Gene flow risk assessment in centres of crop origin and diversity

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Background

With the constant development of transgenic technology for a wide variety of major and minor crops, there is a need for comprehensive, easily accessible baseline information to evaluate the potential of gene flow and introgression between crops and their wild relatives, particularly in centres of crop origin and diversity.

Purpose and Objectives

Compile gene flow information to assist well-informed decision-making on the ecological risk of releasing genetically engineered (GE) crops in their centres of origin and/or diversity

- Assemble baseline gene flow research data for the 20 most important crops
- Identify sexually compatible CWR for each crop
- Identify crop-specific factors to consider for evaluating the potential of gene flow and introgression
- Evaluate the potential of gene flow and introgression between the 20 most important crops and their crop wild relatives
- Map gene flow "hot-spots"
- Identify knowledge gaps and research needs

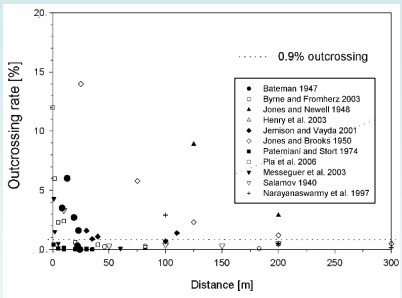


Fig. 2. Published studies on maize pollen flow show a rapid decrease of outcrossing rates within the first 50 m. However, in several occasions outcrossing rates beyond 100 m far exceed the 0.9% threshold defined by the European Union. The isolation distances of 200 m recommended in many countries should therefore not be further reduced.

Crops included

- Banana & plantain
- Barley
- Cassava
- Chickpea
- Cotton
- Cowpea
- Common bean
- Finger millet
- Groundnut
- Maize
- Oat
- Oilseed rape
- Pearl millet
- Pigeon pea
- Potato
- Rice
- Sorghum
- Soybean
- Sweet potato
- Wheat

Gene flow indicators

- **Biological information**
  - Centre(s) of origin, centre(s) of diversity
  - Mating system, vegetative regeneration
  - Flowering
  - Pollen dispersal and viability
  - Seed dispersal and persistence, seed banks
  - Volunteers, ferals
  - Persistence, weediness
- **Pollen flow and separation distances**
- **GE technology – state of development**
  - State of GE technology, GE traits
  - Total crop area, % GE crop area
  - Commercial GE production (countries)
  - GE field trials (countries)
- **Sexually compatible crop wild relatives**
- **Hybridization potential**
- **Geographical distribution → risk mapping**

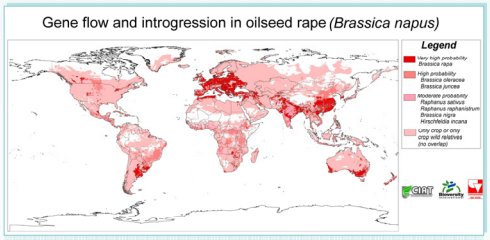


Fig. 3. Hot-spots of gene flow risk for oilseed rape and its wild relatives

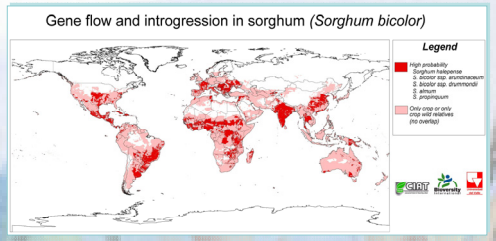
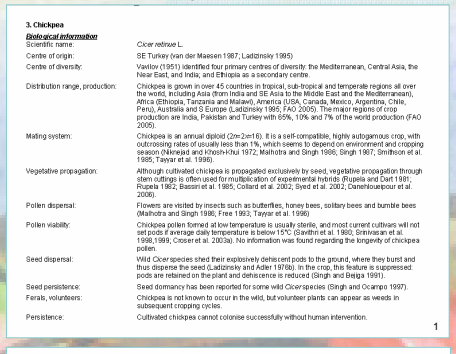


Fig. 4. Hot-spots of high gene flow risk for wild relatives of sorghum

Outputs

- Comprehensive baseline information to assist the evaluation of the gene flow and introgression potential of 20 crops and their sexually compatible crop wild relatives (e.g., Fig. 1 and 2)
- World maps per crop, identifying "hot-spots" for gene flow between the crop and its wild relatives to assist the decision on further risk assessment by analyzing other determinant factors (e.g., Fig. 3 and 4)
- Knowledge and research gaps that need to be addressed for adequate risk assessment
- Publication in form of a book, consisting of an introductory chapter (overview of hybridization, gene flow, introgression, ecological impact, risk assessment etc.) and 20 crop-specific chapters (relevant factors for assessing the risk of gene flow and its ecological implications and impact), including coloured world maps to identify at a first glance regions with high, moderate and low gene flow potential.

