3 Biology of Safflower (*Carthamus tinctorius*)

This chapter deals with the biology of safflower (*Carthamus tinctorius*). It contains information for use during the risk/safety regulatory assessment of genetically engineered varieties of safflower intended to be grown in the environment (biosafety). It includes elements of taxonomy, centres of origin, cultivation, reproductive biology, genetics, hybridisation and introgression, as well as ecology. Annexes present safflower's common pests and pathogens, and current biotechnology developments.

Introduction

This chapter was prepared by the OECD Working Party on the Harmonisation of Regulatory Oversight in Biotechnology, with **Australia** as the lead country. It was initially issued in 2020 as the Consensus Document on the Biology of Safflower (*Carthamus tinctorius* L.). Production data have been updated in this publication, based on FAOSTAT.

Species and taxonomic groups

Classification and nomenclature

Cultivated safflower (*Carthamus tinctorius* L.) is an annual oilseed crop (Figure 3.1) that is a member of the family Asteraceae (Compositae), tribe Cardueae (thistles) and subtribe Centaureinae (Bérvillé et al., 2005). Asteraceae is recognised as the largest family of flowering plants and contains more than 1 500 genera and 22 000 species ranging from annual herbs to woody shrubs. Safflower is known by many other names, such as kusum, kasunmba, kusumbo, kusubi, kabri, ma, sufir, kar/karar, sendurgam, agnisikha, hebu, su, suban and others. The Arabic usfur is thought to have been the root for the English name via a number of other terms – affore, asfiore, asfrole, astifore, asfiori, zaffrole or zaffrone, saffiore to, finally, safflower – while in the People's Republic of China (hereafter 'China') it is known as *hung-hua* or "red flower" (Chavan, 1961, and sources cited therein) and under many other names around the world as summarised by Smith (1996).

Figure 3.1. Safflower crop

Source: muratart/Shutterstock.com.

The taxonomy of *Carthamus* has changed substantially as data for this group has been obtained and interpreted (McPherson et al., 2004; Sehgal and Raina, 2011). There have been as few as four species in the genus (with related species in a separate genus) to as many as 25 species and subspecies divided into up to five sections. The sections were based on five chromosome groups identified by Ashri and

Knowles (1960), being n = 10, 11, 12, 22 and 32. Safflower belongs to the *Carduncellus-Carthamus* complex. Morphological and cytological characteristics have not been sufficient to delimit the species into discrete sections and genera. Depending on the taxonomist and the emphasis on particular morphological characteristics, species have been moved between the genera *Carthamus* and *Carduncellus* (McPherson et al., 2004). Determining species relationships is made more difficult by the low levels of genetic variation that occur when clear morphological differences are present (Mayerhofer et al., 2011).

The classification scheme followed in this document is that of López-González (1990), as shown in Table 3.1, which recognises 16 species within *Carthamus* and another closely related species, *Femeniasia balearica.* The species have been further divided into three sections based on chromosome numbers, the section *Carthamus* (n = 12), section *Odonthagnathis* (n = 10 or 11), section *Atractylis* (n = 22 or 32) and two species of uncertain placement.

Carthamus oxyacanthus and *Carthamus persicus* were thought to be the parent species of *C. tinctorius* (Ashri and Knowles, 1960). More recent genetic analysis and geographic evidence indicate that *Carthamus palaestinus* is the wild progenitor of safflower and originated in the Middle East, and is fully cross-compatible with safflower (Pearl et al., 2014).

Table 3.1. Taxonomic groups of *Carthamus* sensu

Section	Species	Number of chromosomes
Carthamus L.	*C. tinctorius* L.	2n = 2x = '24,' n = 12
	C. oxyacanthus Bieb.	2n = 2x = '24,' n = 12
	C. palaestinus Eig	2n = 2x = '24,' n = 12
	C. persicus Willd. (basionym *C. flavescens* auct.)	2n = 2x = '24,' n = 12
	C. curdicus Hanelt.	2n = 2x = '24,' n = 12
	C. gypsicolus Ilj.	2n = 2x = '24,' n = 12
Odonthagnathis (DC.) Henelt	*C. divaricatus* Beguinot & Vacc.	2n = 2x = '22,' n = 11
	C. leucocaulos Sm.	2n = 2x = '20,' n = 10
	C. glaucus Bieb.	2n = 2x = '20,' n = 10
	C. tenuis (Boiww. & Bl.) Bornm.	2n = 2x = '20,' n = 10
	C. dentatus (Forssk.) Vahl	2n = 2x = '20,' n = 10
	C. boissieri Halácsy	2n = 2x = '20,' n = 10
Atractylis Reichemb.	*C. lanatus* L.	2n = 4x = '44,' n = 22
	C. creticus L. (syn *C. baeticus* (Boiss & Reuter) Nyman)	2n = 6x = '64,' n = 32
	C. turkestanicus Popov	2n = 6x = '64,' n = 32
Uncertain placement	*C. nitidus* Boiss.	2n = 2x = '24,' n = 12
	Femeniasia balearica Susanna	2n = 2x = '24,' n = 12

Source: Based on the classification proposed by López-González, G. (1990), "Acerca de la clasificación natural del género" Carthamus" L., s. l.", *Anales del Jardín Botánico de Madrid*, Vol. 47, pp. 11-34.

Description

Safflower is one of humanity's oldest crops yet it remains a minor crop compared to other oilseeds (FAOSTAT, 2022). Safflower is now mostly cultivated for the production of vegetable oil (Kumar et al., 2015).

Safflower is an erect, herbaceous, highly branched, spiny, thistle-like annual plant that grows from 30 to 150 cm in height (Singh and Nimbkar, 2006; Kumar and Kumari, 2011). Young safflower plants form a rosette and remain in this vegetative state for many weeks, during which leaves and a deep taproot system develop. This deep taproot system, with abundant thin horizontal roots, allows the plant to extract water and nutrients from deeper layers of soil than many other crop plants (Li and Mündel, 1996; GRDC, 2010). The rosette stage is followed by rapid stem elongation, extensive branching then flowering, with leaves being arranged on both sides of the stem (Li and Mündel, 1996; Singh and Nimbkar, 2006). The flower colour of cultivated safflower is typically brilliant orange (Figure 3.2). Leaf size varies with variety and position on the plant, although typical leaves are 2.5-5 cm wide and 10--15 cm long. The leaf morphology is described as alternate, sessile and ovate-lanceolate (Teotia et al., 2017). Upper leaves often develop hard spines, while those lower on the stem are usually spineless. These spines make the crop difficult to walk through but act as a deterrent to larger animals such as pigs and kangaroos (GRDC, 2010). As plants mature, they become stiff, woody and resistant to some environmental stressors such as hail and wind. Safflower growth cycle, floral biology and pollination are considered in greater detail in the reproductive biology section below.

Figure 3.2. Flowers of cultivated safflower

Source: High Montain/Shutterstock.com.

Positive identification of safflower plants is important to ensure not only the purity of seed at harvest but also to prevent outcrossing with wild relatives. Safflower has a similar morphological appearance to some close relatives and also to other thistle species. An identification guide and their respective global distribution are shown in Table 3.2. Unfortunately, many of the distinctions can only be made once the plants have reached flowering.

Table 3.2. Guide to the positive identification of *Carthamus tinctorius* L.

Species (common name)	Identification by morphology	Global distribution
Carthamus tinctorius (cultivated safflower)	Brilliant orange flowers, with traces of red and yellow (Figure 3.2)	Cultivated globally (Figure 3.3)
Cirsium vulgare (spear thistle)	Pink or purple flowers	Germany, France, Spain, Japan
***Carduus* sp.** (sheep, slender and plumeless thistles)	Pink or purple flowers	France, Germany, Spain, Netherlands, Sweden, United Kingdom, Japan
Carthamus lanatus (saffron/distaff thistle)	Divided leaves and lighter yellow flowers	Spain, France, Italy, Portugal, United States, Japan
Centaurea solstitialis (Barnaby star thistle)	Yellow flowers; small, round and spiny capitula	France, Germany, United States, Spain, Australia, Greece, Netherlands, Italy, Japan
Centaurea melitensis (Maltese cockspur or Malta star thistle)	Narrow and non-spiny leaves; yellow flowers; small and round capitula	Spain, Australia, United States, France, Portugal, Argentina, Mexico, South Africa, Japan
Scolymus hispanicus (golden thistle)	Denticulate leaves; yellow flowers; flat seeds	Spain, France, Portugal, Australia, Greece, Italy, Israel
Scolymus maculatus (spotted golden thistle)	Obovate leaves; yellow flowers	Spain, Israel, France, Portugal, West Bank and Gaza Strip, Australia
Carthamus dentatus (toothed thistle)	Pink or purple flowers	Australia, Greece, Turkey
Carthamus leucocaulos (Whitestem distaff thistle)	Purple flowers	Greece, Australia, United States
Carthamus glaucus (glaucous star thistle or Mediterranean thistle)	Purple flowers	Israel, West Bank and Gaza Strip, Turkey, Syrian Arab Republic, Lebanon, Greece, Australia

Sources: HerbiGuide (2014a), *Safflower*, (accessed 13 May 2020); HerbiGuide (2014b), *Weeds*, (accessed 13 May 2020); GBIF (2020), *Global Biodiversity Information Facility*, https://www.gbif.org/ (accessed 13 May 2020).

Geographic distribution, natural and managed ecosystems and habitats, cultivation and management practices, and centres of origin and diversity

Geographic distribution

Safflower is a dryland oilseed crop but was traditionally grown for the extraction of dyes for textiles and food (Weiss, 1971; Zohary, Hopf and Weiss, 2012) throughout South and Central Asia and the Mediterranean (Weiss, 1971; Li and Mündel, 1996; Zohary, Hopf and Weiss, 2012). Today, the cultivation of safflower occurs in arid and semi-arid conditions wherever the crops have established a tolerance to hot and dry conditions. The geographical distribution of safflower cultivation is depicted in Figure 3.3.

Figure 3.3. Recorded global distribution of cultivated safflower (*Carthamus tinctorius* L.) from 1795 until 2019

Note: Yellow (or light grey) dots indicate georeferenced occurrences.
Source: GBIF Backbone Taxonomy (2017), "*Carthamus* L.", in *GBIF Secretariat*, *licensed under CC BY 4.0* https://creativecommons.org/licenses/by/4.0/legalcode.

Ecosystems and habitats where the species occurs natively and where it has naturalised

A naturalised species is one that has the potential to be self-sustaining and exhibits population spreading without human assistance but does not necessarily impact the environment. The capacity for a species to naturalise in foreign environments is a good indicator of its weed potential (Randall, 2017). Safflower has been found to naturalise in many of the countries where it is commonly cultivated including Australia, Chile, China, Croatia, Estonia, Italy, Japan, the Democratic People's Republic of Korea, the Lao People's Democratic Republic, Mexico, Norway, Portugal, Romania, the Russian Federation (hereafter 'Russia'), Ukraine, the United Kingdom and the United States (Randall, 2017).

Agronomic ecosystems where the species is grown, including management practices

Production regions

Traditionally, safflower was grown in hot arid dry regions but it is a highly adaptable plant. In the Americas, commercial production extends from southern Canada, south into Argentina (Li and Mündel, 1996). Although safflower is considered a minor crop compared to other oilseed crops, it is grown in over 20 countries, occupying over 700 000 hectares of agricultural land and producing around 650 000 tonnes of seed in 2020 (FAOSTAT, 2022). The top four producers of safflower from 2018 to 2020 consistently included, in decreasing order, Russia, Kazakhstan, Mexico and the United States. Other significant producers of safflower include Turkey, India, Argentina and China. Worldwide, yields generally range from approximately 0.5 to 1.7 tonnes per hectare (t/ha) (FAOSTAT, 2022). Trial data has shown that safflower yields are variable, dependent on many factors such as planting date (winter vs. spring), sowing rates, temperature, cultivars and water availability (Wachsmann et al., 2008).

Agronomic practices

Safflower is an annual plant with a long growing season. The sowing dates vary among different countries, summarised in Figure 3.4. Similar to other oilseed crops, the sowing date has been shown to affect seed oil content (Mirshekari et al., 2013). Safflower may be sown later than other winter crops, which allows it to be used for weed management or as an option when earlier planted winter crops have failed to establish (GRDC, 2010).

Figure 3.4. Sowing and harvest dates of major global safflower growers

Region	Month of harvest											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
India												
United States												
Mexico												
Argentina												
Australia												
China												
Africa												

Note:
Sowing period.
Harvest period.
Source: Adapted from Gilbert, J. (2008), "International safflower production - An overview", Paper presented at "Safflower: Unexploited Potential and World Adaptability, 7th International Safflower Conference", Wagga Wagga, NSW, Australia.

Sowing rates of safflower depend on the region and moisture availability. The sowing rates have a broad range from 12-15 kg/ha in northern Australia (drier conditions) and 18-24 kg/ha in southern Australia (irrigated conditions), with plant densities being 20-25 plants/m^2 and 30-40 plants/m^2 respectively (GRDC, 2010). Safflower in the United States is sown at a high seeding rate of 28-39 kg/ha, although the crop develops at a significantly higher density of approximately 65 plants/m^2, promoting better weed competition (Oelke et al., 1992).

Ideally, sowing should be into moist soil, typically between 2 and 5 cm deep but this will vary with soil type and conditions. Delayed emergence and reduced early vigour can occur due to deeper sowing, leaving plants susceptible to pests, diseases and competition from weeds (Mikkelsen et al., 2008). Safflower is normally planted with standard cereal sowing equipment in rows 18-36 cm apart. Narrower rows help suppress weeds, whilst wider spacing allows for better airflow for disease control (GRDC, 2010).

