



## LETTER TO THE EDITOR

## The Sea Beet (*Beta vulgaris* L. ssp. *maritima*) of the Adriatic Coast as Source of Resistance for Sugar Beet

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### INTRODUCTION

The crosses among different species of the Genus *Beta* has played an important role in the breeding of sugar beet (*Beta vulgaris* L. subsp. *vulgaris*). In interspecific crosses, the first problem is in obtaining viable F1 plants.

Crosses with cultivated beet, listed by Lange *et al.*, 1999, in the Section *Beta* (syn. *Vulgares*), were carried out using species belonging to the Sections *Corollinae* and *Procumbentes*. Similar work have not been carried out with the *Beta nana*, the only specie of the fourth and last Section *Nanae*. Obviously, slight difficulties were encountered in crossing the species and subspecies of the Section *Beta* (tab. 1). The sea beet, classified by the aforementioned authors as *Beta vulgaris* L. subsp. *maritima* (L.) Arcang., is the most important and the easiest to cross with sugar beet.

In the Section *Corollinae* the species display a variable chromosomic number,  $2n=18$ ,  $2n=36$ ,  $2n=54$  (Scheibe, 1934; Walther, 1961, 1963). Attempts of crosses with the sugar beet have so far produced poor practical results (Seitz, 1935, 1936, 1938; Stehlik, 1947). The only successful hybridation involving species of the Section *Corollinae* was carried out by Helen Savitsky in 1969, when she crossed sugar beet  $4n$  genotypes with *Beta corolliflora* that was also  $4n$  and resistant to the "curly top" virus (BCTV). The experience did not produce any practical developments, but the methodology adopted in the experiments proved valuable for later works (Coons, 1975).

Many breeders have obtained hybrids using species of the Section *Procumbentes* and those of the Section

*Beta* (Oldemeyer, 1954; Johnson *et al.*, 1961). In 1965 Savitsky and Price obtained a significant quantity of hybrids  $2n$ ,  $3n$ , and  $4n$  with species of the Section *Beta*, without passing through bridging hosts. In short, the lack of vitality found in interspecific crosses with the Section *Procumbentes* can be resolved by crosses between sea beet and *Beta procumbens*, which can produce viable F1 hybrids. These hybrids can be used in subsequent crosses with cultivated beet (Oldemeyer *et al.*, 1956).

Further experiments by Savitsky in 1973 and 1975 transferred an alien gene-carrying chromosome for resistance to cyst nematode from the *Beta procumbens* to the sugar beet. Through repeated crossing, monosomic additions were obtained that transmitted the resistance to their progenies with a frequency of about 10%.

This material was used by Heijbroek *et al.*, 1988, to obtain a homozygotic diploid line which contained a fragment of alien chromosome carrying the resistance to cyst nematode. If the diploid material is used as pollinator to produce resistant varieties, it very easily loses the genes of resistance. Therefore, every individual plant has to be checked using recombinant DNA techniques to ensure the gene is present. For the moment, this

Table - 1 : Taxonomy of Section *Beta* (Lange *et al.*, 1999)

<i>Beta vulgaris</i> L.
subsp. <i>vulgaris</i> (cultivated materials)
subsp. <i>maritima</i> (L.) Arcang.
subsp. <i>adanensis</i> (Pamuk) Ford-Lloyd & Will
<i>Beta macrocarpa</i> Guss.
<i>Beta patula</i> Ait.

represents a significant problem in producing commercial resistant varieties. A second source of resistance to cyst nematode was found in a sea beet biotype collected in France (Hjner, 1951). The character is recessive and multigenic (Heijbroek, 1977) and was used for the same purpose by Lange *et al.* in 1994.

As stated before, the greatest successes have been obtained in crosses with sea beet, considered by many experts to be the progenitor of the cultivated beets (Hjerdin *et al.*, 1994).

