




Review

Chickpeas' Tolerance of Drought and Heat: Current Knowledge and Next Steps

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Abstract: The global increase in temperature and associated meteorological disruptions, such as the earlier onset of high temperatures and disruptions in precipitation, are becoming severely limiting factors in crop cultivation. Chickpea, as a cool season crop, is under the direct influence of heat and drought stress that is not only affecting this crop in its podding stage but, with current climate trends, the drought and heat are now also affecting earlier stages, such as flowering. The deteriorating effects of heat and droughts include reduced flowering, abortion of flowers and absence of podding; thus, this is severely affecting crop yield. Further research has been conducted to identify the genes correlated to higher stress tolerance and to utilize them in developing more tolerant varieties. Different alleviation approaches have been also tested and it has been determined that some positive effects can be seen in supplementation with Zn through melioration of water relations, seed priming and some transgenic and genome editing approaches. Breeding strategies for future chickpea varieties have been focused on the identification of varieties with more tolerant traits for an improved yield under stressed conditions. In this review, we have reviewed recent strategies and biotechnological approaches that have been used with chickpea crops to address the two major abiotic stresses (heat and drought) linked to future climate change.

Keywords: chickpea; heat; drought; stress; tolerance



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1. Introduction

With high temperatures breaking world records each year, global climate change-related research has become of crucial importance. In 2022, a heat wave in July caused a record-breaking high temperature across Europe [1] which, in combination with the drought, severely affected crop growth and yield. Current trends of global climate change will increase global temperature and drought intensity bringing additional stress through an earlier onset of high temperatures and disruptions in precipitation [2].

Most of the important crops cultivated worldwide are facing increasing threats due to climate change. Drought and heat stress are the most common abiotic stresses that are severely affecting the yields and quality of crops, especially when they occur at critical phenological stages during the crop lifecycle. Crop growth and performance are modulated by a complex network of many environmental (E) and management (M) (agronomic) factors that relate to climate change. The complex relationship between management and the environment explains a significant proportion of a crop's trait variability [3,4]. The interactions of plant genotype (G) with E and M, are important in breeding and agronomic activities [5]. Indeed, this highlights the need to spend more resources for a better evaluation of broad biodiversity among genotypes of crops (such as legumes) to identify which one is better adapted to specific climatic and pedological conditions. It is

important that breeders exploit the steps undertaken by natural and previous artificial selection to speed up the process of the obtainment of improved genotypes for a sustainable approach to agriculture that limits the use of non-renewable and dangerous inputs. G*E*M interactions are responsible for the modulation of any physiological processes controlled by quantitative trait loci such as water and nutrient uptake or transport, yield production and partitioning of nutrients, organ development, flowering and ripening. More investigation into the genetic and physiological interconnections underlying crop responses to climate change is important to optimize crop adaptive responses to exploit all the yield potential [6].

These abiotic stresses are also limiting to legumes, which are essential crops in sustainable agricultural approaches to enhance soil fertility through symbiosis with efficient rhizobia. In addition, when they are optimized in the agricultural rotations and systems, these crops can provide a significant profit to growers as well as healthy foods for the consumer community. Food legumes (or pulses) are cultivated on 80.3 million hectares of crop area [7]. They have been the backbone of different agro-ecosystems (Mediterranean basin, Middle East, South America) since ancient times. However, the use of a restricted number of cultivars has limited the study of unique and wide biodiversity which has not been sufficiently valorized, especially by North-African countries. Legumes are ideal crops for sustainable land use (greening), as indicated by the Common Agricultural Policy (CAP) from the European Union (EU) because they have several beneficial effects on the agro-ecosystems: (1) enhance water use efficiency, (2) limit the employments of anthropogenic inputs maintaining a high level of soil fertility, (3) favor pollination and ecological balance with flora and fauna, (4) protect close-by wildland ecosystems, (5) improve other ecosystem services (e.g., biotic stress management) and (6) provide healthy and highly nutritive food, with high levels of proteins.

