quality was estimated by measurement of germination percentage, germination energy and the time to reach the 50% of full germination (T50). The results evidenced that conservation of celeriac dried seeds of "Friulano" cultivar at -20°C for one year maintained a good level of seed germination (ranging from 38% to 53%), not statistically diverse from the that of commercial cultivar, for both short hydro- and osmopriming treatments. The germination percentage increased when primed seeds have been treated in Petri dishes (up to 70%-80%). Furthermore, osmopriming for 24 h positively affected the germination energy, similarly to that observed in commercial cultivar, being statistically higher compared to hydropriming- and prolonged osmopriming-treated samples. Accordingly, T50 in short osmopriming treated seeds was low, indicating that this treatment induced a rapid and uniform germination, being most of the seeds germinated within one week. In conclusion, these findings represent an important informative step in amelioration of *ex situ* conservation of a rare autochthonous horticultural cultivar in North-eastern of Italy.

**Keywords:** *Apium graveolens* var. *rapaceum* (Miller) Gaudin; Seed germination; T50; Priming; NaCl

**Abbreviations:** DW: Dry Weight

## Introduction

Celeriac or celery root (*Apium graveolens* var. *rapaceum* (Miller) Gaudin) cultivar "Friulano" represents an old autochthonous local cultivar, selected some decades ago deriving from the Dutch cultivar "Gigante di Praga" by local family farmers in Northern Italy. This biennal cultivar showed a good resistance to cold and pest, and it is characterized by a bitter and slightly spicy taste of tap root (ERSA). The species, however, presents poor and not homogenous germination rate [1], causing a great limitation in its germplasm preservation and in the expansion of its horticultural market. According to seed germination manual rules [2], *Apium graveolens* seeds stored for six months in dry conditions showed 100% of germination within 6 to 14 d, whereas prolonged storage at 21.1°C (up to 3 years) drastically reduced germination level to 3% in 10-18 d.

For many horticultural species and crop seeds, the technique of seed pretreatments (e.g. water, salts, or osmolytes) prior to germination, named seed priming, has been revealed to be an effective operation which allows to induce a uniform and synchronized germination rate, an improved stand establishment, more tolerance to drought or salinity, and an increased crop yield [3,4].

Few past studies reported some improvements on T50 (days to reach the full germination level) of the celery seeds, without noticeable reduction in germination percentages, by using cryopreservation technique for conservation or priming pre-treatments with salt solutions of $NaNO_3$ or $KNO_3$ or osmopriming [5,6]. Osmopriming with polyethylene glycol 6000 solution for up to 10 days, followed by drying back, substantially lowered mean time germination time of fresh celery seeds, compared to hydropriming [7]. For both primed or pelleted celery and celeriac seeds devoted to genebank establishment, Groot et al. [8] suggested that avoidance of oxygen should be taken into account during dry and cool conservation, in order to prolong the longevity of seed.

In order to evaluate the tolerance of seeds of the local cultivar "Friulano" to prolonged storage at temperature of -20°C for one year, celeriac dry seeds harvested in 2014 were compared in germination performances to those of a commercial seed lot, maintained at room temperature. Different hydro- and osmopriming treatments were tested in seeds germinated both in potted soil or Petri dishes for 46 d at 25°C and 70% RH with 16 h light period).

## Materials and Methods

### Plant material

Experiments were conducted at the facility of Botanical Garden "Orto Botanico Friulano", in Udine (IT) on spring 2016, starting by two lots of seeds of *Apium graveolens* var. *rapaceum* (Miller) Gaudin: "Friulano" seed lot provided by ERSA (Ente Regionale per lo Sviluppo Agricolo). Lots were harvested in 2014 (20 mg FW), stored, after drying at room temperature for 1 week, at -20°C in a hermetic glass jar; "Sedano rapa gigante di Praga" commercial seed lot (L'Ortolano, Italy), harvest season 2014 (39 mg FW), was stored at room temperature in sealed paper bag.

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## Priming

Only for the "Friulano" lots, three aliquots of seeds were subjected to 3 priming treatments in sterile conditions: (i) incubation in 2% NaCl for 24 h (short osmopriming); (ii) incubation in bidistilled water for 24 h (short hydropriming); (iii) 2.1% NaCl for 7 d in aerated conditions (prolonged osmopriming). After priming, the seeds were briefly dried over filter paper before sowing. The commercial seed lot was not subjected to priming treatment.

## Seed germination

Four replicates (40 seeds each) for each priming treatment were sowed in 4 expanded polystyrene trays of 20 pots (2 seeds each pot), with potted soil and incubated for 46 d at 70% (RH), with interval of 12 h under light at 21°C, and 12 h under dark at 19°C (Figure 1). Every 2 d, at the time of seed observation and counting, soil was sprayed with bidistillated water, in order to prevent dehydration.

