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Research Note

Germination under aseptic conditions of different ecotypes of wild beet (*Beta vulgaris* L. ssp *maritima*)

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Summary

The characterization of the wild beet relatives, as well as their use for the selection of useful traits, is limited by difficulties associated with the germination of these plants. Currently available germination protocols deliver low percentage, unsynchronised and lengthy germination. We tested several procedures likely to weaken the woody/corky structure where the seeds are enclosed, the glomerule. Our results show that with an H_2O_2 treatment [30% (v/v) twice for one hour], followed by aseptic hand scarification and incubation in half-strength Murashige and Skoog it is possible, within days, to establish a synchronized culture due to successful germination, 40 to 90% depending on the ecotype.

Experimental and discussion

The *Beta* genus (Chenopodiaceae family) is native to Europe and adjacent areas, and has been diversifying and spreading northward and eastward since prehistoric times. In Portugal there are only representatives of the Beta and Procumbentes sections, the first one represented by *B. vulgaris* L. ssp. *maritima* (L.) Archang., *B. macrocarpa* Guss. and *B. patula* Aiton.

The beet complex is of particular interest since *B. vulgaris* spp. *maritima* is considered the main progenitor of the existing crop cultivars. One of the youngest cultivated forms, the sugar beet, has become a cash crop of worldwide importance. As the sugar beet was probably selected from one single cultivated population, its genetic background and variability is supposed to be very narrow (Bartsch and Ellstrand, 1999). For the enrichment of the gene pool of beet crops, the wild relatives are considered to be of great value, since they are characterized by genetic and phenotypic variability. Their adaptive

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capacity allows them to grow even on salty soils, and under conditions of water scarcity. In addition to being a potential source of resistance to abiotic stress, the wild beet is already currently being used as a source of genetic resistance to sugar beet diseases such as *Cercospora* and *Rhizomania* (Luterbacher and Smith, 1998). The wild relatives of sugar beet are therefore important for future breeding programs of crops of the *Beta* genus.

The characterization of the wild beet relatives, as well as their use for the selection of useful traits, is limited by difficulties associated with the germination of these plants (low percentage, unsynchronised and lengthy). McGrath *et al.* (2000) indicated that incubation of sugar beet glomerules in dilute hydrogen peroxide solutions stimulated germination, and methods of vegetative propagation of beet plants have also been proposed, for instance from buds and inflorescence pieces (Miedema, 1982; Zong *et al.,* 1993), respectively. Since these propagation methods are neither simple nor practical, we decided to further analyze the germination of wild beets. It is known that germination of weed beet is low and highly extended in time due to the effect of several inhibitory factors (Sester *et al.,* 2006), some of which reside in the woody/corky nature of the seedball. This structure and the ovary cap can act as a physical barrier to germination but inhibitory substances are also involved, as it has been shown for beetroot (Heydecker *et al.,* 1971). So, with the aim of reaching a suitable germination of the several ecotypes under study we directed our attention onto the glomerule, trying to recreate conditions that are likely to contribute to decrease the effects imposed by the glomerule structure on the seeds. Those conditions included: temperature cycles, drying and re-watering cycles, centrifugation of water imbibed glomerules, swirling with wet sand, scarification and incubation in hydrogen peroxide.

For the several pre-treatments, we used glomerules from *Beta vulgaris* ssp. *maritima* ecotypes, collected at Comporta salt marsh, Ponta do Sal cliffs, Cabrela, Vaiamonte and Oeiras and stored in the Germplasm Bank EAN (PRT 005) with the following accession numbers 4268, 4277, 5251, 5252 and 5253. Information concerning those accessions was integrated on the Portuguese National Inventory on plant genetic resources for food and agriculture and can be found at http://eurisco.ecpgr.org.

Germination was defined as radicle emergence and the following pre-treatments were evaluated:

1) Hydrogen peroxide (H_2O_2) – glomerules were immersed in 0.3% v/v H_2O_2 for 48h with agitation in the dark (McGrath *et al.,* 2000). Half of the glomerules were hand scarified (treatment 1A) afterwards, and the other half were not (treatment 1B), and then placed on wet filter paper in Petri dishes of 9 cm diameter.
2) Alternating temperatures – glomerules were placed on wet filter paper in Petri dishes of 9 cm diameter and submitted to alternating temperatures of 4°C (seven days) and 22°C (seven days) for four cycles (56 days in all).
3) Alternate drying and re-watering – glomerules were placed in Petri dishes submitted to repeated air-drying periods (24 hours drying, 24 hours re-watering) over 90 days. Promptly affected by the alternate cycles, seed moisture content (determined by the low constant temperature oven method) reached its maximum after 2 drying and re-

watering cycles, changing from 14.3% (± 1.4) to 93.1% (± 6.6) (Oeiras ecotype), and from 10.4% (± 0.3) to 79.3% (± 1.4) (Cabrela ecotype).

