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> *Beta vulgaris* L.

The Biology of *Beta vulgaris* L. (Sugar Beet)

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Biology Document BIO2002-01: A companion document to Directive 94-08 (Dir94-08), Assessment Criteria for Determining Environmental Safety of Plant with Novel Traits

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Part A - General Information

A1. Background

The Canadian Food Inspection Agency (CFIA) is responsible for regulating the field testing of plants

with novel traits (PNTs) in Canada. PNTs are defined as a plant variety/genotype possessing characteristics that demonstrate neither familiarity nor substantial equivalence to those present in a distinct, stable population of a cultivated species of seed in Canada and that have been intentionally selected, created or introduced into a population of that species through a specific genetic change. Familiarity is defined as the knowledge of the characteristics of a plant species and experience with the use of that plant species in Canada. Substantial equivalence is defined as the equivalence of a novel trait within a particular plant species, in terms of its specific use and safety to the environment and human health, to those in that same species, that are in use and generally considered as safe in Canada, based on valid scientific rationale.

PNTs may be developed using traditional plant breeding techniques or other methodologies such as recombinant DNA technologies. Regulated field testing is necessary when PNTs are: 1) considered unfamiliar when compared with products already on the market; 2) not considered substantially equivalent to similar, familiar plant types already in use and regarded as safe.

Before PNTs may be authorized for unconfined release, they must be assessed for environmental safety. **Regulatory Directive 94-08: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits** describes the criteria and information requirements that must be considered in the environmental assessment of PNTs to ensure environmental safety in the absence of confined conditions.

A2. Scope

The present document is a companion document to **Dir94-08: Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits**. It is intended to provide background information on the biology of *Beta vulgaris* L., its centres of origin, its related species, the potential for gene introgression from *B. vulgaris* into relatives, and details of the life forms with which it interacts.

Such species-specific information will serve as a guide for addressing some information requirements in Part D of Dir94-08. Specifically, it will be used to determine if there are significantly different/altered interactions with other life forms, resulting from the PNTs novel gene products which could potentially cause the PNT to become a weed of agriculture, invasive of natural habitats, or be otherwise harmful to the environment.

The conclusions drawn in this document about the biology of *B. vulgaris* only relate to plants of this species with no novel traits.

Part B - The Biology of *Beta vulgaris*

B1. General Description

The sugar beet (*B. vulgaris* L.) belongs to the Chenopodiaceae family. This family includes approximately 1400 species divided into 105 genera (Watson and Dallwitz, 1992). Members of this family are dicotyledonous and usually herbaceous in nature. Economically important species in this family include sugar beet, fodder beet/mangolds, red table beet, Swiss chard/leaf beet (all *B. vulgaris*), and spinach (*Spinacia oleracea*).

Sugar beet is normally a biennial species, however under certain conditions it can act as an annual (Smith, 1987). The sugar beet plant develops a large succulent taproot in the first year and a seed stalk the second year. Typically sugar beet root crops are planted in the spring and harvested in the autumn of the same year. For seed production, however, an overwintering period of cold temperatures of 4 - 7°C (vernalization) is required for the root to bolt in the next growing season and for the reproductive stage to be initiated (Smith, 1987).

During the first growing season, the vegetative stage, the sugar beet plant is described as having glabrous leaves that are ovate to cordate in shape and dark green in colour. The leaves form a rosette from an underground stem. A white, fleshy taproot develops, prominently swollen at the junction of the stem (Duke, 1983). During the second growing season, the reproductive stage, a flowering stalk

elongates (bolts) from the root. This angular seed stalk forms an inflorescence and grows approximately 1.2-1.8 metres tall. A large petiolate leaf develops at the base of the stem with small leaves, further up the stem there are less petiolate leaves and finally sessile leaves developing. At the leaf axils, secondary shoots develop forming a series of indeterminate racemes (Forster *et al.*, 1997). These flowers are small, sessile and occur singly or in clusters. Sugar beets produce a perfect flower consisting of a tricarpellate pistil surrounded by five stamens and a perianth of five narrow sepals. Petals are absent and each flower is subtended by a slender green bract (Smith, 1987).

