Seed increase protocol for *Beta* and *Patellifolia* species

Updated version of a protocol written in 1996 to support partners of the GENRES CT95 042 project

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Beta germplasm collections consist of rather different types of accessions. Whereas sugar, fodder and garden beet of Northwest European origin fit quite well into the usual seed production scheme of breeders, other germplasm is more difficult to handle. This seed-increase protocol summarizes our experiences with seed production in wild and cultivated *Beta* germplasm. Since climatic conditions and length of day as well as technical facilities may differ considerably from those at the location Braunschweig, the following descriptions should be taken as guidelines and not as a recommendation valid for all seed multiplication sites.

If plants are grown in field isolations the use of herbicides may become necessary. According to our experience, 3-4 l/ha Betanal can be applied in wild beets. We do not recommend the application of Goltix.

The term ‘flowering’ used in this text is defined as number of days from sowing to that date when about 10% of the plants have opened flowers. If flowering data have been recorded previously they are provided along with the passport data list of accessions to partners multiplying seed samples.

At Braunschweig young plants are produced routinely during autumn, winter and early spring. All information given below refers to that cultivation method. In order to minimize the loss of genes, 50-60 plants per accession should be transplanted to an isolation plot or greenhouse compartment. All species of section *Beta* require isolation. Apomictic species such as *B. trigyna* and *B.intermedia* can be grown without isolation. All other species like *B. corolliflora, B. macrorhiza, B. lomatogona* and *Patellifolia* species should also be isolated. If greenhouses are used for isolation, *Patellifolia* accession can be jointly grown with accessions of *Beta* section *Beta* and *Beta* section *Corollinae*. Outcrossing between section *Corollinae* and *Beta* is possible but unlikely and of little practical significance for routine seed increase.

Please note that in annual wild species of section *Beta* seed shattering is often linked with prostrate to procumbent growth habit.

The seed multiplication work within GENRES CT95 42 aims at the production of sufficient seeds for evaluation (about 250 gr. / accession) and replenishment of germplasm seed stock (150 gr. / accession) and seed regeneration of highly endangered material.

According to common experience the species listed below produce the following amount of seeds per plant (in brackets range between accessions within species).

Species	Amount in gr.	Species	Amount in gr.
B.vulgaris ssp.maritima	30 (4-110)	*B.corolliflora*	30 (25-50)
B.vulgaris ssp.adanensis	20 (13-75	*B.macrorhiza*	10 (1-21)
B.macrocarpa	20 (8-57)	*B.lomatogona*	5 (1-26)
B.patula	12	*B.intermedia*	20 (15-25)
B.vulgaris Leaf beet	70 (15-170)	*B.trigyna*	30 (25-50
B.vulgaris Garden beet	40 (15-70)	*P.procumbens*	20 (2-80)
B.vulgaris Fodder beet	50 (15-70)	*P.webbiana*	20 (2-80)
B.vulgaris Sugar beet	50 (15-70)	*P.patellaris*	20 (2-80)
Beta nana	0.7 (0.1-2.0)		

50 -60 plants per population, except for *B. nana,* should therefore suffice to produce 250 gr. and more seed yield.

Beta section Beta (wild and cultivated beets)

The seed samples are disinfected, then usually sown in trays, transplanted into 8 cm pots soon after emergence and, if needed, again transplanted to 10 to 12 cm pots. Supplement light is given for 14 hours a day during warm cultivation from mid-October to mid-January. Temperature at sowing is about 20 °C and young plant cultivation is done at 10 / 16 °C. Vernalization in the cold greenhouse starts mid-January. Our greenhouse cannot be chilled actively. We try to keep the temperature at 6 °C. If the winter is not very cold germplasm which presumably requires strong vernalization is put in late March into a cold storage room (constant temperature 4°) for 10 to 14 days to complete the vernalization process.

After vernalization the material is transferred to isolation greenhouses as early as possible (transplanted into 10 l pots) or to the field.

Please note that we start sowing rather early. The intention is to bring the material as early as possible into the small isolation greenhouses which get fairly hot even in spring. If you intend to transplant the material to hemp field isolations, you may start sowing later.

1. *Beta vulgaris* ssp. *maritima*

1.1 North Atlantic material (Sweden, Denmark, Germany, Ireland

Sowing date is from mid to end of October. Samples from Sweden and Ireland flower 220 to 260 days after sowing in autumn in the greenhouse with subsequent vernalization in the cold greenhouse. Vernalization is continued outdoors when spring temperatures allow hardening of material. Plants can bear light night frost temperature around 0 °C.

1.2 Northwest Atlantic material (The Netherlands, Belgium, Northwest France, England)

Seeds are planted in mid-January in the greenhouse and cultivated with supplement light. After plants have produced 6-8 true leaves the plants are kept in the cold greenhouse. Vernalization treatment is continued outdoors if spring temperatures allow hardening of material. Plants can bear light night frost temperature around 0 °C.

