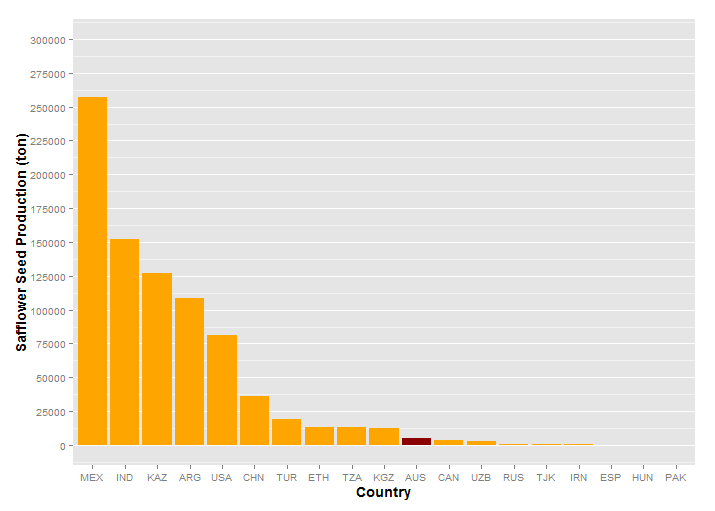
1. **Safflower; its history and cultivation, vernalisation response, and genetics**
   1. *History and cultivation*

Safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae family of flowering plants and is native to the eastern and southern Mediterranean, the Middle East and India (Refs). Safflower has been cultivated in these regions for thousands of years, with safflower arrangements and safflower-based dyes found in Pharaoh Tutankhamen’s tomb as well as at sites of archaeological importance that date back to the ancient Mesopotamia (Zohary & Hopf, 1993). It was originally cultivated for its edible seeds, and for the dyes that can be created from processing the vibrant yellow and orange flowers of a safflower plant. Safflower seed oil is chemically similar to sunflower seed oil (*Helianthus annuus* L; a closely related asteraceae family member); both have similar melting points and fatty acid composition (Chempro Technoatvation Pvt. Ltd. n.d.). However, safflower seed oil tends to be less susceptible to oxidation, lending itself for use as a base for varnishes, paints and industrial lubricants (Işigigür et al., 1995; Gecgel et al., 2007). In addition, after oil extraction, the remaining safflower meal can be used as an animal feedstock (Ref). In 2012, just over 833,793 tonnes (t) of safflower seed was harvested globally (**Fig. 1**), with approximately 65% (536,651 t) of the global total produced in Mexico (30.9%, 257 451 t), 18.2% from India (152,000 t) and 15.2% from Kazakhstan (127,200 t) (United Nations 2014). Australian produced safflower seed accounted for just over 0.5% (4,800 t) of the 2012 global production, as it is more commonly grown as a ‘break crop’, to break up hard clay soils or to remove excess water from soils before the cultivation of more traditional crops, such as wheat and barley (Knights, 2010).



**Figure 1.** 2012 global safflower production as ordered by quantity produced per nation. Australia (AUS; indicated by dark red coloured column) was ranked as the 11th highest producer of safflower seed in 2012, producing an estimated 4,800 t.