The ovary forms a fruit which is embedded in the base of the perianth of the flower. Each fruit contains a single seed whose shape varies from round to kidney-shaped. The ovaries are enclosed by the common receptacle of the flower cluster (Duke, 1983). A monogerm seed is formed when a flower occurs singly. The multigerm beet seed is formed by an aggregation of two or more flowers (Cooke and Scott, 1993).

B2. Use as a Crop Plant

There is no seed production of sugar beets in Canada. Most sugar beet seed imported into Canada is produced in Oregon, USA (Webster, 2001). Sugar beet roots are processed into white sugar, pulp and molasses for food, feed or industrial applications and are rarely used as a raw commodity. A typical sugar beet root consists of 75.9% water, 2.6% non-sugars, 18.0% sugar and 5.5% pulp. In the sugar fraction 83.1% is recovered as crystalline sucrose, 12.5% is recovered as molasses (Bichsel, 1987). Sugar is a multi-purpose carbohydrate that contributes significantly to the flavour, aroma, texture, colour and body of a variety of foods. In addition to processing pure sugar, sugar factories also produce a by-product known as dried sugar beet pulp. This pulp is used as feed for cattle and sheep, and is produced and shipped in pressed plain dried, molasses dried, and pelleted forms. Another important by-product is sugar beet molasses, a viscous liquid containing about 48% saccharose, which cannot be economically crystallized. Sugar beet molasses is used for production of yeast, chemicals, pharmaceuticals, as well as in the production of mixed cattle feeds..

Currently, sugar beet is the major sugar crop grown in temperate regions of the world. In Canada, sugar beets are grown in the provinces of Alberta and Ontario. In 1998, 42,000 acres were planted in Alberta and 6,500 acres were planted in Ontario. Acreage of sugar beet in Canada was significantly higher in 1998 than in previous years suggesting that future acreage may be on the rise (OMAFRA, 1998). In 1997, 650 thousand tonnes of sugar beet were produced in Canada worth over \$43 million (Canadian Sugar Beet Producers Association, 1997). Sugar beet production in Canada represents about 10 to 15 percent of total domestic sugar consumption (Canadian Federation of Agriculture, 1998).

B3. The Centres of Origin of the Species

The centre of origin of beet (*Beta*) is believed to be the Middle East, near the Tigris and Euphrates Rivers. It is thought that wild beets spread west into the Mediterranean and north along the Atlantic sea coast. Geographic isolation of wild beets on the Canary Islands led to the creation of several distinct species (*B. patellaris*, *B. webbiana* and *B. procumbens*) that are largely annual (Cooke and Scott, 1993). The dispersal of wild types north into the mountains of Turkey, Iran, and the Caucasus Mountains of Russia, also led to the establishment of the species *B. trigyna*, *B. lomatogona*, and *B. macrorhiza* (Cooke and Scott, 1993). These species are somewhat perennial in growth habit. Finally, wild beet spread east through most of Eastern Asia. Cultivated sugar beet is likely to have originated from wild maritime beet (*B. vulgaris* subsp. *maritima*) through breeding selection (Cooke and Scott, 1993).

Historically, beets have been used for both livestock and human consumption. The first recorded use of beets is from the Middle East. Records dating to the 12th century contain the earliest descriptions of sugar beets as plants with swollen roots (Toxopeus, 1984). It was not until the late 18th century, that German scientists began to breed beets to increase the sugar content of their roots (American Sugar beet Growers Association, 1998). Original forms of sugar beet were derived from white Silesian beet, which had been used as a fodder crop and contained only about 4% sugar. Repeated selection and breeding have raised the sugar content to its present level.

B4. Brief Outlook at Breeding, Seed Production and Agronomic Practices for Sugar Beet

Early breeding techniques for sugar beet were developed by the USDA and include cytoplasmic male sterility, monogerm seeds and hybrid vigour (Panella, 1996). Today, all U.S. sugar beet cultivars are monogerm hybrids. The use of monogerm sugar beet seed has greatly reduced the need to thin clusters of sugar beet seedlings, a requirement when multigerm seed was planted (Smith, 1987). Private seed companies now dominate sugar beet breeding concentrating on varieties which produce high sucrose concentrations, have disease and pest resistance as well as herbicide tolerance.

