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Chapter

Advanced Breeding Approaches for Cold-Tolerant Chickpea and Lentil in Dryland Areas

Hamid Hassaneian Khoshro and Ramin Lotfi

Abstract

Chickpea and lentils are the two most economically important food legumes in dryland areas. They are traditionally sown in the spring of cold dryland areas of the Mediterranean regions. Therefore, the grain yield of the crop is affected by drought and high thermal stresses at the end of the season. Autumn cultivation of these crops by cold-tolerant varieties could increase grain yield up to 50%, then spring cultivation through higher availability of soil water. Breeding for cold-tolerant chickpea and lentil that is widely adaptable to autumn cultivation in cold regions and various growth conditions is the best strategic approach but requires a fine-tuned combination of advanced phenotyping and genotyping methods. However, breeding and selection of suitable cold-tolerant chickpea and lentil genotypes is complex by its narrow genetic base, which limits the sources of novel alleles. This chapter illustrates the morphological, physiological, and molecular effects of cold stress on chickpea and lentil growth and development. It will be also elaborated on conventional and advanced breeding approaches and application of advanced genotyping and phenotyping tools commonly used to develop cold-tolerant chickpea and lentil cultivars. The following, about key crop cold-tolerance traits that can be easily screened by using genotypic and phenotypic technologies are discussed.

Keywords: chickpea, cold tolerant, lentil, molecular techniques, plant breeding, physiological traits

1. Introduction

The term "stress" is defined as any disturbance that adversely influences plant growth [1–6]. Plants in nature deal with abiotic/biotic stresses. Abiotic stresses, such as low or high temperature, deficient or excessive water, high salinity, heavy metals, and ultraviolet radiation, are hostile to plant growth and development. In most crop species, suboptimal temperatures can be divided into chilling and freezing ranges. According to Graham and Patterson [7] for chickpea plant temperature below −1.5°C is the typical freezing point, and between −1.5°C and 15°C is chilling range temperatures. Temperatures up to 15°C have been demonstrated to cause flower and pod abortion in parts of the world [3, 8]. Freezing range temperatures during the seedling and early vegetative stages of crop growth are considered an important problem for winter-sown chickpea in the countries surrounding the

Mediterranean Sea, the tropical highlands, and temperate growing regions [8]. Cold-sensitive crops are damaged through temperatures below −1.5°C. Ice forming within the intercellular spaces could damage sensitive plants. The rigid ice lattice structure enlarges with reducing temperature and may creep into cellular membranes and disrupted the normal cell function [9]. The upper and lower leaves of the plant canopy, stems, meristems and roots have different responses to the freezing stress [10]. Antifreeze proteins and ice nucleators control the initial formation of ice. Tolerance to freezing is often associated with mechanisms at the cellular level, including increased membrane fluidity and osmotic adjustment [11] as well as supercooling without ice nucleation [12]. Wery et al. [11] found that selected wild *Cicer* species had more freezing tolerance than well-known cold-tolerant cultivars. The effects of cold and freezing temperatures during growth stages of legume crops need to study by observing physiological, biochemical, and molecular traits to develop cold-temperature-tolerant cultivars.

2. Cold stress effects on legume plants

2.1 Morphological aspects

Freezing range temperatures are detrimental to chickpea yield. At the vegetative stage, freezing temperatures have a severe negative effect on plant growth and development. Freezing range temperatures even during a low period can disrupt germination, decline the early growth and biological yield of the plant, and can destroy plants, especially those at the late vegetative or reproductive growth stages. During germination, chilling range temperatures result in poor crop establishment, increased susceptibility to soil-borne pathogens, and reduced seedling vigor. Walia et al. [13] demonstrated that low temperature (10°C) decreased the germination rate of chickpea seeds. The recommended threshold temperatures range for chickpea germination that varies from 5 to 35°C and the optimum germination temperature is 20°C [11]. Chickpea, along with many other chilling sensitive species, is prone to "imbibitional chilling injury" [14]. In the field, chilled seeds are often vulnerable to infestation by soil organisms, which reduces seedling survival. At the seedling stage, long periods of chilling range temperatures can retard the growth of the plant and, in severe cases, cause plant death. Isolated frost events during the reproductive stage commonly result in flower or pod abortion [3]. Less dry matter production reduces the reproductive sink that the plant can support, which, in turn, reduces potential yield. Flower, pod, or seed abortion are further symptoms of chilling range temperatures. Causal observations have indicated that freezing can reduce seed size, probably due to stress conditions affecting the mobilization of plant resources. In addition, the seed coat can be discolored [3]. Exposure at the mature pollen stage delayed anther dehiscence and induced partial pollen sterility [15]. A low period of freezing temperatures induced pollen sterility of plants. It depends on the age of the flower; older flowers are so resistant to the amount of sterile pollen than younger flowers. Pollen were completely sterilized under low temperature at young microspore stage whereas, at vacuolated microspore stage about 23.59% and at vacuolated stage 52.4% of pollen were viable and at finally mature stage 65.5% of pollen were viable [15]. Chilling stress at reproductive stage could negatively affect flower number, pod set, seed growth and development in chickpea [3, 16]. In comparison to that, low temperature impairs seed filling processes, which influence seed size of chickpea [16].