Safflower has a deep root system, which makes it ideal for rainfed cropping systems (Singh and Nimbkar, 2006). Tap roots from safflower may extend 2-3 m into the soil (Oyen and Umali, 2007; Heuzé et al., 2015). Well-drained, deep, fertile, sandy loam soils provide maximum safflower yields (GRDC, 2010). In Australia, due to its deep tap root system, safflower is often used on problem soils to break up hard pans and to improve both water and air infiltration in the subsoil (GRDC, 2010).

Although safflower has high water requirements, it does not tolerate waterlogging well. Safflower has the ability to extract water from deeper layers of soil compared to many other crop plants due to its taproot and thus is considered quite drought tolerant (Li and Mündel, 1996; GRDC, 2010). Irrigation can extend the growing season by two weeks, whereas drought, salinity, increased temperatures or day length will hasten maturity. Safflower is considered to have moderate to high salinity tolerance, being similar to barley or cotton (GRDC, 2010). Safflower is also moderately frost tolerant during the rosette stage but is susceptible

to frost damage from the stem elongation stage to maturity. It is also relatively resistant to hail or wind damage (Mündel et al., 2004).

One tonne of safflower seed removes 25 kg of nitrogen, 4.3 kg of phosphorous and 4 kg of sulphur from the soil. Most soils (with the possible exception of sandy soils) contain adequate levels of potassium and sulphur (GRDC, 2010). Although safflower can access nutrients from deeper in the soil profile than cereal crops, fertilisers tend to increase yields and oil levels, especially in irrigated or higher rainfall areas. Fertiliser application rates are dependent on expected yields based on available soil moisture (or irrigation), which also varies significantly between different cultivars. For safflower grown in Pakistan, a study of different nitrogen application rates determined that plant height, number of branches, number of capitula and total seed yield were all significantly increased with the application rate of nitrogen at 120 kg/ha (Siddiqui and Oad, 2006).

Safflower is a poor competitor with weeds, particularly during emergence through to the rosette stage of development, and weed management is essential when growing this crop. It is important to control the number of weeds as a means of reducing the potential negative impacts on yield. Cultivation can be used to control weeds when the safflower plants are seedlings, measuring 7-15 cm tall. There are some registered herbicides available for use in safflower cropping systems, which are typically used as either pre-planting or pre-emergence herbicides. These herbicides are used for the control of in-crop grass and broadleaf type weeds (see sub-section “Weediness of safflower crops”).

Harvest

Safflower sown in winter is usually ready for harvest four to six weeks later than wheat sown at a similar time. Safflower is ready for harvest once all the leaves have turned brown and the latest flowering heads are no longer green. At maturity, the seeds should be white and easily threshed by hand (Oelke et al., 1992). For the major global safflower growers, the harvest dates are variable, summarised in Figure 3.4, which helps to ensure the supply of safflower seed throughout the year. In Australia, the recommended seed moisture at the time of harvest should be less than 8% to avoid overheating and mould formation during processing and storage. It is also recommended that harvest occurs as soon as possible as rain can cause staining or early sprouting of the seed, both of which reduce the value of the seed (Oelke et al., 1992; Bockisch, 1998; GRDC, 2010). In parts of Canada, the seed is harvested at a moisture content of 12-15% and then dried by aeration (Mündel et al., 2004).

Safflower is generally harvested without swathing. Safflower is suitable for harvest by direct heading since the capitula do not shatter easily. The same machinery used for cereals can be used for safflower but ground speeds are slower to reduce seed loss (Oelke et al., 1992; Thalji and Alqarallah, 2015). Periodic cleaning of equipment to remove bristles from radiators and hot engine components may be necessary to minimise the risk of fire (GRDC, 2010). In addition, harvesting in cooler or more humid parts of the day is recommended both to reduce the risk of fire and to increase seed cleanliness (Jochinke et al., 2008). In Australia, seed loss during harvest (direct heading) is about 3-4% (GRDC, 2010).

Centres of origin and diversity

Safflower is an ancient crop that is believed to have a single origin of domestication from approximately 4 000 years ago in the Fertile Crescent (Pearl et al., 2014). This region ranges from southern Israel to western Iraq (Chapman et al., 2010). Safflower has been grown for centuries in India, China and northern Africa.

Seven “centres of similarity”, or “centres of culture”, were identified by Knowles (1969a), namely the Far-East, India-Pakistan, the Middle-East, Egypt, Sudan, Ethiopia and Europe. Ashri (1971) added more centres, however, these were not centres of diversity or origin but of very similar safflower types. Considerable genetic diversity exists across different genotypes. When 60 representative genotypes from

India and other countries were examined it was observed that plant height, seed yield, branching height and seed weight accounted for 80% of the diversity (Patel et al., 1989). Patel et al. (1989) identified 14 clusters of genetic diversity but distribution into clusters was random showing that geographic isolation is not the only factor causing genetic diversity. Up to ten centres of similarity throughout the world were identified based on morphology. Nuclear microsatellite analysis of accessions suggests the presence of five genetic clusters, one in each of the following regions: Europe; Turkey-Islamic Republic of Iran (hereafter 'Iran')-Iraq-Afghanistan; Israel-Jordan-Syrian Arab Republic (hereafter 'Syria'); Egypt-Ethiopia; and Far East-India-Pakistan (Chapman et al., 2010).

The different species of *Carthamus* are all believed to have one common ancestor, probably from Iraq and north-western Iran. With the exception of cultivated safflower, the species are all spiny weeds that grow in the wild. There appear to be three wild species that are closely related. *Carthamus flavescens* (= *C. persicus*) is usually found in wheat fields in Lebanon, Syria and Turkey. *C. oxyacanthus* is a serious weed in the area from western Iraq to north-western India and northward into the southern parts of some former republics of the Union of Soviet Socialist Republics (USSR). *C. palaestinus* is found in the desert regions of Iraq, Israel and Jordan. These species readily cross with *C. tinctorius* to produce fertile progeny. It is thought that early in its evolution, safflower spread to Egypt, Ethiopia, South Asia and the Far East, where distinct types have evolved (as reviewed by Smith, 1996).

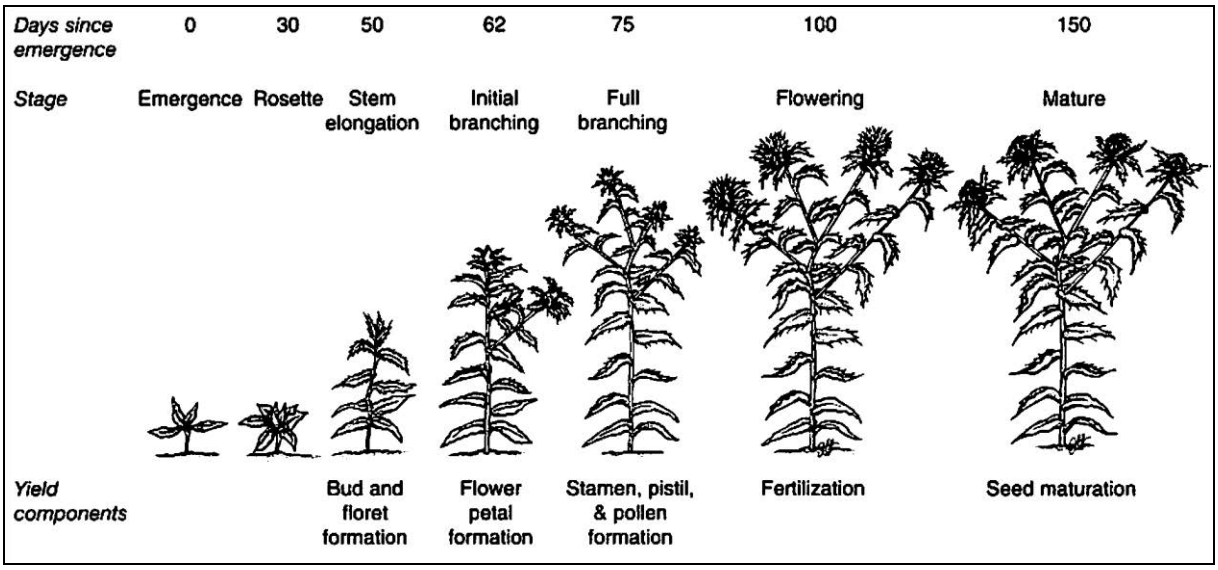
Domestication of safflower has resulted in traits such as reduced shattering, smooth seeds, reduced duration of the early vegetative growth stage, restriction of branching to the upper part of the stem and reduced seed dormancy (Bérvillé et al., 2005, and references cited therein). Breeding programmes have resulted in the release of cultivars with higher oil content and/or increased disease resistance (GRDC, 2010).

Reproductive biology

Generation time and duration under natural circumstances and where grown or managed

Traditionally safflower was grown in the Mediterranean regions but today cultivation of safflower occurs in arid and semi-arid conditions, wherever the crops have established a tolerance to the hot and dry conditions (Weiss, 1971; Li and Mündel, 1996; Kumar et al., 2015). Typically, the generation time of safflower is within the range of 182-217 days (Figure 3.5), although there have been reports of growing seasons being as short or long as 81 days and 239 days respectively (Cerioni et al., 1999, as cited in Bellé et al., 2012). Generation time is influenced by variety, management practices and environmental conditions. Safflower cultivation during the fall/winter or the spring/summer season has a significant effect on the generation time (Bellé et al., 2012).

Figure 3.5. Development stages and development timeline of a safflower plant



Source: Kaffka, S.R. and T.E. Kearney (1998), "Safflower production in California", *University of California Agriculture and Natural Resources*, Vol. 21565, as adapted from GRDC (2010), *Raising the Bar with Better Safflower Agronomy*, ACT, Australia, Grains Research and Development Corporation.

Safflower emerges 1-3 weeks after sowing. Emergence takes longer under cooler temperatures, increasing the risk of damage by insects and disease. The first emerging leaves form a rosette. The duration of this vegetative rosette stage determines the generation time of safflower. This stage generally lasts between 20 and 39 days post-emergence but the duration varies with variety and growing conditions (temperature and photoperiod for example) and can be as long as several months (Anderson, 1987; Corleto, 2008; Emongor, 2010). During the rosette stage, the deep tap roots begin to develop but no stem is formed. The large tap root system can elongate up to three metres (Li and Mündel, 1996; Bockisch, 1998).

The rosette stage is followed by rapid stem elongation and extensive branching (Li and Mündel, 1996; Singh and Nimbkar, 2006). As temperature and day length increase the stem begins to elongate and branch. Lateral branches develop on stems that are about 20-40 cm high and these lateral branches may branch further to produce secondary and tertiary branches. The branching habit is classified as narrow, with branching angles (branch to stem) ranging from 30 to 75° in respect to the primary stem (Singh and Nimbkar, 2006). The level of branching is greatly influenced by the variety, environment and also plant density (Bockisch, 1998; Bellé et al., 2012). Significantly more branching occurs when plants are sown at lower densities than when sown at higher plant densities (Weiss, 1971; Kaffka and Kearney, 1998).

Higher seed yields can be achieved with a greater number of branches per plant since each branch ends in a flower head. The timing of flowering is mainly influenced by day length, requiring long days to initiate flowering (Gilbert, 2008). After flowering, the time to maturity is around four weeks.

Reproduction (production of flowers and seeds)

Floral biology

Safflower reproduction occurs through the development of seed (USDA-APHIS, 2008). Safflower flowers are typically brilliant orange (Figure 3.2), yellow or red, or more rarely white. The inflorescence is of the composite type characteristic of the family Asteraceae, with each plant producing 3-50 or more flowering

heads, called capitula, on the ends of the branches. Capitula on the primary branches flower first, followed by those on secondary and tertiary branches. The flowering of the individual florets in each capitulum starts at the margin of the head and proceeds inward over 3-5 days. Each head normally contains between 20 and 180 individual florets (GRDC, 2010), although there can be as many as 250 florets, with bristles being interspersed between the flowers (Singh and Nimbkar, 2006).

Each flower is composed of five petals which are all attached to a corolla tube. There are also five fused anthers attached to the corolla tube, which surrounds both the style and stigma. It may take between 10 and 45 days for all flowers on a plant to reach anthesis, during which pollen can be shed (Li and Mündel, 1996). Safflower anthers contain 150-300 pollen grains (Pandey and Kumari, 2008). The stigma is receptive for approximately 32-56 hours post-anthesis, after its exertion from the corolla tube (Knowles, 1980). At the base of the corolla tube, it is attached to an inferior ovary, which develops into a single-seeded fruit called an achene (seed) following pollination.

Pollination, pollen dispersal, pollen viability

Pollination

Safflower is primarily self-pollinating and cross-pollination rates vary between lines (Knowles, 1969a). Australian commercial varieties are largely self-pollinating with cross-pollination rates of less than 10% (GRDC, 2017). Self-pollination is predominant because the style and stigma grow through the surrounding anther column; after elongation, the stigma is usually covered with pollen from the same floret (Claassen, 1950). Individual safflower florets are largely self-pollinating, as safflower florets produce pollen that will outcompete with adjacent florets. However, an un-pollinated elongated stigma can remain receptive for several days, and outcrossing rates and seed set can be increased by insect pollinators (Claassen, 1950; Li and Mündel, 1996; GRDC, 2010). Outcrossing rates vary depending mainly on insect pollinators but also on variety, pollen source size and environment. Intra- and interspecific cross-pollination are considered in greater detail in sub-sections on intraspecific crossing and natural facility of interspecific crossing respectively.

