The Biology of *Lens culinaris* Medikus (Lentil)

Biology Document BIO2003-12: A companion document to the 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's (CFIA) Plant Biosafety Office (PBO) is responsible for regulating the intentional introduction of plants with novel traits (PNTs) into the Canadian environment.

PNTs are plants containing traits not present in plants of the same species already existing as stable, cultivated populations in Canada, or are expressed outside the normal statistical range of similar existing traits in the plant species.

PNTs that are subject to an environmental safety assessment are those plants that are potentially not substantially equivalent, in terms of their specific use and safety for the environment and for human and animal health, to plants currently cultivated in Canada, with regard to weediness/invasiveness, gene flow, plant pest properties, impacts on other organisms and impact on biodiversity.

Consistent with the Canadian approach, the CFIA recognizes that it is the presence of a novel trait in a plant that potentially poses environmental risk, and hence is subject to regulatory oversight, as opposed to how the traits are specifically introduced, e.g., introduction of novel traits by traditional breeding, mutagenesis, recombinant DNA techniques, etc.

Before PNTs may be authorized for unconfined environmental release, they must be assessed for environmental safety. Directive 94-08 (Dir94-08), *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.

The assessment criteria are designed to be used in conjunction with species-specific biology documents that describe the biology of the species to which the modified plant belongs, including details of other life forms with which it interacts. The assessment is part of the continuum of research, development, evaluation and potential commercialization of plants with novel traits.

A2. Scope

The present document is a companion document to **Dir94-08**. It is intended to provide background information on the biology of *Lens culinaris*, its centre of origin, its related species and the potential for gene introgression from *L. culinaris* into relatives, and details of the life forms with which it may interact. Such species-specific information will serve as a guide for addressing some of the information requirements of Part D of Dir94-08. Specifically, it will be used to determine if significantly different/altered interactions occur with other life forms resulting from the PNT's novel gene products, which could potentially cause the PNT to become a weed of agriculture, become invasive of natural habitats, or be otherwise harmful to the environment.

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

Part B - The Biology of *L. culinaris*

B.1 General Description, Cultivation and Use as a Crop Plant

From the tribe *Viceae*, *Lens* is a genus in the family Leguminosae (Fabaceae), commonly known as the legume family. The genus *Lens* consists of the species:

- *Lens culinaris* Medikus,
- its progenitor *L. orientalis* (Boiss.) Hand.-Maz.,
- *L. nigricans* (M. Bieb.) Grand.,
- *L. ervoides* (Bring.) Grand.,
- *L. odemensis* Ladiz.,
- *L. lamottei* Czefranova, and
- *L. tomentosus* Ladiz. (Ladizinsky *et al.*, 1984; van Oss *et al.*, 1997).

Most accessions of *L. orientalis* cross readily with *L. culinaris*, and both are genetically isolated from the other species. Crosses are possible between *L. culinaris* and the remaining species, but they are characterized by a high frequency of hybrid embryo abortion, albino seedlings and chromosomal rearrangements that result in hybrid sterility, if these seedlings reach maturity (Ladizinsky, 1993).

All *Lens* species are annual, herbaceous diploids, 2N = 14. The lentil plant has a fine stem, rarely grows more than 45 cm tall and has an indeterminate growth habit (Saskatchewan Pulse Growers, 2000). The first two nodes are vestigial and occur at or below the soil surface. If apical dominance is destroyed or if growing conditions are favourable, new shoots will arise from the dormant buds at the uppermost of these two nodes. The lentil plant may produce up to four basal branches and will produce up to five aerial branches at the five uppermost nodes just below the first flower. Under extremely favourable growing conditions, the aerial branches may also produce secondary branches. The first flower on the main stem is borne in the axis of the 11th to 13th node above the first two vestigial nodes. The leaves are pinnate with up to 10 pairs of leaflets. One or two small lens-shaped seeds are borne in small, flattened pods (one or two) on a short pedicel arising in the leaf axils above the 11th to 13th node. The seed coat colour ranges from white (zero tannin) to pale green to gray to brown to black, often with purplish flecks of varying sizes (Vandenberg and Slinkard, 1990). Seed weight ranges from 30 to 70 g/1000 seeds in Canadian cultivars, but is much lower in Indian cultivars and in the wild species.

Lentil was one of the first crops to be cultivated, with archeological evidence dating from the early Stone Age. Today, lentil remains an important source of dietary protein in India, Southwest Asia and the Mediterranean with India and Turkey leading the world in lentil production. Main lentil production areas in North America are found in Saskatchewan, and in the Palouse area of Washington and Idaho.

Lentils perform best on at a soil pH of 6.0 to 8.0. They do not do well on waterlogged soils and will not tolerate flooding or salinity. Lentil is well adapted to the dark brown soil zone of western Canada (Saskatchewan Pulse Growers, 2000).

Canadian lentil production is primarily for export. While lentil is grown mainly for the seed to be harvested as a food export, the straw can also be used as a high quality animal feed or as a source of organic material for soil improvement (Saskatchewan Pulse Growers, 2000).