2.2 Physiological aspects

Low-temperature stress (5°C for 3 days) inhibited root growth and the capacity for water and mineral uptake to subsequently impact the nutritional influences on plant growth [17, 18]. Photosynthesis is the principal process of capturing light energy to form carbohydrates and is sensitive to low temperatures [19, 20]. Chlorophyll (Chl) fluorescence is a direct tool for detecting photosystem II (PSII) efficiency, as the ratio of Fv to maximal fluorescence emission (Fv/Fm) [21, 22]. Photo-inhibition could decline the efficiency of the electron transport chain during the light phase of photosynthesis, and this event disrupts photosynthetic apparatus in response to stress; its key characteristics are a reduction in maximum potential quantum efficiency of PSII and dissipation of light energy as heat. Despite the reduction in photosynthetic capacity, it is often accompanied by enhancement of sugar accumulation, which is a typical stress response in all plants [21–24]. In the northern hemisphere, low temperatures during the winter and early spring are usually followed by intense PAR. These conditions can cause degradation of the thylakoid structure and distortion in light-dependent photosynthetic reactions [25]. Cold stress also affects ChlF parameters. For example, a decrease was observed in chlorophyll content, OEC efficiency on the donor side of PSII, photochemical quenching, and efficiency of open PSII reaction centers exposed to cold stress [26]. Some plant species are known for their tolerance to low temperatures, showing less photoinhibition of PSII. For example, under cold stress plants show only small modifications in ChlF parameters [27]. Low temperatures (17.6/4.9°C; day/night for 26 days during reproductive phase) resulted in a reduction in relative leaf water content, possibly due to a decline in root hydraulic conductivity, oxidative and membrane damage, and chlorophyll loss [28]. Low temperatures (5/5°C for 4 days) also reduced the leaf water content because the stomata are unable to close [29]. Generally, cold stress causes damage to PSII and reduces the stability of chloroplast membranes and photosynthesis. We conducted a study on cold-tolerant of 24 wild chickpea genotypes in DARI, Iran. According to the field result, those genotypes were divided into three groups as a response to cold stress (3 sensitive genotypes, 11 tolerant genotypes, and 10 resistant genotypes). Four selected genotypes were evaluated under 22°C, 4°C, and −4°C temperatures in a controlled cold room by chlorophyll a fluorescence (ChF) parameter. As a general phenomenon, at −4°C Fm, Fv/Fm, Fv/Fo, and PIabs significantly reduced. However, ABS/RC and Fo/Fm were increased. Maximum Fm and Fv/Fm and minimum ABS/RC were recorded in the ILWC109 genotype, similar to Aana as a newly released cold-tolerant chickpea variety (**Table 1**). It seems, ILWC109 genotype under −4°C has been could increase the number of active RC of PSII and by absorbing photons, the electron transfer chain is done more efficiently (under press by the authors). This claim is confirmed by the improvement of Fv/Fm and PIabs under −4°C.

Chlorophyll a fluorescence (ChF) allows us to evaluate the photosynthesis efficiency of plants. It is useful to study the effects of environmental stresses on plants' photosynthetic function of plants. Therefore, chlorophyll a fluorescence could help us to identify different stresses effects on plant growth, health, or integrity of the internal apparatus during photosynthesis [30, 31]. The fast ChlF technique also represents a useful tool to monitor PSII thermostability. The most efficient approach is to estimate the critical temperature, i.e., the threshold level above which there is a sharp increase/decrease of the observed parameter [32]. Low temperature affects the activity of enzyme ribulose activate (RCA), changes the availability of large and small subunits of rubisco, disrupts PSII oxygen-evolving complex (OEC), and damages the structure and functioning of D1 and D2 polypeptides of PSII [33]. Georgieva and Lichtenthaler [34] found on two pea cultivars that ChF and the