* 1. *Vernalisation response*

Of particular current interest in safflower is the coordination of flowering time, a trait that if modifiable, is likely to have enormous impact on safflower’s adaptability to climate change, an increased range of cultivatable land, and total yield. Research in the late 1970s characterised ‘winter’ and ‘spring’ safflower ecotypes *(or cultivars? Pick most appropriate and stick to it throughout)* and specifically described a low survival rate for spring ecotypes when planted in winter, and a converse poor performance of winter ecotypes when planted in spring (Yazdi-Samadi & Zali, 1979). Research in *Arabidopsis thaliana* (*Arabidopsis*), and in cereals (*state studied species*), indicates that molecular modifications to a plant’s vernalisation response, is the primary classifier of a winter or spring ecotype within these species (*Refs*). Therefore, it is hypothesised, and this hypothesis is strongly supported by previous research (Johnson et al., 2006; *Refs*), that “winter hardy” varieties of safflower will possess and express molecular phenotypes similar to those previously reported in other species. However, it is also important to note, that while some safflower ecotypes rapidly progress to flowering following vernalisation, it is not a necessary environmental cue for flowering in safflower, instead; vernalisation is seen as a *facultative* response in safflower (Salisbury & Ross, 1992). While it has been documented in other agronomically important crop species, such as wheat, that vernalisation closely relates to total crop yield, the genetic mechanism(s) of vernalisation in safflower, and the effect of an extended cold treatment on safflower ecotypes of interest (specifically in relationship to safflower ecotypes that display desirable phenotypic characteristics under Australian growth conditions), remains to be characterised.

* 1. *Genetics*

Safflower is a dicotyledonous plant with a diploid genome consisting of eleven paired chromosomes (Knowles, 2012). The haploid (the estimated size of safflower’s 11 unpaired chromosomes) genome size of four safflower ecotypes, namely Ljubljana, Uzbekistan, S-2190 and Huesca has been approximately calculated to be 1.34 Gb (1 Gb = one Giga base = 1,000,000,000 base pairs), 1.38 Gb, 1.39 Gb and 1.40 Gb, respectively (Garnatje *et al*., 2006). However, to date, despite there being a number of publically accessible online transcriptomic and expressed sequence tag (EST)-based datasets (Li *et al*., 2011; Li *et al*., 2012; Lulin *et al*., 2012; NCBI - National Center for Biotechnology Information), currently no ‘draft’ genome sequence for safflower, or any other Asteraceae species, exists. In addition, little is currently known about the molecular evolution of Asteraceae family members despite this family being one of the largest and most successful amongst flowering plants, especially within the context of vernalisation.

* *This section just ends here……..needs something else to finish it off…….. an additional paragraph or two!!!!*
* *Maybe even expand this section to talk more about the lack of genetic and molecular data for safflower which is becoming an increasingly important oil crop???*

1. **Vernalisation; its history and biology, and, in *Arabidopsis* and cereals**

Vernalisation, and its effect on harvest time and total crop yield, has been a central research focus of the plant biology community for over 150 years. Vernalisation is characterised by a prolonged – greater than two weeks – exposure to low, yet non-freezing temperatures (*refs*). In addition, in *which plants????*, it has been demonstrated that the resulting time to flowering is directly proportional to the period of vernalisation (Sheldon *et al*., 2000), and furthermore, in numerous plant species, planting time has been optimised to increase the period of time a plant remains in the vegetative growth stage. For example, carrots (*Daucus carota*)store carbohydrates in their root organ during their optimised growing season, and then following a ‘wintering’ period will transition into reproductive growth and utilise this stored source of energy for flowering and seed production (Ingram *et al*., 2008). While flowering is often attributed to increased day length, day length is not solely responsible for the invocation of flowering (*refs*). Exposure of a seedling to an extended period of cold is also responsible for triggering an early transition from vegetative to reproductive growth, and this transition is largely been attributed to the initiation of the molecular flowering pathway (*refs)*. Vernalisation is therefore an important determinant of flowering time, and flowering time itself is a central component of overall yield. Furthermore, vernalisation responses also protect delicate organs (such as those created during flowering and required for reproduction) from damage to cold exposure, restricting development until after winter has passed (*refs*).