Chickpea is considered a cool-season crop and in normal growth conditions, higher temperatures and drought are present at the end of chickpeas' life cycle. Under Mediterranean and semiarid environments, chickpea is normally exposed to drought and higher temperatures during pod setting and seed filling, known as terminal drought. Current trends of early onset of drought and higher temperatures are imposing drought and heat stress during the growth and flowering of chickpea, affecting the plants' yield [8]. The chickpeas' growth is additionally affected through the generation of oxidative stress due to high temperatures and subsequent damaging of chlorophyll structures that affect photosynthetic performance and leaf structure [9], and further impairment of related metabolic pathways [10].

Droughts and heat affect basic physiological processes and are among the most limiting factors of chickpea yield [11]. High temperatures and heat stress can impair all aspects of chickpea development from germination and seedling establishment to seed production [11]. In most areas of chickpea cultivation, heat stress specifically occurs during the reproductive phase. Flowering, as one of the most sensitive processes, is severely affected by heat, consequently resulting in decreased flowering rate or even flower abortion, leading to a severely affected yield [12]. Temperatures above 35 °C can cause yield losses of up to 39% [13] with heat affecting anthers and pollen viability and stigma function, leading, subsequently, to the decrease in fertility and pod setting [14].

From the agricultural point of view, a drought can be described as a state in which the evapotranspiration demand is higher than the amount of available water to be used by the crop. In this sense, drought stress occurs when the soil water content is low, and it works as a limiting factor for plant transpiration. For this reason, we should consider drought stress not only as caused by an extreme weather event, such as the reduction in the soil water reservoir, but can also be caused by diurnal fluctuation of environmental factors, in combination with heat and light intensity, that drives an imbalance between root water uptake and plant transpiration [15]. Water deficit affects photosynthesis through the decrease in leaf water potential, affecting CO₂ availability as well as stomatal conductance and respiration rate [11]. Consequently, drought conditions strongly affect seed number and size, leading to yield losses of as much as 80% [16]. These conditions are particularly

detrimental while occurring during the reproductive stage of the plants [17], but drought stress occurring at the vegetative stage can have an even greater impact on chickpeas' yield [18]. Besides yield, drought also alters the nutritional value of chickpea seeds. A decrease in starch, protein, fat, and fibre contents ranging from 25 to 67% and depending on the cultivar was recorded under water stress, while the accumulation of soluble sugars was increased by the stress [19].

2. Genes Associated with Heat and Drought Tolerance in Chickpea

Different approaches have been undertaken to shed light on which genes are contributing to the tolerance to heat and drought in chickpea crops. Previous approaches used key genetic factors for the improvement of stress responses in cropping environments well-characterized by stress regimes. Although it is known that drought tolerance/response is a complex quantitative trait, previous studies have focused on the modulation of single genes for the improvement of abiotic stress tolerance, inducing mutations or gene editing [20,21]. Other ways focused on the selection of genes involved in plant architecture which favor a post-flowering balance between supply and demand of water allowing the stay-green trait that uses some genetic determinants such as the PIN-formed protein (PIN) genes and the vernalization response (VRN) gene family [22]. Genetic variation is correlated to the tolerance of abiotic stress, such that heat and drought is connected to variation of genes involved in key processes such as crop growth rate, reproductive organs development, enzymatic activity, plant growth duration, reproductive growth and accumulation of ABA (abscisic acid) in seed or pod [23]. Investigations of allele diversity gave insights into candidate genes that could be correlated to heat and drought tolerance in chickpeas (Table 1). A quantitative trait locus (QTL) research identified specific different genome regions comprising different genes that could be linked to different phenotypic traits related to plant performance (growth, yield) under heat stress by creating QTLs maps and markers [24–26].

Table 1. Candidate genes for chickpea heat and drought tolerance.