Treatments	PIabs	ABS/RC	Fv/Fo	Fv/Fm	Fo/Fm	Fm	Fo
4°C	2.75b	1.07b	1.86b	0.64b	0.36b	694.25b	244.25a
−4°C	0.94b	1.42a	1.06c	0.51c	0.49a	481.25c	234.25a
22°C	9.75a	0.91b	3.71a	0.79a	0.21c	1134.13a	242.50a
ILWC109	3.14a	1.16a	2.27a	0.672a	0.329b	830.67a	251.16a
ANA	5.16a	1.10a	2.28a	0.65ab	0.35ab	770ab	233.33a
ILWC119	5.89a	1.01a	2.31a	0.653ab	0.348ab	762.50b	231.66a
ILC533	3.72a	1.25a	1.95a	0.597b	0.403a	716.33b	245.16a

Different letter in each column indicates significant difference at $p \leq 0.05$.

Table 1.
Chlorophyll a fluorescence parameter changes of chickpea genotypes under different temperatures.

Chl/Car ratio reduced, while the Chl a/b ratio increased under cold stress. In soybean plants, photosynthetic efficiency declined by more than 50% when subjected to only one night of chilling treatment [35, 36]. Respiration in plants is a temperature-sensitive process and an initial increase in response to chilling has been reported [37]. A 68% decrease in cellular respiration was reported in chickpea [38] at freezing range temperature (5°C/13°C), possibly due to altering in mitochondrial structure, less kinetic energy, and damage structure of housekeeping proteins and enzymes related to cytochrome activity, ubiquinone synthesis, and phosphorylation reactions related to ATP-dependent metabolism [39]. Freezing tolerance is related to the process of cold acclimation in plants. Acclimation is a process resulting from both metabolic and physiological alterations in plants during low temperatures [40]. Cellular and metabolic changes occur during cold acclimation include increasing of sugars, soluble proteins, prolines, and organic acids as well as the appearance of new isoforms of proteins and altered lipid membrane composition [41, 42]. Autumn planting chickpea is exposed to decreasing photoperiods and temperatures during the fall session to early winter. Therefore, seedlings of fall-planted chickpea have a possibility of acquiring some degree of tolerance to moderate subzero temperatures.