Pollen dispersal

Wind

Safflower pollen is yellow and relatively large with a mean diameter of 53-56 µm (USDA-APHIS, 2008). It is not transferred significantly by wind (Claassen, 1950; Li and Mündel, 1996). Claassen (1950) examined outcrossing rates for safflower plants grown either with or without insect exclusion cages. Depending on the cultivar, uncaged plants had outcrossing rates averaging 8.2-35% (range 6.3-58%), whereas the caged plants averaged 0.4-1.2% outcrossing (range 0-3.2%). The author acknowledged that the outcrossing observed in the caged plants could have been due to wind or to insect pollination of a few stigmas that had grown through the cage. In a glasshouse study, which excluded insects, no outcrossing was detected among the safflower plants (Claassen, 1950).

In the same study, pollen traps were placed at heights of 46, 76 and 122 cm above ground level while the safflower plants were in full flower. Safflower pollen was only detected at 46 cm, which was below the level of some of the flowers (Claassen, 1950). The height of the safflower plants was not given. Based on the assumption that some flowers were at or near the 46 cm height, there was no wind-dispersed pollen detected at distances of about 30-76 cm from the flowers. The results of these studies suggest that wind does not facilitate significant outcrossing or transport of safflower pollen and outcrossing is primarily due to insect-mediated pollen movement.

Insect pollinators

Safflower florets are largely self-pollinating but outcrossing rates and seed set can be increased by insect pollinators (Claassen, 1950; Li and Mündel, 1996; GRDC, 2010). Cross-pollination is thought to occur in safflower at approximately 10% but this is highly variable and honey bees, bumblebees, beetles and other insects can increase the level of cross-pollination (Emongor, 2010).

Pollination studies showed that honey bees (*Apis mellifera* and *Apis* spp.) are the major pollinators of safflower crops (Kumari and Pandey, 2005; Pandey and Kumari, 2008) but other insects such as other species of bees and non-hymenopterous insects do forage in safflower (AOSCA, 2012). In studies in the United States, 80-90% of insects observed visiting safflower plants were honey bees and over 80% of these observations occurred between 8 am and noon (Boch, 1961; Levin and Butler, 1966; Bukero et al., 2015). Greatly depleted pollen loads of safflower stigmas were observed in the late mornings, explaining the timeframe of honey bee foraging activity (Langridge and Goodman, 1980).

Bumblebees (*Bombus* spp.) play a role in the transfer of pollen in the northern hemisphere where they represent less than 10% of insect pollinators in safflower (Cresswell, 1999, 2000). Other insects which have been observed to be involved in the pollination of safflower include species from the families of Halictidae (Apoidea bees) and Syrphidae (flies, particularly hoverflies) (Langridge and Goodman, 1980).

Pollinator behaviour

Safflower ranks highly among the commercial crops which are preferred by honey bees. Chaney (1985, as cited in Van Deynze, Sundstrom and Bradford, 2005) found that honey bee pollen collectors bypass cotton and fly 8 km to safflower while nectar collectors forage in nearby cotton. Conclusions from a Californian trial were that the population density of bees in trial crops (onion, carrot and safflower) was primarily a function of the quality and quantity of foraging resources and secondarily a function of competition from nearby colonies (Gary et al., 1977). Nectar gatherers were observed to be the predominant visitors in Australia on “Gila” safflower fields but many were well dusted with pollen (Langridge and Goodman, 1980). The distance of pollen dispersal or movement is dependent on pollinator behaviour and also on plant density, for example sparse areas of plants receive fewer pollinator visits (Kunin, 1997). Long-distance bee foraging has been documented with 1 bee (of 2 000 marked) collected in a safflower field 7.1 km from the hive (Gary et al., 1977). Foraging distances of pollen-collecting honey bees is longer in simple sparse landscapes than complex landscapes with ample vegetation (AOSCA, 2012).

Studies of the foraging habits of honey bees on safflower fields in India observed honey bees making foraging trips that lasted 15 minutes, visiting 5 to 8 flowers per trip, with 15 seconds to 2 minutes spent per flower (Kumari and Pandey, 2005; Pandey and Kumari, 2008). In a study of safflower fields (variety Gila) in Australia, honey bees were observed to visit on average 9 flowers per head, usually visiting 1 head per plant and spending 12.2 seconds per plant. One bee visited 54 plants in 15 minutes while another visited 48 plants in under 8 minutes (Langridge and Goodman, 1980).

Pollen viability

The likelihood of successful pollination or cross-pollination is both dependent on pollen dispersal and on how long the pollen grain remains viable. In general, pollen viability is dependent on a number of factors including temperature and humidity. Safflower is usually grown in dry conditions, where pollen is expected to desiccate rapidly (USDA-APHIS, 2006). There is limited information on safflower pollen viability. However, one study indicated that safflower pollen has a short life, showing a reduction in viability to 73.6% only 24 hours after anthesis (Pandey and Kumari, 2008).

Seed production and natural dispersal of seeds

Seed production

Each safflower head or capitulum usually produces 15-60 seeds. Safflower seeds are contained within a thick hull, this type of fruit is known as an achene, which matures 4-5 weeks after flowering (Li and Mündel, 1996; Singh and Nimbkar, 2006). The composition of mature safflower seed is described as 27-32% oil, 5-8% moisture, 14-15% protein, 2-7% ash and 32-40% crude fibre (Weiss, 2000). The seeds are usually white but can also be striped. Safflower seeds are relatively large measuring 6-10 mm long, tetragonal in shape, with an average weight of 30-40 mg (25 000 seeds/kg) (Bockisch, 1998; GRDC, 2010; Bellé et al., 2012).

The white hulled varieties are used for the birdseed and pet food markets. Seed with brown stripes or with mould or staining is not acceptable (Mündel et al., 2004). Seeds are typically smooth but some varieties have tufts of hairs (pappus) on the ends, which is not desirable in commercial cultivars (Li and Mündel, 1996). Therefore, most seeds of cultivated safflower lack a pappus or, if present, it is reduced (Bérvillé et al., 2005). Since safflower seeds are typically smooth, due to the absence of or reduced pappus, the likelihood of dispersal through wind or adherence (to human clothes or animal fur) is significantly minimised (Vander Wall, Kuhn and Beck, 2005; Wichmann et al., 2008; Mayerhofer et al., 2011).

Natural dispersal of seeds

Wind

Safflower seed is not appreciably dispersed by wind. During domestication of safflower, traits that increased seed recovery at harvest were selected and as a result, cultivated safflower is highly shatter-resistant compared to its wild relatives (Bérvillé et al., 2005; McPherson et al., 2009b). Safflower does not lodge readily but branches/flower heads could be dispersed by strong winds, particularly if the plants or stems were weakened due to pathogen infections or damaged through the activity of birds or other animals (McPherson et al., 2009b; GRDC, 2010). The distance of safflower seed dispersal by wind has not been investigated, although studies with *Brassica* sp. seed can provide indicative information. The wind dispersal of *Brassica* sp. seed was low, dispersing seed less than 250 m due to their spherical shape and high terminal velocities (Bullock and Clarke, 2000; Wichmann et al., 2008). It would be expected that this distance would be significantly less for safflower seeds due to their tetragonal shape and increased seed weight (Bockisch, 1998; Bellé et al., 2012).

Water

No data has been found on the seed transport rates by water of safflower seed. Overall, the dispersal of seed by water has not been widely studied (Wichmann et al., 2008). It is likely that seed could be carried by heavy rains and flooding either shortly after planting or at harvest. If there were heavy rainfalls, the transported seed is likely to germinate because safflower seed has little or no dormancy. Safflower is sensitive to excess moisture/water either as heavy rainfalls, standing water (waterlogging) or humidity. This is due to the increased chance of disease (e.g. *Phytophthora cryptogea*) under these conditions and can lead to substantial yield losses (Nimbkar, 2008; GRDC, 2010), hence it would be expected that dispersal by water has minimal contribution in the dispersal of safflower.

Humans

Human-mediated dispersal can take many forms. Spillage during movement of seed on equipment for planting, harvest or post-harvest storage and/or shipping provides the greatest potential for dispersal of safflower seed. Seed could be spilled during transport and may also be dispersed if inadvertently transported on the machinery (e.g. on muddy wheels). It is also possible for small amounts of seed to be transported on or in clothing (e.g. pockets and cuffs) or boots (especially muddy boots) of workers. Detailed

information in regards to the frequencies and distances of human-mediated seed dispersal is still unknown, although some research has focused on the dispersal distances associated with walking (Bullock and Primack, 1977; Mack and Lonsdale, 2001; Wichmann et al., 2008). It has been reported that seed retention and dispersal via clothing (e.g. shirts and trousers) can occur up to 250 m (Bullock and Primack, 1977). Small seeds of some plant species may persist on shoes for more than 5 km, with the predicted potential to be over 10 km (Wichmann et al., 2008). However, for germination and establishment to occur, the seeds must be located in a suitable environment.

Animals

Safflower seeds are a food source for a range of species including mammals, birds and invertebrates. Secondary seed dispersal may also occur and some seeds may be transported intact by ants, dung beetles or scatter-hoarding rodents (Vander Wall, Kuhn and Beck, 2005). Safflower seeds are firmly held within the seed heads and are highly shatter-resistant, therefore limiting access by rodents. Post-harvest dispersal of seeds by small mammals, i.e. rodents, is most likely with predation of seeds present on the soil surface. Safflower seeds may be either dispersed or hoarded by rodents.

For some larger animals such as cattle, foraging or grazing is minimal due to the spiny nature of mature safflower plants (Cummings et al., 2008) but sheep and goats are not irritated by the spines. Feral pigs or boars are destructive and difficult to exclude from fields (Rao et al., 2015). Native animals may also feed on safflower. The viability of safflower seed after passing through the digestive gut of grazing animals is poorly understood.

Safflower dispersal by birds is most likely as some safflower seed varieties are sold as birdseed. Small birds, such as sparrows, can feed on maturing safflower seeds and larger birds, such as cockatoos, can chew safflower plants at the base in order to access seeds (GRDC, 2010). Safflower seed dispersal by several bird species (blackbirds, mallard ducks, pigeons and pheasants) was examined and it was observed that seed did not pass through the digestive tract but did remain viable in the oesophagus and gizzard regions for several hours. The safflower seed viability was measured as a percentage of germination, where the germination rate was in the range of 16-30% and 4-29% for seed collected from the oesophagus and gizzards of birds respectively (Cummings et al., 2008). A few seeds were also transported externally on soil attached to feet or legs of pheasants and pigeons (Cummings et al., 2008; Vazačová and Münzbergová, 2013). Seeds did not attach to plumage possibly due to the fact that safflower seeds are smooth. The researchers also mentioned other bird species that hoard or cache seeds such as ravens, jays and crows as potential transport vectors of safflower seeds.

Seed viability, longevity and dormancy, natural seed bank, germination and seedling viability

Seed longevity, dormancy and germination

Safflower seed has been selected for reduced dormancy during domestication (Bérvillé et al., 2005; McPherson et al., 2009b). Seeds of modern cultivars generally lack dormancy and can germinate in the head if rainfall occurs at harvest time (Zimmerman, 1972; Li and Mündel, 1996). A study was conducted to examine the germination of freshly harvested seed from 1973 accessions from over 50 countries, with seed germinated at 20°C. The average time to achieve at least 60% germination was 60 hours for approximately 99% of the accessions. The remaining 1% required more than 120 hours to reach at least 60% germination (Li et al., 1993, as cited by Li and Mündel, 1996). Low levels of dormancy have been observed in safflower, with some variation between cultivars; however, this low level of dormancy was lost during storage. For example, dormancy was lost after 24 weeks of storage at room temperature (Kotecha and Zimmerman, 1978).

Safflower is ideally sown into moist soil at a depth of 2.5-4 cm. Shallow sowing promotes uniform emergence, while deeper sowing increases the susceptibility of the seed to *Pythium* (Oelke et al., 1992; GRDC, 2010). Germination can occur at temperatures as low as 2-5°C and takes between 3 and 8 days, depending on the temperature (Li and Mündel, 1996; Emongor, 2010). However, germination is poor when soil temperatures are below 5°C. Safflower seedlings are frost resistant to about -7°C. Sowing depth, light, temperature and moisture all have an influence on germination (McPherson et al., 2009b). The timing of emergence also depends on temperature but, generally, plants emerge 1-3 weeks after sowing (GRDC, 2010; Bellé et al., 2012).

Seed banks/persistence

Dormancy can affect the persistence of seeds in the soil but, as discussed above, safflower generally has no or little long-term seed dormancy which limits its persistence in seed banks (Bérvillé et al., 2005).

In Australia, safflower seed loss during harvest is about 3-4% (GRDC, 2010). Similarly, harvest losses in California (United States), were estimated at 3-4%, or 192-384 seeds/m^2 on yields of 2 200 to 3 400 kg/ha (Knowles et al., 1965). In one study conducted over 6 sites in Alberta (Canada), seed losses ranged from 230 to 1 070 seeds/m^2 with 80-520 viable seeds/m^2, representing a range of 26% to 84% viable seed depending on the site (McPherson et al., 2009b). It is not rare that a large portion of seed lost during harvest is non-viable. Combine harvester settings (e.g. sieve size, wind speed) are normally such that low weight and small-sized seed are dispersed during harvest. Such seed is usually immature and is unlikely to be viable. However, these levels are relatively high and represent up to five times the recommended seeding rate for that region. The researchers did state that similar pre-harvest and harvest losses are found in wheat fields. Despite these large losses, densities of safflower volunteers emerging in spring ranged from 3 to 11 seedlings/m^2. Volunteers did not survive in fields under chemical fallow. In only 3 of 10 cereal fields surveyed, a few volunteers (0.05-0.33 plants/m^2) survived the first year and generated viable seeds (1-4 seeds per plant). However, volunteer populations did not persist beyond two years (McPherson et al., 2009b).

