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GENETICALLY MODIFIED SUGAR BEETS - SURVEY OF BENEFITS, PROSPECTS AND RISKS

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ABSTRACT

Genetically modified (GM) sugar beets offer several advantages for growers, food industry, consumers and for the agricultural environment. Transgenic beets tolerant to total herbicides (tHT) are discussed and their advantages, based on field experiments, are presented along with prospects, limits and putative risks.

Key words: sugar beet, genetic modification, herbicide resistance

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INTRODUCTION

Transgenic, or genetically modified (GM), crops raise comprehensible interest among producers - farmers and gardeners, as well as consumers. The first group hopes to benefit from marketing new products showing improved quality traits, higher yield or else produced at lower cost. The second one, however, manipulated by the media and their exaggerated misgivings, often shows anxiety expressed sometimes by severe protests and destruction of GM field experiments [3,16]. A lack of common public acceptance, at least in Europe, produces serious difficulties in registration and marketing transgenic cultivars. Regulations cause additional delays [17]. Transgenic bacteria producing human insulin, growth hormones etc. have been accepted much more easily and with no public opposition. In the US, Canada, Argentina and some other non-European countries, GM crops, such as soybean, maize, tobacco, cotton, rape, potato and vegetables, are successfully grown on the area of dozens of millions of hectares [13].

Different sophisticated methods of genetic transformation are applied in biotechnological laboratories, vector methods mainly [14]. As regards dicots, it is usually *Agrobacterium tumefaciens,* responsible for root tumours, which is used. The gene determining this trait can get incorporated into the genome of a host plant. By recombinant-DNA techniques which make use of specific enzymes (restriction endonucleases), the gene can be removed and substituted by a desirable gene derived from the donor - another bacteria, plant or even an animal. Some cells of host plant leaf discs, cultured *in vitro* in a suspension of such transformed bacteria can incorporate into their genome a DNA fragment containing the donor gene. The genome of plants regenerated from such cells becomes permanently modified by the presence of an alien gene.

Plant genetic transformation aims at the development of cultivars showing desirable traits which cannot be achieved by traditional methods, such as hybridisation and selection, as they are available neither within a given species nor among related ones. Most frequently resistance to pests and virus diseases is involved along with improved quality traits (e.g. responsible for a longer tomato shelf life and for transport damage resistance), resistance to non-selective herbicides, discussed further on for sugar beets. Sometimes transgenic crops show unusual traits, e.g. lettuce with anti-icterus vaccine properties, developed in the Poznań Institute of Bioorganic Chemistry, or else rice with a high content of vitamin A.

TRANSGENIC (GM) SUGAR BEETS

As regards transgenic sugar beets, the research is targeted at developing cultivars showing such traits as:

- **Resistance to nematodes**. Up till now the efforts aimed at transferring this most desirable trait from wild *Beta* species of *Procumbentes* section into cultivated beets have not given satisfactory results which would be measured with a marketing success, whereas genetic transformation offers a chance of successful transfer and expression of the resistance gene [40];

- **Resistance to aphids and other pests.** No resistant cultivars have been developed yet. The results of genetic transformation are very promising [34];

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- **Resistance to virus diseases.** A decrease in losses caused by virus diseases is feasible at present mainly employing chemical control of insect vectors, including aphids, beet lace bugs, and beet leafhoppers, the last one in the US. A genetic manipulation opens a possibility for the development of varieties with a permanent expression of resistance [22];

- **Resistance to abiotic stress** - low temperature [19], drought, high soil salinity

- **Faster growth** due to an increased production of indole-3-acetic acid (IAA) and specific cytokinins, as well as **desirable modifications of quality traits** [9,35];

- **Production of raw materials to obtain bioplastics.** Synthetic polymers pose a serious environmental threat due to their long period of natural breakdown. Genetically modified beets may constitute a valuable source of non-toxic and fast disintegrating substitutes - bioplastics [31];

- **Production of modified assimilates.** Overproduction of sugar calls for a search for cultivars storing alternate forms of carbohydrates. The gene determining synthesis of 1-ISST enzyme, which converts saccharose into polyfructans, has been successfully transferred to sugar beet from garden artichoke [33] and Jerusalem artichoke [32]. Polysaccharides with a different degree of polymerisation are used in food and chemical industries for the production of low-calorie sweeteners, organic detergents and enamels. Such modified sugar beets have been registered under the commercial name of Fructanbeet™.