2.3 Biochemical aspects

Each plant has different enrichment pathways in different periods of cold stress. In cold-tolerant chickpea genotypes, the content of unsaturated fatty acids increased during low-temperature exposure (10°C for 5 days followed by 4°C for 2 days), which possibly contributed toward the maintenance of membrane integrity during cold stress. Reactive oxygen species (ROS) are produced in response to cold stress in chickpea [43] and damage vital molecules in cells, including membranes. Generally, lipid peroxidation and hydrogen peroxide concentrations are measured as markers of temperature-induced oxidative stress [44]. A positive correlation was observed between lipid peroxidation and malondialdehyde (MDA) concentration in *Cicer* occidentalis [45]. Plant cells have different mechanisms (anti-oxidative) to combat oxidative damage by activating ant oxidative systems that include both non-enzymatic (e.g., tocopherols, ascorbate, proline) and enzymatic (e.g., superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX)) [46]. A few studies in chickpea have identified an increase in the double bond index due to enhanced lipoxygenase (LOX) activity, suggesting that increased LOX activity plays an important role in providing cold tolerance in chickpea [47]. The upregulation of various types of antioxidants has been correlated with cold tolerance in chickpea [48].
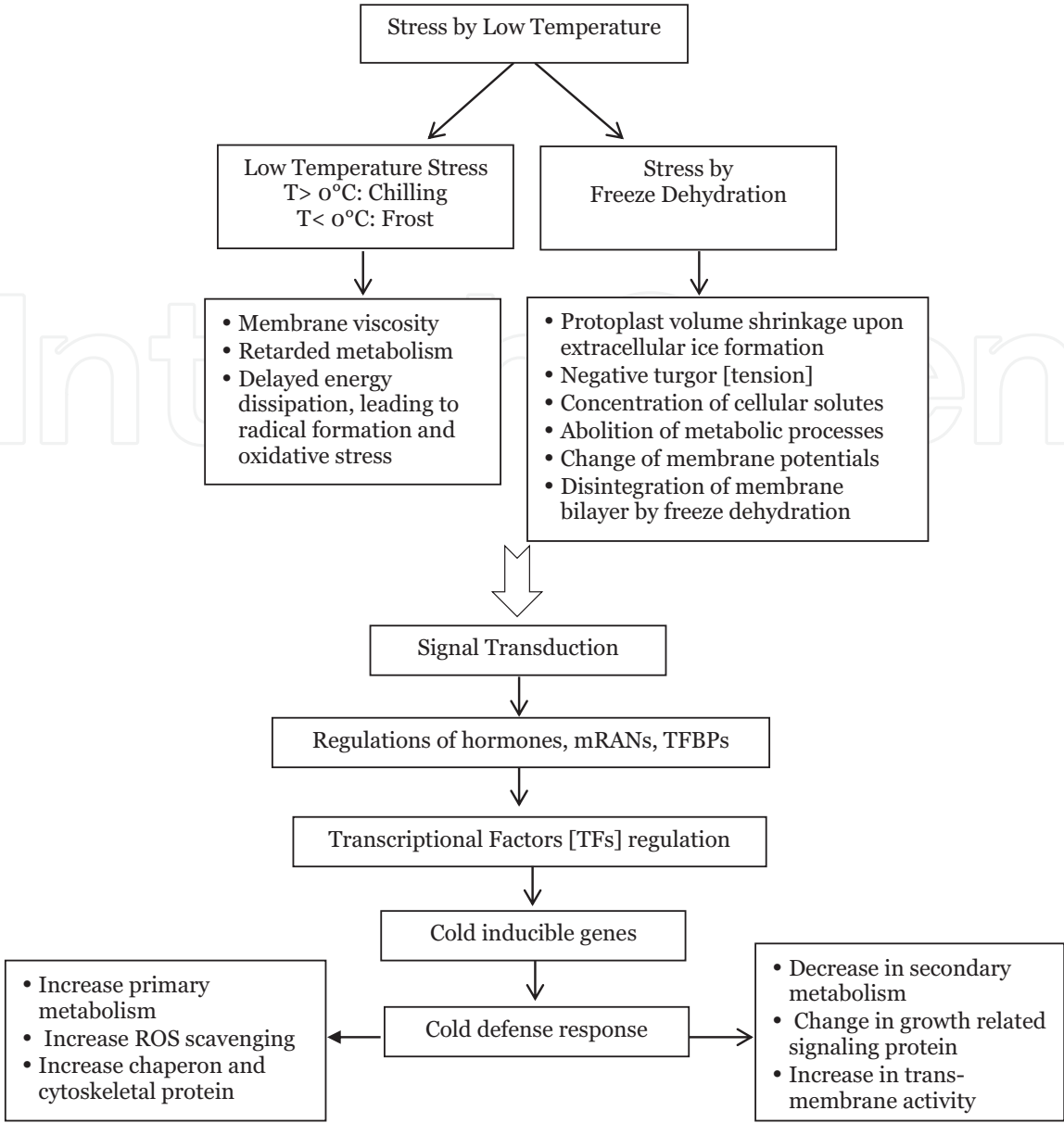
Figure 1.
A schematic diagram of cold stress response in chickpea and lentil.

Glycine betaine (GB) protects the activities of enzymes and proteins and stabilizes membranes and photosynthetic apparatus under chilling (12–14/3–4°C day/night) and freezing stress at reproductive stages [38]. Cold stress (12–14/3–4°C day/night at bud stage) decreased the endogenous GB concentration in chickpea leaves and flowers, resulting in the loss of pods [48]. Exogenously applied GB to chickpea plants at bud and pod filling stages during cold stress improved flower function, pollen germination, pollen tube growth, stigma receptivity, and ovule viability, leading to floral retention, pod set, and pod retention [38]. Also, the application of GB at reproductive stages improved grain yield/plant, the number of grain/100 pods. Low-temperature tolerance induced by GB may be related to an enhancement in relative water content (RWC), chlorophyll and sucrose, and a decrease in ABA and active oxygen species (MDA and hydrogen peroxide) [18, 45].

2.4 Molecular aspects

Several studies display those genotypes of chickpea and lentil has different molecular responses under low-temperature conditions [49–52]. This event needs an enormous gene expression reprogramming, which results in the adjusted