Only for short hydro- and osmopriming, four replicates (40 seeds each) for each priming treatment were sowed in Petri dish over a wet Whatman filter and incubated for 46 d at 70% (RH), with interval of 12 h under light at 21°C, and 12 h under dark at 19°C (Figure 2). When filter paper appeared to dry, plate was opened and new bidistillated water was added for wetting the filter.

During germination, the amount of germinated seeds was counted every 2 days and the following parameters were recorded: germination capacity, as % germination=(number of germinated seeds after 46 d/ total number of seeds) x 100; germination energy, as % of germinated seeds after 8 d/ number of germinated seeds after 46 d; 50% germination (T50) was calculated according to the following formula of Farooq et al. [9]:

$$T50 = \frac{\left[\left(\frac{N}{2} - N_1\right) x \left(T_2 - T_1\right)\right]}{N_2 - N_1} + T_1$$

Where N is the final number of germination and $N_1$, $N_2$ cumulative number of seeds germinated by adjacent counts at times $T_1$, and $T_2$ when $N_1<N/2<N_2$.

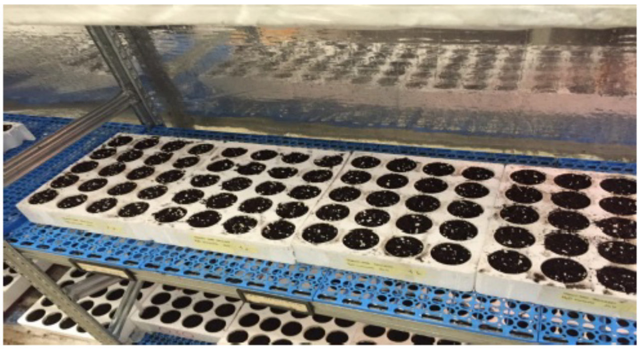

**Figure 1:** Potted soil trays for germination experiments on stored primed celeriac seed lot "Friulano". Four trays of 40 celeriac seeds each were used for each priming treatment and placed in the germination chamber at Orto Botanico Friulano facility.

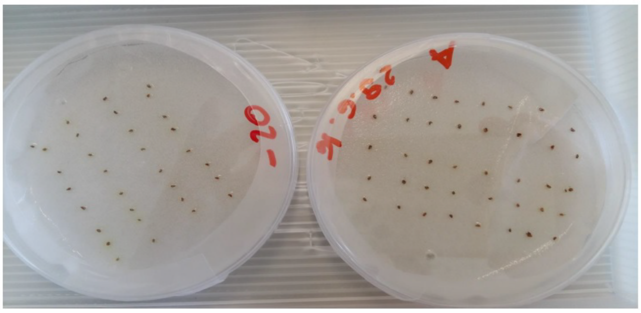

**Figure 2:** Petri dishes for germination experiments on stored primed celeriac seed lot "Friulano". Four sterile Petri plates containing 40 celeriac seeds each, over a wet Whatman filter, were used for two priming treatments and placed in the germination chamber at Orto Botanico Friulano facility.

## Data analysis

Preliminarily, data were pooled and analyzed as mean of the four independent replicates. The effect of different priming treatments compared to commercial cultivar seed lot (no priming treatment) in potted soil was considered significant, by using a one way ANOVA test. A Tukey pairwise test was afterward applied to detect significant differences between treatment levels ($p<0.05$). The effect of short osmopriming versus hydropriming in Petri dishes was done by T test. The distribution of residuals was checked using visual diagnostic plots and variable were log-transformed when assumptions were violated [10]. All statistical analyses were performed using the R software ver. 3.4.3 [11].

# Results and Discussion

Germination of freeze-stored "Friulano" seeds was checked every two days for a period of 46 d, chosen as the sufficient interval of time for reaching the maximum number of germination in this cultivar, as observed in previous experiments (result not shown). Seeds were counted as germinated, when green cotyledons appeared (Figures 3 and 4). All primed seeds of "Friulano", except samples treated by long osmopriming, started to germinate very quickly, just after 6 d into the potted soil (Figure 5) and the total germination %, at the end of the period, was as good as that of commercial seed lot. As it can be observed in Table 1, germination rate reached approximately 50%, near to the value of 59% found in untreated commercial seeds. This finding was different from the values reported in specific handbooks for seed conservation of wild species [2], where it has reported that drastic reduction in germination (3%) occurred if seeds were stored for 2-3 years at 21°C. To the best of our knowledge, tolerance of celeriac seeds "Friulano" to storage temperatures under 4°C was not studied so far.