Cytoplasmic male sterility (CMS) allows the breeder to develop male-sterile or female parental lines. These lines are a key factor in the breeding of hybrid cultivars (Forster *et al.*, 1997). Commonly, a monogerm O-type (or maintainer) line will be hybridized with the monogerm male-sterile equivalent of another line to produce a monogerm male-sterile F₁. The F₁ then is used as the seed parent in crosses with diploid or tetraploid pollinator lines (Forster *et al.*, 1997).

Data indicates that there is an inverse relationship between the weight of sugar beets produced per unit area and the percentage of sugar produced (Smith, 1987). Recurrent and reciprocal recurrent selection techniques have not changed this relationship (Hecker, 1978). Originally sugar beet was a diploid with 18 chromosomes (2x). Commercial exploitation of polyploidy in sugar beets began in Europe in the 1940s with the development of anisoploid varieties. Such varieties were actually mixtures including diploid, triploid and tetraploid individuals, and were produced by interpollination of diploid and tetraploid seed-parents (Forster *et al.*, 1997). The use of cytoplasmic sterility in conjunction with polyploidy allowed the production of triploid varieties. Currently there are diploid, triploid, and anisoploid varieties available (Forster *et al.*, 1997). Higher ploidy levels have been produced experimentally but have had limited usefulness. In Canada, the diploid hybrid cultivars are the dominant cultivars in use.

For seed production in Europe, small vegetative plants known as stecklings are produced in the first season. The following season they are transplanted into the field where seed production will take place. In the United States, 90-95 % of seed production is through the direct seed method. Seed is planted in August, overwinters, and the seed is harvested in July of the following year. Typically seed production areas are planted with 2 rows of pollinator stecklings followed by 4-8 rows of CMS stecklings. After flowering and pollen dispersal, the pollinator plants are removed in order to optimize seed quality.

Sugar beet is sensitive to cold temperatures and is killed by frost at temperatures below -5°C. Hence, in Canadian agricultural practice, sugar beet is handled as an annual crop with the roots being harvested for sugar after 5-7 months of growth. Yields of roots range from 10 to 35 tonnes per acre where sucrose concentrations range from 12-20% or more. The sugar content in the root is affected by nitrogen availability. Nitrogen should be applied early, as an excess late in the season reduces sugar content. To optimize sucrose storage in the roots, plants should exhaust the available nitrogen supply 4-6 weeks prior to harvest.

Sugar beet is a poor competitor with weeds, particularly early in the season. Weed control is critical from the cotyledon to 12 leaf stage of seedling growth. In fields where weeds are never controlled and consist of tall growing species such as *Chenopodium album* (lambsquarters), yield loss can be as great as 95% (Scott and Wilcockson, 1976). This, however, is unlikely in a commercial situation and a typical yield reduction due to weeds is generally 6-10% when weed control is used.

A number of insects can attack the developing plant. Sugar beet root maggot is a major pest in Alberta and can be reduced by applying a suitable soil applied insecticide. Other invertebrate pests include cutworms, wireworms, flea beetles, grasshoppers, sugar beet root aphid, beet webworm, beet leaf miner and sugar beet cyst nematode. The primary control for sugar beet cyst nematode and diseases affecting sugar beet is crop rotation, with sugar beet grown no more frequently than every fourth year with a grain or hay crop in the rotation (Alberta Agriculture, Food and Rural Development, 1998).

B5. The Reproductive Biology of *B. vulgaris*

Sugar beet seed is only produced by biennial flowers during the second year although certain conditions during the first year may cause premature bolting.