metabolic-structural alterations. However, the efficient adjustments are dependent on suitable cold signal transduction. Cold stress signal perception that is carried out by different pathways is the first stage. The cascades of transcriptional are the next players, which act through ABA-independent and ABA-dependent pathways to persuade cold-regulated (COR) gene expression, and the result is increasing in the levels of hundreds of metabolites, in which some of them are recognized to have defensive effects against the damaging effects of cold stress and some like reactive oxygen species (ROS), photosynthetic metabolites, and soluble sugars are thought to operate as signaling molecules and regulate specific COR genes [53, 54]. The different aspects of these phenomena are displayed in **Figure 1**. Different receptors at the cellular level are involved in receiving the external signals and, in turn, transfer them intracellularly. Thermal reactions in plants in the face of cold stress include molecular regulation and complex intracellular machinery. Two key transcriptional pathways are activated in reaction to cold stress, CBF/DREB-independent and C-repeat (CRT)/ dehydration responsive element (DRE)-binding factor (CBF/DREB)-dependent [55]. The transcription factor, CBF, operates as a master regulatory player and is induced by the binding of trans-acting factors to the promoter regions of the CBF gene [53]. The constitutive expressed ICE1 (Inducer of CBF Expression 1) binds to the corresponding cis-elements on the CBF promoter and elicits the ICE1-CBF cold-responsive pathway, which is conserved in diverse plant species [53, 55].

3. Breeding strategies for improvement of cold tolerance

3.1 Conventional breeding

Conventional breeding involves crossing, the selection from landrace genotypes, simple backcrosses to a recurrent parent forms the backbone of breeding and has been widely used to introduce novel traits within breeding programs and produce chickpea and lentil cultivars suitable for targeted environments and cropping systems. Through conventional breeding, lines of varying maturity can be selected that are suitable for production in different agroecological regions. In the last 10 years at DARI, significant improvement has been achieved in crop yield and productivity through conventional breeding, which has donated to the development of high-yielding chickpea cultivars tolerant to cold stress and suitable to autumn sowing in cold regions of Iran such as FLIP 00-86C (Saral), Flip05-42C (Soufi), FLIP 02-51C (Nosrat), x03TH148 (ATA), and x03TH130 (ANA). These cultivars have been selected from the ICARDA breeding materials and registered as new cultivars [51, 52, 56].

3.1.1 Screening for freezing tolerance in the field

Based on survival and killing percent, various scales including 1–3, 1–5, or 1–9 have been developed and used by numerous workers. Attempts were made to develop a more reliable field screening technique for evaluation of cold tolerance in chickpea and lentil at ICARDA, Tel Heldya, Syria, and the main research site of ICARDA at Aleppo, Syria [57], and a screening procedure was developed. They also developed a more precise 1–9 scale (**Table 2**), using a combination of percent plants killed and visual damage on leaflets and branches on individual plants, which can be used to evaluate even individual plants.

Later, Saccardo and Calcagno [58] used a 0–5 scale (0 = all plants killed; 5 = all plants survived) to screen chickpea material for cold tolerance and to develop lines for winter sowing in Italy. They identified 27 lines as cold-tolerant, ones at the site where the minimum temperature was −12°C and the plant survival rate

Scale	Category	Reaction
1	—	No visible symptoms of damage
2	Highly tolerant	Up to 100% of leaflets show withering and drying, no killing
3	Tolerant	11–20% leaflets show withering and upto 20% of branches show withering and drying, no killing
4	Moderately tolerant	21–40% leaflets and up to 20% of branches show withering and dryings, no killing
5	Intermediate	41–60% leaflets and 21–40% branches show withering and drying, up to 5% plant-killing
6	Moderately susceptible	61–80% leaflets and from 41 to 0% branches show withering and drying, to 25% plant-killing
7	Susceptible	81–99% leaflets and 61–80% branches show withering and drying, 26–50% plant-killing
8	Highly susceptible	100% leaflets and 81–99% branches show withering and drying, 51–99% plant-killing
9	—	100% plant-killing

Table 2.
Scoring of cold tolerance in field conditions in chickpea and lentil [57].

was 50–70%. Wery [59] and Kanouni and Khalily [52] reported variation among the chickpea cultivars, which were evaluated for frost resistance (minimum temperature −10°C to −18.5°C) and suggested that the phenological stage as most important in determining the response of the crop to cold (**Figure 2**); cold resistance decreased with progress in growth from germination to the flowering stage. They used a "frost resistance ratio" (the number of plants at harvest/the number of plants that emerged) as a parameter for cold tolerance and grouped the genotypes in following categories: "fall type" (frost resistance); "winter type" (frost-tolerant); and "spring type" (susceptible to frost) and also confirmed that early sowing dates are more suitable for screening for cold tolerance under Mediterranean areas.

