CHAPTER 5

Chickpea and temperature stress: An overview

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5.1 Introduction

Chickpea is an important food grain legume and an essential component of crop rotations throughout the world. However, the adaptation and productivity of chickpea is often limited by low and high temperatures. Cold stress generally occurs in the late vegetative and reproductive stages across the geographical areas of chickpea production. Cold and freezing temperatures (−1.5°C to 15°C) are considered a major problem during the seedling stage of winter-sown chickpea in Mediterranean areas and autumn-sown crops in temperate regions (Singh, 1993). South Australia and parts of north India are most affected by chilling temperatures at flowering (Berger et al., 2011). On the other hand, high day and night temperatures (>30/16°C) may cause damage during the reproductive stage on winter-sown chickpea in Mediterranean areas and autumn-sown crops in temperate regions (Singh, 1993). South Australia and parts of north India are most affected by chilling temperatures at flowering (Berger et al., 2011). In chickpea, temperature is a major environmental factor regulating the timing of flowering thus influencing grain yield (Summerfield et al., 1990; Berger et al., 2004). Both low and high temperatures can limit the growth and grain yield of chickpea at all phenological stages.

The FAO climate change technical paper and the Intergovernmental Panel on Climate Change (IPCC) have provided evidence of climate change linked to human activity. Global temperature has been increasing at the rate of 0.74°C per 100 years (IPCC, 2007a). Over the past 50 years, the linear warming trend has been nearly twice the rate of the previous 100 years (FAO, 2009). Projections to the end of the 21st century estimate a rise in global mean temperature of between 1.8 and 4°C, depending on greenhouse emissions and changes in rainfall patterns (IPCC, 2007a,b). Such changes in climate will impact crop production and some estimates suggest a grain yield decrease of between 8 and 30% (ICRISAT, 2009).

Changes in seasonal temperature and rainfall patterns and their subsequent impact on yield may change the geographic distribution of chickpea production. In Australia, chickpea could expand in new production areas where the frequency of low temperatures (<15°C) is higher during early crop growth (Maqbool et al., 2010). However, temperatures lower than 10°C at flowering can reduce grain yield by 15–20% (Chaturvedi et al., 2009). In contrast, the frequency of high temperatures (>30°C) during the reproductive stage is often higher in the Australian chickpea production areas of northern New South Wales (NSW) (Devasirvatham et al., 2012a) and any increase in the frequency and duration of these temperatures will limit productivity. A decrease in chickpea yields of 53 kg/ha was observed in north India per 1°C increase in seasonal temperature (Kalra et al., 2008). In south India, the yield loss was estimated to be 10–15% for every 1°C increase beyond the optimum temperature (Upadhyaya et al., 2011). The effect of high and low temperatures on grain quality (grain size and seed coat colour) is also a recognized problem (Wery et al., 1994).

Considerable progress in the improvement of chickpea adaption to stressful environments has been made. Screening the germplasm in the field and controlled
environments for stress response has increased our knowledge of plant responses to stress in chickpea, and this information has been used in crop improvement. Physiological response (e.g., canopy temperature) and male (pollen) and female (ovary) reproductive function under stress have been investigated to determine their suitability as stress screening techniques (Clarke & Siddique, 2004; Ibrahim, 2011). Sources of tolerance to temperature stress identified using these methods can be used to develop genetic populations to increase our understanding of inheritance. These populations can be used to map quantitative trait loci (QTL) and the resulting linked markers used for marker-assisted selection (MAS). This chapter explores plant responses to high and low temperatures and the implications for stress tolerance breeding in chickpea.

