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**CONSEQUENCES OF THE GENE FLOW FROM CULTIVATED
BEET TO THE WILD BEET RELATIVES IN UKRAINE**

Gene flow via seed or pollen is a basic biological principle in plant evolution. The ecological and genetic consequences of gene flow depend on the amount and direction of gene flow as well as on the fitness of hybrids. Since wild relatives of cultivated plants are important plant genetic resources, the conservation of wild plants has become an important task in biosafety of transgenic plants. The assessment of potential risks has to be done before the background that also conventional crops do cross with wild plants. In case of sugar beet, unintended products of these crosses are weed beets that bolt in their first year of planting. Weed beets cause harvest delays and yield losses. Gene flow is hard to control in wind-pollinated plants. We present here the first data of the geographical distribution and diversity of wild *Beta* population in the Ukraine. We found substantial evidence for crop to wild gene flow in the Crimean Peninsula area. The precautionary approach in risk management makes it necessary to monitor the local wild populations that might be affected by transgene escape.

Introduction It is clear that biosafety research on environmental effects should not only target the probability of gene flow, but must also focus on the consequences (and potential hazards) of successful transgene flow to relatives of transgenic crops. Gene flow via seed or pollen is a basic biological principle in plant evolution. The ecological and genetic consequences of gene flow depend on the amount and direction of gene flow as well as on the fitness of hybrids (Bartsch et al. 1999). The assessment of potential risks has to be performed taking into account that also conventional crops cross with wild plants (Saeglitz and Bartsch, 2002). This means that biosafety research should address the phenotype (especially the fitness phenotype) of the transgenic hybrid versus that of non-transgenic controls.

Biosafety research cannot solve every open and basic question of general ecology (Kareiva et al. 1997). After the best pragmatic use of the case-by-case

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and step-by-step approach, a well-designed monitoring program is necessary **after commercialisation**. This monitoring must prove, on a **larger** scale, the **prognostic** assumptions made by former biosafety research and assessment (Marvier et al. 1999). We know for sure that containment strategies do not work **properly** and provide no justification to avoid monitoring (Sukopp and Sukopp 1993; Saeglitz et al. 2000). Monitoring must **be** flexible enough to recognize **unforeseeable** phenomena such as pleiotropic effects. Currently, we have no **evidence** that transgenic plants systematically express more pleiotropic effects **than** plants from classical breeding programs (Bartsch and Schuphan 2002).

Beet gene flow studies Beets (*Beta vulgaris* ssp *vulgaris*) have been cultivated for more than 2000 years in the eastern Mediterranean region, but the best known cultivar of this species - sugar beet - is a relatively young crop introduced for planting less than 200 years ago. On a geographical scale, Ukraine is the biggest sugar beet market in the world. Approximately 1 million ha of sugar beets are grown by farmers from year to year for sugar producing. In addition, about 9000 ha are seed production areas that are mainly concentrated in the Black Sea region using direct plantation methods and some areas in the Central part using indirect method of stecklings cultivation. Domesticated beet seed production in the Black Sea region has been going more than 100 years, with an intensification of sugar beet seed producing since the 1970's. All subspecies of *B. vulgaris* are usually wind-pollinated. In Crimea, commercial sugar beet seeds are produced on 4.500 ha, each ha containing approximately 100.000 flowering plants (Fig. 1). Furthermore, small farmers in the region grow red beet and Swiss chard for private -seed production, which may be an additional source of gene flow.

We examined wild beet accessions of Ukrainian origin. We have used allozymes to characterize the genetic diversity of wild beet populations on the Crimean Peninsula and to compare it with the diversity of the cultivated *Beta* and putative wild progenitors that have already been screened in a previous study (Bartsch and Ellstrand 1999). Seed material was obtained from seed companies, from international plant genetic resource collections or from collecting directly from wild populations (Bartsch et al. 1999). Accessions were selected so that most of the geographical range of wild and cultivated beets in Europe, USA was represented. (Table 1). That comparison provides indirect estimations of gene flow between cultivated and wild beet in Ukraine. Seed material of Crimean wild beet was sampled in 2001 at several locations of the Crimean Peninsula.