HERBICIDE RESISTANT (tHT) SUGAR BEETS

However the growers are mostly interested in **sugar beets tolerant to non-selective herbicides** (tHT - transgenic herbicide tolerant). Currently there are known two types of such beets; Roundup Ready® (RR), resistant to glyphosate, an active compound of the Roundup herbicide, produced by Monsanto. Transgenic RR cultivars have been developed by Hilleshög, a plant breeding centre and an affiliate of Syngenta AG (product of a fusion of the agricultural branch of Novartis and agrochemical branch of AstraZeneca), and Danisco. Recently also KWS Saat has joined the RR gene users.

The other tHT sugar beet type, Liberty Link® (LL), is resistant to ammonium glufosinatee, an active compound of Basta and Liberty herbicides, produced by Hoechst-Schering, while breeding projects unite the efforts of Aventis, the company which originated from a fusion of AgrEvo and the agricultural branch of Rhône-Poulenc, and KWS Saat (sugar beet) as well as Trifolium (fodder beets).

Herbicides kill the plants by blocking synthesis of an enzyme essential for a regular course of one of the metabolic tracks vital for growth and development, e.g. respiration or assimilation. As for selective herbicides, this is true for the processes specific for one taxonomic group, only (e.g. monocots or dicots), or else for only one genus (e.g. *Beta*, beet). Non-selective or total herbicides block such processes common for all green plants. The resistance mechanism is based on the ability to produce enzyme with the same physiological properties as the blocked one yet tolerating the presence of a given herbicide or on the production of an enzyme inactivating the active compound of the herbicide.

The first mechanism has been applied in developing RR beets. One of the vital proteins, essential in the assimilation, is the EPSP enzyme (enolpyrovylshikimat-3-phosphat-synthase) whose synthesis is blocked by glyphosate. A tHT beet was developed by transferring a dominant gene of bacterial origin, which determines the production of cp4-EPSP protein which is of the same physiological activity as EPSP but resistant to glyphosate, to the beet genome [21].

There has been incorporated into LL beet genome, however, the PAT gene, similarly of bacterial origin, determining the production of PAT enzyme, phosphinothrin transpherase, which causes the acetylation and thereby inactivation of glufosinate [28].

BENEFITS

Both RR and LL systems are equal in efficacy - beets with an alien gene remain unharmed after being sprayed with the respective herbicide. Their cultivation would bring several benefits for producers, sugar industry and for sustainable agriculture:

- **Limitations on the spraying number.** Usually two or three sprayings are advised, exclusively after emergence, whereas for selective herbicides very seldom are two sprayings sufficient, more often three or even four of them are needful [6];

- **More freedom in the spraying timing.** Contrary to selective herbicides, whose effectiveness is conditioned as a rule by rigorous meeting the spraying guidelines as well as by weather conditions and the phenological phase of both weeds and beet seedlings [41,27];
- **First spraying can be delayed,** even up to the 6 - 8 leaf phase and 10 cm-height of the weeds, which usually follows 5 - 6 weeks after drilling. In this way the population of aphids on beet seedlings becomes limited as aphids readily feed on young weeds. At the same time the biodiversity of entomofauna in agricultural environment increases [8]. Also the soil becomes protected against wind erosion [25]. The weeds have to be eliminated, however, before they start to compete with beets for light, water, and nutrients;
- **Comprehensive weed killing**, including perennials, with one herbicide only (Roundup or Liberty). Selective herbicides show a different effectiveness in relation to particular weed species, which makes it necessary to apply several products, either successively or else in mixtures [41,23];
- **Professional advisory services are not necessary.** At present more than 20 sugar beet herbicides are registered in Poland, and the number of their combinations, doses and mixtures suggested for use, depending on the number and botanical composition of weed flora, exceeds 100 [26]. Therefore the choice of the best method often requires help and advice of specialised agrochemical extension services. In case of RR or LL beets, however, only one herbicide is applied in the dose recommended by the producer (usually 6 $l \cdot ha^{-1}$ divided between two or three sprayings at two-week intervals);
- **No repression of seedling growth is observed as a rule** (particularly in RR system), even when overdosed. Selective herbicides, specially in case of overdosing, inadequate timing or under unfavourable weather conditions, often cause inhibition of

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beet seedling growth and/or transient yellowing of leaves, which may result in a decreased root yield [4];

- **Effective control of secondary weeds.** If need be, the herbicides can be applied until the inter-row close-up, resulting in an increased yield and in a lower dirt tare of roots delivered to the sugar-processing plant [23];
- **Less hazard to the environment** due to a lower toxicity and faster complete degradation in the soil of both glyphosate and glufosinate, unlike in the majority of selective herbicides with a high toxicity and long degradation period [41,42];
- **Lower costs of chemical weed control.** A total calculation, including all costs borne and benefits, is hardly possible due to a lack of some data such as the seed price as well as root and sugar yields obtained by the grower of tHT cultivars [10]. However, the cost of herbicides only in RR and LL systems is reduced by half, as compared with the costs of traditional herbicides [24].