Material from Bretany (France) flowers under these conditions 100-120 days after sowing.

1.3 Southwest Atlantic (Portugal) and Mediterranean wild and primitive ‘vulgaris’ germplasm

The length of the vegetative phase considerably varies within this group (40 to about 200 days). Some of the material from the East Greek Islands shows an extremely short vegetative phase of 40 to 50 days from sowing to flowering. The whole Mediterranean material has a great phenotypic plasticity. At Braunschweig it is usually sown in mid to end of March and transferred from 8 cm pots to 10 l pots in the isolation greenhouse. Even if the young plants have already started bolting they recover after transplanting and can form larger, flowering plants. Vernalization is usually not necessary.

B. vulgaris ssp. *maritima* from Iran shows a similar reaction (90 to 100 days).

Seed shattering occurs frequently in Mediterranean *Beta* germplasm with a short vegetative phase, as well as in *Corollinae* and *Patellifolia* germplasm. Even green to light green seed balls may shatter.

2. *B. macrocarpa, B. patula* and *B. vulgaris ssp. adanensis*

B. vulgaris ssp. *adanensis* may show seed dormancy and strong seed shattering. This germplasm is sown at the beginning of April and transplanted from 8 cm into 22 cm pots. All three germplasm types need to be cultivated in the greenhouse under northwest European climatic conditions (40 - 60 days).

Contrary to *B. vulgaris* ssp. *adanensis*, *B. patula* and *B. macrocarpa* retain the seeds much better.

3. *B. vulgaris* cultivated germplasm

The same cultivation methods as for sugar beets can be applied for northwest European Swiss chard and Garden beet accessions. There is much more variation in Mediterranean cultivated germplasm with respect to vernalization requirement and day length reaction in garden beets and particularly in Swiss chards.

Garden beets

When sown at the end of April in the field, Greek garden beets tend to bolt early. This year we have observed up to 50 % bolter in Greek accessions. The garden beet germplasm of Greek origin bolts, when sown in mid-March in the greenhouse, end of April and starts flowering 90 to 100 days after sowing.

Swiss chards

The time from sowing to flowering varies between 90 and 240 days in Spanish material and 70 to 220 days in Greek germplasm.

Material from China obviously shows day length reaction. If sown in mid October it takes 200 to 240 days until flowering starts. If sown in the field end of April the material behaves as annual germplasm and produces seeds in August.

Beta section Corollinae

All species are hard-seeded. The pericarp cap has to be mechanically removed before sowing. For regeneration purposes up to 300 seed balls are prepared by carefully cutting off the cap with a specific knife used for wood carving (see Fig.1 on last page).

The material is frost resistant if covered with a peat layer. The species are perennial. If the first seed increase fails due to incomplete bolting the plants can be kept in a sheltered placae for a third or fourth year.

Under northwest European conditions, these species should all be cultivated in the greenhouse where they are not exposed to long periods of rainfall during flowering.

According to our records it takes about 400 to 450 days from sowing to flowering.

4. *B. corolliflora*, *B. trigyna* and *B. intermedia*

Seeds should be sown at a depth of 1.5 to 2 cm. If sown too shallow the seedlings will not be able to strip off the fruit. It sometimes takes 3-4 weeks until all viable seeds have emerged. Early sowing in March in the greenhouse is required to produce sufficiently old seed parent plants. Contrary to *Beta vulgaris* young plants of *Corollinae* species resist vernalization. After transplanting in 8 cm pots plants may be further cultivated in 12 to 16 cm pots or transplanted to the field.
At Braunschweig we keep the mother plants in 10 l pots in an unheated greenhouse where they are sheltered but yet exposed to winter temperature conditions.

At Quedlinburg *B. lomatogona,* and plants of *B. macrorhiza* from Turkey as well as Dagestan, survived temperatures as low as -15 °C in the field under snow or thatch cover in three consecutive winters.

Seed production of both species is almost without problems if the vernalization has been sufficient. Plants are in general vigorous and healthy. The material starts bolting early and can already flower in May even in the field isolation plots.

5. *B. macrorhiza*

B. macrorhiza is a more difficult species. Sowing and seed parent production is similar to *B. corolliflora*. *B. macrorhiza* suffers from *Alternaria* leaf disease throughout its growth and from Powdery Mildew when plants reach seed parent stage. Fully vernalized plants start flowering very early in the season (March). Some populations of the species are very bad seed producers. Plants often have to be kept and the seed increase repeated the next year.

The growth habit is more procumbent and the plants size of seed parents rather small compared to *B. corolliflora*. If grown in pots, at watering the foliage should be kept dry to improve seed set.