Gene	Correlation to Stress
Aquaporins gene family (CaAQPs) [27]	Biotic and abiotic stress
CarERF116 [28]	Abiotic stress response
CarLEA4 [29]	Plant developmental processes
Abscisic acid stress and ripening gene (ASR) [30]	Reproductive processes
Drought responsive element binding protein (DREB) [31]	Heat and drought stress response
Dehydration responsive element binding (DREB1) [31]	Induced by dehydration and high-salt stresses
CAP2 gene (DREB2A) [31]	Regulates expression of water stress-inducible genes
SNF-1-related protein kinase (AKIN) [31]	Response to nutritional and environmental stresses in plants
Amino aldehyde dehydrogenase (AMADH) [31]	Osmotic stress, dehydration, and salt stress tolerance
CAP2 promoter [31]	Induce a set of abiotic stress-related genes
Dehydrin (DHN) [31]	Induced by environmental stress, dehydration, or low temperature
ERECTA (fragment 7F-5R) [31]	Mediates plants' responses to disease and stress
ERECTA (fragment 8F-8R) [31]	Mediates plants' responses to disease and stress
Myb transcription factor [31]	Response to biotic and abiotic stresses
Sucrose synthase (SuSy) [31]	Sugar metabolism pathway
Sucrose phosphate synthase (SPS) [31]	Induced by drought and mannitol
Heat shock proteins [32]	Heat stress resistance
Pollen-specific leucine-rich repeat extensin-like protein 1 [32]	Heat stress resistance
Transcription factor CAULIFLOWER A-like [32]	Heat stress resistance
Heat shock protein-binding protein [32]	Heat stress resistance
Heat shock amino-terminal domain protein [32]	Heat stress resistance
PHOTOPERIOD-INDEPENDENT EARLY FLOWERING isoform X1 [32]	Heat stress resistance
Heat shock protein/heat shock factor protein HSF24-like [32]	Heat stress resistance
Calmodulin-binding heat-shock protein [32]	Heat stress resistance

3. Chickpeas' Fight against Heat and Drought Stress

3.1. Alleviation by Supplementation

Effects of abiotic stress are often emphasized by different mineral deficiencies in the soil; one of the elements crucial for chickpea growth and yield is zinc [32]. There are some reports that supplementation with zinc can contribute to higher drought and heat tolerance in chickpea plants. Supplementation with Zn can lead to improved plant growth and PSII efficiency, and improve overall water relations [33,34]. The positive effect of Zn supplementation can be correlated to the mode of Zn absorption in plants, where passive uptake of Zn is undertaken together with water molecules and the hyper-polarisation of root cells' plasma membranes, facilitating transport through non-selective cation channels [35]. Additionally, active transport of Zn includes the activity of transport proteins: ZIP, heavy metal ATPase family (HMA) and metal tolerant (MTP) protein families [36]. Zn supplementation under drought stress maintains membrane permeability and improves plant growth, photosynthetic activity and ROS scavenging [37,38]. Regulation of water relations by Zn supplementation is due to Zn's role in osmolyte accumulation and its protective role in leaf tissues preventing effects of water deficiency, contributing to the stimulation of antioxidant activity and decrease in electrolyte leakage that improves membrane stability [39]. Combining Zn supplementation with Fe in the form of nanoparticles as fertilisers can have positive effects on antioxidant activity and photosynthesis [40]. Additionally, the application of micronutrients in the form of a foliar spray can alleviate heat stress, as recorded for lentils [41], and the foliar application of zinc had a similar effect in chickpeas [42].

Besides micronutrients, supplementation/application of other molecules can have an alleviating effect on chickpeas under drought stress as well. The application of different antioxidants, such as ascorbic acid, glutathione and proline, can help alleviate the stress in plants subjected to droughts and heat stress due to the upregulation of antioxidant enzyme genes [43]. The application of proline not only stimulates the production of antioxidant enzymes but also stimulates the production of osmolytes alleviating drought stress through two pathways: reactive oxygen (ROS) scavenging and osmoregulation.