B2. Breeding, Seed Production and Agronomic Practices for Lentil

The first Canadian lentil cultivar was "Laird" lentil, registered in 1978. Laird lentil soon became the standard of quality in many lentil markets, due primarily to its extra large seed size and bright green seed coat colour. Laird lentil was grown on over 70% of the Canadian lentil acreage from 1984 to 1999. However, it has become susceptible to ascochyta blight, caused by the fungus; *Ascochyta lentis*, which became more aggressive as the acreage devoted to lentil increased over the years (Andrahennadi, 1997). Ascochyta blight was first reported in western Canada in 1978 (Morrall and Sheppard, 1981). The teleomorph of *Ascochyta lentis* is *Didymella lentis* (Kaiser and Hellier, 1993), which was first found in Saskatchewan in 1999. The other two major lentil cultivars grown in 1999 were Eston lentil (small seeded) and CDC Richlea lentil (medium seed size), both of which are susceptible to ascochyta blight. Ascochyta resistant versions of all three of these cultivars are in the pedigreed seed system and commercial seed for the small-seeded cultivar CDC Milestone, the medium-seed-sized CDC Vantage and the large-seeded CDC Glamis is now available. All of these cultivars have yellow cotyledons, but the majority of the lentils produced and consumed in the world have red cotyledons. Three red cotyledon cultivars with resistance to ascochyta blight, CDC Redwing, CDC Redcap and CDC Robin, are registered. CDC Robin also has resistance to anthracnose, caused by *Colletotrichum truncatum*, and has seeds that are easier to dehull and split due to their more nearly spherical shape.

Average yield of lentil is about 1400 kg/ha, but yields as high as 3600 kg/ha have occurred. Lentil is often considered a drought-tolerant crop, but it has a rather low water use efficiency as compared to spring wheat. Lentil plants have a low dry matter yield and the seedlings use very little soil moisture up to about June 25 (the first flower stage). However, a drought stress in the seedling stage will result in dwarfing of the plants and reduced yields. Timely rains just before flowering and for the next three weeks will result in above average seed yields.

Lentil is direct seeded into cereal stubble early in the spring. The planting rate is 132 seeds per square meter (12 seeds per square foot), which is about 45 kg/ha for the small-seeded Eston lentil, 60 kg/ha for the medium-seed-size CDC Richlea lentil and 90 kg/ha for the large-seeded Laird lentil. Lentil seeds can germinate in light or dark, and in fluctuating temperatures. However, rates of germination vary strongly with temperature, and depend on cultivar and seed size.

Lentils are most often grown in rotation with cereal crops such as spring or durum wheat. Lentil is susceptible to ascochyta blight and anthracnose, and crop rotation impacts on the risk of these diseases. Sclerotinia may be a problem if lentil follows other susceptible broadleaf crops such as canola, mustard or pea. Volunteer canola or mustard may be difficult to control in lentil. Volunteer cereal seeds such as barley or durum wheat are difficult to remove from lentil (Saskatchewan Agriculture and Food, 2000).

Lentil has a relatively high requirement for phosphorus, which is needed to promote the development of extensive root systems and vigorous seedlings. Phosphorus also plays an important role in the nitrogen-fixing process. Generally, nitrogen (N) fertilizer application is not required if N fixation is optimized. Micronutrient deficiencies in lentil production have not been identified (Saskatchewan Agriculture and Food, 2000).

A major challenge in lentil production is weed control. Lentil seedlings are short and slow-growing in relation to many weed species and therefore compete very poorly. Effective chemical weed control is necessary for commercial viability. Soil-incorporated dinitroaniline herbicides have been used for control of many annual broadleaf and grassy weeds with metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] used to control annual weeds of the mustard family. Most graminicides are registered for use on lentils also.

Due to its indeterminate growth habit, the lentil crop is often desiccated with diquat (1,1'-ethylene-2,2'-bipyridilium dibromide salt) in order to dry down the top growth rapidly and minimize exposure of the mature seeds to the elements. The seed coat of lentil contains polyphenolic compounds (tannin precursors), which oxidize rapidly if the mature seed is exposed to moisture and sunlight for even a short period of time. The seed coat turns brown in a tanning reaction and this is a down grading factor, resulting in a lower price for the crop. After the desiccated top growth has dried down, the lentil crop is direct harvested and stored in a bin until sold. Safe seed moisture level for storage is 14 % or less (Canadian Grain Commission, 1993). Lentil seed should not be stored over more than one summer since the polyphenolic compounds in the seed coat will slowly oxidize, resulting in seed coat browning and down grading.