Fig.1. Screen shots of an example chapter: Gene flow in chickpea (*Cicer arietinum* L.)



3. Chickpea

Biological information

Scientific name: *Cicer retinue* L.

Centre of origin: SE Turkey (van der Maesen 1987; Ladizinsky 1995)

Centre of diversity: Vavilov (1951) identified four primary centres of diversity: the Mediterranean, Central Asia, the Near East, and India; and Ethiopia as a secondary centre.

Distribution range, production: Chickpea is grown in over 45 countries in tropical, sub-tropical and temperate regions all over the world, including Asia (from India and SE Asia to the Middle East and the Mediterranean), Africa (Ethiopia, Tanzania and Malawi), America (USA, Canada, Mexico, Argentina, Chile, Peru), Australia and S Europe (Ladizinsky 1995; FAO 2005). The major regions of crop production are India, Pakistan and Turkey with 65%, 10% and 7% of the world production (FAO 2005).

Mating system: Chickpea is an annual diploid (2n=2x=16). It is a self-compatible, highly autogamous crop, with outcrossing rates of usually less than 1%, which seems to depend on environment and cropping season (Nikneiad and Khosh-Khui 1972; Malhotra and Singh 1986; Singh 1987; Smithson et al. 1985; Tayyar et al. 1996).

Vegetative propagation: Although cultivated chickpea is propagated exclusively by seed, vegetative propagation through stem cuttings is often used for multiplication of experimental hybrids (Rupela and Dart 1981; Rupela 1982; Bassiri et al. 1985; Collard et al. 2002; Syed et al. 2002; Danehloueipour et al. 2006).

Pollen dispersal: Flowers are visited by insects such as butterflies, honey bees, solitary bees and bumble bees (Malhotra and Singh 1986; Free 1993; Tayyar et al. 1996)

Pollen viability: Chickpea pollen formed at low temperature is usually sterile, and most current cultivars will not set pods if average daily temperature is below 15°C (Saxthiti et al. 1980; Srinivasan et al. 1998,1999; Croser et al. 2003a). No information was found regarding the longevity of chickpea pollen.

Seed dispersal: Wild *Cicer* species shed their explosively dehiscent pods to the ground, where they burst and thus disperse the seed (Ladizinsky and Adler 1976b). In the crop, this feature is suppressed: pods are retained on the plant and dehiscence is reduced (Singh and Beiga 1991).

Seed persistence: Seed dormancy has been reported for some wild *Cicer* species (Singh and Ocampo 1997).

Ferals, volunteers: Chickpea is not known to occur in the wild, but volunteer plants can appear as weeds in subsequent cropping cycles.

Persistence: Cultivated chickpea cannot colonise successfully without human intervention.

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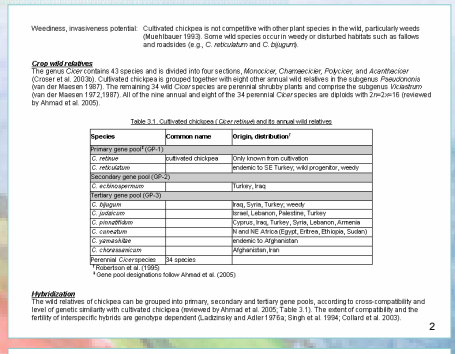

Weediness, invasiveness potential: Cultivated chickpea is not competitive with other plant species in the wild, particularly weeds (Muehlbauer 1993). Some wild species occur in weedy or disturbed habitats such as fallows and roadsides (e.g., *C. reticulatum* and *C. bijugum*).

Crop wild relatives

The genus *Cicer* contains 43 species and is divided into four sections, *Monocicer, Chamaecicer, Polycicer,* and *Acanthocicer* (Croser et al. 2003b). Cultivated chickpea is grouped together with eight other annual wild relatives in the subgenus *Pseudonovis* (van der Maesen 1987). The remaining 34 wild *Cicer* species are perennial shrubby plants and comprise the subgenus *Viciastrum* (van der Maesen 1972,1987). All of the nine annual and eight of the 34 perennial *Cicer* species are diploids with 2n=2x=16 (reviewed by Ahmad et al. 2005).