Seed viability of safflower on the soil surface and buried at two different depths was also examined (McPherson et al., 2009b). The viability of the seed was evaluated after burial in artificial seed banks or spreading the seed on the surface. Seeds did not persist beyond 2 years at the soil surface and beyond 1 year if buried at 2 cm or 15 cm. Thus, the authors recommended tillage to reduce the persistence of the seed bank because the buried seed lost viability faster than the seed on the soil surface. The authors also demonstrated that chemical fallow is an effective control measure, eliminating the presence of safflower volunteers from the fields (McPherson et al., 2009b).

Asexual propagation (apomixis, vegetative reproduction)

Safflower reproduces by seed and is not known to reproduce asexually (USDA-APHIS, 2008).

Genetics

Relevant detailed genetic information on the species

Genetic composition

Cultivated safflower (*C. tinctorius* L.) is a genetically diverse diploid (2n = 2x = 24) with the genus consisting of 16 species (further discussed in the section “Hybridisation and introgression”). In recent years there has been extensive research concentrating on the genetics and genomics of safflower to develop an understanding of both diversity and trait mapping to enable crop improvement through breeding.

The haploid genome size for safflower is approximately 1.4 gigabases (Gb) (Ali et al., 2019), although the genome size varies among populations from different origins (Garnatje et al., 2006). Analysis of genome sizes for those species within the *Atractylis* section reveals that, through the development of allopolyploids, the nuclear DNA content is either the sum of the parental genomes or non-additive, resulting in a smaller hybrid genome size than predicted (Table 3.3). These non-additive changes in genome size function to stabilise polyploidy genomes, which is an adaptive pre-programmed response to genomic stress induced by hybridisation and allopolyploidy (Ozkan, Tuna and Arumuganathan 2003). It was demonstrated that the monoploid genome size ($1C_X$) decreases with increasing ploidy levels (Garnatje et al., 2006). The sum of the nuclear DNA contents can be used to evaluate the origins and the evolution of hybrid species. For example, the 2C value for *Carthamus creticus* is lower than the sum of the hypothesised parents being *Carthamus lanatus* and *Carthamus leucocaulos*. Similarly, this was also observed for the allopolyploid *Carthamus turkestanicus*, a hybrid of *C. lanatus* and *Carthamus glaucus* (Garnatje et al., 2006).

Table 3.3. Nuclear DNA content and other karyological features

Taxa	2C ± s.d. (pg)	2C (Mbp)	2*n*	Ploidy level	1Cx
Section *Atractylis*					
C. alexandrines	3.02 ± 0.20	2 953.56	20	2×	1.51
C. anatolicus	2.96 ± 0.03	2 894.22	20	2×	1.48
C. boissieri	2.94 ± 0.01	2 875.32	20	2×	1.47
C. criticus	6.89 ± 0.07	6 738.42	64	6×	1.15
C. dentatus	2.70*	2 640.60	20	2×	1.35
C. glaucus	3.00 ± 0.08	2 934.00	20	2×	1.50
C. lanatus	4.75 ± 0.05	4 645.50	44	4×	1.19
C. leucocaulos	2.26 ± 0.02	2 210.28	20	2×	1.13
C. nitidus	2.44 ± 0.04	2 386.32	24	2×	1.22
C. tenuis	2.74 ± 0.07	2 679.72	20	2×	1.37
C. turkestanicus	7.32 ± 0.11	7 158.96	64	6×	1.22
Section *Carthamus*					
C. gypsicolus	2.71 ± 0.06	2 650.38	24	2×	1.36
C. oxyacanthus	2.62 ± 0.06	2 562.36	24	2×	1.31
C. palaestinus	2.82 ± 0.06	2 757.96	24	2×	1.41
C. persicus	2.65 ± 0.06	2 591.70	24	2×	1.33
C. tinctorius	2.77 ± 0.04	2 709.06	24	2×	1.39

Source: Garnatje, T. et al. (2006), "Genome size variation in the genus *Carthamus* (Asteraceae, Cardueae): Systematic implications and additive changes during allopolyploidization", *Annals of Botany*, Vol. 97, pp. 461-467.

Repetitive DNA sequences may influence both chromosome structures and recombination events, hence playing an active role in the process of evolution through genome differentiation. Consequently, their abundance, sequence divergence and chromosomal distribution are all important factors in acquiring a complete understanding of genome organisation (Yan et al., 2002). The repetitive elements within the safflower genome have been investigated to better understand their characteristics including size, sequence, location on chromosomes and whether they are unique to safflower (Raina et al., 2005). The location of one element (pCt*Kpn*I-1) in the subtelomeric region of many safflower chromosomes (Raina et al., 2005), a region involved in recombination events during mitosis, suggests a role for this element in the genetic diversity of safflower and its environmental adaptability (Brown et al., 2010). The homology of

another element (pCt*Kpn*I-2) with a gene family of *Centaurea stoebe* (Asteraceae) has suggested a role in driving tissue-specific gene expression (Macas, Navrátilová and T. Mészáros et al., 2003; Raina et al., 2005). Further investigations utilising these sequence repeats may help to develop a better understanding of evolution within the Asteraceae family, specifically the *Carthamus* species.

Genetic diversity

The genetic diversity of safflower has been investigated through various molecular techniques including random amplified polymorphic DNA (RAPD), sequence-related amplified polymorphism (SRAP), single nucleotide polymorphisms (SNPs), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR), of which the AFLP technique was found to be the most accurate measure (Sehgal et al., 2009). AFLP fingerprinting was further utilised to elucidate associations between genetic differentiation and geographical distribution of globally sourced safflower accessions and cultivars (Kumar et al., 2015).

The Far East region has been described as one of the most conserved centres for safflower, which was confirmed by analysis of genetic diversity, highlighting that most of the accessions analysed formed isolated clusters. Proposed as centres of origin (Knowles, 1969a), the Near East and Iran-Afghanistan regions exhibited high levels of genetic diversity with accessions being distributed across many clusters. It has been suggested that the increased diversity may have been facilitated through genetic exchanges between wild and cultivated germplasm (Ashri, 1971). The accessions from Turkey were fragmented into two clusters that were genetically similar to either accession from the Near East or Iran-Afghanistan regions. A high level of genetic diversity was found within accessions from the Indian subcontinent, with accessions being distributed across multiple clusters. However, the Indian commercial cultivars were found to cluster together, highlighting the untapped potential for the local germplasm to be used for crop improvement by means of introgression. Breeding lines from America also are clustered with the same geographical accessions, indicating low genetic diversity (Kumar et al., 2015). The use of molecular markers, such as AFLP fingerprinting, reflects the diversity of safflower at the DNA level as opposed to morphological markers, thus eliminating the environmental element of observed phenotypes.

Chromosome pairing and cytomixis

During diversification of the safflower cultivars, quantitative genome changes can occur through the exchange of genetic information between chromosome arms, showing variation in DNA content from 2.68 to 2.79 pg (Garnatje et al., 2006; Sheida, Sotoode and Nourmohammadi, 2009). Approximately 75% of this variation can be attributed to mean chromosome length and the lengths of both the short and long arms of chromosomes. The exchange of genetic information occurs during chromosome pairing and the formation of chiasma, where chromosomes crossover, following the chromosomal decondensation phase of meiosis. Genetic linkages are formed during translocation at the point of chiasma, which is mostly associated with chromosome 3 (Pillai, Kumar and Singh 1981). Consequently, increases in chiasma frequencies would enable enhanced genetic diversity. The shedding of elements in the synaptonemal complex,[1] modification of histone proteins and the adaptation to adverse environmental conditions are proposed reasons for genetic diffusion. Simple translocations can also be artificially induced using gamma-irradiation (Singh, Pillai and Kumar 1981).

Methods of breeding

Classical breeding

As with other crops, the ultimate goal of safflower breeding is to accumulate favourable traits into a cultivar. The most commonly utilised breeding method for the development of safflower cultivars is selection for desired traits. This is a multi-step process, which begins with the selection of parents having desirable traits. Examples of desirable traits include seed yields, seed oil content and disease resistance (Singh and

Nimbkar, 2006). Consequently, the selection of parents plays a crucial role in determining the success of any crop improvement breeding programme (Joshi, 1979; Singh and Nimbkar, 2006). The parent plants are then crossed to generate a breeding population. This first hybrid generation (F_1) is allowed to self-pollinate. The traits of interest segregate in the F_2 population. The next step of the breeding process is to select the best performing individuals from within the F_2 and subsequent generations and then to let them self-fertilise in order to generate homogenous lines (homozygous genotypes) exhibiting fixed traits. Homogenous lines are evaluated at multiple geographical locations to identify which ones are best adapted to different environments.

The different safflower varieties and their wild relatives provide the starting material for new crop cultivars. When a new breeding programme is initiated, the selected parental varieties are crossed. Crossing generates genetic variation through genetic recombination at meiosis. Since safflower is mostly self-pollinated, the crossing of the parental lines to generate hybrids would most likely occur in the controlled environment of a glasshouse. Another reason for performing breeding programmes in glasshouses is that it eliminates the likelihood of unknown or unwanted insect-mediated outcrossing that may occur in the field (Li and Mündel, 1996). Another method to ensure that only planned crosses occur is to emasculate the flowers by removing the anther tubes in the late budding stage. Once the styles have elongated, the emasculated florets are then fertilised with pollen from another preselected flower (Knowles, 1980). The F_2 and subsequent generations are processed by a selection process, which is a method of determining the relative worth of individuals in a segregating population. The selective breeding methods are described below (Singh and Nimbkar, 2006).

- **Pedigree selection:** In this method of breeding, individual plants from the F_2 population (5-10% of the population) are further propagated, with the genealogy of each line being recorded. The selected lines are self-fertilised for each generation to ensure the development of homozygous progeny. The pedigree breeding method is the most labour-intensive method but provides the greatest detail of genetic information. It is generally used to create new lines and cultivars that combine the best traits from elite parental lines. This method has been used to breed in desirable traits such as improved seed yields and increased seed oil content (Knowles, 1969b; Ranga Rao, Ramachandram and Arunachalam 1977).
- **Bulk selection:** In this method, plants are chosen which express individual advantages and a sample of the collective seed is propagated in the next inbreeding cycle. The breeder often relies extensively on natural selection or relatively simple selection techniques within the bulk population for removing unwanted types or retaining desirable types, as the population is harvested *en masse* with no individual progeny testing. Consequently, the strong natural selection pressure favours the development of higher-yielding varieties. Another advantage of this method is that breeders are able to handle multiple bulk populations concurrently.
- **Single-seed descent selection:** Involves self-fertilisation of a random sample of F_2-derived plants in each generation and advancing only one seed per plant, with the intent to achieve homozygosity whilst practising minimal selection. When inbred lines have been produced, selection can be based on data from replicated field trials for desirable attributes including agronomic performance, biotic and abiotic stress tolerance, and/or end-use quality testing. This method is usually applied when crossing elite safflower cultivars in which many of the desirable alleles are already fixed.
- **Recurrent selection (backcrossing):** Backcrossing is a method of recurrent selection, used to introduce a desirable trait into a specific genetic background, typically a widely adopted variety (referred to as the recurrent parent). The parental source of the desirable trait is designated the donor parent and the parent in which the trait is introduced is the recurrent parent. After numerous backcrosses, the recurrent parent will have acquired the new desired trait. After the final backcrossing cycle, the selected elite plants are self-fertilised to produce progeny that is both homozygous for the new trait and similar to the recurrent parent. The backcrossing method has been used effectively as a breeding strategy to incorporate dominant genes for the control of

devastating diseases, such as root rot caused by *Phytophthera drechsleri* (Thomas, Rubis and Black 1960; Rubis, 2001) and in the development of high oleic acid safflower (Knowles, 1968; Hamdan et al., 2009).

If a trait of interest does not occur in the existing genetic resources, there are methods to generate genetic variation. Mutagenesis is a technique that induces changes in the genomic DNA sequence, which can be induced by exposing safflower seeds to chemical mutagens or ultraviolet or ionising radiation. TILLING (Targeting Induced Local Lesions IN Genomes) is one example of a mutagenesis technique that uses ethyl methanesulfonate (EMS) to induce short insertion/deletion (INDELS) mutations (Sikora et al., 2011; Kashtwari, Wani and Rather 2019). This mutagenesis is non-targeted, that is genes are mutated at random and this may generate a trait of interest. To date, this technique has not yet been explored for the potential crop improvement of safflower, although it has been used for *Helianthus annus* L. (sunflower), another member of the Asteraceae family (Sabetta et al., 2011).

Hybrid breeding

Hybrid breeding, often referred to as hybridisation, is mainly practised as a method to integrate the desirable traits of two or more varieties into one elite cultivar (Ashri and Knowles, 1960; Baydar, Gökmen and Friedt 2003). Similar to classical breeding methods, parental selection is critical in determining the success of crop improvement breeding programmes involving hybrid breeding (Joshi, 1979; Singh and Nimbkar, 2006). The existence of heterosis for capitula numbers, seed yields and other commercially important traits makes safflower a suitable candidate crop for the exploration and exploitation of hybrid vigour (Urie and Zimmer, 1970).

The very high linoleic acid (*lili*) content in safflower, controlled by recessive alleles at a single locus (*Li*), is a unique trait that is not found in any other commercial oilseed crop (Mattson, Sun and Koo 2004; Hall, 2016). A close genetic repulsion-phase linkage has been demonstrated between traits of nuclear male sterility (NMS; controlled by the gene *Ms*) and very high linoleic acid content (Hamdan et al., 2008). When the safflower parental lines of CL-1 (NMS; linoleic content of 74%) and CR-142 (high linoleic: 88%) were crossed, the recombination frequency of these two genes was evaluated to be 10%, which resulted in most of the progeny being both male-sterile and having an intermediate linoleic acid content. For breeding programmes that involve the very high linoleic acid trait, this genetic linkage enables simple selection of the trait through only progressing fertile progeny (Hamdan et al., 2008).

