Evidence for gene exchanges between sugar beet (*Beta vulgaris* L.) and wild beets: consequences for transgenic sugar beets

The production of hybrid seeds of sugar beet is essential to obtain regular high yields and, as much as possible, plant homogeneity. Hybrid seed production involves a complex scheme for organizing the multiplication of the cytoplasmic male-sterile (CMS) female lines, the construction of the male population, and to set up the hybrid seed field production distant from wild beets or other cultivated beets.

To maintain a beet variety, it is important to prevent it from coming into contact with other beet varieties and with contaminating wild beet pollen. Wind dispersal of beet pollen is so efficient that the Groupement National Interprofessionnel des Semences (GNIS) recommends an isolation distance of 1000 m of the seed field production site from any *Beta* plant [1]. In addition, the use of CMS females increases the contamination risk. Contaminants include plants belonging to wild forms (*B. maritima*, *B. macrocarpa*), cultivated forms (any other sugar beet types, forage beets, table beets and Swiss chards) and annual weed beets such as *B. maritima* [2].

The presence of bolting plants in sugar beet field production may also affect the yield and furthermore they are commercially unattractive. It has been proposed that the bolting plants either could be the result of crosses with *B. maritima*, or flower induction in plants that are easily vernalized [3]. The first genetic system is simple: the *B* allele (B for bolting) from *B. maritima* is dominant and therefore rapidly eliminated through the breeding programmes. The second genetic system, however, involves a wide range of genotypes which may bolt depending upon environmental conditions. Although breeders carefully screen varieties before commercialization to determine the percentage of bolting plants, the bolting phenotype of such individuals cannot directly indicate whether bolting is due to the presence of the *B* allele, suggesting that such individuals originated from a cross with an annual form, or be-

cause their allelic composition makes them sensitive to flowering.

Four ribosomal DNA unit types (V-11-2.9, V-11-2.6, V-11-2.3, and V10.4-2.3) have been found in twelve *B. maritima* accessions while in root beets [4] only the V-11-2.9 unit type is present, and being 11 kb long, it is unique to *B. vulgaris*. It contains a 2.9 kb *Eco* RI fragment which is hybridized by a probe consisting of the 6.1 kb rDNA sunflower fragment [5]. We propose that the V-11-2.9 unit array was selected for together with the domestication process of root beets, therefore indicating that its presence in wild beets may be due to pollination by cultivated beets [4] since the V-11-2.9 is not found in wild beet populations isolated from cultivated beets [4].

To estimate possible gene flux in both directions between wild and cultivated beets, wild beets were collected in the vicinity of the seed production fields and bolting plants were harvested in sugar beet production fields.

Although several meticulous searches to eliminate wild beet plants had already been done by breeders, thirteen plants resembling *B. maritima* var. *maritima* were found around the sugar beet seed production fields and were harvested in order to determine the rDNA unit type. Some of the plants were found to carry anthocyanin genes, expressed either along the axil or in petiole or both. Most of these individuals carried at least the V-11-2.9 allele (12 out of 13), four were found to be homozygous for the V-11-2.9, while three carried a V-11-2.6 and four a V-11-2.3. Therefore, irrespective of the reproduction conditions, the frequency of homozygous individuals for the V-11-2.9 allele is expected to be high, while the proportion of individuals homozygous for the other alleles is small (here $^1/_{13}$). Therefore the domesticated rDNA allele found in wild beets with the bolting genes has escaped from the cultivated beets to wild beets.

Bolting plants were harvested from fields containing five different sugar beet varieties. Their rDNA *Eco* RI profiles are shown in Fig. 1 and Table 1. Randomly selected non-bolting individuals of sugar beet varieties were never found to carry any *B. maritima* alleles in our fields. However, in contrast, from four varieties of bolted test plants, many of the individuals clearly carried two major *Eco* RI fragments of 2.9 and 2.3 or 2.6 kb, while we did not detect the presence of any *B. maritima* allele in the fifth variety. We suggest that plants displaying the (2.9 + 2.3) or (2.9 + 2.6) profile are the result of crosses between sugar and wild beets. Because of codominance, with the rDNA RFLP, we detected all the variants. Since we were unable to recognise such a hybridisation in one variety, we propose that the contaminating wild beets have been already intercrossed with