Table 3.1. Cultivated chickpea (*Cicer reticulum*) and its annual wild relatives

Species	Common name	Origin, distribution[1]
Primary gene pool[2] (GP-1)		
C. reticulum	cultivated chickpea	Only known from cultivation
C. reticulatum		endemic to SE Turkey; wild progenitor, weedy
Secondary gene pool (GP-2)		
C. echinospermum		Turkey, Iraq
Tertiary gene pool (GP-3)		
C. bijugum		Iraq, Syria, Turkey; weedy
C. judaicum		Israel, Lebanon, Palestine, Turkey
C. pinnatifidum		Cyprus, Iraq, Turkey, Syria, Lebanon, Armenia
C. cuneatum		N and NE Africa (Egypt, Eritrea, Ethiopia, Sudan)
C. yamashitae		endemic to Afghanistan
C. chorassanicum		Afghanistan, Iran
Perennial *Cicer* species	34 species	

[1] Robertson et al. (1999)
[2] Gene pool designations follow Ahmad et al. (2005)

Hybridization

The wild relatives of chickpea can be grouped into primary, secondary and tertiary gene pools, according to cross-compatibility and level of genetic similarity with cultivated chickpea (reviewed by Ahmad et al. 2005; Table 3.1). The extent of compatibility and the fertility of interspecific hybrids are genotype dependent (Ladizinsky and Adler 1976a; Singh et al. 1994; Collard et al. 2003).

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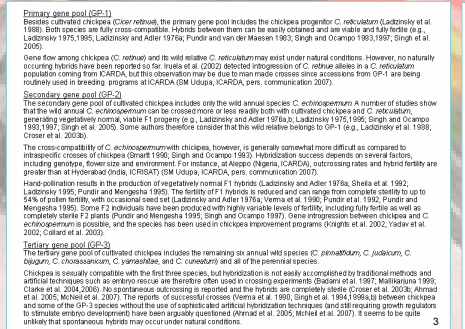

Primary gene pool (GP-1)

Besides cultivated chickpea (*Cicer retinue*), the primary gene pool includes the chickpea progenitor *C. reticulatum* (Ladizinsky et al. 1998). Both species are fully cross-compatible. Hybrids between them can be easily obtained and are viable and fully fertile (e.g., Ladizinsky 1975,1985; Ladizinsky and Adler 1976a; Pundir and van der Maesen 1983; Singh and Ocampo 1993,1997; Singh et al. 2005).

Gene flow among chickpea (*C. retinue*) and its wild relative *C. reticulatum* may exist under natural conditions. However, no naturally occurring hybrids have been reported so far. Ruiela et al. (2002) detected introgression of *C. retinue* alleles in a *C. reticulatum* population coming from ICARDA, but this observation may be due to man made crosses since accessions from GP-1 are being routinely used in breeding programs at ICARDA (SM Udupa, ICARDA, pers. communication 2007).

Secondary gene pool (GP-2)

The secondary gene pool of cultivated chickpea includes only the wild annual species *C. echinospermum*. A number of studies show that the wild annual *C. echinospermum* can be crossed more or less readily both with cultivated chickpea and *C. reticulatum*, generating vegetatively normal, viable F1 progeny (e.g., Ladizinsky and Adler 1976a,b; Ladizinsky 1975,1995; Singh and Ocampo 1993,1997; Singh et al. 2005). Some authors therefore consider that this wild relative belongs to GP-1 (e.g., Ladizinsky et al. 1998; Croser et al. 2003b).

The cross-compatibility of *C. echinospermum* with chickpea, however, is generally somewhat more difficult as compared to intraspecific crosses of chickpea (Smartt 1990; Singh and Ocampo 1993). Hybridization success depends on several factors, including genotype, flower size and environment. For instance, at Aleppo (Nigeria, ICARDA), outcrossing rates and hybrid fertility are greater than at Hyderabad (India, ICRISAT) (SM Udupa, ICARDA, pers. communication 2007).

Hand-pollination results in the production of vegetatively normal F1 hybrids (Ladizinsky and Adler 1976a; Sheila et al. 1992; Ladizinsky 1995; Pundir and Mengesha 1995). The fertility of F1 hybrids is reduced and can range from complete sterility to up to 54% of pollen fertility, with occasional seed set (Ladizinsky and Adler 1976a; Verma et al. 1990; Pundir et al. 1992; Pundir and Mengesha 1995). Some F2 individuals have been produced with highly variable levels of fertility, including fully fertile as well as completely sterile F2 plants (Pundir and Mengesha 1995; Singh and Ocampo 1997). Gene introgression between chickpea and *C. echinospermum* is possible, and the species has been used in chickpea improvement programs (Knights et al. 2002; Yadav et al. 2002; Collard et al. 2003).