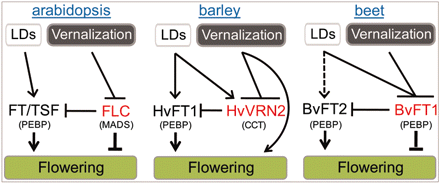
* 1. *The history of vernalisation*

The necessity of vernalisation for some ‘winter’ cereal cultivars to flower was initially demonstrated as early as 1857 (Klippaert, 1857), and this initial demonstration was further investigated in other crop species by Gassner in 1918 (*refs*). The term ‘vernalisation’ was coined by Lysenko in 1928 (Latin: *vernum* meaning *spring*), who conducted a vast volume of agricultural research in the Soviet Union (reviewed in Chouard, 1960). The early studies by Lysenko and others showed that some cereal cultivars only germinated as the weather warmed even though they were planted prior to, or during winter (*refs*). However, Lysenko incorrectly concluded that the progeny of a ‘vernalised’ cereal maintained the attributes of the vernalised parental plant(s), and did not require re-exposure to winter temperatures for germination. In addition, in many early Australian colonies, seed that had been transported from Europe struggled under the warmer conditions and mild winters of the Australian environment and this lead to widespread hunger for the early settlers as the resulting crops failed (*refs*). It was not until almost *XXX* years later through the work of William Farrar that many ‘Australian wheats’ were developed via a traditional cross breeding approach using European wheats as the breeding lines and selecting those progeny best suited for the Australian climate (Macindoe & Brown, 1968).

* 1. *The biology of vernalisation*

Grafting was originally used to demonstrate the transmissible characteristic of vernalised plant tissue. Namely, when a vernalised shoot apical meristem (SAM) was grafted onto a non-vernalised root stock, the grafted plant flowered as if the entire plant had been exposed to the vernalisation treatment. Conversely, when a non-vernalised SAM *(or is it a shoot tip – very different – need to be specific)* was grafted onto a vernalised root stock, the opposite was observed (reviewed in Chouard, 1960). This initial finding has been consistently reported for both facultative (vernalisation decreases the time period to flowering, but is not essential for flowering) and absolute (vernalisation is required for the plant to transition from vegetative to reproductive growth) vernalisation sensitive species. However, contemporary research has shown that the regulatory and genetic mechanisms of vernalisation are species-specific (*see* **Fig. 2**). For example, vernalisation in *Arabidopsis*,and in many other dicotyledonous species, is centrally regulated by the floral repressor FLOWERING LOCUS C(FLC) (refs). In cereals, the central role played by FLC is mediated by VERNALISATION2 (VRN2), and in beets, FLOWERING TIME2 (FT2) regulation by FT1, is the central mediator of the vernalisation response (**Fig. 2**). Although the central mediator of vernalisation differs between plant species, the vernalisation pathway of all vernalisation responsive plant species is mechanistically related, being epigenetic in nature, that is; environmental cues can modify gene expression between ecotype and/or cultivars of the same species, that in turn can result in the expression of a different phenotype, without any alteration to the DNA sequence.

* ***This blue coloured section requires additional work.***



**Figure 2.** The central machinery proteins (indicated be red coloured text) of the vernalisation pathway in *Arabidopsis*, barley (*Hordeum vulgare* L.) and beet (*Beta vulgaris*). Figure taken directly from Pin et al. (2010). *You should modify this Figure as it is against university policy to simply copy-and-paste!!!*

* 1. *The Arabidopsis vernalisation pathway*

In *Arabidopsis*,FLC, a MADS-box transcription factor, mediates the transition of *Arabidopsis*, and of many other dicots, from vegetative to reproductive growth (*see* **Fig. 3**). Research has shown that both genetic and epigenetic mechanisms contribute to repressing the expression of the floral repressor *FLC* during vegetative to reproductive growth transition (Boss et al. 2004; Finnegan et al. 2005). Namely, when *FLC* is expressed at high levels, promoted by FRIGIDA (FRI), FRIGIDA-LIKE1(FRL1) and FRIGIDALIKE 2 (FRL2), FLC represses the *FLOWERING TIME* (*FT*) expression, and the expression of the FT homologues, *TWIN SISTER OF FT* (*TSF*), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*). Subsequently, increased FT, TSF and SOC1 levels suppresses *LEAFY* (*LFY*) and *APETALA1* (*AP1*) expression, two primary promoters of floral apical meristem growth (*see* **Fig. 3**; Amasino 2004; *Refs - a single reference from 2004 for all of the above - really???*).