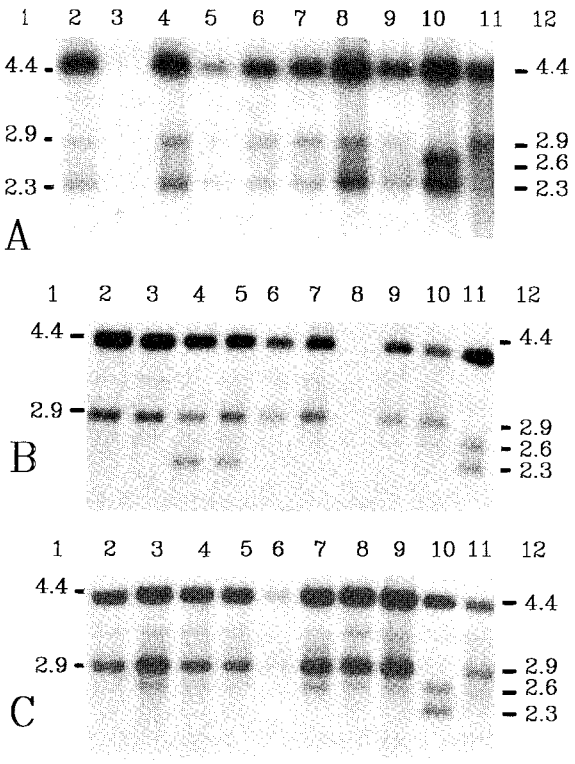

Fig. 1. Hybridization profiles of *Eco* RI-restricted total DNA from bolting plants harvested in sugar beet production fields. Lanes 1 and 14: fragment size in kb. DNA preparation, restriction and transfer to nylon membranes. The methods have already been described [6, 7]. **A.** Variety number 1, lanes 2 to 9: bolting individuals; lane 10: *B. maritima* F4008; lane 11: sugar beet. **B.** Variety number 2; lanes 2 to 9: bolting individuals; lane 10: sugar beet; lane 11: *B. maritima* F4008. **C.** Variety number 4; lanes 2 to 9: bolting individuals; lane 10: *B. maritima*; lane 11: sugar beet. Plants 3A, 8B, and 6C were controlled to be of the hybrid type and of the sugar beet type, respectively.

Table 1. rDNA unit type number found in presumed hybrid bolting plants of five sugar beet production fields.

Varieties	Place of Production	Plants under analysis	rDNA unit type	
			V–11–2.9	V–10.4–2.3
1	East France	8	8	8
2	West France[1]	8	8	2
3	Italy[1]	8	8	2
4	Yugoslavia	8	8	0
5	SW France	4[2]	3	1

[1] Same variety. [2] Four control plants of the same field display the V–11–2.9 profile.

the cultivated beets. Consequently, these beets carry the bolting genes and the cultivated rDNA allele. Thus, in practice the 2.3 and 2.6 kb rDNA fragments allow detection of part of the crosses. Therefore this probe underestimates the crosses between wild and cultivated forms, on the basis of rDNA RFLP only.

Breeders usually use the presence of colour as a marker in order to determine the level of wild beet contamination in commercial seed lots. Use of this marker also underestimates the contamination levels, and moreover, while the anthocyanin gene expression is dependent on structural genes, it is also environmentally regulated. Therefore, the rDNA unit type is a more reliable marker. However a better marker than the rDNA would be one tightly linked to the DNA sequence conditioning bolting.

Our results suggest that the presence of bolting plants are due to uncontrolled pollination by wild annual beets. Therefore crosses between wild and cultivated beets occur both ways.

The data presented above draws attention to a potential problem using genetically engineered beets. Herbicide-resistant beets and disease-resistant beets (rhizomania, nematode) will soon be available to breeders. Although the dissemination of new transgenes to other beet varieties is likely to be restricted by the cost to the plant breeder of buying these genes, our data suggests that the transgenes will nonetheless be able to escape because of the high outcrossing levels. Since the crosses between wild and cultivated beets occur both ways, any genetically transformed sugar beet plant flowering in a field may transmit the new genes to the wild forms, thus giving the weeds new weapons to infest sugar beet fields.

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