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Sugar Beet Improvement using *Agrobacterium*-mediated Transformation technology

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Abstract

Since discovering *Agrobacterium tumefaciens* distinctive capacity to incorporate a specified part of their transfer-DNA (T-DNA) into eukaryotic cells, the bacteria were commonly used for crop transformation originally of dicotyledonous crops and subsequently of nearly all organisms. To achieve this, the tumor-inducing (Ti) plasmid was changed to extract phytohormone and opine biosynthetic proteins (cytokinin and auxin) so as not to interfere with ordinary morphological growth. Overall, the conversion mediated by *Agrobacterium* was easier, more effective and less costly relative to other technologies. It also results in insertions with small copy count. Tumor development in crops has also proved the susceptibility of explants from field-grown sugar beet crops to *Agrobacterium tumefaciens*. Early efforts by *Agrobacterium tumefaciens* to transform sugar beet were unsuccessful, primarily owing to inability to regenerate crops from stably modified callus or suspended cells. A genotype-independent method was defined under which cotyledonary explants of various sugar beet genotypes are inoculated with *Agrobacterium tumefaciens* comprising whether kanamycin tolerance and GUS activity or kanamycin resistance, GUS activity and glyphosate tolerance. GUS expression, NPT dot blot as well as EPSPS assays verified the presence of transgenes; progeny showed Mendelian genetically modified inheritance and glyphosate tolerance at deadly concentrations to control plants. Unfortunately, there was no publication of technical information of the technique. Here we reviewed the concept *Agrobacterium*-mediated transformation and how to be applicable

Keywords: *Agrobacterium tumefaciens*, callus, suspended cells, sugar beet, kanamycin resistance.

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Introduction

Sugar beet is extremely prone to *Agrobacterium tumefaciens* as an instance (1–5). Though, genotype-dependent susceptibility (1,3) can be enhanced by pre-culture explants before inoculation (6) or by extending the length of co-culture (1,3). Krens et al. (4) used *Agrobacterium tumefaciens* strains carrying an isolated cytokinine gene to enhance the development of sugar beet leaf discs, cotyledons and hypocotyls from two-week-old seedlings of nine distinct types. Cotyledon and hypocotyl explants generated low-frequency shoots; however, inoculated leaf discs did not regenerate the shoot. It was impossible to confirm a stable transformation.

Lindsey and Gallois (1) revealed *Agrobacterium tumefaciens* first effective regeneration of genetically modified sugar beet crops. Transgenic seeds were acquired from co-cultivated shoot-base cells with Agrobacterium strain LBA4404 containing a bi-nary plant with a kanamycin resistance gene (nptII) and either chloramphenicol acetyl transferase (cat) or β-lucuronidase (gusA) genes. The frequency of transformation depended on the kinds of explants, genotypes and circumstances of choice.

Halluin et al. (2) who established crops resistant to broad-spectrum herbicides, glufosinate and sulfonylureas, as well revealed sugar beet transformation. Friendly callus, originating from cotyledons, hypocotyls, petioles as well as true leaves of 2-to 3-month-old seedlings cultivated in dark, was carefully cut and inoculated with *Agrobacterium tumefaciens* either a mutant acetolactate synthase (ALS) or a bialaphos resistance (bar) gene powered by multiple promoters, i.e. 35S CaMV, nos, TR1' or TR2.' This genotype-dependent protocol took nearly two years to get grown, which probably explains the morphologies of the aberrant plant.

Latest studies utilizing *Agrobacterium tumefaciens* generated transgenic crops at frequencies that are adequate to produce crops used in experiments to evaluate herbicide tolerance and resistance to disease (7–12). For physiological and molecular research, *Agrobacterium tumefaciens* produced transgenic crops were also used. An endogenous sugar beet GUS (SB-GUS) enzyme has been contrasted to E. coli in leaf disc transformation (13). Variations in patterns of gene expression in roots have been investigated using distinct constructs (14) in another application. In another, development habit and accumulation of sugar were explored after transforming into a patatin gene promoter (15) with a bacterial cytokinin biosynthetic gene. Although various explants of sugar beet, i.e., shoot bases, petioles, leaves and callus, were used, cotyledon explants were often more effective in transformation with efficiencies varying from 0.1 to 1.0 (4,7,9,10,16,17).

With kanamycin selection (18), leaf lamina explants from shoots multiplied from apical meristems of four genotypes of sugar beet produced a 6.2 percent transformation level. Small (1–3 mm) bud tips from various shoot clumps from undeveloped floral buds of five distinct genotypes resulted in hygromycin-resistant shooting rates of 13.3 to 30.6 percent. Over 50% of crops from shoot-base tissue kanamycin-resistant explants produced the hpt gene (19). Finally, a mixture of *Agrobacterium* and void infiltration has been used to promote the transfer of bacterial DNA to callus and crop tissue, resulting in more than 40% transgenic callus clones (20).