3.1.2 Screening under controlled conditions

In addition to field screening, there are several controlled conditions and laboratory-based tests available for the identification of genotypes with tolerance to cold stress. Some of the more common techniques applied in legumes and other plants are summarized (**Table 3**). Whereas these techniques enable segregation of germplasm with high tolerance to special temperature regimes, they do not take into account the other stresses caused by overwintering, for instance, ice heaving or snow cover, and results accordingly will necessary to be acknowledged by screening in the field. Laboratory-based methods may find a wide-ranging application in distinguishing genotypes that have the tolerance to chilling at the stages of reproduction, since conditions of the field for this stress are very replicable. These can also be suitable in screening a restricted number of parental genotypes for a given trait, such as pollen vigor at chilling range temperatures. Appropriate genotypes identified from this screening can then be used in a hybridization program to generate progenies with variable tolerance to either freezing or chilling stress. Recently at DARI, Heidarvand and Maali-Amiri [18] identified two chickpea Sel95Th1716 and Sel96Th11439 as chilling tolerant based on controlled environment and laboratory-based screening techniques. Clarke et al. [69] has developed a method for screening
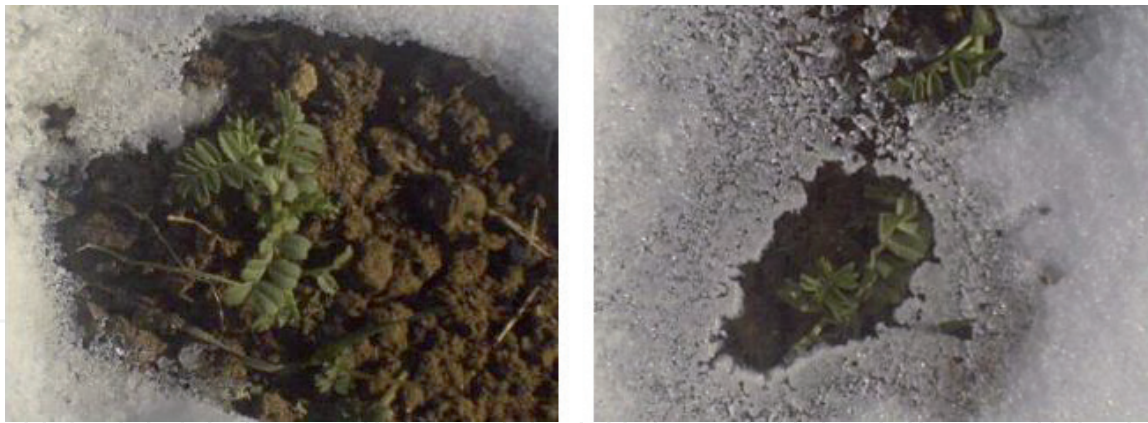
Figure 2.
Saral (FLIP 00-86C) is an Iranian new chickpea cultivar of tolerance to freezing at the seedling stage that can withstand at temperature of −22°C with snow cover in field condition [52].

Technique	Methodology	Example reference/s
Controlled environment frost screening	Plants are subjected to gradually decreasing temperature for 3 weeks, which is increased when 50% of plants show frost damage	[51, 60]
Controlled environment chilling screening	Plants are subjected to chilling temperatures during the flowering period and assessment is based on pod and seed set	[61]
Chlorophyll fluorescence and photosynthesis	Based on the fact that fluorescence emission is storing when leaves are irradiated after a dark period but is reduced if stress has damaged the cells	[28, 33, 39, 62]
Ion efflux	Measurement of ion efflux in leaves	[63]
Controlled freezing tests	Freezing whole plant/parts under a specific regime and then assessing for visible injury	[64]
Triphenyl tetrazolium chloride test (TTC)	A cell viability test based on the reducing capacity of living cells. Healthy, non-injured cells can reduce TTC better than injured cells	[65]
Leachate test	Based on the amount of naturally occurring compounds that diffuse from cells following cold exposure. Larger amounts of leachate are indicated by greater electrical conductivity	[66]
Plasmolysis test	Based on the fact healthy cells plasmolyse in a hypertonic solution such as calcium chloride, whereas injured cells do not	[67]
Pollen tube growth	Cold-sensitive genotypes yield pollen with reduced tube growth and fewer pollen tubes reaching the ovule	[68]
Osmoprotectant, membrane integrity, and enzymatic activity	There is a close relationship between cold tolerance and osmoprotectant (such as sugar, prolin, proteins, fats) metabolism	[18, 43, 45, 69]

Table 3.
A summary of controlled environment and laboratory-based screening techniques for the identification of chickpea and lentil tolerance genotypes to cold stress.

of pollen tube growth to recognize germplasm with chilling tolerance at the stages of reproduction. This method compares pollen tube growth of diverse genotypes at changing temperatures and has been applied to select reputed chilling tolerant lines as parents in the legumes breeding program. Other laboratory-based methods for

identifying tolerant genotypes can be to measure ROS-scavenging systems, including both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic antioxidants such as ascorbate, proteins, fats, and proline [18, 45].