4) Centrifugation – imbibed glomerules (24 hours under agitation) were centrifuged in water at 7840*g* for 10 min at 20°C, and placed on wet filter paper in Petri dishes of 9 cm diameter.
5) Aseptic conditions – glomerules were surface sterilized with 30% v/v H_2O_2 twice for one hour (under agitation) and then washed with sterile water twice for 24 h (under agitation); after which they were hand scarified using a lancet under sterile conditions. The glomerules were germinated in half-strength Murashige and Skoog (MS) medium (without vitamins or hormones) containing 8g/L agar Miedema (1982), in either tubes or Petri dishes, and placed in a growth chamber (22°C and a 16 hour photoperiod of 70-90 µmole m^{-2} s^{-1}). To test the relevance of both the aseptic conditions and growing medium, "Ponta do Sal" glomerules were also placed in non-sterile water imbibed Jiffy-7 peat pellets instead of the aseptic Murashige and Skoog culture medium.

Considering the pre-treatments 1A (0.3% of H_2O_2 plus scarification), 1B (0.3% of H_2O_2 without scarification), 2 (temperature cycles), 3 (drying and re-hydration cycles) and 4 (centrifugation) (table 1), after 60-90 days germination results were erratic and close to zero, except for glomerules which had been centrifuged (17% germination) and for immersion in 0.3% H_2O_2 for 48 hours followed by scarification (20% germination) (table 1). H_2O_2 immersion only was not effective, although such treatment was previously reported to stimulate the germination of sugar beet cultivars (McGrath *et al.,* 2000). It should be noted that the problems we faced with germination of the wild ecotypes are

Table 1. Germination (defined as radicle emergence) of wild beet seeds from Oeiras (accession number 5253) and Cabrela (accession number 5251) ecotypes after the following pre-treatments have been applied: 1A: 0.3% of hydrogen peroxide (H_2O_2) during 48h with agitation in the dark, seeds scarified; 1B: 0.3% of hydrogen peroxide (H_2O_2) during 48h with agitation in the dark; 2: Cycles of 4°C and room temperature of five days each; 3: Air-drying and re-hydration cycles of 24h over three months; 4: Centrifugation at 7840g, in water.

Seed ecotype	treatments	no. of glomerules	germination (%)	no. of days
	1A	25	20	60
	1B	25	0	60
Oeiras	2	25	0	60
	3	25	4	90
	4	30	0	60
	1A	25	8	60
	1B	25	0	60
Cabrela	2	25	0	60
	3	25	4	90
	4	30	17	60

n° of days: days necessary to detect radicle emergence

not normally observed with the commercial cultivars, because such constraints have been eliminated during the plant improvement for agricultural utilization (Snyder, 1963), nowadays seeds being also primed and highly polished during processing.

Given the positive effect of H_2O_2 on the seed germination of the wild ecotypes, it was decided to utilize this particular pre-treatment in subsequent tests. The protocol finally adopted includes 30% (v/v) H_2O_2 together with scarification by hand. When germination proceeded under aseptic conditions in half-strength Murashige and Skoog medium, very good results were obtained with the five ecotypes tested (table 2). Not only was germination relatively high (generally above 40%), but was also considerably faster (within 4 to 5 days). A replicate sample of the ecotype "Ponta do Sal" was placed in water-imbibed Jiffy-7 peat pellets instead of the aseptic Murashige and Skoog culture medium after the scarification step, which decreased germination from 90% to 25%. It also took 22 instead of the 5 days to reach this value (data not shown).

Table 2. Germination (defined as radicle emergence) under aseptic conditions of wild beet seeds from Oeiras (accession number 5253), Ponta do Sal cliffs (accession number 4277), Cabrela (accession number 5251), Comporta salt marsh (accession number 4268) and Vaiamonte (accession number 5253).

Seed ecotype	germination in	no. of glomerules	germination (%)	no. of days
Oeiras	tube	67	46	4
	petri dish	125	75	5
Ponta do Sal	tube	48	90	5
Cabrela	tube	15	47	5
Comporta	tube	50	80	5
	petri dish	43	72	5
Vaiamonte	tube	46	39	5
	petri dish	38	47	5

n° of days: days necessary to detect radicle emergence

Our results show that a H_2O_2 pre-treatment of beet seed from wild ecotypes, together with scarification followed by sowing in an aseptic culture medium, allows for synchronised and successful germination, providing a viable solution to circumvent the difficult task of the laboratory propagation of wild relatives of beet crops.

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