**GEOGRAPHIC DISTRIBUTION AND DESCRIPTION**

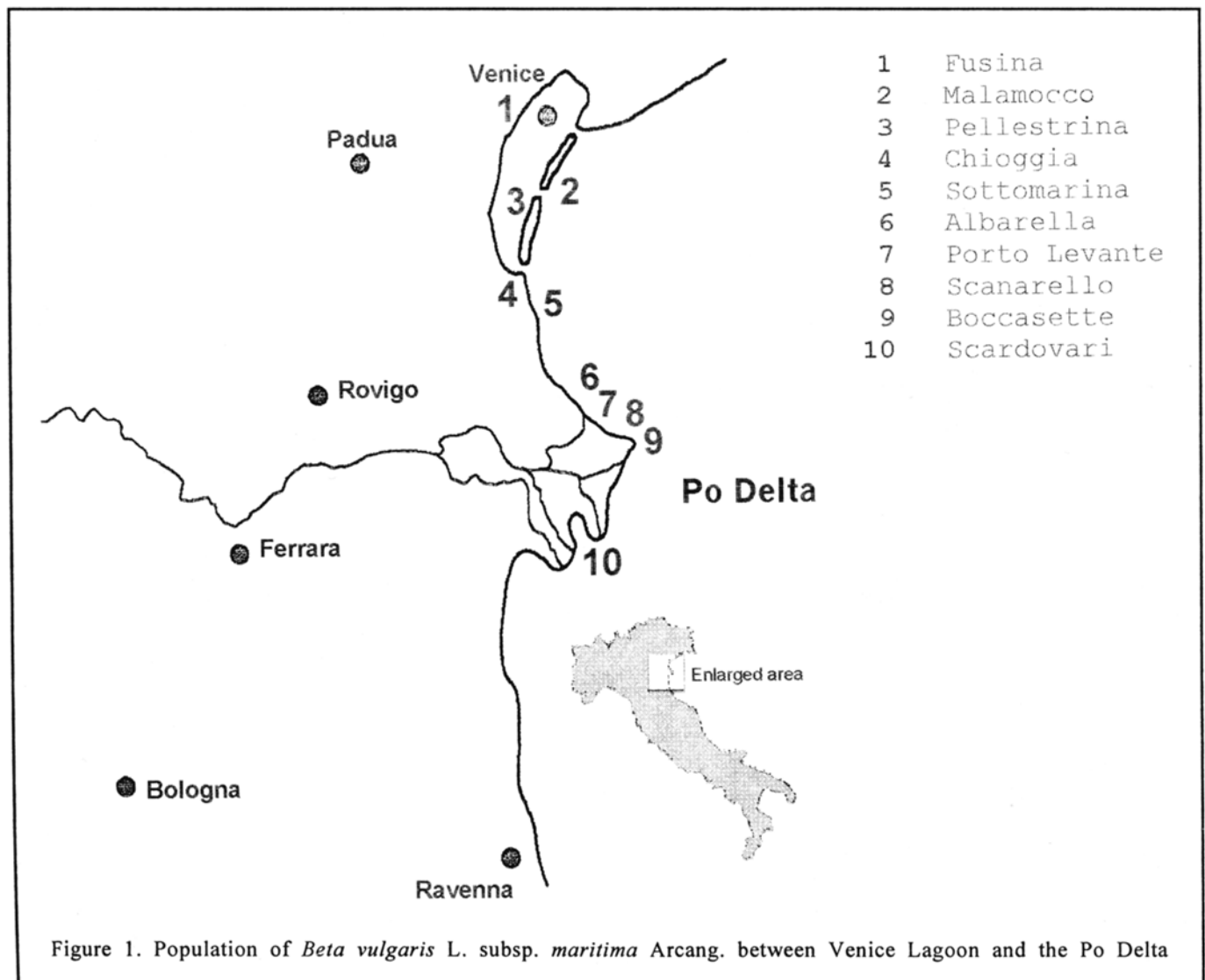
The sea beet grows along the Mediterranean and North Atlantic coasts, from the British Isles to the Canary Islands (Doney *et al.*, 1990). This species is also commonly found in the belt that sweeps down from the Balkans, through Greece and Turkey as far as the Persian

Gulf (Ulbrich, 1934).

The plant is relatively common along the coasts of the Adriatic Sea. The most densely populated area in Italy is between the Venice Lagoon and the Po Delta. This would appear to be because this zone contains the largest area of coast in natural condition (Fig. 1).

During the months of July and August 2000, sea beet populations were mapped along the coasts of Friuli-Venzia-Giulia, Veneto and Emilia-Romagna. The aim of the investigation was (i) to study the genetic variability of the different populations and (ii) identify whether or not there had been any crosses with cultivated beet.