Flowers reach anthesis about 5 to 6 weeks after the initiation of reproductive development. Anthesis continues for a period of several weeks. After dehiscence of the mature anthers the globular pollen is transmitted largely by wind and occasionally by insects. Sugar beet pollen is extremely sensitive to moisture, however, under dry conditions its viability is lost within 24 hours. The primary method of pollination is cross pollination due to the lack of synchrony between pollen release and receptiveness of the stigma. Since the pollen can be carried by the wind over long distances, breeding stock and commercial seed production fields must ensure the isolation of flowering sugar beet plants. According to the Canadian Seed Growers Association (CSGA) regulations, pedigreed sugar beet crops must be isolated by 400 meters from any plants that are a source of contamination through cross pollination.

Sugar beet is strongly self-sterile setting few or no seeds under strict isolation. The underlying genetic mechanisms may be explained by two series of multiple sterility alleles ($S_1 - S_n$, $Z_1 - Z_n$) (J. R. Stander, 1995). The setting of some seeds after selfing, so-called pseudo-compatibility, is due to a break-down of the incompatibility-mechanism (J. R. Stander, 1995).

Pseudo-compatibility is pronounced in varying degrees in different genotypes and is highly influenced by environmental conditions, especially temperature (J. R. Stander, 1995). There is a self-fertility gene which, when introduced, can create plants which are self-fertile (Smith, 1987).

B6. Cultivated *B. vulgaris* as a Volunteer Weed

The occurrence of *B. vulgaris* in Canada is limited to commercial production for harvest of roots in Southern Alberta and Southwestern Ontario. Low temperatures and long day lengths can occasionally cause sugar beet to bolt, and set seed in the first year. This seed has the potential to result in weeds in subsequent crops.

Weed beet are defined as undesirable beet species (within the *Beta* Section) occurring in managed areas. While not a management issue in North America, weed beet is one of the most significant weed problems in European sugar beet production. In Europe, weed beet can arise as a result of contaminating pollen from sexually compatible wild annual relatives, bolting beet plants, dormant seed, and groundkeepers. Groundkeepers are small roots left in the field after harvest, which will flower in the next season if not controlled.

Occasionally, volunteer sugar beets occur in Canada but do not become established as persistent weeds. Canada's cold winter climate does not allow sugar beet roots to survive and any plants produced by seed from bolters do not persist long in the environment. Both lack of annual relatives and colder temperatures in Canadian growing areas, minimize weed beet. In crop production systems, volunteer beets are removed with the production practices that are normally used for crops that succeed beets in rotation. Sugar beet is easily controlled by most broadleaf herbicides, however in some instances, herbicides that are registered for use on sugar beet can cause damage to the beet crop.

B7. Summary of Ecology of *B. vulgaris*

B. vulgaris is not a primary colonizer in unmanaged ecosystems. Seedlings of this species do not compete successfully against plants of similar types for space. In Canada, beets do not survive outside of cultivation for significant periods of time due to cold sensitivity and poor competitiveness.

In crop production systems, volunteers do not compete well with crops used in rotation with sugar beets. In addition, volunteer sugar beets are removed by the typical production practices for crops that are used in rotation with beets, and sugar beets can be easily controlled using chemical and/or mechanical control methods.

B. vulgaris is not listed as a noxious weed in the Weed Seed Order (1986). It is not reported as a pest or weed in managed ecosystems in Canada, nor is it recorded as being invasive of natural ecosystems. In summary, there is no evidence that in Canada, *B. vulgaris* has weed or pest characteristics.

Part C - The Close Relatives of *Beta vulgaris*

C1. Inter-Species/Genus Hybridization

Important in considering the potential environmental impact following the unconfined release of genetically modified *B. vulgaris*, is an understanding of the possible development of hybrids through interspecific and intergeneric crosses with the crop and related species. The development of hybrids could result in the introgression of the novel traits into related species resulting in:

- related species becoming more weedy
- the introduction of a novel trait, with potential for ecosystem disruption, into the related species.

The genus *Beta*, to which *B. vulgaris* belongs, is comprised of 15 recognized species which are divided into four sections: *Beta* (formerly *Vulgares*), *Corollinae*, *Procumbentes* (formerly *Patellares*) and *Nanae* (see Table 1).

