

Gene flow risk assessment in centres of 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

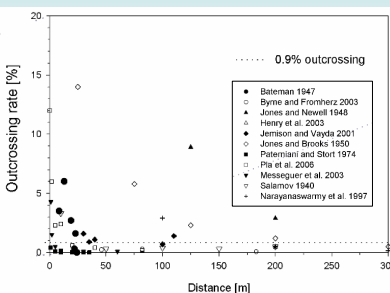


Fig. 2. Published studies on maize pollen flow show a rapid decrease of outcrossing rates within the first 50 m. However, in several occasions outcrossing rates beyond 100 m far exceed the 0.9% threshold defined by the European Union. The isolation distances of 200 m recommended in many countries should 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**

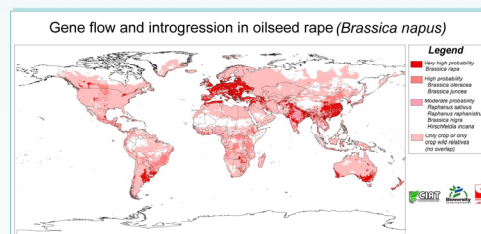


Fig. 3. Hot-spots of gene flow risk for oilseed rape and its wild relatives

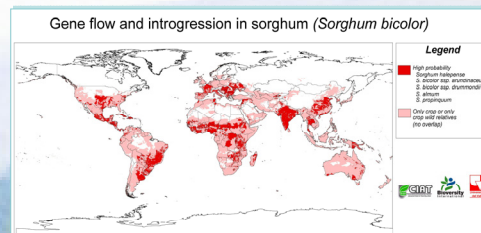


Fig. 4. Hot-spots of high gene flow risk for wild relatives of sorghum

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

3. Chickpea

Biological information

Scientific name: *Cicer reticul.*

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

Centre of diversity: Various (wild) identified for various parts of 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 the tropical, subtropical and temperate regions all over the world, including the Middle East, the Near East, the Mediterranean, the Indian subcontinent, the Pacific Islands, the Caribbean, Mexico, Argentina, Chile, Peru, Australia and 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=20). It is a self-compatible, highly autogamous crop, with outcrossing rates (usually) less than 1%, which seems to depend on environment and cropping season (Mehradji and Khoshdel 1972; Mahitola and Singh 1986; Singh 1987; Smithson et al. 1995; Taylor 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 (Rajeev and Singh 1980; Rajeev 1982; Baseri et al. 1995; Colard et al. 2002; Singh et al. 2002; Chaturvedi et al. 2008)

Pollen dispersal: Flowers are visited by insects such as butterflies, honey bees, solitary bees andumble bees (Mahitola and Singh 1986; Fries 1992; Taylor et al. 1996)

Pollen viability: Chickpea pollen formed above temperature is usually sterile, and most current cultivars will not set pods if average daily temperature is below 15°C (Gallwitz et al. 1980; Bhatnagar et al. 1986, 1988; Colard et al. 2002a). No information was found regarding the longevity of chickpea pollen.

Seed dispersal: Wild *Cicer* species used their explosively dehiscent pods to the ground, where they burst and thus disperse the seed (Ladizinsky and Adler 1974b). In the crop, this feature is suppressed (pods are not to occur in the wild, but volunteer plants can appear as weeds in subsequent cropping cycles)

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

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

Crop wild relatives

The genus *Cicer* contains 43 species and is divided into four sections, *Monocoryn*, *Chamaecicer*, *Poleocicer*, and *Aristocicer* (Crosser et al. 2004). Cultivated chickpea grouped together with eight other annual wild relatives in the subgenus *Pachycroton* (van der Maaten 1987). The remaining 34 wild *Cicer* species are perennial shrubby plants and comprise the subgenus *Viciastrum* (van der Maaten 1987). Other annual and biennial wild *Cicer* species are grouped with *2n=10* (reviewed by Ahmad et al. 2005).

Species	Common name	Origin/distribution
<i>C. arietinum</i> (CWR1)	chickpea	only known from cultivation
<i>C. reticulatum</i>	reticulate chickpea	endemic to SE Turkey; wild progenitor of <i>C. arietinum</i>
<i>C. pavonius</i> (CWR2)	peacock chickpea	Turkey, Iraq
<i>C. echinocarpum</i>	chickpea	India, Syria, Turkey, widely
<i>C. pinnatifidum</i>	pinna chickpea	Iran, Lebanon, Myanmar, Turkey
<i>C. anatolicum</i>	anatolian chickpea	Cyprus, Iraq, Turkey, Syria, Lebanon, Armenia
<i>C. caudatum</i>	caudate chickpea	Iran and NE Africa (Sudan, Eritrea, Ethiopia, Sudan)
<i>C. arabicum</i>	arab chickpea	known to Afghanistan
<i>C. khorasanicum</i>	Khorasan chickpea	Afghanistan, Iran

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 (Ahmad et al. 2005, Table 1). The closest compatibility and the highest introgression potential are observed between chickpea and its wild relatives in the primary gene pool (CWR1).