Regarding the priming treatments studied, the germination percentage at the end of 46 d was not statistically different among treatments, however the seed quality parameters, germination energy

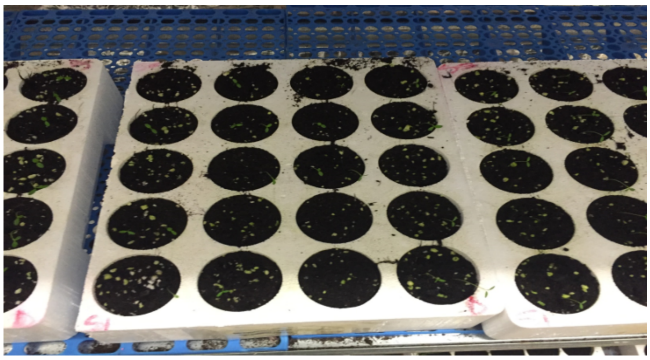

**Figure 3:** Germinated seeds of stored primed celeriac seed lot "Friulano" on potted soil. Celeriac seeds were counted as germinated, when green cotyledons appeared.

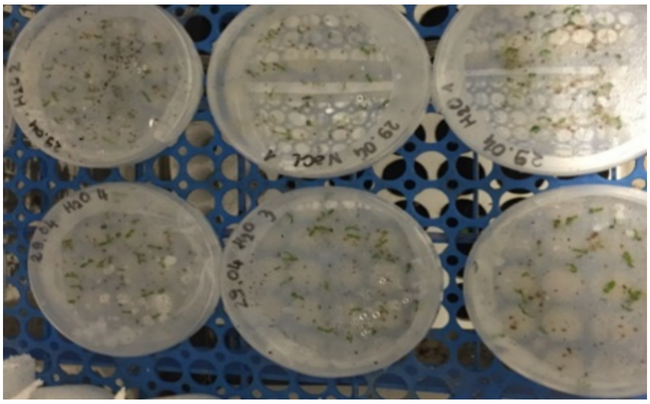

**Figure 4:** Germinated seeds of stored primed celeriac seed lot "Friulano" on Petri dishes. Celeriac seeds were counted as germinated, when green cotyledons appeared.

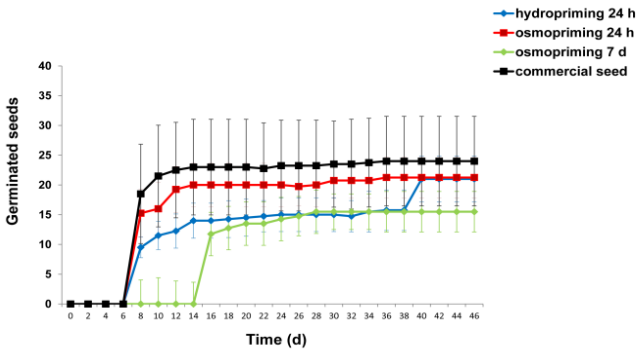


**Figure 5:** Seed germination on potted soil of stored primed celeriac seed lot "Friulano" and untreated commercial seed lot. Celeriac seed lots "Friulano" were subjected to three different priming treatments, while commercial seed lot "Sedano rapa gigante di Praga" was untreated. Both seed lots were stored for 2 years, at -20°C or room temperature, for "Friulano" or "Sedano rapa gigante di Praga", respectively. Potted soil was used as substrate. Values are means of 4 independent replicates ± standard deviation.

and T50, allowed to better discriminate their effects (Table 1). As a result, short osmopriming (24 h) induced a highly significant increase in germination energy (71.4% for "Friulano", comparable to 75.5% value for the commercial unprimed seed lot) and decrease in the time needed to reach 50% of total germination, being reduced from 8 or 14 d (hydropriming for 24 h or osmopriming for 7 d) to 7 d. These results indicate that priming caused most of the seeds to germinate synchronically after 6-8 d. The effect of priming on germination performance could be explained by a partial hydration of seed, causing the activation of processes related to cell cycle and reduction in mechanical restraint of cap endosperm, thus conditioning the embryo to a lag phase ready to germinate [12]. Accordingly, it has been recently suggested that osmopriming could also induce a sort of 'priming memory' on pre-sowed seeds, by stimulating a variety of stress responses which are able to greatly increase not only the potential capacity of germination but also the stress-tolerance of germinating primed seeds [13]. However, as shown in this study, a careful investigation of the right time of seed exposure to the osmoticum has to be considered for each species and variety.