Tertiary gene pool (GP-3)

The tertiary gene pool of cultivated chickpea includes the remaining six annual wild species (*C. pinnatifidum, C. judaicum, C. bijugum, C. chorassanicum, C. yamashitae,* and *C. cuneatum*) and all of the perennial species.

Chickpea is sexually compatible with the first three species, but hybridization is not easily accomplished by traditional methods and artificial techniques such as embryo rescue are therefore often used in crossing experiments (Badiani et al. 1997; Mallikarjuna 1999; Clarke et al. 2004,2006). No spontaneous outcrossing is reported and the hybrids are completely sterile (Croser et al. 2003b; Ahmad et al. 2005; McNeil et al. 2007). The reports of successful crosses (Verma et al. 1990; Singh et al. 1994,1998a,b) between chickpea and some of the GP-3 species without the use of sophisticated artificial hybridization techniques (and still requiring growth regulators to stimulate embryo development) have been arguably questioned (Ahmad et al. 2005; McNeil et al. 2007). It seems to be quite unlikely that spontaneous hybrids may occur under natural conditions.

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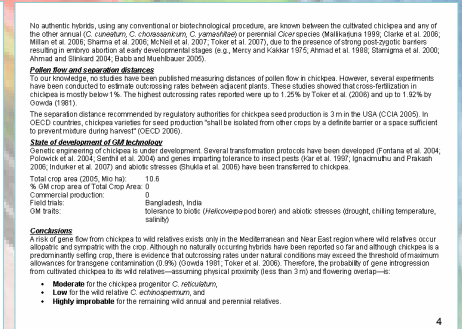

No authentic hybrids, using any conventional or biotechnological procedure, are known between the cultivated chickpea and any of the other annual (*C. cuneatum, C. chorassanicum, C. yamashitae*) or perennial *Cicer* species (Mallikarjuna 1999; Clarke et al. 2006; Millan et al. 2006; Sharma et al. 2006; McNeil et al. 2007; Toker et al. 2007), due to the presence of strong post-zygotic barriers resulting in embryo abortion at early developmental stages (e.g., Mercy and Kakkar 1975; Ahmad et al. 1998; Starrigma et al. 2000; Ahmad and Slinkard 2004; Babb and Muehlbauer 2005).

Pollen flow and separation distances

To our knowledge, no studies have been published measuring distances of pollen flow in chickpea. However, several experiments have been conducted to estimate outcrossing rates between adjacent plants. These studies showed that cross-fertilization in chickpea is mostly below 1%. The highest outcrossing rates reported were up to 1.25% by Toker et al. (2006) and up to 1.92% by Gowda (1981).

The separation distance recommended by regulatory authorities for chickpea seed production is 3 m in the USA (CCIA 2005). In OECD countries, chickpea varieties for seed production "shall be isolated from other crops by a definite barrier or a space sufficient to prevent mixture during harvest" (OECD 2006).

State of development of GM technology

Genetic engineering of chickpea is under development. Several transformation protocols have been developed (Fontana et al. 2004; Polowick et al. 2004; Senthil et al. 2004) and genes imparting tolerance to insect pests (Kar et al. 1997; Ignacimuthu and Prakash 2006; Indurker et al. 2007) and abiotic stresses (Bhalla et al. 2006) have been transferred to chickpea.

Total crop area (2005, Mio ha): 10.6
% GM crop area of Total Crop Area: 0
Commercial production: 0
Field trials: Bangladesh, India
GM traits: tolerance to biotic (*Helicoverpa*-pod borer) and abiotic stresses (drought, chilling temperature, salinity)

Conclusions

A risk of gene flow from chickpea to wild relatives exists only in the Mediterranean and Near East region where wild relatives occur allopatric and sympatric with the crop. Although no naturally occurring hybrids have been reported so far and although chickpea is a predominantly selfing crop, there is evidence that outcrossing rates under natural conditions may exceed the threshold of maximum allowances for transgene contamination (0.9%) (Oswalt 1981; Toker et al. 2006). Therefore, the probability of gene introgression from cultivated chickpea to its wild relatives—assuming physical proximity (less than 3 m) and flowering overlap—is:

- **Moderate** for the chickpea progenitor *C. reticulatum*,
- **Low** for the wild relative *C. echinospermum*, and
- **Highly improbable** for the remaining wild annual and perennial relatives.

4

Thanks for financial support:

Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH

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