3.2 Molecular breeding

3.2.1 Marker-assisted breeding

Molecular markers are now considered better than physiological and morphological characters because of unaffected by environmental factors, theoretically unlimited, being stable, and simply detectable without distinction of growth and stages of development. They are also ideal for the identification of QTLs, genetic diversity analysis, tagging of useful genes, fingerprinting, construction of genetic and physical maps, evolutionary studies, positional cloning of useful genes, and marker-assisted selection [70–72]. Molecular markers are reaching a stage where they can be applied cost-effectively in breeding programs. QTL analysis, genomics research, and genotyping platforms are used to speed up the breeding process through exploiting variation at the genome level [73]. Several studies reveal the successful application of molecular markers in the improvement of chickpea and lentil cold tolerance cultivars [74–76]. Clarke and Siddique [77] found that molecular markers based on amplified fragment length polymorphisms (AFLPs) have been linked to the trait using bulked segregant analysis for F2 progeny of a cross between the chilling-sensitive cultivar amethyst and the chilling-tolerant ICCV 88516 [77]. Putative markers linked to traits for both chilling sensitivity and chilling tolerance prevail the limitations of the dominant AFLP marker system. Six pairs of specific 18–24-mer primers (AFLP-based markers) were applied to amplify the defined DNA fragment from genomic DNA of individual F4 progeny with known phenotypes in an effort to develop Sequence Characterized Amplified Regions (SCAR) markers [78]. The foremost promising primers were based on a 560-bp fragment containing a simple sequence repeat (SSR), with 10 repeats within the tolerant parent and 9 within the susceptible parent [77]. Their results also showed three-base differences on a vertical acrylamide gel, which was very suitable within the selection of chilling-tolerant progeny resulting from crosses between ICCV 88516 and amethyst [77]. Results of Amini et al. [79], based on cDNA AFLP analysis of transcripts, represented different groups of genes involved in metabolism pathways, cellular defense, cell connections and signaling, transcriptional regulation, and chromatin architecture in chickpea during cold stress.

A new method developed for marker-assisted breeding in *lupins* [80] could also be considered for chickpea and lentil in the future. Microsatellite-anchored fragment length polymorphism (MFLP) is highly efficient in producing DNA polymorphisms, and many MFLP markers can easily be converted into sequence-specific, simple PCR-based codominant markers. Difficulties in screening and breeding for tolerance to low temperatures are further confounded by low genetic variability within cultivated chickpea [81, 82]. Relatives of chickpea among the wild *Cicer* species offer a valuable genetic resource to overcome these limitations [8, 83, 84]. Tolerance to cold has been reported in five annual and one perennial species [3, 83, 85]. The original collection and many selections of annual *Cicer* species held in world gene banks were analyzed using DNA molecular markers, which are not affected by environmental influences, providing useful data for the selection of suitable parents for crosses [84, 86]. To a certain extent, it will also be possible to use chickpea-derived Sequence Tagged Microsatellite Site (STMS) markers for the marker-based analysis of wide crosses because many STMS can

be transferred between *Cicer* species [87]. Barriers in wide crosses are also being addressed through international collaboration with the aim to use embryo rescue to overcome incompatibility [77]. In lentil plant, Murray et al. [64] reported 12 QTL for winter hardiness and also, their results indicated that winter hardiness is influenced by several genes and the cumulative effects of cold stress. Target-induced local lesions in the genome (TILLING) of chickpea were used for functional validation of abiotic stress-responsive genes. A TILLING approach based on next-generation sequencing has been used in the mining genes associated with cold tolerance [88, 89]. Glaszmann et al. [90] used eight chickpea genotypes from different origins as parents for the development of a Multi parent advanced generation intercross (MAGIC) population. MAGIC population is one among a next-generation multiple mapping population, which comprised 4–20 parents in cross-combination and source of increasing genetic variability. The use of a MAGIC population is helpful because the inclusion of several parents confirmed the segregation of deployment for understanding complex traits, QTLs for multiple traits, and therefore the detection and description of unique genes [90].