Development of hybrids

Dominant and recessive genetic male sterility (GMS), cytoplasmic male sterility (CMS) and thermosensitive genetic male sterility (TGMS) systems for producing hybrid safflower plants have been developed (Anjani, 2005; Singh, Ranaware and Nimbkar 2008; Meena et al., 2012; Deshmukh, Wakode and Ratnaparakhi 2014). Identification and development of GMS lines have assisted the release of non-spiny (NARI-NH-1) and spiny (NARI-H-15) safflower hybrids in India (Singh, 1996; Singh, Deshpande and Nimbkar 2003), which exhibit increases in both total seed yield and oil content by 20-25%. Similarly, CMS and TGMS lines are also commercially available in India (Meena et al., 2012). The average yield and oil content of CMS hybrid lines were greater than the open-pollinated lines in field trials run across sites in the United States, Canada, Pakistan, Mexico and Spain (Li and Mündel, 1996). In Australia, the comparison of four US derived CMS lines against open-pollinated lines was inconclusive with regard to yield (Wachsmann et al., 2003).

For hybrid seed production and breeding programmes, GMS lines are used as they reduce the manual labour involved in flower emasculation (Knowles, 1980). In naturally occurring GMS lines, male-sterile and fertile plants can only be distinguished at the time of flowering, with identification typically being dependent on flower morphology and the presence of pollen (Singh, 1996). For the female parent, all fertile plants have to be emasculated before flowering to avoid self-fertilisation, hence eliminating the risk of reductions in both seed yields and seed purity. Genetic linkage has been identified between the recessive alleles of

male sterility (*Ms*) and dwarfism (*dw*), which produce dwarf male-sterile (DMS) plants when present in the homozygous state (Singh, 1997). At approximately 30-40 days after sowing, the male-sterile plants are only 5-10 cm tall, whereas the male-fertile plants are significantly taller at 20-25 cm.

Similar to the dwarf trait, a marker-linked GMS (MGMS) line was developed with sterile and fertile plants being distinguishable at the elongation stage, where plants are approximately 40-45 days old (Kammili, 2013), enabling identification approximately 45-50 days prior to the flowering stage. Genetically linked segregation was observed for the male sterility and the non-spiny traits, with sterile plants being identified morphologically by non-spiny leaves, whereas the leaves of fertile plants had spines (Kammili, 2013). The benefits of early identification of male-fertile plants, aided through the traits of either dwarfism or non-spiny leaves, include increased yields, the production of pure hybrid seed and the faster breeding of elite varieties (Singh, 1997; Kammili, 2013).

Intraspecific crossing: Outcrossing and gene flow potential

Vertical gene transfer is the transfer of genetic information from an individual organism to its progeny. In flowering plants, vertical gene transfer mainly occurs via pollen dispersal and cross-pollination between related sexually compatible plants. Intraspecific crossing refers to fertilisation between *C. tinctorius* (safflower) plants (Ashri and Efron, 1964; Imrie and Knowles, 1970). Gene flow captures all of the mechanisms that result in the movement of genes between populations of species that are cross-compatible, whether they are the same or different species or subspecies (Ridley and Alexander, 2016). Outcrossing in safflower is mainly insect-mediated with wind-mediated outcrossing playing a minor role (see sub-section on pollination, pollen dispersal, pollen viability). Honey bees and bumblebees are the main pollinators of safflower. Worldwide, studies show that outcrossing rates appear to be quite variable (Table 3.4) and may depend on a number of factors such as pollen source size and shape, environmental climatic conditions, insect numbers and type and the variety/cultivar.

Table 3.4. Intraspecific crossing rates and gene flow potential in safflower

Study and country	Outcrossing range % (average %)	Distance
Kadam and Patankar (1942): India	1-28 (10)	Close proximity
	0.8-5.9 (1.9)	13.7 m
Claassen (1950): United States	8.3-100 (34.2)	1 m
	0-26 (14.9) low outcrossing lines	1 m
	31.8-93.6 (57.3) high outcrossing lines	1 m
Rudolphi, Becker and von Witzke-Ehbrecht (2008): Germany	6-33 (9.7-18)	Close proximity
	0-11.5 (6.5)	At least 5 m
McPherson et al. (2009a): Canada and Chile	0.48-1.7	0.3-3 m
	0-0.86	≈ 10 m
	0-0.26	≈ 20 m
	0-0.10	≈ 30 m
	0.03-0.16	≈ 40 m
	0.0024-0.04	50 m
	0.01	≈ 100 m
	Nil	≈ 300 m
Cresswell (2010)	0.005-0.05 (mathematical model)	Field to field
Velasco, Fischer and Fernandez-Martinez (2012): Spain	0.5-35.9 (10.3)	1-1.5 m
Nabloussi, Velasco and Fernandez-Martinez (2013): Morocco	8-53 (26.6)	1-1.5 m

Sources: Full reference information listed in the reference section below.

Although safflower is typically considered to be self-pollinating (described in sub-section on pollination, pollen dispersal, pollen viability), if self-pollination- does not occur, pollen may fall from other flowers or pollination may occur through the transfer of pollen from insects such as bees. Due to the limited wind-mediated movement of pollen, less than 1.2 m, cross-pollination of safflower is prominently insect dependent (Claassen, 1950). There are many factors that can influence successful outcrossing including pollinator effects (pollinator species and distance to pollen sources), abiotic factors (distance to compatible plants, wind direction and velocity) and crops characteristics (ploidy level, pollen of donor and receptor plants, pollen longevity, floral synchrony and cross-compatibility) (Kadam and Patankar, 1942; Rudolphi, Becker and von Witzke-Ehbrecht 2008; McPherson et al., 2009a). Although the intraspecific outcrossing potential varies significantly between varieties, consistently it has been demonstrated that the frequency of outcrossing decreases as the distance increases (Kadam and Patankar, 1942; Kumari and Pandey, 2005; Cresswell, 2010). The self-compatibility of different safflower varieties is an important attribute to consider for the evaluation of self-pollination and the potential for intraspecific crossing since self-pollination rates have been shown to range from 9.3% to 81.5% (Claassen, 1950).

One of the earliest studies to examine intraspecific crossing in a number of safflower cultivars, using corolla colour as a morphological marker, was conducted in the United States (Claassen, 1950), with results summarised as follows. Outcrossing levels between rows spaced approximately 1 m apart ranged from 0% to over 50% for some cultivars, although most were less than 10%. Individual plants varied considerably with outcrossing frequencies ranging from 0% to 100%. In inbred varieties selected for high yield and high oil content, the average outcrossing between rows was less than 5%. When outcrossing rates were measured in two different regions within Nebraska, no significant differences were found between the two regions (Claassen, 1950).

In an earlier study conducted in India, also using corolla colour as a marker, cross-pollination rates ranged from 1% to 28%, with an average of 10%, between safflower plants in close proximity (exact distance not given). At a distance of 13.7 m, the average outcrossing rate ranged from 0.8% to 5.9% (average 1.9%) (Kadam and Patankar, 1942).

In 2008, a small study in Germany found the level of outcrossing between plots of safflower ranged from 0% to 33%, with averages of 6.5-18% depending on the location of the sampled plant (Rudolphi, Becker and von Witzke-Ehbrecht 2008). Outcrossing rates were also measured between plants grown together in the same plot and dropped from 63% in 2004 to 30% in 2005. The large variation between the two years of the study may have been due to different environmental conditions (Rudolphi, Becker and von Witzke-Ehbrecht 2008).

A study in Spain, as a model for a typical Mediterranean environment, examined outcrossing from a high oleic content cultivar (CR-6) to a low oleic content cultivar (Rancho) separated by 1-1.5 m. The CR-6 plants were surrounded by Rancho plants and high oleic acid was used as a biochemical marker to estimate outcrossing. The experimental crops were grown at three different times, winter sowing in 2009, winter sowing in 2010 and spring sowing in 2010. Average outcrossing rates of 5.7%, 12.1% and 13.2% were observed respectively. Higher outcrossing frequencies were detected at the single plant level (up to 35.9%) and the single-head level (up to 58.3%) (Velasco, Fischer and Fernandez-Martinez 2012).

Nabloussi, Velasco and Fernandez-Martinez (2013) used the same cultivars and field layout as Velasco, Fischer and Fernandez-Martinez (2012) to determine the outcrossing frequencies under Moroccan conditions. The average outcrossing rate at 1-1.5 m was 26% with a range of 8.3-53% at the plant level. This rate was approximately twice that reported by Velasco, Fischer and Fernandez-Martinez (2012). As this and the Velasco study used the same cultivars and field layout, collectively these studies demonstrate the influence of the environment, and possibly the pollinators, on outcrossing rates.

The frequency of natural intraspecific crossing from genetically engineered (GE) safflower to non-GE safflower was measured under field conditions in three different environments. Outcrossing experiments were conducted in the province of Santiago, Chile (2002) and the Canadian provinces of British Columbia

(2002) and Alberta (2004) (McPherson et al., 2009a). The GE safflower contained the *pat* gene (*phosphinothricin acetyltransferase*), conferring tolerance to the herbicide glufosinate, with this trait used to confirm outcrossing to the non-GE safflower. The three trial sites varied in design layout including the distance from the GE safflower to the first rows of non-GE safflower (0.3-3.0 m), the distance over which outcrossing was measured, and size of the GE pollen source (99-900 m^2) (McPherson et al., 2009a).

The highest rate of outcrossing of 1.67% was detected at the British Columbia site at a distance of 3 m, which was the nearest distance measured. Outcrossing was observed at each distance sampled at this site (from 3 to 101 m), except for a single measurement at 300 m where no outcrossing was detected. At the site in Santiago, outcrossing was observed at nearly every distance (0.7-60.5 m) with the highest outcrossing rate of 0.48% again observed in samples taken at the closest distance of 0.7 m. No outcrossing was detected at most distances measured at the Alberta site (from 0.3 to 49.5 m), the highest outcrossing rate observed was 0.62% at 0.3 m (McPherson et al., 2009a). The highest levels of outcrossing occurred closest to the pollen source and significantly declined over distance for all three sites, with the frequency of outcrossing reduced by 96-100% at 50 m.

Outcrossing frequencies were as heterogeneous between the three sites as they were between blocks (replicates). Researchers indicated this variation may be due to the non-random movement of pollen by insects, as wind is not a significant factor in safflower outcrossing (Claassen, 1950; McPherson et al., 2009a). Additionally, the pollen source size was suggested to be influencing outcrossing. The area of the British Columbia pollen source was about 9 times larger (900 m^2) than either of the other 2 sites (99 and 110 m^2) and outcrossing close to the pollen source at this site was 4 times greater. The larger site also demonstrated a slower decline in outcrossing with distance (McPherson et al., 2009a). Other differences in site design may have affected outcrossing rates. The Alberta site had a barren zone between the GE and non-GE safflower and this may have affected insect-mediated cross-pollination. Differences in insect populations at the sites have been proposed as a possible cause for the lack of outcrossing observed at the Alberta site (McPherson et al., 2009a). Directionality was also considered at the three trial sites and it was noted that there were predominately westerly winds during flowering. However, greater outcrossing was not found on the leeward side of the trial sites, which supports Claassen's (1950) findings that wind-mediated pollination plays a minor role, if any, in outcrossing of safflower.

For the distance range of 0.3-3 m, the intraspecific crossing rates in the study by McPherson et al. (2009a) ranged from 0-1.7%, which is an order of magnitude lower than other studies for distances of 1-1.5 m (see Table 3.3). One reason for this is the environmental differences that can influence outcrossing rates. For example, both Velasco, Fischer and Fernandez-Martinez (2012) and Nabloussi, Velasco and Fernandez-Martinez (2013) used the same cultivars and field designs in different countries (Spain versus Morocco) but had a twofold difference in outcrossing rates. The outcrossing rates could also be influenced by the cultivars included in the study. This was demonstrated through the work by Claassen (1950) where a huge variability in outcrossing was observed (14.9% and 57.3% in low and high outcrossing lines respectively). Additionally, the rate of outcrossing can be influenced by the type and number of pollinators at the trial site.

McPherson et al. (2009a) did point out that this work cannot predict maximum distances of pollen movement by pollinators due to long-distance foraging by bees, as pollen can potentially be dispersed by bees foraging over a range of kilometres. In addition, the researchers found that the outcrossing rate in safflower was spatially heterogeneous as was the case observed by Nabloussi, Velasco and Fernandez-Martinez (2013), indicating that bee and other insect visitations occur in a random and unbalanced way. There is evidence of long-distance insect-mediated pollen transfer in other self-pollinated crops, such as cotton and oilseed rape, due to the long-distance foraging capability of honey bees and bumblebees (AOSCA, 2012).

Bumblebees have been suggested as being more effective at field-to-field pollination of safflower than honey bees. Using a mathematical model of field-to-field gene flow due to insect pollination, the maximum level of bee-mediated gene flow between large fields was estimated at 0.005-0.05% (Cresswell, 2010). The highest value occurred when it was assumed that fields were pollinated exclusively by bumblebees. Values for the model were determined using observations of honey bee and bumblebee behaviour on a 40-ha field of safflower in Canada. Bees made long foraging bouts within the field, making between field pollinations rare. This factor, as well as safflower's high capacity for self-pollination, resulted in the very low estimates of pollinator mediated gene flow between fields (Cresswell, 2010).

Hybridisation and introgression

The natural facility of interspecific crossing (extent, sterility/fertility)

Interspecific crossing refers to the outcrossing of safflower to related species (Ashri and Efron, 1964; Imrie and Knowles, 1970; Garnatje et al., 2006). This hybridisation of different species or subspecies needs to be considered with respect to potential evolutionary and ecological consequences (Ridley and Alexander, 2016).