* You need to modify **Fig. 3** (again this is just a copy-n-paste job = NOT COOL) to only include the proteins that you are referring to in your text!
* Nomenclature for plant genes, mRNAs and proteins
* Genes are capital italics (e.g., *FLC*)
* mRNAs are in capital italics (e.g., *FLC*)
* proteins are in capitals (e.g., FLC)
* I think that you could combine Figs 3 and 4 into a single, two part (3A and 3B) Figure to allow the reader to focus in on the message you are trying to convey in your text!
* The below could then be included in the same paragraph as above – currently this paragraph in disjointed by the inclusion of two Figures, one of which you have shoved right in the middle of a paragraph – very lazy!

Upon *FLC* expression induction, the VERNALISATION2(*VRN2*)/Plant Homeodomain Polycomb Repression Complex2 (PHD-PRC2) complex that consists of *VRN2* and PHD-PRC2 proteins, CURLY LEAF (CLF),SWINGER(SWN), and FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) (Köhler & Villar 2008), is constitutively bound to the *FLC* locus. The binding of the VRN2/PHD-PRC2 complex to *FLC* maintains the locus in an open confirmation, allowing transcription machinery to access *FLC* regulatory sequence to promote *FLC* expression, via histone H3 acetylation (*refs*). During vernalisation, *VERNALISATION INSENSITIVE3* (*VIN3*) levels increase. VIN3, along with VERNALIZATION5/VIN3-LIKE1 (VEL1) and VRN5, bind to the PHD-PCR2 complex to promote histone H3 deacetylation and VRN1 and VRN2-directed methylation of H3K9 and H3K27 (**Fig. 3B**). Histone methylation of the *FLC* locus closes the open confirmation of *FLC*, blocking transcription machinery access to *FLC*, repressing *FLC* expression. This epigenetic repression of *FLC* is irreversible, and ensures that the vernalised plant transitions from vegetative to reproductive growth (Levy *et al*. 2002; Sung & Amasino, 2004). Reduced FLC, increases *SOC1* and *FT* expression, and increased SOC1 and FT levels in turn enhances the expression of the floral promoters, *LFY* and *AP1* (**Fig. 3A**) *– fix your referencing up here too.*

The External Coincidence Model, also referred to as the Photoperiod Pathway, mediates the transition to flowering by exposure to increasing day length, and without the requirement of a period of vernalisation (Hayama & Coupland, 2004). Increased periods of day light promotes the expression of *CONSTANS* (*CO*), and CO in turn overrides the repressive effects of FLC, via CO-mediated activation of *FT* and *SOC1* expression (Golembeski *et al*., 2014). Even without exposure to cold or increasing day length, the Autonomous Pathway can trigger the floral transition of *A. thaliana* by down regulating *FLC* expression. *FCA* with *FY*, *FLA* and *FPK* are all independently involved with *FLC* RNA processing, *FLD* and *FVE* deacetylate histones at the *FLC* locus. The result is similar gene regulation as seen with vernalisation but initiated and progressing at a much slower rate (Simpson 2004). In *A. thaliana*, summer ecotypes possess allelic variations, but not necessarily in specifically vernalisation genes. The *A. thaliana* ecotype Landsberg *erecta* (Ler-0) contains an allele of *FLC* which is unresponsive to up regulation by *FRI*, meaning there is no repression of *FT*, leading to early flowering of L*er*-0. Therefore, the necessity to decrease *FLC* expression by vernalisation and increased expression of *VRN1* and *VRN2* (or the necessity for long day conditions to increase the expression of *LD*) to down regulate *FLC* is no longer required (Michaels & Amasino 1999).There are many other *A. thaliana* ecotypes that can be categorised as “summer” and “winter” based on their response to vernalisation (Nordborg & Bergelson 1999).

* This blue coloured section requires a lot of work – it is currently very poorly written, making it quite confusing!