3.2. Alleviation by Seed Priming

Seed priming involves the process of the imbibition of seeds under controlled conditions for metabolism activation and seed redrying prior to radicle protrusion. By this process, different metabolic changes including some epigenetic processes are activated resulting in a specific "primed" state, where seeds incorporate epigenetically induced changes resulting in "primed memory" [44]. Only a small number of research papers investigate how seed priming could contribute to the alleviation of heat and drought stress and how seed priming can affect later stages of plant development. It has been recorded that seed priming using $ZnSO_4$ can enhance seed germination performance and subsequently contribute to drought and heat resistance of grown plants [45]. Experiments with faba bean under drought stress demonstrated that zinc seed priming can improve emergence and growth through regulation of α -amylase activity and soluble sugar content [46]. Seed priming using gibberellic acid can contribute to better tolerance of drought in chickpea, as recorded by Shariatmadari et al. [47], again, the positive effects could be correlated to α -amylase activity leading to the assumption that α -amylase activity is a key process included in drought tolerance of chickpea. Alongside their role in the regulation of α -amylase activity, gibberellins (GA) are involved in growth processes and immune responses to stress conditions such as heavy metal stress [48]. Gibberellins have been successfully used for the enhancement of seed performance and seedling vigour in chickpeas [49], as well as a rescue seed pre-treatment for the tolerance of chilling [50].

Alleviation of drought stress can be achieved through osmopriming using mannitol, where seedlings developing from osmoprimed seeds show significantly increased growth compared to control in a water deficit (Figure 1). Seedlings from primed seeds showed better α -amylase and invertase activity [51], again pointing out the importance of sugar degrading enzymes in the response of chickpea to drought stress. Hydropriming can also

be used to improve chickpea growth and yield, where seeds are soaked in water from 2 to 10 h with no correlation between the duration of priming and plant growth and yield [52].