5.2 Impacts on productivity

5.2.1 Temperature stresses during the vegetative period

Cold temperature (<15°C) at emergence reduces crop establishment and results in plants with low vigour. In some sensitive genotypes, cold temperature causes whole plant necrosis and plant death. During chickpea germination, cold temperature also increases susceptibility to soil-borne pathogens thus retarding plant growth and reducing dry matter production (Wery et al., 1994). A plant survival score can be used as an index to describe genotype tolerance to low temperature under field conditions. Chickpea genotypes (Sel 95Th1716 and Sel 96Th11439) were identified as cold tolerant based on plant survival scores in northwestern Iran (Heidarvand et al., 2011). Genotypic differences in chickpea establishment were identified in Australia, and the cold-tolerant cultivars CPI 562896, Semsen and Sombrero showed improved establishment under low temperatures (Wery et al., 1994).

Cold temperatures decrease membrane stability, modify proteins and lipids, and cause changes in respiration and photosynthesis (Croser et al., 2003). Abscisic acid (ABA) content was observed to increase in seedlings at temperatures of 1–7°C compared with the control (23°C) (Nayyar et al., 2005a). The sugar and proline contents also increased under cold stress in the same study. These observations suggest that manipulation of ABA could improve cold tolerance in chickpea.

Field screening for cold tolerance during the late vegetative stage where plants were exposed to −7.4°C for 3 weeks was effective in identifying cold-tolerant genotypes (Malhotra & Singh, 1991). These authors scored materials on a scale of 1 to 9 and concluded that the method was effective in identifying tolerant, moderately tolerant and sensitive genotypes. This method was used to screen cultivated and wild chickpea in the Mediterranean region (Toker, 2005).

Cold temperature generally encourages prolonged vegetative growth in chickpea. Temperature is the main determinant for flower initiation in most environments, although some authors have linked flower initiation in chickpea to a photothermal response (Roberts et al., 1985). In northern NSW, Australia, flower initiation can commence at ≤15°C, although the occurrence of flower abortion will likely be high (Jenkins & Brill, 2011).

The minimum temperature for germination is 10–15°C (Ellis et al., 1986). At high temperatures, greater than 42.5°C, germination decreases significantly (Ibrahim, 2011) and above 45°C no germination is observed due to lack of embryo growth (Singh & Dhaliwal, 1972). Similarly, high temperature affects photosynthesis, transpiration rate and plant growth (Singh & Dhaliwal, 1972) and the length of vegetative period is generally reduced. In other words, phenology can be modified under high temperature (Summerfield et al., 1984). At high temperatures (>35°C), the vegetative period was reduced by 10 to 15 days compared with optimum temperature (28°C) at Kanpur, India (ICRISAT, 2011). High temperature therefore accelerates flowering and reduces the overall crop growth period.

High temperatures can cause cellular abnormalities such as oxidative stress, and denaturation of proteins and enzymes. Oxidative injury occurs as lipid peroxidation, and hydrogen peroxide content tends to increase in heat-sensitive genotypes at day and night temperatures of over 40/30°C compared with heat-tolerant genotypes (Kumar et al., 2012a). ABA remains high at 40/35°C but was observed to decline at 45/40°C (Kumar et al., 2012b). A membrane injury test based on electrolyte leakage from leaves was shown by Ibrahim (2011) to be an effective measure of high temperature sensitivity in chickpea, with sensitive types displaying high degrees of membrane injury. Therefore, heat stress injury can be measured using a combination of oxidative stress assessments, ABA level and membrane injury in chickpea.
Both high and low temperature stresses can affect seed germination, seedling survival, photosynthesis, membrane function, and protein and hormone function. Cold stress encourages a prolonged vegetative period but high temperatures reduce the vegetative period.