RESULTS OF FIELD TRIALS

The economic evaluation of transgenic cultivars has been based on plot experiments, only. As an example, mean results of field tests commissioned by KWS Saat and Novartis (harvest plot size ~ 17 and 9 m^2, respectively, four replications and four locations for each firm), performed in 1999 in experiment stations of COBORU (Research Centre for Cultivar Testing), are presented in Tables 1 and 2. New tHT cultivars KWS 8191 and KWS 9193, both representing Z (high sugar) type, reached sugar yield equal or higher than the standard varieties, particularly when treated with Liberty, a non-selective herbicide. Applying conventional herbicides (Betanal + Goltix) resulted in a somewhat lower yield, most probably due to a transient restriction of seedling growth directly after spraying.

Table 1. Economic value of registered and transgenic sugar beet cultivars

Cultivar	Herbicide	Root yield	Sugar content	Sugar yield
Standard*		75.3 $t \cdot ha^{-1}$	17.9 %	11.7 $t \cdot ha^{-1}$
Diadem	Betanal + Goltix	104	98	103
Nilla	Betanal + Goltix	100	101	100
Cordelia	Betanal + Goltix	109	96	105
Kutnowska	Betanal + Goltix	88	104	92
Saskia	Betanal + Goltix	98	108	103
Sonja	Betanal + Goltix	99	102	102
Kassandra	Betanal + Goltix	98	102	101
KWS 8191	Betanal + Goltix	99	106	106
KWS 9193	Betanal + Goltix	99	105	104
KWS 8191	LIBERTY	99	107	107
KWS 9193	LIBERTY	100	108	108
$LSD_{0,05}$		8.1	3.3	9.0

***Standard - mean calculated for the cultivars of 'Diadem', 'Nilla', 'Cordelia' and 'Kutnowska'**

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Table 2. Economic value of registered and transgenic sugar beet varieties mean values from Novartis field trials performed by COBORU, Poland, 1999)

Cultivar	Herbicide	Root yield	Sugar content	Sugar yield
Standard		63.7 t·ha^{-1}	17.6 %	9 t·ha^{-1}
Diadem	Betanal + Pyramin T	104	99	103
Nilla	Betanal + Pyramin T	102	101	102
Cordelia	Betanal + Pyramin T	103	97	99
Kutnowska	Betanal + Pyramin T	92	103	95
HM 1744	Betanal + Pyramin T	103	97	98
HM 1745	Betanal + Pyramin T	93	101	94
HM 1976	Betanal + Pyramin T	102	98	100
HM 5512	Betanal + Pyramin T	93	106	100
HM 1744	ROUNDUP	105	98	101
HM 1745	ROUNDUP	91	100	92
HM 1976	ROUNDUP	102	99	101
HM 5512	ROUNDUP	94	106	103
$LSD_{0.05}$		6.9	2.3	7.9

***Standard - mean calculated for the cultivars of 'Diadem', 'Nilla', 'Cordelia' and 'Kutnowska'**

Similarly, in Novartis tests sugar yield of three transgenic varieties (out of the four tested) proved similar to that of the standard ones, a bit higher when Roundup replaced conventional products (Betanal + Pyramin T). A special attention should be paid to HM 5512 variety representing Z type with a very high processing quality.

Table 3 shows the results of similar tests performed in the Netherlands yet no standard varieties are included. Also here the sugar yield of transgenic RR varieties was higher when Roundup Ultra was applied in different doses (similar to hand-weeding), as compared with spraying with conventional, selective herbicides. Moreover, even a double Roundup dose (12 l·ha^{-1} instead of 6 l·ha^{-1}) did not decrease the sugar yield, whereas a double dose of selective herbicides decreased the yield by 3%. In LL experiment 3×2 l·ha^{-1} Liberty treatment was considered standard. Increased doses and/or number of treatments increased the sugar yield. All such trends were evident yet non-significant.

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Field experiments performed by the Plant Protection Institute (IOR) also confirmed the advantages of RR and LL systems over the conventional ones, both as regards the efficiency of weed control and the root yield [1, 2].