To evaluate the origin of Crimean wild beets, we first constructed an UPGMA dendrogram to elucidate the genetic relationships among 11 major groups of wild and cultivated beet populations based on Nei's (1978) genetic distances (Fig. 2). The dendrogram generally separates the accessions according to their taxonomic designations. Measured genetic evidence suggests that Crimean wild beets belong to two different taxa: *B. vulgaris* ssp *maritima* and

B. trigyna. As a first conclusion of our allozyme analysis, genetic distance of Ukrainian sea beets is relatively far from European sea beets, and therefore seems to be a special plant genetic source.

The results clearly revealed significantly higher genetic diversity of Ukrainian sea beet accessions in comparison to other European accessions (Fig. 3). Our genetic information is insufficient to determine that extent to which hybridization of cultivated beet with sea beet and/or direct introduction of: *B. vulgaris* ssp *maritima* from outside Ukraine may have contributed to wild beet evolution in Crimea. However, highest genetic identity of Ukrainian sea beet was found in comparison to the Southern European sea beet group.

Based on the genetic diversity statistics, gene flow measured as N_m within Ukrainian accessions seems to be higher than in other accession groups (Table 2). Moreover, quantitative allozyme marker gave evidence for introgression of cultivar alleles into the gene pool of local sea beet populations. We found one allele MDH2-1 that is specific to sugar beet, relative to other cultivated types, and a second ACO1-2 that is in high frequency in Swiss chard and red beet. Both alleles are typically rare in sea beet populations that are distant from seed production areas, but both are common in those that are near the Italian and Crimean cultivated beet seed production regions - supporting the contention that gene flow from the crop to the wild species can be substantial when both grow in proximity.

Abbreviations for gene diversity statistics include N (average number of plants sampled per accession), A (average number of alleles per locus), A_p (average number of alleles per polymorphic locus), P (Proportion of loci polymorphic), H (estimated heterozygosity), and U (number of unique alleles per group with the *B. vulgaris* alleles). F_{st} = Nei's (1978) summary F statistics for population differentiation between populations within *Beta vulgaris* and *B. macrocarpa*, N_m = estimated genetic migration from $0.25 (1-F_{st}) / F_{st}$ (Slatkin and Barton 1989).

Table. 1:
Genetic diversity statistics for seven major groups of genus *Beta*

	<i>N</i> *	<i>I</i>	<i>Ля</i>	<i>P</i>	<i>H</i>	<i>U</i>	<i>Fst</i>	<i>N_m</i>
<i>p. vulgaris</i> (all)								
Sugarbeet (16 varieties)	29.8	2.08	2.36	0.846	0.300	27	0.171	1.21
Swiss chard (4 varieties)	30.0	1.76	2.22	0.692	0.200	23	0.140	1.54
Red beet (5 varieties)	24.0	2.00	2.56	0.692	0.195	26	0.189	1.07
Weed beet (one accession)	30.0	2.00	2.40	0.769	0.260	26	.	.
Sea beet Europe North (11 accessions)	22.8	2.31	2.63	0.846	0.196	30	0.371	0.42
Sea beet Europe South (27 accessions)	21.7	2.85	3.18	0.846	0.294	37	0.309	0.56
Sea beet Ukraine (6 accessions)	20.0	2.23	2.45	0.846	0.320	29	0.091	2.51
Sea beet USA (13 accessions)	15.5	2.23	2.60	0.769	0.247	29	0.142	1.52
<i>R. macrocarpa</i> N.America (10 accessions)	30.4	2.46	3.22	0.682	0.057	25	0.107	2.10
<i>R. macrocarpa</i> Mediterr. (4 accessions)	23.6	1.62	2.50	0.462	0.063	16	0.199	1.01
<i>B. trigyna</i> Crimea (1 accession)	7.0	1.92	2.33	0.692	0.335	19	.	.
<i>B. webbiana</i> (1 accession)	11.0	1.38	2.17	0.462	0.222	6	.	.
<i>B. patellaris</i> (1 accession)	10.0	1.31	2.00	0.462	0.273	9	.	.
<i>B. procumbens</i> (1 accession)	2.0	1.31	2.00	0.462	0.273	6	.	.