Studies have revealed that safflower can hybridise with other *Carthamus* species to produce allopolyploid plants (Sheidai, Sotoode and Nourmohammadi 2009), typically associated with differences in the DNA content (Table 3.3; sub-section on genetic composition). During meiosis, chromosome migration can occur within cytomictic channels (inter-meiocyte connections) of the anther, which can lead to aneuploidy of meiocytes. The aneuploidy meiocytes are precursors to the formation of unreduced (2*n*) pollen grains, hence enabling the production of plants with higher levels of ploidy (Sheidai, Sotoode and Nourmohammadi 2009).

Natural interspecific hybridisation between safflower and its wild relatives can only occur if there is synchronous flowering (temporal sympatry) and proximity (spatial sympatry) (Ellstrand, Prentice and Hancock 1999). Hybridisation between safflower and wild *Carthamus* species has probably played a role in the evolution of *C. tinctorius* in the Mediterranean and Asia where they are sympatric (McPherson et al., 2004). Spatial sympatry can be seen in Table 3.5., which summarises the geographical distribution of all *Carthamus* species (McPherson et al., 2004; GBIF Backbone Taxonomy, 2017). Successful experimental (artificial) hybridisation of any two species is not an accurate measure of success in nature, although it does describe the potential for cross-compatibility. The self-compatibility and compatibility with *C. tinctorius* have been summarised in Table 3.6.

Table 3.5. Geographical distribution of *Carthamus tinctorius* L. (cultivated safflower) and related species

Taxon	Geographical distribution
Section *Carthamus* (2n = 24)	
C. curdicus Hanelt	Iran only
C. gypsicolus Iljin	Iran, Iraq, Kazakhstan, Azerbaijan, Armenia, Lebanon, Turkey, Syrian Arab Republic, Uzbekistan
C. oxyacanthus Bieb.	Pakistan, Iran, Afghanistan, Iraq, Turkey, India, Uzbekistan, Azerbaijan, Armenia, Australia
C. palaestinus Eig.	Israel, Iraq
C. persicus Willd. (syn. *C. flavescens* Spreng.)	Israel, Turkey, Iraq, Syrian Arab Republic, Ethiopia, Lebanon, Jordan, Iran
C. tinctorius L.	Widely cultivated (safflower, *refer to Figure 3*)
Section *Odonthagnathis* (DC.) Hanelt (2n = 20, 22)	
C. boissieri Halácsy	Greece, France, Cyprus
C. dentatus Vahl	Australia, Greece, Turkey, Bulgaria, Cyprus, Hungary, Iran, Macedonia
C. divaricatus Beguinot and Vacc.	Libya
C. glaucus Bieb.	Israel, West Bank and Gaza Strip, Turkey, Syrian Arab Republic, Lebanon, Greece, Azerbaijan, Afghanistan, Egypt, Ukraine, Armenia, Jordan, Iraq, Russia, Australia
C. leucocaulos Sm.	Greece, Australia, United States, Germany, Turkey, Argentina
C. tenuis (Boiss. and Bl.) Bornm.	Israel, West Bank and Gaza Strip, Lebanon, Greece, Cyprus[3], Jordan, Egypt, Syrian Arab Republic, Turkey
Section *Atractylis* Reichenb. (2n = 44, 64)	
C. creticus L.	Greece, Spain, United States, Portugal, Denmark, Morocco, New Zealand, Australia, France, Egypt, Iraq, Turkey
C. lanatus L.	Spain, France, Italy, Portugal, United States, Greece, Argentina, Ethiopia, Morocco, Turkey, Germany, Brazil, Netherlands, India, Pakistan, Australia
C. turkestanicus Popov	Afghanistan, Iran, Armenia, Turkey, Uzbekistan, Pakistan
Uncertain placement (2n = 24)	
C. nitidus Boiss	West Bank and Gaza Strip, Israel, Jordan, Syrian Arab Republic, Saudi Arabia, Lebanon, Egypt

Sources: McPherson, M.A. et al. (2004), "Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World", *Canadian Journal of Plant Science*, Vol. 84, pp. 923-934; GBIF Backbone Taxonomy (2017), "*Carthamus* L.", in *GBIF Secretariat*.

Table 3.6. Assessment of self-compatibility, compatibility with *C. tinctorius* L. and genomic formulae for *Carthamus* spp.

Taxon	Self-compatibility	Compatibility with *C. tinctorius*	Fertility comments	Genomic formula
Section *Carthamus* (2n = 24)				
C. curdicus Hanelt	Compatible	Unknown	–	–
C. gypsicolus Iljin	Compatible	Unknown	–	–
C. oxyacanthus Bieb.	Both known	Yes	Fertile	BB
C. palaestinus Eig.	Compatible	Yes	Fertile	B_1B_1
C. persicus Willd. (syn. *C. flavescens* Spreng.)	Incompatible	Yes	Fertile	B_1B_1
C. tinctorius L.	Compatible	Yes	Fertile	BB
Section *Odonthagnathis* (DC.) Hanelt (2n = 20, 22)				
C. boissieri Halácsy	Unknown	Unknown	–	–
C. dentatus Vahl	Incompatible	No	–	A_1A_1
C. divaricatus Beguinot and Vacc.	Incompatible	Yes	Fertile self-incompatible hybrids	–
C. glaucus Bieb.	Unknown	Yes	Infertile hybrids	AAA_3A_3
C. leucocaulos Sm.	Compatible	Yes	Infertile hybrids	A_2A_2
C. tenuis (Boiss. and Bl.) Bornm.	Unknown	Unknown	–	–
Section *Atractylis* Reichenb. (2n = 44, 64)				
C. creticus L.	Compatible	Yes	Fertile	$A_1A_1B_1B_1A_2A_2$
C. lanatus L.	Compatible	Yes	Infertile hybrids	$A_1A_1B_1B_1$
C. turkestanicus Popov	Compatible	Yes	–	$A_1A_1B_1B_1A_3A_3$
Uncertain placement (2n = 24)				
C. nitidus Boiss	Compatible	Yes	Infertile hybrids	–

Source: McPherson, M.A. et al. (2004), "Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World", *Canadian Journal of Plant Science*, Vol. 84, pp. 923-934, and references cited therein.

Section *Carthamus* (n = 12)

- Natural hybrids have been identified between *C. tinctorius* and *C. oxyacanthus* and *C. palaestinus*, which are all members of the *Carthamus* section (Table 3.1) (Ashri and Knowles, 1960). *C. oxyacanthus* and *C. tintorius* have a relatively high rate of natural hybridisation when grown side by side and the F_1 plants showed hybrid vigour (Deshpande, 1952). Natural hybrids between these species have been identified in both India and Pakistan where they are sympatric. In contrast, hybrids between *C. tinctorius* and either *C. oxyacanthus* or *C. palaestinus* did not demonstrate any hybrid vigour, increased fitness or weediness (Mayerhofer et al., 2011).
- A review by Knowles and Ashri (1995) indicates that *C. flavescens* (= *C. persicus*), *C. oxyacanthus* and *C. palaestinus* can easily be artificially crossed with *C. tinctorius* and occasionally will form natural hybrids. Hybrids of *C. tinctorius* and *C. oxyacanthus* have been documented in greenhouses and in the field in India and Pakistan where they are sympatric (McPherson et al., 2004, and references cited therein). *C. oxycanthus* is rated as one of the top ten weeds in Pakistan. Hybrids of safflower and *C. palaestinus* have been found in Israel where the two species are sympatric (Knowles and Ashri, 1995). Hybrids of these two species were also found where alternate rows of *C. tinctorius* and *C. palaestinus* were planted in field trials. Seeds from the plants were collected and planted in the field in the following seasons and hybrids with either species

as the female parent were identified morphologically (Ashri and Rudich, 1965). The review also noted that the possibility of natural hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* had not been determined (Knowles and Ashri, 1995).

Section *Odonthagnathis* (n = 10, 11)

- Naturalised populations of wild safflower species, specifically, *C. leucocaulos, C. dentatus* and *C. glaucus*, have been reported in Australia (Groves et al., 2003; GBIF Backbone Taxonomy, 2017). *C. leucocaulos* is a noxious weed in Australia and California (the United States) (Mayerhofer et al., 2011). There are no reports of species within this section crossing with *C. tinctorius* under natural conditions.
- The potential for natural crossing between *C. tinctorius* and *C. tenuis* or *C. boissieri* (both n = 10) has not been determined.

Section *Atractylis* (n = 22, 32)

- Naturalised populations of *C. lanatus* (n = 22) have been reported in Australia (Groves et al., 2003) and has also been reported as a noxious weed in both Australia and the United States (California) (Mayerhofer et al., 2011). Hybridisation between species with either n = 10 or n = 12 with *C. lanatus* all produce infertile hybrids as a result of the irregular pairing of chromosomes during meiosis (McPherson et al., 2004 and references cited therein), hence the probability of a fertile hybrid occurring naturally is highly unlikely.
- Artificial crosses between *C. tinctorius* and *C. creticus* have resulted in the production of fertile F_1 hybrids, thus it is likely that natural interspecific crossing could occur between these two species if both temporal and spatial sympatry existed (McPherson et al., 2004).

Species of uncertain placement (n = 12)

- Crosses between *C. tinctorius* and *C. nitidus* result in the production of F_1 hybrids which are infertile (Knowles and Schank, 1964).

Experimental crosses

Cross-compatibility has been demonstrated with some of its weedy and wild relatives (McPherson et al., 2004; Garnatje et al., 2006; Mayerhofer et al., 2011; Ali et al., 2019). Both the selfcompatibility and outcrossing potential of safflower with its related species have been investigated, with results summarised in Table 3.6 (Ashri and Efron, 1964; Knowles and Schank, 1964; Imrie and Knowles, 1970; Estilai and Knowles, 1976; Heaton and Klisiewicz, 1981; McPherson et al., 2004; Garnatje et al., 2006; McPherson et al., 2009a; Mayerhofer et al., 2011). Typically experimental crosses are performed by using emasculation and hand-pollination (Mayerhofer et al., 2011). Although hand-pollination is not an appropriate technique for investigating the potential for outcrossing, since the process does not simulate natural pollination and seed production (Ellstrand, Prentice and Hancock, 1999), it does provide information on cross-compatibility.

Section *Carthamus* (n = 12)

- Most *Carthamus* species with n = 12 chromosomes (*C. tinctorius*, *C. oxyacanthus* and *C. palaestinus*) can be crossed successfully to produce fertile progeny (Ashri and Knowles, 1960; Mayerhofer et al., 2011). As discussed in the sub-section on the natural facility of interspecific crossing, natural hybrids of these species have also been identified. The success rate of these interspecific hybridisations occurring under artificial conditions was 30% with *C. palaestinus* and 56% with *C. oxyacanthus*. In comparison, *C. tinctorius x C. tinctorius* control crosses occurred at a rate of 40% (Mayerhofer et al., 2011).

- Crosses between *C. tinctorius* and *C. flavescens* (= *C. persicus*) produced fertile F_1 and F_2 progeny (Imrie and Knowles, 1970), while a review by Knowles and Ashri (1995) indicates that *C. flavescens* (= *C. persicus*), *C. oxyacanthus* and *C. palaestinus* can easily be artificially crossed with *C. tinctorius*. The possibility of artificial hybrids occurring between *C. tinctorius* and *C. gypsicolus* or *C. curdicus* was not determined (Knowles and Ashri, 1995).

Section *Odonthagnathis* (n = 10, 11)

- Safflower has also been crossed with four species outside the section *Carthamus*, to produce viable hybrids. *C. tinctorius* has been artificially crossed with *C. divaricatus* (n = 11) and produced self-sterile F_1 hybrids which show some female fertility in backcrosses with *C. tinctorius*, although at low rates (Knowles and Ashri, 1995). However, backcrossing these hybrids with *C. tinctorius* results in offspring with low fertility (Estilai and Knowles, 1976).
- Artificial crosses between *C. tinctorius* and other members of the species with = 10, are reported to be difficult to achieve and the F_1 hybrids are highly sterile (Knowles and Ashri, 1995; McPherson et al., 2004). Ashri and Knowles (1960) crossed *C. tinctorius* with *C. tenuis* and *C. glaucus*, obtaining sterile hybrids in both cases. Crosses of *C. tinctorius* with *C. leucocaulos* or *C. glaucus* were performed (Mayerhofer et al., 2011). The cross with *C. leucocaulos* resulted in sterile offspring (seed was produced but would not germinate). Although the cross with *C. glaucus* produced fertile F_1 plants, the authors noted that there was some uncertainty about the identity of the *C. glaucus* seeds used. Different regional variants of *C. glaucus* behave differently in interspecific crosses, therefore some subspecies or varieties may produce viable hybrids with *C. tinctorius* (McPherson et al., 2004). Hybrid vigour or increased fitness or weediness was not observed in the F_1 hybrids (Mayerhofer et al., 2011).
- Artificial crosses were performed to investigate the potential for outcrossing between genetically engineered safflower, containing resistance to glyphosate (*pat* gene), and wild relatives. All experimental crosses produced F_1 hybrids that retained the intact transgene, except for one species and demonstrated that hybrid fitness was equal to or greater than the respective parents involved (Ellstrand, Prentice and Hancock, 1999; Mayerhofer et al., 2011). The transgene was completely deleted in approximately 21% of the F_1 progeny resulting from crosses between transgenic *C. tinctorius* and *C. glaucus*, which suggests that some *Cathamus* species possess a negative selection mechanism against foreign DNA (Mayerhofer et al., 2011). The transfer of any gene in nature is typically controlled by selective advantage, a trait that promotes a better chance of both selection and survival (Haygood, Ives and Andow 2003; Chapman and Burke, 2006).
- The potential for artificial or natural crossing between *C. tinctorius* and *C. dentatus* or *C. boissieri* (both n = 10) has not been determined. However, cytogenetic analysis of the interspecific hybrids within this section showed a high frequency of chromosome pairing at meiosis, indicating the close relationship among them (see review by Kumar, 1991). In contrast, analysis of crosses between *C. leucocaulos* or *C. tenuis* (both n = 10) with *C. tinctorius* (n = 12) showed very low chromosome pairing at meiosis, poor pollen stainability and a failure of the hybrids to produce seeds. A review of the potential for safflower to hybridise with other *Carthamus* species indicated that crosses between species with n = 10 and *C. tinctorius* produced sterile hybrids (McPherson et al., 2004). Similarly, Knowles (1980) indicated that most n = 10 species will cross *C. tinctorius* but the hybrids are highly sterile. Thus, it is highly likely that crosses between *C. tinctorius* and *C. dentatus* or *C. boissieri* will also have very low levels of chromosome pairing at meiosis and generate sterile offspring.