Hybridization between *B. vulgaris* and specific members within the *Beta* section (i.e. fodder beet, red beet, leaf beet, Swiss chard) can occur. One study reported successful hybridization events between *B. vulgaris* and relatives of Western European origin; *B. maritima*, *B. macrocarpa* and *B. atriplicifolia* (BRIDGE, 1993, Appendix 2). Hybrids of *B. vulgaris* and *B. maritima* are fertile and show compatibility at the chromosomal level (Forster *et al.*, 1997). Hybrids between *B. macrocarpa* and *B. vulgaris* have caused weed problems in European sugar beet fields (McFarlane, 1975). Other studies detected genetic barriers between these two species, resulting in partial pollen sterility and embryo abortion in the hybrid (Abe, *et al.*, 1986). Similarly, lower hybridization levels between *B. vulgaris* and *B. macrocarpa* were found in the BRIDGE study (BRIDGE, 1993).

Artificial hybrids have been produced with species in the section *Corollinae*. However, such hybrids are highly sterile and few plants set seed when backcrossed to sugar beet. It is generally not necessary to use bridge species, although *B. vulgaris* ssp. *maritima* has been successfully used to introduce traits from *B. trigyna* into sugar beet.

Artificial hybrids between sugar beet and members of the section *Procumbentes* have been produced with great difficulty. The hybrids become necrotic and die at the seedling stage. Successful hybrids can be produced by grafting hybrids onto sugar beet plants or by using fodder beets, mangolds, *B. vulgaris* ssp. *maritima* as bridge species. These hybrids are almost completely sterile and fertile plants produce little seed upon backcrossing with *B. vulgaris*. Pollen sterility in F₁ and BC₁ generations is the result of abnormal meiosis. Chromosome lagging, multiple spindles, bridges and ejected chromosome have been frequently observed causing lack of fertility or embryo abortion. The chromosomes of the species of section *Procumbentes* do not pair with those of section *Beta* (Van Geyt *et al.*, 1990). No hybrids between cultivated beets and *B. nana* of section *Nanae* have been reported.

In conclusion, within the Family Chenopodiaceae, all crosses between cultivated sugar beet and species from sections other than *Beta*, are highly improbable

C2. Potential for Introgression of Genetic Information from *B. vulgaris* into Relatives

All evidence demonstrates that *B. vulgaris* only forms hybrids with specific members of the Chenopodiaceae within the *Beta* section (De Bock, 1986). Natural hybridizations between cultivated beet and some wild or weedy forms of section *Beta* can occur in areas where both are present. Of the wild relatives that can hybridize with sugar beet, only *B. vulgaris* ssp. *maritima* and *B. vulgaris* ssp. *macrocarpa* are present in North America. These isolated populations are limited to California and are not found near sugar beet seed production areas in that state.

C3. Occurrence of Related species of *B. vulgaris* in Canada

There are no wild *Beta* species in Canada. The occurrence of *B. vulgaris* is limited to commercial

production for root harvest. Occasionally, plants and seedlings grow as volunteers, but these do not become established in Canada as persistent weeds. *B. vulgaris* is not reported as a weed in Canada.

Part D - Potential Interactions of *Beta vulgaris* with Other Life Forms

Table 1. Taxonomic Division of the Genus *Beta* (based on DeBock, 1986)

SPECIES	CHROMOSOME NUMBER	DISTRIBUTION
Section 1: <i>Beta</i> (syn: <i>vulgares</i>) <i>B. vulgaris</i> subsp. <i>vulgaris</i> L.	18	Global (cultivated) ¹
<i>B. vulgaris</i> subsp. <i>maritima</i> L.	18	N. Africa, Portugal, Spain, Egypt, Israel, Jordan, Syria, Turkey, Albania, Belgium, Bulgaria, Denmark, France, Germany, Greece, Ireland, Italy, Netherlands, Sweden, U.K., Yugoslavia ¹
<i>B. atriplicifolia</i> (Rouy)	18	Europe ¹
<i>B. patula</i> (Ait.)	18	Portugal ¹
<i>B. orientalis</i> (Roth.)	18	India (cultivated) ¹
Section 2: <i>Corollinae</i> <i>B. macrorhiza</i> (Stev.)	36	Turkey, Iran, Caucasus Mountains ³
<i>B. lomatogona</i> (Fish et Mey.)	18, 36	Caucasus, W Asia ²
<i>B. corolliflora</i> (Zos.)	18	Turkey, Iran, Caucasus Mountains ³
<i>B. trigyna</i> (Wald et Kit.)	36, 45, 54	Caucasus, W Asia, E Europe, SE Europe ²
<i>B. intermedia</i> (Bunge)	18	Turkey ³
<i>B. foliosa</i> (Hauskn.)	?	
Section 3: <i>Nanae</i> <i>B. nana</i> (Bois. Et Held.)	18	Greece ³
Section 4:		