No authentic hybrids, using any conventional or biotechnological procedures, are known between the cultivated chickpea and any of the other annual *C. conephense*, *C. chiosense*, *C. viciastrum* or perennial *Cicer* species (Mehradji 1989; Clarke et al. 2005; Milar et al. 2006; Sharma et al. 2006; Mehal et al. 2007; Fries et al. 2007), due to the presence of strong post-polliniferous barriers resulting in embryo abortion at early developmental stages (e.g. Mery and Kiskar 1975; Ahmad et al. 1998; Sharma et al. 2000; Ahmad and Shrestha 2004; Shaha and Kumbhar 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 related taxa. These studies showed that cross-fertilization in chickpea is mostly below 1%. The highest outcrossing rates reported were up to 1.26% by Tokar 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 (CICR 2005). In OECD countries, chickpea varieties for seed production have been isolated from other crops by a distance barrier or a space sufficient to prevent wind dispersal (OECD 2005).

State of development of GM technologies

Genetic engineering for crop improvement. Several transformation protocols have been developed (Fontana et al. 2004; Potiwika et al. 2004; Sethi et al. 2004) and genes imparting tolerance to insect pests (Jain et al. 1997; Jaganmohan and Prakash 2005; Indurkhya et al. 2007) and abiotic stresses (Sharma et al. 2006) have been transferred to chickpea.

Total crop area (2005, Mio/ha): 0.6
 % GM crop area of Total GM Area: 0
 Commercial production: 0
 Field trials: 0
 GM traits: tolerance to biotic (Rhizoctonia root rot) 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 adjacent and sympatric with the crop. Although no natural 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 allowed introgression (0.9%) (Gowda 1981; Tokar 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:

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

Primary gene pool (CWR1)

Relevant cultivated chickpea (*Cicer reticulatum*), the primary gene pool includes the chickpea progenitor *C. reticulatum* (Ladizinsky et al. 1996). 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 1974a,b; Ladizinsky and Adler 1974b; Ladizinsky 1975, 1986; Singh and Colard 1992, 1997; Singh et al. 2002).

Gene flow among chickpea (*C. reticulatum*) and its wild relative *C. reticulatum* may exist under natural conditions. However, no naturally occurring hybrids have been reported so far. Hybrids of *C. reticulatum* and *C. reticulatum* are being routinely used in breeding programs at ICARDA (CIM UoG, ICARDA, pers. communication 2007).

Secondary gene pool (CWR2)

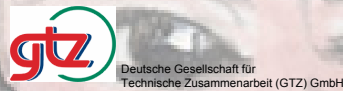
The secondary gene pool of cultivated chickpea includes only the wild annual species *C. echinocarpum* and can range from complete sterility to up to 54% of viable fertile, with occasional seed viability under 100% (Ladizinsky and Adler 1974a,b; Fries et al. 1992; Fries and Ladizinsky 1995). Some F2 individuals have been produced with high viable levels of fertility, including fully fertile as well as completely sterile F2 plants (Fries and Ladizinsky 1995; Singh and Colard 1997). Gene introgression between chickpea and *C. echinocarpum* is possible, and the species has been used in chickpea improvement programs (Singh et al. 2002; Yadav et al. 2002; Colard et al. 2005).

Tertiary gene pool (CWR3)

The tertiary gene pool of cultivated chickpea includes the remaining annual wild species (*C. pavonius*, *C. judaicum*, *C. caudatum*, *C. anatolicum*, *C. arabicum*, and *C. khorasanicum*) 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 or seed rescue (Ahmad et al. 1998; Singh et al. 1997; Mehradji 1989; Clarke et al. 2004, 2005). No spontaneous outcrossing is reported and the hybrids are completely sterile (Crosser et al. 2003b; Ahmad et al. 2005; Mehal et al. 2007). The reports of successful crosses (Vermis et al. 1980; Singh et al. 1994, 1996a,b) between chickpea and some of the *C. 2n=10* species without the use of sophisticated artificial hybridization techniques and still requiring growth regulators to stimulate embryo development have been highly questioned (Ahmad et al. 2005; Mehal et al. 2007). It seems to be quite unlikely that spontaneous hybrids may occur under natural conditions.

Thanks for financial support:



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.

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