Section *Atractylis* (n = 22, 32)

- Successful crosses between *C. tinctorius* and *C. lanatus* (n = 22) have been achieved, especially with *C. tinctorius* as the female parent, but all resulting F_1 plants are sterile (Ashri and Knowles, 1960; Heaton and Klisiewicz, 1981; Mayerhofer et al., 2011). Fertile hybrid plants could only be

achieved by treating rescued embryos with colchicine (Heaton and Klisiewicz, 1981). The F_1 hybrids did not exhibit any hybrid vigour or increased fitness or weediness (Mayerhofer et al., 2011).

- Experimental crosses between *C. tinctorius* and the two members of the section *Atractylis*, *C. creticus* and *C. turkestanicus* (both n = 32), produced viable fertile offspring (McPherson et al., 2004; Bérvillé et al., 2005) but with low success rates of < 2% and 0.3% respectively (Mayerhofer et al., 2011).

Species of uncertain placement (n = 12)

- *C. nitidus* (n = 12) has been artificially crossed with *C. tinctorius* with the F_1 hybrid being sterile (Knowles and Ashri, 1995). Attempts to cross *C. nitidus* with other *Carthamus* species produced viable but sterile hybrids (Knowles and Schank, 1964; Knowles, 1989). There is no information on the potential for crossing between *C. tinctorius* and *F. balearica.*

Information and data on introgression

Knowledge, access and exploitation of available genetic diversity in domesticated and wild relatives are essential for expanding the genetic base of safflower cultivars to achieve increases in both crop stability and performance (Sujatha, 2008). Interspecific hybridisation experiments for safflower have typically been targeted towards the assessment of cross-compatibility relationships and the characterisation of F_1 hybrids (see sub-section on experimental crosses). Wild *Carthamus* species potentially possess a wealth of genetic diversity with respect to traits of environmental adaptation, biotic and abiotic stress resistance, and oil content and quality. The largest barrier to the introgression of desirable traits from wild safflowers into cultivated safflower is the difference in basic chromosome number (2*n*; see Table 3.6), hence sexual incompatibility.

The Australian Wildlife Conservancy (AWC) collection was developed through simultaneous open pollination of the thin-hulled safflower line, A4138, with 12 different *Carthamus* species including *C. alexandrines*, *C. arborescens*, *C. creticus*, *C. caeruleus*, *C. dentatus*, *C. flavescens*, *C. glaucus*, *C. oxyacanthus*, *C. syriacus*, *C. palaestinus*, *C. tenuis* and *C. lanatus* (Rubis, 1981). Following flood treatment of the resulting progeny, the thin hull phenotype facilitated the recurrent selection of lines that demonstrated resistance to *P. dreschsleri* root rot. For example, the line PI 537690 exhibited 95% survival, whereas commercial variety checks were 100% killed by disease. The exact pedigree of the surviving plants is unknown, although the plant and seed characteristics indicate that the introgressive germplasm most likely came from either *C. flavescens* or *C. oxyacanthus* (Rubis, 1981).

A disease-resistant allopolyploid was developed from a cross between *C. tinctorius* and *C. lanatus* (Heaton and Klisiewicz, 1981). The resulting allopolyploid contained 34 chromosomes. It is proposed that 22 came from *C. lanatus* and 12 from *C. tinctorius*, with the doubled haploid being 2*n* = 64 chromosomes. The progeny exhibited morphology similar to *C. lanatus*, and demonstrated resistance to a variety of important safflower pathogens, including *Alternaria carthami*, *Fusarium* spp., *Verticillium dahliae*, and bacterial blight. The resulting allopolyploid is self-fertile but is unable to backcross to *C. tinctorius* due to the sterility associated with the majority of chromosomes being non-homologous (Heaton and Klisiewicz, 1981).

General interactions with other organisms (ecology)

Interactions in natural and agronomic ecosystems

Pollination studies showed that honey bees (*Apis mellifera* and *Apis* spp.) are the major pollinators of safflower crops (Kumari and Pandey, 2005; Pandey and Kumari, 2008). Studies in the United States observed that 80-90% of insects visiting safflower plants were honey bees. Safflower ranks highly among

the commercial crops which are preferred by honey bees. Honey bees have been found bypassing cotton and corn fields, flying distances more than 8 km, to collect pollen from safflower plants, while nectar collectors remain foraging in nearby cotton (Gary et al., 1977; Van Deynze, Sundstrom and Bradford, 2005). Honey bees that were located in an alfalfa seed field-collected alfalfa pollen until the nearby safflower flowered, after which the honey bees preferentially collected safflower pollen (Torchio, 1966; Wichelns, Weaver and Brooks 1992).

The dense and aggressive root structure of safflower penetrates deeper into the soil than many other crops, having the ability to utilise surplus water from deep in the soil profile. Consequently, safflower can be used to dry saturated soil profiles, for example following irrigated crops such as cotton (GRDC, 2010). Drying the soil profile has additional benefits of disease control in the following crop, for example, root rot caused by *Rhizoctonia solani* (Cook, Schillinger and Christensen 2002; GRDC, 2010). However, safflower does have a high water consumption value, which may result in decreased water availability from the water table for subsequent crops (Pfister et al., 2011). The channels created by safflower roots are able to improve the movement of air and water through the effects of cracking and aeration, which facilitates improved root development of succeeding crops (Gilbert, 2008; GRDC, 2010).

Safflower often requires less pest management than other crops. Growers have found large numbers of beneficial insects such as ladybirds (*Coccinellidae* spp.), spiders and green lacewing (*Chrysoperla carnea*) in safflower fields. These beneficial insects feed on the pest insects (described in sub-section on pests and also listed in Annex Table 3.A.1) and thus reduce the need for spraying insecticides (Hanumantharaya et al., 2008; GRDC, 2010).

Pests and diseases

Safflower is usually grown as a rainfed crop which means the incidence of disease is relatively low. However, safflower has developed from wild species growing in arid desert environments and is particularly susceptible to a large number of insects (especially in regions where it evolved) (Li and Mündel, 1996), to foliar diseases (favoured by moist environments) and root rot organisms (favoured by irrigation), summarised in Annex 3.A. If grown under irrigation, humid conditions and waterlogging favour the development of disease (GRDC, 2010).

Pests

Insects

The most serious crop damage by insects usually occurs as a result of infestations either at the time of germination or flowering, where young seedlings or developing capitula are the targets of attack (Esfahani et al., 2012; Vaani, Udikeri and Karabhantanal, 2016b).

Aphids are a major pest in many countries, having a severe rating of incidence in India, the Middle East, Asia, Russia, Africa, Spain, Australia and the United States (Li and Mündel, 1996; Esfahani et al., 2012) and infestations have caused yield losses of up to 84%, through a combination of affecting both total seed yield and seed oil content (Nimbkar, 2008; Vaani, Udikeri and Karabhantanal, 2016b). In Australia, the main insect pests of safflower are aphids (plum, green peach, leaf curl), cutworms (*Agrotis* spp.), native budworm or heliothis (*Helicoverpa* spp.), Rutherglen bugs (*Nysius vinitor*), red-legged earth mites (*Halotydeaes destructor*) and blue oat mite (*Penthaleus major*), all of which can be readily controlled with insecticides and some with biological control (GRDC, 2010; Vaani, Udikeri and Karabhantanal, 2016a).

In Iran, the serious insect pests that are associated with safflower include the safflower capsule fly (*Acanthiophilus helianthi*), aphids (*Uroleucon carthami*), capsule borer (*Helicoverpa peltigera*), spider mites (*Tetranychus urtica*) and caterpillars (*Perigaea capensis*) (Esfahani et al., 2012). Similarly, the most prevalent pests associated with safflower grown in India include aphids, the capsule borer and caterpillars

(Hanumantharaya et al., 2008). The safflower capsule fly, aphids and capsule borer are the most important pests as they can cause extensive damage to the plants and significant loss of crop yields (Saeidi et al., 2011a). Heavy infestations of the safflower capsule fly is typically associated with the reproductive phase as eggs are laid inside the developing heads, on the inner side of the bracts (Saeidi, Mirfakhraei and Mehrkhou 2012), throughout flowering. The hatched larvae then feed on the capitula bracts or seeds, which has severe impacts on both seed quality and yield, and also seed marketability (Ricci and Ciriciofolo, 1983). The safflower fly is also one of the main limiting factors on production of the crop in several countries, including countries within Africa, Asia and Europe (Saeidi et al., 2011a; Saeidi, Mirfakhraei and Mehrkhou 2012). Resistance to safflower fly has been found in wild accessions of *C. oxyacanthus* and may be used in breeding programmes to develop fly-resistant safflower cultivars (Sabzailian et al., 2010).

Other animals

The majority of crop yield loss occurs as a result of either insects (sub-section on pests) or disease (sub-section on diseases), with damage often being devastating. During a cropping season, safflower seeds can provide a food source for a range of mammals, birds and invertebrates and damage to crops can occur while they are searching for food. For some larger animals such as cattle, grazing is minimal due to the spiny nature of mature safflower plants being a deterrent (Cummings et al., 2008) but sheep and goats are not irritated by the spines. Feral pigs or boars can be destructive and have proven difficult to exclude from fields (Rao et al., 2015).

Damage to safflower crops by animals is most likely to be caused by birds, whether by feeding on the developing capitula or by chewing plants off at the base to access either developing or mature seed (GRDC, 2010; Hall, 2016). Small birds, such as sparrows, can feed on maturing safflower seed, whereas larger birds, such as cockatoos, can chew safflower plants at the base in order to access seeds (GRDC, 2010). Several other bird species have been identified by researchers as potential safflower pests including blackbirds, mallard ducks, pigeons, pheasants, ravens and crows (Cummings et al., 2008; Vazačová and Münzbergová, 2013).

Diseases

When under irrigation, diseases are much more prevalent than if purely rainfed (Nimbkar, 2008; Mirshekari et al., 2013). Safflower is susceptible to many fungal, bacterial and viral diseases and some of these can cause considerable damage (Singh and Nimbkar, 2006), with fungal disease being the most prevalent. Outbreaks of disease can devastate safflower crops.

Leaf blight, caused by the fungus *A. carthami*, is a major disease for safflower grown in India and Australia, having the potential to cause significant seed yield losses in the range of 10-50% (Irwin, 1976; Jackson, Irwin and Berthelsen 1982; Sehgal and Raina, 2011; Taware, Gholve and Dey, 2014). The disease is identifiable from the small brown to dark spots with concentric rings that form on the lower leaves of the safflower plants. These spots can coalesce and form irregular lesions. Seeds can also be infected with this fungus, identified by dark sunken lesions on the testa. If infected seeds germinate, the same spots and concentric rings will become visible on the cotyledons (Taware, Gholve and Dey 2014). The disease is favoured by temperatures in the range of 25-30°C and relative humidity of 80% (Murumkar et al., 2008).

Wilt, a seed-borne disease caused by the fungi *Fusarium proliferatum* and *F. oxysporum*, has been identified as a serious disease for safflower crops grown in India, affecting 40% to 80% of the annual crops (Singh and Kapoor, 2018). This disease has also been documented in Egypt, Australia and the USnited States (Zayed et al., 1980; GRDC, 2010) and more recently in crops grown in Korea (Kim et al., 2016). Safflower crops have been reported as having disease incidence up to 80%, resulting in significant seed yield losses. The severity of the disease significantly affects the extent of seed yield loss, which can vary from 7.2% to 100% (Govindappa, Rai and Lokesh 2011). The disease is visually identified early by the yellowing of leaves and brown discolouration of stems and roots, followed by wilting and dropping of the

leaves (Govindappa, Rai and Lokesh 2011). White fungal masses can also be found in the base of the stem. As the disease progresses the infected plants may wither and die. Severe infection is typically associated with delayed flowering and in many cases, the ovaries will fail to develop seeds (Govindappa, Rai and Lokesh 2011; Kim et al., 2016). Disease resistance has been proposed as the most efficient strategy of controlling the disease (Sastry and Chattopadhyay, 2003).

Sclerotinia sclerotiorum causes head rot in safflower, which can lead to significant losses in both total seed yield and oil content (Mündel, Huang and Kozub 1985). Sclerotinia head rot is an important agronomic disease in Canada, India and the United States (Morrall and Dueck, 1982). The disease is typically isolated to the developing capitula, with diseased capitula easily identified by the discolouration of the bracts. Crop rotation is recommended to assist in the control of sclerotinia head rot, although this practice has limited success due to both the persistence in soil and the broad range of hosts including sunflower, rapeseed and soybean (Hoes and Huang, 1976; Huang and Hoes, 1980). The severity of disease was positively correlated with seed yield losses which varied significantly between different cultivars, indicative of potential resistance to sclerotinia head rot. Healthy plants, compared to their diseased controls, also had an average increased seed oil content of 4.4% (Mündel, Huang and Kozub 1985).