<i>Procumbentes</i> (syn. <i>Patellares</i>) <i>B. procumbens</i> (Chr. Sm.)	18	Canary Islands, S Spain, NW Africa ³
<i>B. webbiana</i> (Moq.)	18	Canary Islands, S Spain, NW Africa ³
<i>B. patellaris</i> (Moq.)	36	Macaronesia, N Africa, SE Europe, SW Europe ²

¹ From Terrell, E.E. 1986.

² From Wiersema, J.H and León, B. 1999

³ From USDA web publication, 2001

Table 2. Examples of Potential Interactions of *B. vulgaris* with Other Life Forms During its Life Cycle

Table 2 is intended to guide applicants in their considerations of potential impacts the release of the PNT in question may have on non-target organisms, but should **not be considered as exhaustive**. Where the impact of the PNT on another life form (target or non-target organism) is significant, secondary effects may also need to be considered.

Other Life Forms (Common Name)	Interaction(s) with <i>B. vulgaris</i> (Pathogen, Symbiont or Beneficial Organism, Consumer, and/or Gene Transfer)
Beet Curly Top Virus (BCTV)	Pathogen
<i>Cercospora beticola</i> (Cercospora Leaf Spot)	Pathogen
Fungal seedling diseases (e.g., <i>Pythium ultimum</i> , <i>P. aphanidermatum</i> , <i>Aphanomyces cochlioides</i>)	Pathogen
<i>Rhizoctonia solani</i> (Rhizoctonia Root Rot)	Pathogen
<i>Rhizomania</i> (Beet necrotic yellow vein virus (BNYVV))	Pathogen
Virus Yellows (e.g., Beet Yellows Virus (BYV), Beet Western Yellows Virus (BWYV), Beet Mosaic Virus (BMV))	Pathogen
<i>Pegomya hyoscyami</i> (Beet Leaf Miner)	Consumer
<i>Loxostege sticticalis</i> (Beet webworm)	Consumer
<i>Euxoa</i> spp.	Consumer

(Cutworms)	
<i>Psylliodes punctulata melsheimer</i> (Flea Beetles)	Consumer
<i>Metanplus</i> spp. (Grasshoppers)	Consumer
<i>Meloidogyne arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i> , <i>M. hapla</i> (Root Knot)	Consumer
<i>Nacobbus aberrans</i> , <i>N. dorsalis</i> (False Root Knot)	Consumer
<i>Silpha bituberosa</i> (Spinach Carrion Beetle)	Consumer
<i>Heterodera schachtii</i> (Sugar beet cyst nematode)	Consumer
<i>Pemphigus populivenae betae</i> (Sugar beet Root Aphid)	Consumer
<i>Tetanops myopaeformis</i> (Sugar beet Root Maggot)	Consumer
<i>Elateridae</i> (Wireworms)	Consumer
Pollinators	Consumer; Symbiont or Beneficial Organism
Mycorrhizal Fungi	Symbiont or Beneficial Organism
Birds	Consumer
Animal browsers	Consumer
Soil Microbes	Symbiont or Beneficial Organism
Earthworms	Symbiont or Beneficial Organism
Soil Insects	Consumer
Other <i>Beta</i> spp. (<i>B. vulgaris</i> ssp.. <i>maratima</i> , <i>B. vulgaris</i> ssp.. <i>macrocarpa</i>)	Gene Transfer

Part E - Acknowledgements

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