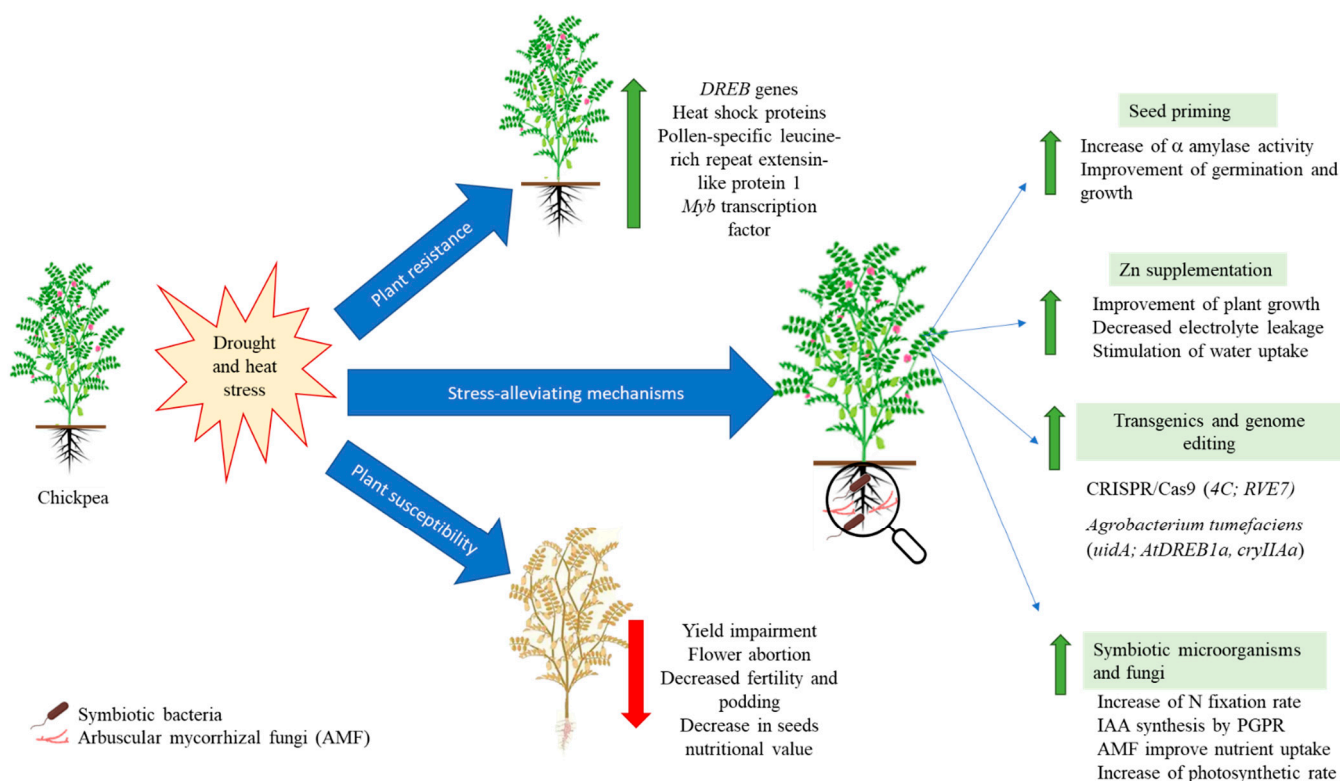


Figure 1. Chickpea tolerance of drought and heat and alleviation strategies.

Hydropriming for 12 h and osmopriming for 24 h has been shown to have beneficial effects on seed germination of chickpea, especially in cold climates, suggesting the importance of seed priming for the alleviation of temperature stress [53].

Soil salinity can be responsible for drought with similar effects on chickpea; due to high ionic content in the water, chickpea plants can experience physiological drought-restriction of water uptake [54]. Alleviation of physiological drought can be achieved by salicylic acid application and positive examples have been recorded. Using 0.2 mM salicylic acid solution it is possible to prime the seed inducing higher germination and growth rate under salt stress [55,56].

Improvement of chickpea germination and plant growth has also been obtained in seeds primed with boron and/or bacteria. In the case of combined seed priming with boron and *Bacillus* sp. MN54 improved seedling establishment, plant growth, yield and increased boron concentration in chickpea grain [57]. There are already some optimised procedures of seed coating with boron and inoculation of *Bacillus* sp. MN54 for biofortification of chickpea seeds resulting in improved nodulation, growth, yield and grain biofortification [58]. Combining GA and *Rhizobium* bacteria for plant priming processes by exogenous application resulted in synergetic effects leading to significant improvements in growth, yield and nutrient contents of chickpea [59]. How the seed would perform under temperature and/or drought stress is yet to be explored.

3.3. Role of Symbiotic Microorganisms and Fungi in Heat and Drought Alleviation

Climate-related environmental stresses cause huge losses in crop growth and yield [60]. Legumes, however, can benefit from their association with rhizobia. This group of soil bacteria are well known for the nitrogen-fixing activity of root symbiotic nodules, which is responsible for approximately 80% of the biologically produced fixed nitrogen [61–63]. The conversion of atmospheric nitrogen is the source of ammonia for the plant metabolism,

allowing for better growth and crop production. Rhizobia nodulation is also adversely affected by different abiotic stresses [64]. For this, various metagenomic studies were performed to better characterize the species as more resilient to the environmental modification [65–67]. The use of plant growth-promoting rhizobacteria (PGPR) is nowadays a current practice to improve plant resistance under abiotic stress conditions, using the most resistant bacteria, such as *Bacillus* spp. and *Pseudomonas* spp. [68,69]. In the last two decades, a lot of studies were carried out to find that the molecular basis of rhizobia modulated improvements in plants' survival. Genes with a differential expression profile were found, under drought stress, connected to various metabolic pathways [70,71], such as nitrogen fixation [72], hormone production [73,74] and even genes involved in the cell structure of different plant species [75].