B3. The Reproductive Biology of *L. culinaris*

L. culinaris is predominantly self-pollinating with less than one per cent cross-pollination being reported (Wilson and Law, 1972). Modest spatial isolation (3 metres) is required to minimize the possibility of outcrossing in pedigreed seed production in Canada (Canadian Seed Growers Association, 2000). The stamens consist of ten tiny anthers (9:1 arrangement). The pistil consists of the stigma, the style and the ovary, usually with two ovules. Pollination normally occurs just before the flower opens (Muehlbauer *et al.*, 1980).

B4. The Centre of Origin of the Species

L. orientalis is the progenitor species (Ladizinsky *et al.*, 1984). The centre of origin for *L. culinaris* is the Near East (Zohary, 1972) and the species was first domesticated in the Near East (Zohary and Hopf, 1973). Cubero (1981) concluded that lentil first spread to the Nile from the Near East, to Central Europe and then to the Indian subcontinent and the Mediterranean Basin by the end of the Bronze Age. It was introduced to South and Central America in more recent times, presumably with the arrival of the Spanish. Subsequently, it was introduced into the United States before WW II and into Canada in 1969.

B5. Cultivated Lentil as a Volunteer Weed

Lentil pods shatter and release some of their seeds on the ground, even if the crop is harvested in a timely manner. However, the lentil seeds germinate readily in the presence of moisture and do not survive the winter. Consequently, very few lentil seedlings emerge as weeds in the succeeding crop. The tiny, slow growing seedlings compete poorly with other cultivated crops or weeds and, thus, volunteer lentils are rarely a problem. In addition, lentil plants are readily killed by most broadleaf herbicides. The incidence of "hard" seeds is near zero in currently grown cultivars.

Part C - The Close Relatives of *L. culinaris*

C1. Inter-Species / Genus Hybridization

Important in considering the potential environmental impact following the unconfined release of genetically modified *L. culinaris* 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 allow the introgression of the novel traits into these related species and result in:

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

L. culinaris can hybridize with the progenitor species, *L. orientalis*, and produce fully fertile offspring. However, *L. orientalis* is not found in Canada, being located only in the Near East (the centre of origin) and the surrounding area from the western Mediterranean to Ethiopia. It is the only species included in the primary gene pool of *L. culinaris*. Crosses have also been made between *L. culinaris* and most of the other wild species of Lens, but most of them are characterized by a high frequency of embryo abortion (Ladizinsky, 1993).

C2. Potential for Introgression of Genetic Information from *L. culinaris* into Relatives

In areas where both *L. culinaris* and *L. orientalis* occur naturally (The Mediterranean Area, northern Africa, and the Near East), *L. orientalis* may occasionally be found growing as a weed in a cultivated lentil field. Under these conditions, an outcross between these two species could result in introgression between these two species. The potential for this to outside of the laboratory in the Western Hemisphere, however, is highly unlikely since no *L. orientalis* is found in Canada.

C3. Occurrence of Related Species of *L. culinaris* in Canada

No related species of *Lens* grow naturally in Canada.

C4. Summary of Ecology of Relatives of *L. culinaris* in Canada

All wild species of *Lens* are winter annuals in their centre of origin. *L. culinaris* has been grown as a summer annual in western Canada, but the wild species likely would not survive here, if they were introduced into the wild. Even in their centre of origin, the wild species of Lens occur as scattered components of the native vegetation and compete poorly with the other species.

Part D - Potential Interactions of *L. culinaris* with Other Life Forms during Its Life Cycle

Table 1. Examples of potential interactions of *L. culinaris* with other life forms during its life cycle in a natural environment in Canada.

Table 1 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.

	Interaction with *L. culinaris*

Other life forms	(Pathogen; Symbiont or Beneficial Organism; Consumer; Gene transfer)
Ascochyta blight (*Ascochyta lentis*)	Pathogen
Anthracnose (*Colletotrichum truncatum*)	Pathogen
Fusarium root rot (*Fusarium solani*)	Pathogen
Fusarium wilt (*Fusarium oxysporum*)	Pathogen
Seedling blight (*Fusarium avenaceum*)	Pathogen
Rhizoctonia seedling rot (*Rhizoctonia solani*)	Pathogen
Pythium seed and root rot (*Pythium ultimum*)	Pathogen
White mold (*Sclerotinia sclerotiorum*)	Pathogen
Grey mold (*Botrytis cinerea*)	Pathogen
Lentil rust (*Uromyces fabae*)	Pathogen
Powdery mildew (*Leptosphaeria* sp.)	Pathogen
Pea seedborne mosaic virus	Pathogen
Grasshoppers	Consumer
Lygus bugs	Consumer
Birds	Consumer
Animal browsers	Consumer
Soil insects	Symbiont or Beneficial Organism or Consumer
Soil microbes	Symbiont or Beneficial Organism or Consumer
Mycorrhiza	Symbiont or Beneficial Organism
Rhizobium leguminosarum (N inoculant)	Symbiont or Beneficial Organism
Other *L. culinaris*	Gene Transfer

Part E - Acknowledgements

A. E. Slinkard and A. Vandenberg, Crop Development Centre, University of Saskatchewan developed this document.

Part F - Bibliography

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