5.2.2 Temperature stresses during the reproductive period

Temperature stress at or around flowering is considered a major challenge to yield in many chickpea production areas. Mean daily temperatures at or exceeding 15°C can cause flower abortion (Clarke & Siddique, 1998). Temperatures of less than 10°C during flowering induce flower shedding, low pod set and ultimately poor seed set. Poor pollen viability and germination are the main reasons for low pod set (Savithri et al., 1980). The field and controlled environment screening at ICRISAT identified chickpea genotypic variation for temperature stress during the flowering stage. Plants exposed to mean daily temperatures of 20°C produced more pods than at 15°C. These experiments identified cold-tolerant genotypes such as ICCV 88502 and ICCV 88503 (Srinivasan et al., 1998). Cold temperature also reduces partitioning of assimilates to the vegetative parts, resulting in reduced harvest index (HI). This reduction in harvest index is more common in south Asia and Australia than other production areas (Siddique & Sedgley, 1986; Saxena, 1990).

Poor pod set in chickpea can occur due to the failure of male or female floral parts, or both. Low temperatures at flowering can affect anther dehiscence. Mean daily temperatures of 15°C can also reduce anther dehiscence and pollen load on stigma (Srinivasan et al., 1999). However, at a similar temperature, pollen viability and pollen germination on the stigma were higher in the tolerant lines ICCV 88501, ICCV 88502 and ICCV 88503 than in the sensitive cultivars Chafa and Annigeri (Srinivasan et al., 1999). Pollen function was clearly more sensitive to temperature change than pistil function (esterase activity). Clarke and Siddique (2004) showed that pollen viability and pollen germination on the stigma were the primary reasons why pod set in chickpea was reduced during low-temperature stress. Pollen sensitivity to low temperatures was identified at 5 and 9 days before anthesis (Clarke, 2001). Clarke and Siddique (2004) and Srinivasan et al. (1999) also observed that low temperature did not affect the pistil function, i.e. esterase activity. However, pollen tube growth on the styles of sensitive genotypes was retarded due to cold temperatures. Short pollen tube length at low temperature (15°C) in in vitro germination tests was observed at 20°C and 25°C by Savithri et al. (1980). Ultimately pod set is reduced by low temperature as observed by Srinivasan et al. (1999). They found that pod set was reduced at a low temperature regime of 15/5°C compared with the control (25/15°C).

High temperature during the reproductive stage is a major cause of yield loss due to partial or complete pollen sterility. In chickpea, temperatures at or exceeding 35°C affected male reproductive tissue (anther and pollen), function (pollen germination and tube growth) and pod set. Both anther and pollen showed more structural abnormalities under stress including changes in anther locule number, anther epidermis wall thickening, and pollen sterility rather than functional abnormalities (e.g. in vivo pollen germination) (Figure 5.1) (Devasirvatham et al., 2013). Pollen abnormalities can also be found at high temperature, including leakage of pollen protoplast, zigzag pollen tube growth, pseudogermination and bulbous tip formation in the pollen tube (Devasirvatham et al., 2013). Heat-tolerant chickpea genotypes had clear pollen tube growth on the style following pollen germination and this was confirmed by pod set (Figure 5.2). In heat-sensitive genotypes no pollen germination on the stigma was observed due to complete pollen sterility at temperatures at or exceeding 35°C (Devasirvatham et al., 2012b, 2013) (Figure 5.2). Pollen sterility in the heat-sensitive genotypes is a function of lower sucrose levels, resulting in poor pollen function and pod set (Kaushal et al., 2013).

At very high temperature (45/35°C) both pollen fertility and stigma function can be affected. Observations by Kumar et al. (2012b) indicate that oxidative stress in the leaves results in poor fertilization. Devasirvatham et al. (2012b, 2013) concluded that the critical temperature affecting pod set was ≥37°C for heat-tolerant genotypes (such as ICCV 92944, ICC 1205 and ICC 15614) and >33°C for heat-sensitive genotypes (ICC 5912, ICC 4567 and ICC 10685).