Table. 2:
Introgression of cultivars alleles. Malatdehydrogenase (Mdh)
Isozyme 2-1 and Aconitase (Aco) Isozyme 1-2 were analysed
according to Bartsch et al. 1999.

Isozyme frequency / group (%)	Mdh2-1	Aco 1-2
Sugar beet (14 varieties)	100	57
Swiss chard (5 varieties)	0	100
Red beet (7 varieties)	14	100
Sea beet (Europe/Egypt, 19 accessions)	21	53
Sea beet (NE Italy, 20 accessions)	65	100
Sea beet (Ukraine, 6 accessions)	100	100

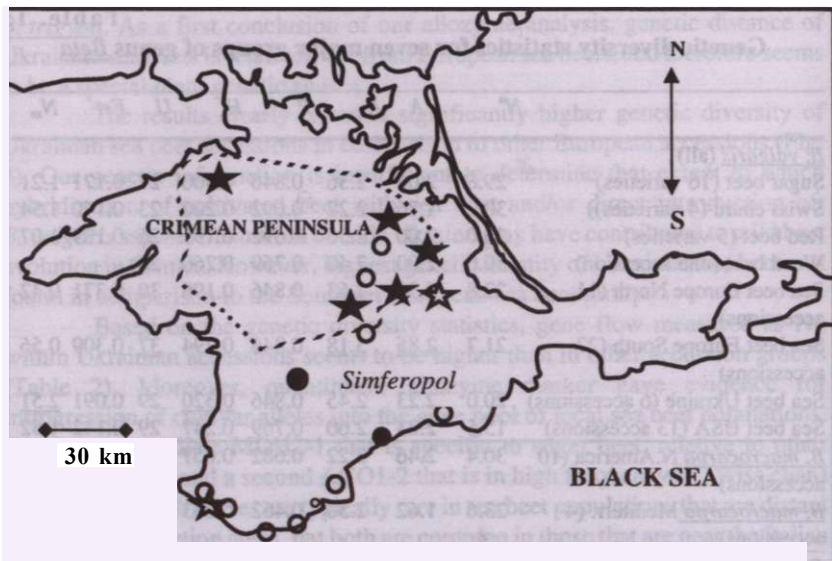


Fig 1: Geographic distribution of wild *Beta* accessions on the Crimean Peninsula: stars = *Beta maritima*, dots = *Beta trigyna*. Filled symbols represent accessions that were used for allozyme analyses. Sugar beet seed production area is surrounded by dotted line

- 11
- 60
- Sugar beet (1-16)
- Sea beet N Europe (27-37)
- Wedge beet K Europe (2, 6)
- p Swiss chard (17-20)
- L Red beet (21-25)
- P Sea beet S Europe (38-64)
- L Sea beet USA (65-76)
- Sta beet Crimea (11, 2)
- Beta trigyna* Crimea (100)
- i *Beta macrocarpa* Mediterra. (93-9)
- ' *Beta macrocarpa* N America (13-92)

Fig. 2: UPGMA dendrogram of systematic relationships among 11 major groups (with accession number) of wild and cultivated beet based Nei's (1978) genetic distances derived from allele frequencies at 13 polymorphic allozyme loci. *See text for explanations of these designations.

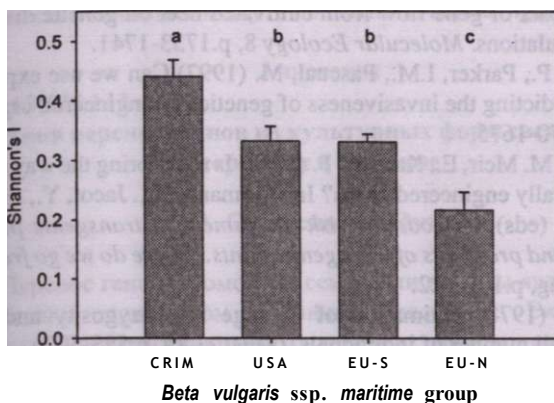


Fig. 3: Shannon's diversity index of a single population in comparison of different geographic origins.