One of the primary effects that plants encounter under drought is an overproduction of reactive oxygen species (ROS). Microorganisms can play a key role in plant protection from oxidative damage [76,77], mainly contributing to the regulation of the amount of superoxide dismutase (SOD). Concerning hormone production, indole-3-acetic acid (IAA) was found to have a key role in plant survival under abiotic stress conditions; PGPRs, indeed, can synthesize a huge amount of this phytohormone available for plant growth promotion [71,78]. The advantage of inoculating crops with PGPRs under environmental critical conditions is reported to contribute specifically to legumes survival due to the release of many nutrients [79] and the alteration of several molecular and physiological mechanisms [69]. Some molecular studies related to PGPRs were performed, under abiotic stress, on chickpeas [80–85] due to their food industry relevance [86]. The association between PGPRs and chickpeas was revealed to promote chlorophyll production and the content in protein and sugars of the entire plants, even in drought conditions, thanks to the involvement of various Gram-positive and negative species that colonize the rhizosphere [83,84,87].

The inoculation of chickpeas with the *Pseudomonas putida* strain MTCC5279 was reported to ameliorate every stage of the chickpeas plant cycle. These PGPRs were shown to contribute to the modulation of various transcripts involved in the drought stress response, mainly transcription factors expressed in response to abiotic stress (*DREB1A*, *NAC1*), genes implicated in the macromolecule's protection (*LEA*, *DHN*) and genes for antioxidant translation (*CAT*, *APX*, *GST*) [83]. Similar results were obtained by Kumari et al. [77] using F2 generation of chickpeas produced from F1 treated with symbiotic species of bacterial and fungi, which were shown to be more resistant to drought stress when compared to the F2 generation derived from F1 uninoculated plants. The main genes regulated by this association were referred to as oxidative damages and the general responses to environmental stresses [77].

Recently, other studies also highlighted the relevance of the non-rhizobia endophytic community in strengthening crop stability under abiotic stresses [82,88,89]. The principal molecular mechanisms involved in this cooperation are like that promoted by rhizobia: hormones regulation (in particular with the decrease in ethylene concentration affecting senescence), enzymes production, micronutrients and nitrogen provisioning and changes in the plant physiology of shoot and root [90–94].

Chickpea plants also establish mutualistic relationships with arbuscular mycorrhizal fungi (AMF). AMF play a key factor in the adaptation of plants to different ecosystems. The symbiotic relationship between AMF and plants is expressed through the formation of intraradical and extra radical structures such as hyphae, vesicles and the formation of hypha's branches called arbuscules. Forming a hyphal network of extra radical hyphae that allows extending the root absorption area [95].

Arbuscular mycorrhizal fungi can improve host plant tolerance under stressful growth conditions, like drought stress, stimulating growth and bringing a modification of the root architecture for improving access to water and nutrients such as N, P, K, Ca, Zn and S from the soil [96,97]. In chickpeas, AMF beneficial effects include their role in hormone production and biological control of plant pathogens, like *Ascochyta rabiei* [98]. A recent

study reported a positive correlation between the amount of mycelium, vesicles, arbuscules, nodule number, nodule fresh weight and leghemoglobin indicating synergistic interaction with nitrogen fixers, P solubilizers and plant growth-promoting rhizo-microorganisms. The presence of AMF also increases the content of chlorophyll a, chlorophyll b, carotenoid and, in general, the photosynthetic rate in drought-stressed chickpea plants relative to control [99].

The alleviation of stress effects through AMF colonization depends on AMF species and the type of chickpea. Sohrabi et al. [100] showed that inoculation of AMF species of *Glomus* genera in two different chickpea types (Desi and Kabuli type) significantly increased the activity of polyphenol oxidase (PPO), peroxidases (POD) and ascorbate peroxidase (APX) enzymes. Most of the POD activity was recorded for inoculated plants with *Glomus etunicatum* and *Glomus versiform* species, and the highest APX activity was observed in chickpea inoculated with *Glomus intraradices*. Inoculation with *G. intraradices* had a more positive effect on chlorophyll a content in Desi variety, compared to Kabuli. In general, the symbiotic association between plants and AM fungi had been reported to have a positive effect on the plants, even though this will depend on the host plant species as well as on the AM fungi involved [100,101].