5.2.3 Temperature stresses during post-anthesis period

Post-anthesis temperature stress, particularly after commencement of pod set, can cause significant pod abortion and decreased grain filling. In chickpea, cold stress decreased the rate and duration of grain filling and
produced smaller seeds (Nayyar et al., 2007; Kaur et al., 2008). At 13/5°C, chickpea average seed weight and size decreased by 41% and 24%, respectively, compared with 28/17°C, largely because seed filling duration reduced from 20 days (non-stressed) to 14 days (cold stressed) (Nayyar et al., 2005b). Similarly, low post-anthesis temperature reduced yield by 1.3 t/ha in northern NSW, Australia, during 2009 (Moore et al., 2010).

In a controlled environment study, Wang et al. (2006) reported a grain yield reduction of 33–39% for post-anthesis heat stress compared with pre-anthesis heat stress. This was possibly due to poor remobilization of

Figure 5.1 Heat-sensitive genotype anther structural abnormalities: anther stained with Alexander’s stain. (a) Locule number changed (ICC 4567). (b) Anther epidermis wall is thickened (ICC 4567). (c) Anther shows fertile and sterile pollen grain (ICC 5912). Fertile – red in colour; sterile – green in colour. Scale: 10 μm.
photosynthates to the grain, thus lowering seed weight and seed number per plant (Wang et al., 2006). Both temperature stress extremes influenced seed shape and seed coat colour (Figure 5.3). Generally, temperature stress reduces cotyledon cell number, cell expansion, grain filling rate and ultimately seed weight (Munier-Jolain & Ney, 1998).

Temperature stress can influence grain filling by altering the concentration of hormones, particularly abscisic acid (ABA) and enzymes, in plant tissue. As discussed earlier, ABA plays a significant role in both cold and heat stress tolerance in chickpea (Nayyar et al., 2005a; Kumar et al., 2012a) and is generally downregulated under stress. Exogenous application of ABA increased tolerance to cold stress by improving survival rate through the reproductive stage (Nayyar et al., 2005a). The exogenous application of ABA decreased electrolyte leakage and increased pollen viability, germination, flower retention, pod set, seed size and grain yield (Kumar et al., 2008). Similarly, Kumar et al. (2012b) showed that ABA treatment reduced oxidative injury in chickpea under high temperature. Clearly, exogenous application of ABA will improve grain filling under temperature stress and hence grain yield.

5.3 Impacts on nutritional and processing quality

Environmental stresses during seed development have a negative effect on the quality of chickpea seeds (Behboudian et al., 2001). However, comparatively few studies have dealt with the effect of temperature on seed development and quality in chickpea. Nayyar et al. (2005b) reported that under cold stress grain sugar
concentration increased in chickpea but the accumulation of storage proteins, starch and several amino acids decreased. However, the effect was influenced by the stage of seed development. There was a greater reduction of starch, proteins, soluble sugars, fat, crude fibre and storage protein fractions when cold stress occurred in late pod-filling compared to early pod-filling stages (Kaur et al., 2008). However, seed germination was inhibited when plants were stressed at early pod-filling.

The effects of high temperature stress were generally similar to cold stress. High sucrose synthase and low invertase activity were observed in the seeds of heat-tolerant genotypes compared with heat-sensitive types during early pod filling (Chickpea Technical Report, 2011). Generally, high temperature during grain filling reduces dough and baking quality in grain crops (Stone & Nicolas, 1994). However, the available information on grain quality under temperature stresses in chickpea is limited. There is clearly a need to extend our knowledge of grain quality including baking quality under both high and low temperature stresses.

### 5.4 Breeding for tolerance to temperature stresses

Chickpea improvement has focused on yield potential and regional adaptation through resistance and tolerance to abiotic and biotic stresses, plant type and grain characteristics. At present, the selected bulk method is the most common selection technique used in chickpea breeding (Gaur et al., 2007). The selected bulk method is relatively inexpensive to employ and the response to selection is generally not inferior to more labour-intensive methods such as pedigree selection (Salimath et al., 2007). This section describes some of the breeding strategies used to improve temperature tolerance in chickpea and explores options for future breeding.