Conclusions

- Wild beets in Ukraine have two different origins, from European sea beet (*Beta vulgaris ssp maritima*) or from *Beta trigyna*.
- Population level patterns of allozyme variation for wild Crimean beets revealed a significant higher genetic diversity than other European or USA accessions.
- Estimations on gene flow based on F-statistics suggest that level of hybridization and introgression is high for sea beet.
- Quantitative allozyme marker gave evidence for introgression of cultivar alleles into the gene pool of local sea beet populations.
- Since sugar beet seed production at the Crimean Peninsula offers opportunities for recombinant genes to escape to the wild relative, consequences of such gene flow must be assessed and monitored for the protection of plant genetic resources.

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Анотація

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Наслідки перенесення генів з культурних форм буряків у їх дикі родичі в Україні

О.А. Сливченко, D. Bartsch

Перенесення генів за допомогою насіння чи пилку є основним біологічним принципом еволюції рослин. Екологічні та генетичні наслідки цього явища залежать від кількості та напрямку перенесення генів, а також переваг одержуваних гібридів. Так як дикі родичі культурних рослин є важливим генетичним джерелом, збереження дикорослих рослин є актуальним завданням біобезпеки трансгенних рослин. Для встановлення потенційного ризику ГМ рослин слід керуватись механізмом звичайних схрещувань культурних рослин з дикорослими формами. Небажаним продуктом таких схрещувань між цукровими буряками та дикими видами є бур'янова форма, що характеризується цвітущістю в перший рік життя, призводячи до втрат урожайності коренеплодів. Безумовно, перенесення генів важко проконтролювати у перехреснозаплідних рослин. В цій публікації ми хочемо показати перші результати наших досліджень з моніторингу географічного поширення та різноманітності диких Beta популяцій в Україні. Попередня спроба навчитись керувати ризиком,

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Перенесення генів за допомогою насіння чи пилку є основним біологічним принципом еволюції рослин. Екологічні та генетичні наслідки цього явища залежать від кількості та напрямку перенесення генів, а також переваг одержуваних гібридів. Так як дикі родичі культурних рослин є важливим генетичним джерелом, збереження дикорослих рослин є актуальним завданням біобезпеки трансгенних рослин. Для встановлення потенційного ризику ГМ рослин слід керуватись механізмом звичайних схрещувань культурних рослин з дикорослими формами. Небажаним продуктом таких схрещувань між цукровими буряками та дикими видами є бур'янова форма, що характеризується цвітущістю в перший рік життя, призводячи до втрат урожайності коренеплодів. Безумовно, перенесення генів важко проконтролювати у перехреснозаплідних рослин. В цій публікації ми хочемо показати перші результати наших досліджень з моніторингу географічного поширення та різноманітності диких Beta популяцій в Україні. Попередня спроба навчитись керувати ризиком,

пов'язаним з ГМО, пояснює необхідність моніторингу дикорослих популяцій, які потенційно можуть потрапити під вплив "трансгена-втікача".

Аннотация

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Последствия переноса генов из культурных форм свеклы в их дикие сородичи в Украине

О.А. Сливченко, D. Bartsch

Перенос генов с помощью семян или пыльцы является основным биологическим принципом эволюции растений. Экологические и генетические последствия этого явления зависят от количества и направления переноса генов, а также преимуществ полученных гибридов. Так как дикие сородичи культурных растений являются важным генетическим источником, сохранение дикорастущих растений - актуальное задание биобезопасности трансгенных растений. Для установления потенциального риска ГМ растений следует руководствоваться существующим механизмом обычных скрещиваний культурных растений с дикорастущими формами. Нежелательным продуктом таких скрещиваний между сахарной свеклой и ее дикими видами является сорняковая форма свеклы, характеризующаяся цветущностью в первый год жизни, приводя к потерям урожайности корнеплодов. Безусловно, перенос генов трудно проконтролировать у перекрестно-опыляемых растений. В данной публикации мы хотим показать первые результаты наших исследований по мониторингу географического распространения и разнообразия дикорастущих *Beta* популяций в Украине. Предварительная попытка научиться управлять риском, связанным с ГМО, объясняет необходимость мониторинга местных дикорастущих популяций, которые потенциально могут попасть под влияние «ускользнувшего» трансгена.