On the basis of previous survey (Barstch *et al.*, 1999), 13 sites were located containing populations with variable consistency (Fig. 1). Populations separated by natural barriers are considered distinct, as are those divided by



a distance of at least 15-20 km (Doney *et al.*, 1990). For populations consisting of more than 30 individuals, a sample of 30 individuals was taken at random at a distance of 0.5 m from one another. For smaller populations, 2-3 young leaves were taken from each plant, and high-molecular weight genomic DNA has been prepared from each leaf sample according to Shagai-Maroff *et al.* (1984). The samples have been analysed by the AFLP technique (Vos *et al.*, 1995), and the DNA fragments generated and P<sup>33</sup>-labeled have been separated on sequencing gels. These preliminary data show a high number (about 60 per sample) of scorable, reproducible bands, and a high degree of polymorphism both within each sea beet population and between them. A possible development of this technique might be necessary if the gene flow between cultivated and wild populations is to be studied; in this case, it is advisable to identify the loci represented by DNA fragments with a technique allowing to discriminate the homozygosity from the heterozygosity at each locus. This development will be carried out by analysing AFLP bands with the fluorescence-based, capillary electrophoresis system used by the ABI Prism 310 Genetic Analyzer; this system allows the discrimination of the allelic state at each locus on the basis of the fluorescence peak intensity, and therefore the direct estimation of allele frequencies and of heterozygosity

In Italian conditions the sea beet can grow for 6-7 years, and is therefore classified as a perennial species which flowers in the first year of its life (Munerati *et al.*, 1913). The plant grows best in wild zones, close to the sea, on banks and pebble beaches. It can grow on soils with high levels of salinity, where, in normal conditions, it is not subjected to competitive pressures from other species. Rarely has the sea beet been found on sand or not in the proximity of sea water (Biancardi and De Biaggi, 1980). It grows alongside other wild species and in many cases with *Brassica* spp. (Doney, 1990). It most greatly fears competition from the *graminaceae* (Biancardi and De Biaggi, 1980). It often grows alone where survival conditions are very hard.

The sea beet populations display significant morphological variability. The leaf apparatus consists of leaves of varying forms and size. They are deep green in colour, but in a number of individuals the stalks and veins reveal red streaking.

It has a tap root with very irregular forms when growing on stony or very compact soils. When sown in cultivated soils, the roots of the sea beet become far more regular than those found in the wild, but they are more elongated and with less evident root groove than sugar beet.

It is striking to observe the variability of the reproductive structure that can consist of one or more flower stalks that are produced from the end of March onwards. The inflorescences are composed by as many as 4-5 or more flowers, while single flowers are rarely found. The pollen has a diameter of 15-20  $\mu$ m, the same as for cultivated beet with the same ploidy level. Sea beet is largely wind pollinated (Biancardi and Mandolino, 1997). After pollination, the flowers gradually bind themselves to form a single woody structure glomerule.

Flower formation is staggered, as is the seed maturation which begins towards the end of May and ceases around the end of July. The mature seed is easily removed from the stalk, especially when there is low air humidity. The plant disperses its seed for at least two months, and this is believed to be made easier by the proximity to the sea water which can transport the seeds for long distances from its location of origin.

During this investigation, it was noted that the populations of sea beet and the natural environments suitable for their survival, are reducing in number compared with previous survey (Bartsch *et al.*, 1999). The likely cause is the increasing exploitation of coastal areas for tourism. However, greater care should be given to the preservation of this species which has provided the cultivated beet with useful sources of genetic variability.

#### **Resistance to *Cercospora* leaf spot**

The crosses between sugar beet and sea beet are easy, due to the affinity between the subspecies. In the summer of 1909, the author collected a good quantity of seed on the right bank of the Po di Levante river, close to its mouth (Munerati *et al.*, 1913). The seed was forwarded to many breeders, among which the author cited Townsend, a researcher working for the U.S. Department of Agriculture.

A cycle of mass selections began on the plants sown in cultivated soil near Rovigo, followed by inbreeding, with the main objective being to fix the bienniality. Using the first predominantly biennial lines, he began the crosses with the sea beet, which was followed by a number of back-crosses to eliminate the negative traits of the wild parental (fangy and fibrous root, tendency to bolting, etc.). Munerati doesn't mention the specific program to improve the resistance to CLS, to which the sea beet of the Po Delta is sometimes very sensitive. But as early as in 1925, he was in possession of material able to reduce the development of the fungus on the leaves, when compared with the normal varieties (Coons *et al.*, 1955; Skaracis and Biancardi, 2000).