#### 5.4.1 High temperature tolerance

A simple but effective field screening technique for heat tolerance at the reproductive stage in chickpea has been developed at ICRISAT (Gaur et al., 2013, 2014). It involves advancing sowing date to synchronize the reproductive phase of the crop with the occurrence of higher temperatures (≥35°C). This method was effective in identifying heat-tolerant germplasm at ICRISAT and several other locations in India (Gaur et al., 2013, 2014).

A few heat-tolerant chickpea cultivars (ICCV 88512 and ICCV 513) were identified more than a decade ago (Dua, 2001). However, heat tolerance research in chickpea has only received significant attention in recent years. More recently, Krishnamurthy et al. (2011) identified 18 stable heat-tolerant genotypes (e.g. ICC 1205, ICC 637 and ICC 15618) by field screening a reference set of chickpea from southern and central Indian field trials. Short-duration, high-yielding, heat-tolerant genotypes (ICC 5597, ICC 5829, ICC 6121, ICC 7410, ICC 11916, ICC 13124, ICC 14284, ICC 14368 and ICC 14653) were identified by Upadhyaya et al. (2011). A heat-tolerant breeding line, ICCV 92944, has been released in Myanmar (as Yezin 6) and in India (as JG 14) and is performing well under late-sown conditions (Gaur et al., 2013). Several breeding lines with higher yields under heat stress than the standard cultivar ICCV 929944 have been identified (Gaur et al., 2013, 2014). Outside India, Kaloki (2010) identified ICCV 92318 as a source of heat tolerance in the semi-arid environments of Kenya through the African Climate Change Breeding Program.

Devasirvatham et al. (2012b) confirmed the heat tolerance of ICCV 92944 using a pollen selection method. Devasirvatham et al. (2013) also confirmed the heat tolerance of germplasm identified earlier by Krishnamurthy et al. (2011) (ICC 1205, ICC 15614) using pollen viability in the field and controlled environment studies, and suggested using this technique to develop heat-tolerant cultivars. These materials have been incorporated into chickpea improvement at ICRISAT and new heat-tolerant progeny are under development as genetic mapping populations (Gaur et al., 2013). Diversity arrays technology (DArT) (Mace et al., 2008) markers with good genome coverage were associated with traits targeted for high temperature tolerance in chickpea, and many genomic regions linked with phenology and grain yield have been identified (Devasirvatham, 2012), thus demonstrating the feasibility of applying genetic association analysis to explore complex traits in future. While there is clearly significant variation for high temperature tolerance in adapted chickpea, there is a compelling need to extend the search for new genetic diversity to provide additional allelic variation for temperature tolerance. The wild annual *Cicer* sp. is a possible source of variation and
could be exploited. This new allelic variation would allow plant breeders to lift the current reproductive temperature limits on chickpea.

5.4.2 Low temperature tolerance

Low temperature stress breeding generally aims to develop materials adapted to the temperature range −1.5 to 15°C at the reproductive stage and less than −1.5°C at the vegetative growth (Groser et al., 2003). Different sources of resistance to cold tolerance are reported by Chaturvedi et al. (2009), and several cold-tolerant breeding lines such as ICCVs 88502, 88503, 88506, 88510 and 88516 have been developed that set pods at less than 15°C in India (ICRISAT, 1994). The Indian Agricultural Research Institute (IARI) has also developed a few cold-tolerant genotypes (BGD 112 green, BG 1100, BG 1101, PUSA 1103, BGD 1005, PUSA 1108, DG 5025, DG 5027, DG 5028, DG 5036 and DG 5042) (Gaur et al., 2007). Using pollen as a selection method, Clarke et al. (2004) confirmed the cold tolerance of ICCV 88516 and 88510 and the sensitivity of Amethyst, Dooen, Tyson and FLIP84-15C in Western Australia. Accessions of cultivated and wild Cicer sp. were screened for cold tolerance at ICARDA (Singh et al., 1995). These authors reported cold tolerance in the lines ILC 8262, ILC 8617 (a mutant) and a FLIP 97-82C from cultivated Cicer along with wild annual chickpea such as C. bijugum and C. reticulatum.