Percentage of germination and the other seed quality parameters were also measured by comparing short time hydro- and osmo-priming treatment on seeds germinated on Petri dishes (Figure 6 and Table 2).

| Treatment | Germination % | Germination energy | T50 |
|---|---|---|---|
| Hydropriming 24 h | 52.2 ± 9.6 | 46.0 ± 9.2$^{b}$ | 8.45 ± 0.68$^{b}$ |
| Osmopriming 24 h | 53.3 ± 8.5 | 71.4 ± 12.1$^{a}$ | 7.43 ± 0.25$^{c}$ |
| Osmopriming 7 d | 38.8 ± 1.9 | 0$^{c}$ | 15.18 ± 0.21$^{a}$ |
| Commercial seed | 59.4± 19.8 | 75.5 ± 9.8$^{a}$ | 7.37 ± 0.22$^{c}$ |
| *P* value | 0.31 | <0.001 | <0.001 |

Notes: Numbers are referred to means of three independent ± standard deviation and statistical significance was calculated by ANOVA test. Different letters denote significantly different values, as evaluated by Post Hoc Tukey test

**Table 1:** Seed germination performance on potted soil of celeriac seed lot "Friulano" and untreated commercial seed lot. Germination %, germination energy and T50 of seed lot "Friulano", subjected to three different priming treatments and of an untreated commercial seed lot. Both seed lots were stored for 2 years, at -20°C or room temperature, for "Friulano" or "Sedano rapa gigante di Praga", respectively. Potted soil was used as substrate.

| Treatment | Germination % | Germination energy | T50 |
|---|---|---|---|
| Hydropriming 24 h | 87.5 ± 15.0 | 34.8 ± 12.6 | 10.35 ± 1.81 |
| Osmopriming 24 h | 76.3 ± 21.8 | 50.3 ± 24.8 | 10.77 ± 6.25 |
| *P* value | 0.43 | 0.32 | 0.90 |

Notes: Numbers are referred to means of three independent ± standard deviation and statistical significance was calculated by T-test

**Table 2:** Seed germination performance on Petri dishes of celeriac seed lot "Friulano". Germination %, germination energy and T50 of seed lot "Friulano", subjected to three different priming treatments. Seeds were stored for 2 years, at -20°C. Wet Whatman filter on Petri dish was used as substrate.

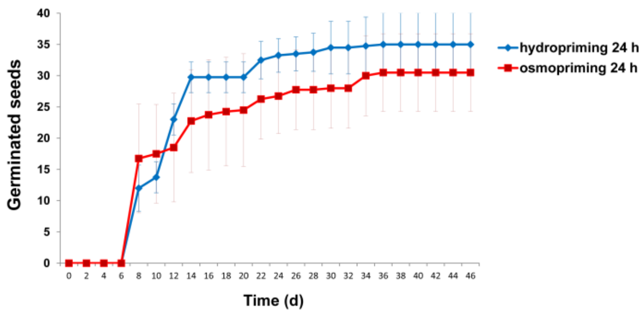


**Figure 6:** Seed germination on Petri dishes of stored primed celeriac seed lot "Friulano". Celeriac seed lots "Friulano" were subjected to two different priming treatments, after storage for 2 years at -20°C. Wet Whatman filter on Petri dish was used as substrate. Values are means of 4 independent replicates ± standard deviation.

In this condition, germination % was even higher than that observed in commercial seed lot on potted soil, reaching values as 87.5% and 76.3%, for osmo- or hydropriming, respectively. The same test on Petri dishes was performed also on commercial and Friulano seed lots without priming treatment. In both seed lots, we observed a remarkably low percentage of germination (61.9 ± 6.25 and 50.0 ± 16.8%, respectively, with $p$=0.154 by T test analysis; data not shown) with respect to those measured in primed seeds. Also levels of germination energy and T50 value were found to be good in both priming treatments, being differences not statistically significant ($p$=0.32 and 0.90, respectively). The amelioration of seed germination parameters observed when primed seeds of "Friulano" were placed on wet Whatman filter inside Petri plates, compared to soil-based condition, could be explained by the more uniform and continuous humidity created inside the sealed Petri plate. In addition, soil-less Petri dish germination, conducted in sterile conditions, could be considered also as a preliminary test to identify the greatest potential germination capacity of the studied celeriac seed cultivar.

## Conclusion

In conclusion, these findings firstly let us to recommend a cold freezing storage of celeriac seed to preserve valuable local seed germplasm in a simple and inexpensive way. Seeds need to briefly dried, before being stored in glass jar in freezer, for prolonging seed longevity at least for two years. The storage time would possibly be extended further, if oxygen absorbers or vacuum packaging could also be applied, as recommended by Groot et al. [8].

Finally, priming pre-sowing achieved by 2% NaCl solution as osmoticum for 24 h, a brief drying of primed seeds before placement on potted soil, and an interval of 12 h photoperiod (at 21°C/19°C) at 70% RH, represent optimal treatments for celeriac seeds to reach uniformly their 50% of full germination, within a very limited period of time. The knowledge acquired in this study could be further improved to better characterize celeriac seed physiology and establishment of good seed bank conservation.

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