Further improvements reduced the tendency to bolting

and after ten years led to the release of the R 581 line, that was considered the first substantial progress against the disease (Coons *et al.*, 1955). The line was immediately distributed to public and private breeding stations, and led directly to a number of varieties classified as CLS resistant. The American variety US 201 is cited as one of the oldest, together with the Italian “Cesena R” and “Mezzano 71”, the Polish “Buszczynski CLR”, the French “Desprez RC2”, and the Dutch “Vanderhaven AC” (Bongiovanni *et al.*, 1958).

In recent years, perhaps because of the increase of global temperature (Schäufele and Wevers, 1996), the CLS is expanding predominantly northwards, leaving only the coldest cultivation zones immune (Holtschulte, 2000). Accordingly, the selection for the CLS resistance has become essential for the main cultivation areas.

The increased effort of the breeding companies has produced an improvement in sugar yield and bolting resistance, which only a few years before were the main negative traits of the resistant varieties.

#### **Rhizomania resistance**

First assessments of commercial varieties in diseased fields began in 1958 (Bongiovanni and Lanzoni, 1964), i.e. before discovery of the disease’s real causes (Canova, 1966). The results of field tests together with those obtained from 1966 onwards from several seed companies (Gentili and Poggi, 1986) showed clearly that the “Alba P” multigermline diploid variety and several others of Italian origin were more productive in rhizomania-infected soils.

The varieties in question also possessed good CLS resistance, so they must have been derived from crosses with the sea beet that Munerati performed at the Stazione Sperimentale di Bieticoltura in Rovigo (Skaracis and Biancardi, 2000). The Italian sea beet biotypes, from which CLS resistance was obtained, probably also provided the first genes with the resistance to rhizomania shown by the “Alba P” (Lewellen and Biancardi, 1990; Biancardi *et al.*, in press).

The same genotypes with CLS resistance were probably used in constituting “Monodoro” (Hilleshøg) and “Ritmo” (Maribo) varieties, registered in Italy in 1979 and 1982. It has been ascertained that the resistance of “Alba type” is governed by different genes with additive effect (Lewellen and Biancardi, 1990). Other authors have confirmed the presence of rhizomania in sea beet biotypes from many countries (Lewellen *et al.*, 1987; Whitney, 1989).

In the period from 1980 to 1985, the “Rizor” variety was bred at the SES-Italia. It contains the gene or genes for qualitative rhizomania resistance. It was much more

productive than the resistant varieties cultivated at the time (De Biaggi, 1987). The variety spread remarkably throughout the areas affected by the disease. Its resistance was later called “Rizor type”.

During a breeding program at the American company Holly Sugar that was not specifically related to the disease, Erichsen discovered two monogerm lines, one O-Type and its CMS, which both had excellent rhizomania resistance. Analysis of the progenies revealed dominant monogenic character in this case too (Lewellen *et al.*, 1987). In 1986 lines with “Holly type” resistance were purchased by European seed producers and integrated into their genetic programs.

The SES continued to improve the resistance of the “Rizor” variety and began a back-crossing program, to introduce both the “Rizor type” and the “Holly type” resistance into varieties suited to the European cultivation environments (Horemans, personal communication).

In the 1990s, many breeders used analyses based on recombinant DNA in order to locate resistance genes on the chromosomes. The argument is relatively clear because the two factors “Rizor” and “Holly” map in the same locus and should be considered components of the same gene (Barzen *et al.*, 1997). The trait has been named Rr or Rz (Lewellen, 1988). Scholten *et al.*, 1999, mapped another gene Rz2, derived from a biotype of sea beet, identified with the code WB 42. It is important to underline that the gene or genes that provide resistance to rhizomania Rz1, Rz2 (Rr1, Rr2) map in the same chromosomal fragment that was very probably derived from sea beet (Barzen *et al.*, 1997).

Biancardi *et al.*, (in press), confirm the hypothesis of the common origin both for the quantitative resistance of “Alba type” and the qualitative resistance of “Rizor type” and “Holly type”. The origin is probably to be found in Italian material with CLS resistance derived from the earliest crosses with sea beet.

#### **CONCLUSIONS**

The sea beet is considered the ancestral species from which the different types of cultivated beet originate. Its hybridation is easy because of its evolutionary proximity, unlike with other species of the Genus *Beta*. These traits have long made the sea beet the subject of research aimed at targeting and isolating its useful characters which could then be transferred to cultivated beet.

The sea beet collected at the mouth of the Po di Levante river in the early 1900s has given more appreciable results than any of the other biotypes from different origins. Indeed, it was from this biotype that today’s most developed genetic resistance to serious

diseases such as cercospora and rhizomania was isolated.

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