Later, Toker (2005) identified chilling tolerance (<−1.5°C) in annual wild Cicer sp. of yamashitae. Heidarvand et al. (2011) identified the genotypes Sel 95Th1716 and Sel 96Th11439 as chilling tolerant based on field screening at the vegetative stage where plants were exposed to −11°C to −25°C at the Dryland Agriculture Research Institute (DARI) of Iran.

Both additive and non-additive gene effects govern cold tolerance in chickpea. Cold tolerance was observed to be dominant over susceptibility for at least five sets of genes (Malhotra & Singh, 1990). Breeding at ICARDA has resulted in the expansion of genetic variability for flowering at low temperatures using cultivated × wild Cicer crosses. The genes responsible for flowering at low temperature have been transferred from wild to cultivated lines (Chaturvedi et al., 2009). These reports suggested that wild relatives of chickpea can be used as a source of tolerance to low temperatures in applied breeding.

5.5 Conclusions

Both high and low temperature stresses cause grain yield loss. Cold stress encourages a prolonged vegetative period while high temperatures reduce the duration of the vegetative period. Reduced pollen viability and pollen germination on the stigma are the primary causes of poor pod set in chickpea following low temperature stress. Similarly, high temperature stress disrupts pollen viability and anther dehiscence. However, stigma receptivity is not affected by either stress. The rate and duration of seed filling are both decreased by cold and high temperature stresses.

Recent chickpea breeding programmes targeting both high and low temperature stresses have been initiated by many countries including India, Australia and Canada, with global centres such as ICARDA and ICRISAT supporting the wider effort through the characterization and exploitation of genetic resources. Screening for tolerance to temperature stresses has identified many promising sources of tolerance to both high and low temperature in chickpea. However, field-based screening is generally based on delayed sowing, and biomass development and the length of the vegetative phase are reduced in such treatments, thus reducing the fitness of plants to survive temperature extremes at flowering. Field-based methods that impose a temperature stress on a normally grown plant should be developed to confirm and validate the response of chickpea lines already identified. The identification of QTLs for temperature stress tolerance and linked molecular markers will undoubtedly improve rates of genetic advance and marker-assisted selection can easily be incorporated into most breeding methods.

Rapid progress has been made in the development of genomic resources for chickpea, and breeders have already started integrating molecular breeding strategies such as marker-assisted backcrossing (MABC) and marker-assisted recurrent selection (MARS) to improving drought tolerance in chickpea (Gaur et al., 2012). Advances in marker systems and genotyping technologies such as DArT and single nucleotide polymorphisms (SNPs) and genotyping by sequencing (GBS) have made genotyping large numbers of materials cost efficient. The integration of genomic technologies in chickpea breeding will greatly improve efficiency of developing chickpea cultivars that are more resilient to changes in temperature. For example, MARS recombines significant gene effects found among
the progeny of a single population in chickpea (Cobos et al., 2007) and will be useful in enhancing tolerance to temperature extremes. Another potential approach is genomic selection (Nayak et al., 2010), where training populations that are representative of the wider gene pool are assembled, genotyped and phenotyped and all the estimated gene effects used to assemble new chickpea cultivars. However, these breeding-by-design approaches are completely dependent upon the accuracy of the phenotyping data used in estimating the gene effects. The screening methods outlined in this chapter offer scope for rapid and accurate phenotyping for chickpea temperature screening methods. The accuracy of the phenotyping data used in estimating the gene effects used to assemble new chickpea cultivars required for an increasingly hostile production environment.

References


Chickpea Technical Report (2011) Improving heat tolerance in chickpea for enhancing its productivity in warm growing conditions and mitigating impacts of climate change. ICRISAT, Patancheru, Andhra Pradesh, India, pp. 1–46