3.2.2 Transcriptomics

Transcriptomics deals with the analytical study of the transcriptome that is the transcribed component of the genetic material. Sequence information and identification of novel genes for agronomically important traits can be done using a number of methods, including EST databases [91]. Next-generation sequencing and Sanger sequencing methods have been used for transcriptomic studies of chickpea. Initially, EST abundance was assessed for development-related expression, tissue-specific expression, and stress-responsive expression. Chickpea genotypes were grown under cold; salt and drought stresses and complementary DNA libraries were generated, which comprised 20,162 ESTs [92]. Gene discovery is very limited in chickpea, and few efforts have been made to identify the ESTs associated with stress responses through transcriptomic studies [92]. Mantri et al. [93] studied the transcript profiling in chickpea genotype under drought cold and salinity stress and concluded that transcriptional change of more than twofold was observed for 109, 210, and 386 genes after drought, cold, and high-salinity treatments, respectively. Deokar et al. [94] studied the differential downregulation and upregulation of the transcriptome in tolerant and susceptible chickpea genotypes subjected to abiotic stress.

In silico expression, studies were carried out to know the differential expression of tolerant and susceptible chickpea genotypes under abiotic stress [92]. Microarray, suppression subtractive hybridization, EST sequencing, and super serial analysis of gene expression (SAGE) have been used for functional genomics analysis of chickpea genotypes in stress responsive conditions [95, 96]. Sharma and Nayyar [96] used DDRT-PCR analysis to identify anther genes involved in cold tolerance in chickpea genotype ICC16349 (cold-tolerant). Their results showed cold stress altered expression of 127 ESTs in anthers, about one-third (35) belonged to several functional categories such as transcription, pollen development, ion transport, translation, signal transduction, carbohydrate metabolism, energy, and cell division. More than two-third (92) of them were novel with unknown protein identity and function. The combination of next-generation sequencing techniques with SAGE is cumulatively known as deep SuperSAGE, which makes the tool even more precise. Transcriptome analysis of chickpea roots was carried out using deep SuperSAGE under normal and abiotic stress conditions and 17,493 unique transcripts were identified which were stress responsive [97].

4. Conclusion

Chickpea and lentil improvement programs targeting the insulation of varieties against low temperature/cold stress have been initiated by many centers globally. In Iran at the Dryland Agriculture Research Institute (DARI), Saeed et al. [54], Kanouni and Khalily [52], and n and Maali-Amiri [18] identified and introduced chickpea genotypes namely FLIP 00-86C (Saral), FLIP 02-51C (Nosrat), x03TH148 (ATA), x03TH130 (ANA), Sel95Th1716, and Sel96Th11439 as chilling tolerant based on field screening and controlled environment and laboratory-based screening techniques at the vegetative stage where plants were exposed to −14°C to −25°C (**Figure 3**). Screening against low temperature has been taken up vigorously in recent years. At the Center for Legumes in Mediterranean Agriculture (CLIMA), in Australia, chilling tolerance has transferred from ICCV 88516 and two desi chickpea varieties WACPE2075 (Sonali) and WACPE2095 (Rupali) have been developed [77]. Breeding efforts made at ICARDA, Syria, have demonstrated the release of more genetic variability for flowering at low temperatures using cultivated x wild *Cicer* crosses. This shows that genes responsible for flowering at low temperatures should be transferred from wild to cultivated species, *Cicer arietinum*. Cold tolerance at flowering can also be achieved through accelerated breeding programmed based on haploid selection. Development and identification of molecular markers and QTLs offer promise for mitigating low-temperature stress at the genetic level. Molecular markers-assisted breeding can be a viable option in targeting the desired gene(s) or QTLs. Good scope exists for the exploitation of transgenic technology in the development of low-temperature/cold-tolerant genotypes. Per se, tolerance to abiotic stresses appears to be a difficult research aim to be tackled by conventional breeding due to several technical limitations. In changing climatic conditions where the crop has to face abrupt low temperature during the reproductive phase, concerted efforts for the development of low-temperature/cold-tolerant chickpea varieties are needed. An integrated approach involving molecular biologists, conventional breeders, physiologists, and agronomists should be adopted to mitigate the low temperature/cold stress for better crop productivity. This may include defining the target environment, development of reliable screening techniques, identification of desirable traits and donors, transferring the targeted gene[s] in desirable agronomic

Figure 3.
ANA (x03TH130) an Iranian new chickpea cultivar that can withstand at temperatures of −24°C with snow cover in field condition.

backgrounds. Critical assessment of cold-temperature genotypes under target areas (proper phenotyping) will certainly help in the identification of high-yielding chickpea varieties for cultivation in low-temperature/cold-prone areas.

Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Hamid Hassaneian Khoshro* and Ramin Lotfi
Dryland Agricultural Research Institute (DARI), Agricultural Research Education and Extension Organization (AREEO), Maragheh, Iran

*Address all correspondence to: h.hosnian@areeo.ac.ir

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