6. *B. lomatogona*

This species occurs on dry growing sites in Turkey, Transcaucasia and Iran. It is least adapted to northwest European growing conditions. The seed balls are monogerm to bigerm and can be prepared for sowing easier than the much more hard-seeded ones like *B.macrorhiza*. At Braunschweig the material often bolts incompletely even in the third year and suffers from seed stalk diseases (or heat stress ?) in our isolation greenhouses during flowering and seed set.

7. *B. nana*

The perennial species is endemic to Greece where it occurs at altitude higher than 1800 m. It is part of the snow patch vegetation. The seed balls are monogerm. At the IPK Genebank, Gatersleben, the seed balls are scarified before sowing the treated seeds in spring in the greenhouse. Seedlings are transplanted and cultivated in a cold greenhouse over winter. The plants flower in between June and September and set seed between September and December. The harvested seeds are desiccated following the genebank's standard procedures and stored under deepfreeze conditions. The germination of wild beets is generally not tested.

At the US National Plant Germplasm System station in Pullman, WA *Beta nana* is decorticated (as per above) before sowing. Decorticated seed is placed in germination media of ½ perlite: ½ vermiculite in spring in a cool greenhouse. After germination, seedlings are transplanted into course

soilless media (e.g. Sunshine Mix #4) and grown until mid September to early October at which time they are transplanted to a sheltered garden. In Pullman, plants flower from late April to mid-June. Seed harvest is from late June to late August. Accessions are isolated using open topped pollen-impervious cloth enclosures. Plants are watered as needed during the summer. Plants in the field are susceptible to root feeding insects and tend to be short-lived (1-3 years). Selfed plants will produce viable seed but cross pollinated plants yield 2-10 times more seed.

Seeded germination pots are kept for many years as we have had germination for up to 5 years following seeding. Vernalization is needed for vigorous flowering if plants are grown in the greenhouse. We did get some flowering on one plant kept at temperatures of 15-21°C day/ 13-15°C night throughout the winter but the number of flowers produced was very low compared to vernalized plants. We used 3 months at 4°C to vernalize a small sample of plants and initiated flowering. Average seed production per field grown is 60 seeds with a range of 10 to 200. Average 100 seed weight is 1.09g.

Patellifolia

8. *P. patellaris, P. procumbens, and P. webbiana*

All species are monogerm and hard-seeded. *P. procumbens* is a nitrophilous plant and grows mostly in lowland areas. It can survive on saline soils close to the spray water zone of coastal areas. In the wild it mostly occurs on alkaline soils with a pH-value around pH 8. The species thrives also on neutral or slightly acidic soils.

As with *B. lomatogona* decapitation of the fruits is comparatively easy. We prepare 150 to 250 seeds per accession. It takes 70 to 80 days from sowing to flowering. Flowering is indeterminate, i.e. fully mature seeds can be harvested and collected by hand while the plant is still flowering. After germination seedling can be transplanted from the tray directly into 16 to 22 cm pots. The material is not frost hardy and must be grown in the greenhouse.

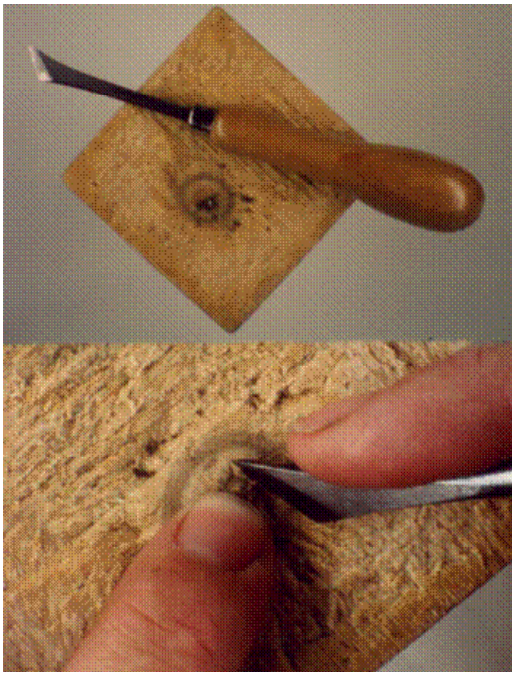

Figure 1: Seed preparation

Pericarp cap is removed using a carving knife. It is not sufficient to cut a small hole, because the root will appear only while the hypocotyl and the cotyledon leaves remain in the seed ball. The seedling will be torn or start rotting. The size of the hole must allow development of the root and cotyledon leaves.
If the pericarp cap is removed correctly, the intact seed with the darkbrown seed skin can be seen. If the starch body is only slightly hurt, the seed can still germinate and develop a plant. In the case of multigerm seed balls it is possible to split the seed ball at the basis of the fruit. This is often sufficient to gain one well prepared seed. The pericarp caps of the remaining seed can then be removed as in the case of genetic mongerm species.