Factors influencing transformation efficiency

Genotype (19), explant origin (21), bacterial strain and incubation time (18) selection technique (22),type of promoter promoting selectable gene (22), light intensity (23), wound with pre-inoculation particle bombardment (10), use of acetosyringone (3), pre-treatment with drugs (6) and , pre- and co-cultivation interval (6). Despite important advancement, the transformation of sugar beet to *Agrobacterium tumefaciens* is still regarded to also be recalcitrant. Because of the failure to absorb and integrate DNA, it is impossible to be due to the small amount of morphologically competent regeneration cells. If they are integrated in big amounts of non-competent cells (24), connection to such competent cells may also be impeded.

Development of transgenic herbicide tolerance

Sugar beet competes badly with weeds, particularly in early growth, leading in dramatically elevated yield losses that can range from 50 percent to total losses (25) unless adequate weed control is attained. Conventional control measures involve herbicide spraying at distinct moments and distances to decrease these losses, making weed control programs complex and challenging.

The development of transgenic sugar beet resistant to broad-spectrum herbicides is therefore a significant alternative. Herbicide tolerance is one of the first features that genetic engineering has effectively brought into several plant species; some types of herbicide tolerant (HT) have been on the market for further than a decade (26). HT crops have been produced using genes from microorganisms or higher plants that confer tolerance by: I changing the active site of the target protein in such a way that converted cells are less susceptible to herbicide; (ii) using an enzyme that transforms the core components of the herbicide into inactive compounds; or (iii) overproducing herbicide target proteins (27).

Agrobacterium-mediated transformation and *in vitro* cell selection have been used to evolve HT sugar beets

tolerant either to non-selective, broad-spectrum herbicides, i.e., glyphosate, Roundup ® active ingredient and glufosinate, Basta ® active ingredient, Liberty ® and Herbiace ® main ingredient, or specific herbicides such as imidazolinone, chlorsulfurone and sulfonylurea (28). In plants, encoded in the nucleus, the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) performs a main part in chloroplast responses arising in synthesis of phenylalanine, aromatic amino acids, tyrosine, and tryptophan.

After finding in the early 1970s that perhaps the analog amino acid glyphosate [N-(phosphonomethyl)-glycine] could preferentially inhibit EPSPS activity, shut down aromatic amino acid synthesis and ultimately kill the plant, strategies are established to produce glyphosate-tolerant plants (or Roundup Ready ®). EPSPS occurs only in crops and certain microorganisms, not in livestock or humans, probably explaining why glyphosate toxicity is exceptionally small in human cells (29).

To create glyphosate-tolerant sugar beet, two methods listed above I and ii) have been utilized. In the first scenario, a gene *Agrobacterium* sp. was used for an altered protein, CP4-EPSPS, which is extremely glyphosate tolerant (30); in vitro assays and in vivo herbicide sprays (16) verified glyphosate tolerance. The second approach used a gene encoding Achromobacter sp. glyphosate oxidoreductase, GOX, and catalyzing glyphosate degradation in and out of non-toxic compounds, glyoxylate and aminomethylphosphonic acid.

However, sugar beet plants were transformed in the second approach with both CP4-EPSPS nd GOX genes (9) and evaluated with distinct Roundup ® spraying systems in the greenhouse and field. Extremely tolerant transformants have been acquired without phytotoxic or any other harmful phenotypic effects (31). Inversely linked with the transgenic copy number (9), herbicide tolerance emerged. Glufosinate (ammonium salt L-phosphinothricin, PPT) and bialaphos (PPT plus two alanines; L-phosphinothricinyl-L-alanyl-L-alanine) are extremely toxic to plant cells; they behave as competing glutamine synthetase inhibitors that are critical to the transformation of glutamine acid and ammonia onto glutamine.

Inhibition contributes to toxic ammonia accumulation, leading to death of the cell. Enzymes encoded in bars and pat and extracted from various Streptomyces sp., detoxify PPT and have been broadly used as selectable markers for the production of transgenic HT plants. Bar, motivated by the proponents of CaMV 35S, nos, TR1' or TR2, was used to achieve glufosinate-tolerant sugar beet crops (2) assessed in the sector of gene flow and agricultural efficiency research (32). Like glyphosate, glufosinate has a very low toxicity to mammals (29).

Joersbo (33) assessed both glyphosate-and glufosinate-tolerant sugar beets environmental efficiency, including economics and utilization flexibility. By deactivating the first enzyme in the pathway, acetolactate synthase (ALS), sulfonylurea compounds prevent the biosynthesis of clustered amino acids, valine, leucine and isoleucine. Sugar beet has been transformed with a mutant sulfonylurea-insensitive ALS gene (2).

In vitro cell selection (34) was also acquired from mutant sulfonylurea-and imidazolin one-tolerant sugar beet crops. However, many HT transgenic beet varieties have been approved for discharge in the U.S. (1996, 1998, 2005), Canada (2001, 2005) and Japan (2007) (26). Roundup Ready sugar beets were grown for the first season in 2008 by sugar beet growers in Michigan. For ten years, Michigan State University researchers have been collaborating with these varieties to determine implementation rates and timing and policies to delay glyphosate-resistant weed growth.

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