Table 3. Actual and relative sugar-yield of the 1997 trials with glyphosate average of three experiments) and with glufosinate-ammonium (one experiment) [41]

Treatment	Sugar yield	
	$t \cdot ha^{-1}$	%
Standard*	7.5	= 100
Double dose of standard	7.3	97
3 x 2 $l \cdot ha^{-1}$ Roundup Ultra	7.9	105
3 x 3 $l \cdot ha^{-1}$ Roundup Ultra	8.0	107
3 x 4 $l \cdot ha^{-1}$ Roundup Ultra	7.9	105
2 x 6 $l \cdot ha^{-1}$ Roundup Ultra	7.8	104
Hand-weeding	7.9	105
$LSD_{0,05}$	ns	
3 x 2 $l \cdot ha^{-1}$ Liberty	12.0	= 100
3 x 3 $l \cdot ha^{-1}$ Liberty	12.3	102
2 x 3 $l \cdot ha^{-1}$ Liberty	13.1	108
2 x 4 $l \cdot ha^{-1}$ Liberty	12.8	106
3 x 2 $l \cdot ha^{-1}$ Liberty + 0.75 ethofumesate	12.4	103
2 x 3 $l \cdot ha^{-1}$ Liberty + 1.0 ethofumesate	13.0	108
$LSD_{0,05}$	ns	

*** Standard treatment – either a low-dose mixture of 0.5 l of phenmedipham 0.5 kg of metamitron, 0.5 l of ethofumeste and 0.5 l of vegetable oil or - 0.75 to 1.0 l of desmediphan, ethofumesate and phenmedipham**
ns – non-significant difference

PROSPECTS, LIMITS AND PUTATIVE RISKS

Despite evident advantages for agriculture, sugar industry and the environment, tHT beet cultivation has still been limited to plot trials. Permission for such trials, i.e. "introduction to the environment", has to be issued by an authorised department of the state administration. The application shall comprise a detailed information about the modified cultivar compliant with the rigorous directive no 90/220 of 23.04.1990 with later modifications and adjustments, issued by the European Community Council [37, 38,39,11]. Finally the application must be reviewed and discerningly examined. Having been accepted, an authorised supervisor monitors the experimental plots throughout the vegetation period. Additionally, in Poland genetically modified organisms are regulated by the decision no 962 of the Ministry of Environmental Protection, Natural Resources and Forestry of 08.10.1999.

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Field experiments with tHT sugar beets started in Denmark back in 1993, in 1994 in Belgium, France, Sweden and Great Britain, in 1995 in Italy and Spain, in 1997 in Germany, the Netherlands, the Republic of Ireland, the Czech Republic and Hungary as well as in Poland. In 1998 - 2000 field tests with GMOs have been permitted in other European countries, including Russia. In England a three-year field experiment has been scheduled for 2000 in order to study a potential environmental effect of GM sugar beet, oil seed rape and maize when included in the crop rotation [8]. Yet introducing tHT beets into commercial cultivation has been allowed in 2000 in the US only. However, due to Japanese and EU restrictions on imported GM beet animal feed pulp none of several American sugar companies has dared to offer such varieties to growers.

This resistance, particularly strong in some European countries, results from objections (sometimes real but in most cases unjustified) which come from a potential hazard to human and animal health or else to the natural environment, caused by transgenic organisms [36]. As for sugar beets, such objections are groundless as commercial sugar consists of over 99% of chemically pure saccharose without any other organic substances. In molasses and pulp all proteins become denatured during the technological process when exposed to high temperature [18], hence in the US no need to mark GM beet sugar. The same is true for other foodstuffs provided that they do not differ from those derived from traditional cultivars [13]. Moreover, as mentioned earlier, non-selective herbicides are environmentally friendly, unlike the traditional ones applied by sugar beet growers.

However, one should not disregard misgivings connected with the appearance of weed beets, (also in Poland) or else annual hybrids of cultivated beet and wild sea beet (*Beta vulgaris* ssp. *maritima*) [15]. An occasional crossing occurred during seed production, mainly in the south of France and north of Italy [30]. As regards the cultivars resistant to non-selective herbicides, such crossing could result in the appearance of resistant weed beets which would present a real threat [5,29,12]. Although the Polish Standard 81/R-74458 on Sugar Beet Roots allows for no weed-beet roots to be supplied to the sugar processing plant, there are no regulations banning weed-beet seeds in the commercial lots. Therefore tHT cultivars should be reproduced in the regions free from annual wild beets and isolated from seed plantations of traditional cultivars [7]. Similarly, all bolters of industrial beet fields ought to be removed and destroyed. On the other hand, "normal" weed beets, tolerant to conventional selective herbicides, could get easily killed with Roundup or Liberty in the fields drilled with RR or LL cultivars, respectively. There is always ‘the other side of the coin’. A fair estimation of potential tHT beet marketing naturally requires a reliable risk/benefit calculation [20]. Drawing on the results of earlier investigations and experiments, one can expect that such a calculation will encourage tHT beet production and probably in a few years tHt beets will appear on European markets. Our agriculture, sugar industry and, first of all, the public opinion should get prepared for this upcoming novelty.

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