Charcoal rot, caused by *Macrophomina phaseolina*, has recently emerged as an important disease affecting safflower (Esfahani, Yazdi and Ostovar 2018), particularly in Iran. This disease has also been identified as a potential problem for safflower crops grown in Australia (GRDC, 2010). This causal fungus is soilborne and has also been attributed to seedling blight and root rot. Symptoms of the disease infection remain latent until the safflower plants approach the stages of flowering or maturity, although the initial infection occurs during the seedling stage (Esfahani, Yazdi and Ostovar 2018). The first symptom is wilting in high temperatures, irrespective of sufficient water. The vascular bundles become covered with fungal microsclerotia, resulting in restrictions of water and nutrient flow to higher parts of the plant. Due to the restricted flow of nutrients, the stress of high temperatures and drought often leads to premature plant death. This fungus can cause the death of approximately 25% of the crop, hence having significant impacts on seed yields (Govindappa, Lokesh and Ravishankar Rai 2005). In the absence of disease-resistant cultivars, the proposed disease management strategies include crop rotation, lowering plant densities and scheduling of both planting and irrigation dates.

Another important disease that affects safflower is root rot, which is caused by a variety of organisms including *Phytophthera cryptogea*, *P. drechsleri*, *Fusarium solani* and *Pythium ultimum* (Nasehi et al., 2013; Esfahani, Yazdi and Ostovar 2018). Although *P. cryptogea* has been reported to be the major cause of root rot (Heritage and Harrigan, 1984), *P. ultimum* has been attributed as the prominent causal agent of seed rot and seedling damping-off (Pahlavani et al., 2009). Reports of the disease have been made in Australia, the United States, Iran, Canada and Argentina (Klisiewicz, 1968; Kochman and Evans, 1969). The yield losses can be high, particularly in conditions where soils with poor drainage coincide with excess water through either irrigation or heavy rainfall. A higher incidence of infections is found when soil temperatures are in the range of 25-30°C (Erwin, 1950; Heritage and Harrigan, 1984; GRDC, 2017). Affected plants are identified by symptoms of vascular wilting, followed by desiccation and collapse of the infected tissues (Thomas, 1970; Esfahani, Yazdi and Ostovar 2018). Early symptoms of stem and root discolouration can appear 4-5 days following rain or irrigation (GRDC, 2017). The best approach to controlling the incidence of root rot and seed rot has been screening for and breeding resistant varieties (Harrigan, 1987; Mailer et al., 2008).

Rust is another fungal disease of safflower caused by *Puccinia carthami*, which has been identified as an important disease in Australia, Italy and Oman (Cappelli and Zazzerini, 1988; Deadman et al., 2005; GRDC, 2017). The disease can lead to significant yield losses, especially when the seeds or soil are contaminated with fungal spores, resulting in the death of seedlings. Significant yield losses can also occur as a result of foliar infections later in the season, leading to the loss of plant biomass (Cappelli and Zazzerini, 1988). Similar to the other fungal diseases affecting safflower, *P. carthami* favours warm and

humid conditions (GRDC, 2017). Rust affected plants are identified by the presence of pustules on the leaves, which can be white, yellow or chestnut brown in appearance (Deadman et al., 2005; GRDC, 2017).

Weeds

Weeds that compete with safflower include grass and broadleaf weeds. Later in the season, many weeds can outgrow safflower in height and the resulting shading can reduce crop yields significantly (Li and Mündel, 1996). Control of weeds in safflower is essential for optimum yields.

Safflower can be sown later than other winter crops which gives farmers more time to control weeds prior to sowing. Harrowing when the safflower plants are 7-15 cm tall can give satisfactory control of small, later germinating weeds but damage to the young plants can occur if the soil is ridged or if the plants were sown too deep (Oelke et al., 1992). Safflower is more tolerant of some pre-emergent herbicides than wheat and knock-down herbicides may be used, as well as cultivation which assists in minimising resistance to selective herbicides (GRDC, 2017). Some herbicides can be used before planting the safflower crop to reduce the weed seed bank on the surface of the soil. Several pre-emergent herbicides control broadleaf and grass weeds. Post-emergent herbicides are used for the control of grass weeds, while others are used for the control of broadleaf weeds (Croissant, Johnson and Shanahan 1986; Oelke et al., 1992; GRDC, 2010). However, in Australia, in-crop herbicide options are limited for safflower, especially with respect to controlling broadleaf weeds (GRDC, 2017). Additionally, care must be taken to ensure sufficient time between the use of herbicides and subsequent planting with safflower crops (GRDC, 2017).

Additional information

Weediness of safflower crops

As with all crops cultivated and harvested at the field scale, some seed may be lost during harvest and remain in the soil until the following season when it germinates either before or following seeding of the succeeding crop. In some instances, the volunteers may provide competition to the seeded crop and warrant chemical and/or mechanical control. Volunteers can also be expected away from the planting site, for example, along roadsides and around storage facilities, as a result of spillage during transport.

Safflower lacks characteristics that are common to weeds, such as very high seed output, high seed dispersal, long-distance seed dispersal, seed shattering, persistent seed banks and rapid growth to flowering. During the rosette stage and early stages of growth, safflower is slow-growing and a poor competitor with fast-growing weeds (Li and Mündel, 1996; GRDC, 2010). Safflower is considered a minor weed of agricultural and natural ecosystems; primarily, it is an agricultural or ruderal weed found in disturbed land use areas such as debris, roadside or disused fields (Groves et al., 2003).

Safflower seed may be inadvertently dispersed into neighbouring fields or non-agricultural areas by water, wind and animals (see sub-section on seed production and natural dispersal of seeds). It is also deliberately and inadvertently spread by humans during transport and on farming equipment.

In a Canadian study, safflower volunteers had reduced plant height, seed heads per plant, seeds per head and per plant, viable seeds per plant, as well as lower seed weight, plant biomass and harvest index, in comparison to safflower crop plants. In addition, the volunteer seed viability was 50% compared to 95% for seed from crops (McPherson et al., 2009b). They were poor competitors with subsequent wheat and barley crops. These studies, conducted over several years in Canada (see sub-section on seed viability, longevity and dormancy) suggest that safflower seed and volunteers would not persist beyond two years and that common herbicide and tillage practices would control any volunteer safflower (McPherson et al., 2009b). Moreover, experienced growers in the areas surveyed were not concerned with the control of safflower in volunteers (McPherson et al., 2009b).

Lack of seed dormancy in safflower (see sub-section on seed viability, longevity and dormancy) reduces the weediness potential and volunteers after harvest are uncommon (USDA-APHIS, 2008). However, some feral populations of safflower have become established in agro-ecosystems in several states of the United States, including California, Illinois, Iowa, Kansas, New Mexico, Ohio and Utah (Bérvillé et al., 2005, and references cited therein). There is little information on how long these populations persist but anecdotal reports suggest safflower does not become established outside of agricultural areas (Bérvillé et al., 2005).

Toxicity and allergenicity

Safflower has a long history of cultivation for seed, oil and meal production primarily, although flowers and pollen are also used. Safflower products are used for food and feed, as food additives, dyes and for medicinal and industrial uses. These uses are discussed by a number of authors (see, for example, Oelke et al., 1992; Li and Mündel, 1996; Mündel et al., 2004; AOSCA, 2012). Although safflower components may contain some toxins and allergens, it is generally considered non-toxic to animals and humans.

Safflower oil is non-allergenic and suitable for use in injectable medications and cosmetics (Smith, 1996). To date, only a single case of Immunoglobulin E (IgE)-mediated response to dried safflowers (occupational asthma) has been reported (Compes et al., 2006).

Annex 3.A. Common pests and pathogens

The tables below summarise the common insect pests (Annex Table 3.A.1) and diseases (Annex Table 3.A.2) that have been associated with significant agronomic importance to the cultivation of safflower. For more information, refer to sub-section on pests and diseases.

Annex Table 3.A.1. Summary of common insect pests that affect *Carthamus tinctorius* (safflower)

Common name	Scientific name(s)	Stage affecting crop	Plant part(s) affected
Agronomically important insects			
Aphids (plum, green peach, leaf curl)	*Aphis fafia* *Brachycaudus helichrysi* *Capitophorus eleagni* *Dactynotus carthami* *Dactynotus orientalis* sp. *Dactyonotus jaceae* *Macrosiphum* sp. *Myzus persicae* *Pleotrichophorus glandolosus* *Uroleucon carthami* *Uroleucon compositae*	Nymphs and adults	Whole plant
Safflower capsule fly	*Acanthiophilus helianthi* *Chaetorellia carthami* *Terellia luteola*	Larvae	Capitula
Capsule borer or Silver moth	*Helicoverpa peltigera*	Larvae	Capitula and leaves
Thrips	*Aeolothrips collaris* *Haplothrips* sp. *Thrips tabaci*	Adults	Capitula and leaves
Grasshopper or leafhopper	*Circulifer haematoceps* *Empoasca decipiens* *Euscelis alsius* *Macrosteles laevis* *Neoaliturus fenestratus* *Psammotettix striatus*	Nymphs and adults	Whole plant
Lygus bug or seed bug	*Lygus hesperus* *Lygus* sp. *Oxycarenus hyalipennis* *Oxycarenus pallens*	Adults	Capitula
Other insects			
Mites: - Red-legged earth mites - Blue oat mite - Spider mites	 *Halotydeaes destructor* *Penthaleus major* *Tetranychus urtica*	Adults	Seedlings and leaves
Native budworm or heliothis	*Helicoverpa* spp.	Larvae	Flower buds, capitula and leaves
Cutworms and caterpillars	*Agrotis* spp. *Perigaea capensis*	Larvae	Leaves and stems
Rutherglen bug	*Nysius vinitor*	Adults	Flower buds, upper stems and capitula

Sources: GRDC (2010), *Raising the Bar with Better Safflower Agronomy*, ACT, Australia, Grains Research and Development Corporation; Saeidi, K. et al. (2011b), "Pests of safflower (*Carthamus tinctorious* L.) and their natural enemies in Gachsara, Iran", *South Asian Journal of Experimental Biology*, Vol. 1, pp. 286-291; Esfahani, M.N. et al. (2012), "The main insect pests of safflower on various plant parts in Iran", *Journal of Agricultural Science and Technology*, Vol. A2, pp. 1281-1288.

Annex Table 3.A.2. Summary of important diseases that affect *Carthamus tinctorius* (safflower)

Disease	Causal organism	Plant part(s) affected
Leaf blight	*Alternaria carthami*	Leaves, stems, capitula and seeds
Wilt	*Fusarium oxysporum* *Fusarium proliferatum* *Verticillium dahlia*	Roots, stems and leaves
Charcoal rot	*Macrophomina phaseolina*	Stem
Rust	*Puccinia carthami*	Leaves
Head rot	*Sclerotinia sclerotiorum*	Developing capitula and bracts
Root rot	*Fusarium solani* *Pythium ultimum* *Phytophthera cryptogea* *Phytophthera drechsleri* *Rhizoctonia solani*	Roots and stems

Sources: Irwin, J.A.G. (1976), "*Alternaria carthami*, a seed-borne pathogen of safflower", *Australian Journal of Experimental Agriculture*, Vol. 16, pp. 921-925; Mündel et al. (1985); Sastry, R.K. and C. Chattopadhyay (2003), "Development of Fusarium wilt-resistant genotypes in safflower (*Carthamus tinctorius*)", *European Journal of Plant Pathology*, Vol. 109, pp. 147-151; GRDC (2010), *Raising the Bar with Better Safflower Agronomy*, ACT, Australia, Grains Research and Development Corporation; Esfahani, M.N., J. Yazdi and T. Ostovar (2018), "The major diseases associated with safflower and some of the resistant sources", *Horticulture International Journal*, Vol. 2, pp. 185-192.

Annex 3.B. Biotechnological developments

The table below lists the genetically engineered safflowers which have been approved, including the type of use(s) for which they are approved, the country in which they are approved and the year in which they were approved.

Annex Table 3.B.1. Approvals of genetically engineered safflowers

OECD unique identifier	Trait(s)	Approving country	Type of approval	Date
GOR-73226-6	Increased production of oleic acid	Australia	Cultivation, Food, Feed, Processing[1]	2018, 2019
GOR-7324Ø-2	Increased production of oleic acid	Australia	Cultivation, Food, Feed, Processing[1]	2018, 2019
IND-1ØØØ3-4	Production of bovine pro-chymosin enzyme; glufosinate tolerance	Argentina	Commercial production[2]	2017
IND-1ØØ15-7	Production of bovine pro-chymosin enzyme; glufosinate tolerance	Argentina	Commercial production[2]	2017
IND-1ØØØ3-4 x IND-1ØØ15-7	Production of bovine pro-chymosin enzyme; glufosinate tolerance	Argentina	Commercial production[2]	2017

Sources:
1. OECD, *BioTrack Product Database*, https://biotrackproductdatabase.oecd.org/ (accessed 13 May 2020); CBD (n.d.), *Biosafety Clearing House Central Portal*, http://bch.cbd.int/ (accessed 13 May 2020); ISAAA, *GM Approval Database*, http://www.isaaa.org/gmapprovaldatabase/default.asp (accessed 13 May 2020); FSANZ (n.d.), *Current GM Applications and Approvals*, https://www.foodstandards.gov.au/consumer/gmfood/applications/Pages/default.aspx.
2. MAGyP (n.d.), *Resolution RESOL-2017-103-APN-SECAV#MA Approving GM Safflower Varieties for Commercial Production*, https://www.magyp.gob.ar/sitio/areas/biotecnologia/ogm/_archivos/RS-2017-31775583.pdf.

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Note

[1] A protein complex that forms during meiosis between homologous chromosomes, which may modulate chromosome pairing, synapsis, and recombination.