3.4. Transgenics and Genome Editing in Chickpea for Drought Tolerance

The genetic transformation and genome editing of chickpeas have shown to be promising approaches for the development of new biotechnological tools to achieve remarkable agronomic traits [102,103]. Although it is not a trivial species for genetic transformation, genome editing and plant regeneration, the chickpea is an important socio-economic crop worldwide that has several agronomic traits that could be improved or implemented, such as abiotic stress tolerance [104]. The genetic engineering associated with traditional breeding can effectively improve several chickpea traits in a short time. Before this can be achieved, protocols with high reproducibility are demanded for the effective tissue culture in vitro of chickpea, embryogenic or organogenic callus production, shoot regeneration, multi-sprouting, plant elongation and rooting. At the same time, protocols with high efficiency for genetic transformation and selection and regeneration of transforming cells and plants are also crucial to obtaining elite transgenic lines or genome-edited plants. In the last 25 years, important advances have been made with the genetic transformation and genome editing using the CRISPR/Cas9 system of different chickpea cultivars (Table 2). Based on these advances achieved with chickpea, more than 55.1% of scientific studies aimed to establish methodologies for genetic transformation and in vitro regeneration of these transgenic plants, 30.6% sought to improve chickpea resistance to insect pests, while 12.2% aimed to improve tolerance to abiotic stresses (Figure 2A). Among these studies, 97.5% used stable genetic transformations of chickpea, 8.1% used composite plants (hairy root genetic transformation) and only 2% used genome editing as an engineering tool (Figure 2B). Among the delivery methods of recombinant DNA (T-DNA, minimal expression cassette, or CRISPR/Cas9 elements), 85.7% used *Agrobacterium tumefaciens* as the DNA-carrier agent, 12.2% used the biolistic as a delivery method, 8.1% used *A. rhizogenes*, 2% used PEG-transformation of chickpea protoplasts and 2% used electroporation of embryogenic axes (Figure 2C). More specifically related to the *A. tumefaciens* strains used in these studies, 42.8% used strain LBA4404, 20.4% used strain EHA105, 16.3% used strain AGL1 while 34.6% used other strains (Figure 1). Furthermore, as in vitro selection markers of chickpea transforming cells, 79.5% used kanamycin, 12.2% used hygromycin, 6.1% used phosphonitrocin (glufosinate-ammonium) while 4% used other sources as a selection agent (Figure 2E). Finally, among the genetic elements present in the constructs used for chickpea genetic transformation, 65.7% of the transcriptional units used the constitutive cauliflower mosaic virus (CaMV) 35S promoter, 20.9% used the nopaline synthase (NOS) promoter while 13.3% used other promoter sequences (Figure 2F). Interestingly, through transgenic approaches that drove the overexpression of AtDREB1a [103]; AtBAG4 and TIBAG [105]; CaHDZ12 and CaWRKY70 [106]; P5CS [107]; and P5CSF129A genes [108] was possible to

make remarkable improvements in abiotic stress tolerance of chickpea. Similarly, the constitutive overexpression of cryIIAa [109], cry1Aabc [110], cry1Ac [111,112], cryIAa3 [113], cry2Aa [114], cryIAC [115], ASAL [116], cry1Ab [117], α AI1 [118] and α -amylase inhibitor genes [119] improved resistance of chickpea to insect pests. Particularly, Badhan et al. [102] showed also that CRISPR/Cas9 NHEJ was efficient to edit the chickpea genome and knock out the 4CL and RVE7 genes in protoplasts, giving clues that chickpea genome editing is viable but still depends on good plant regeneration protocols.

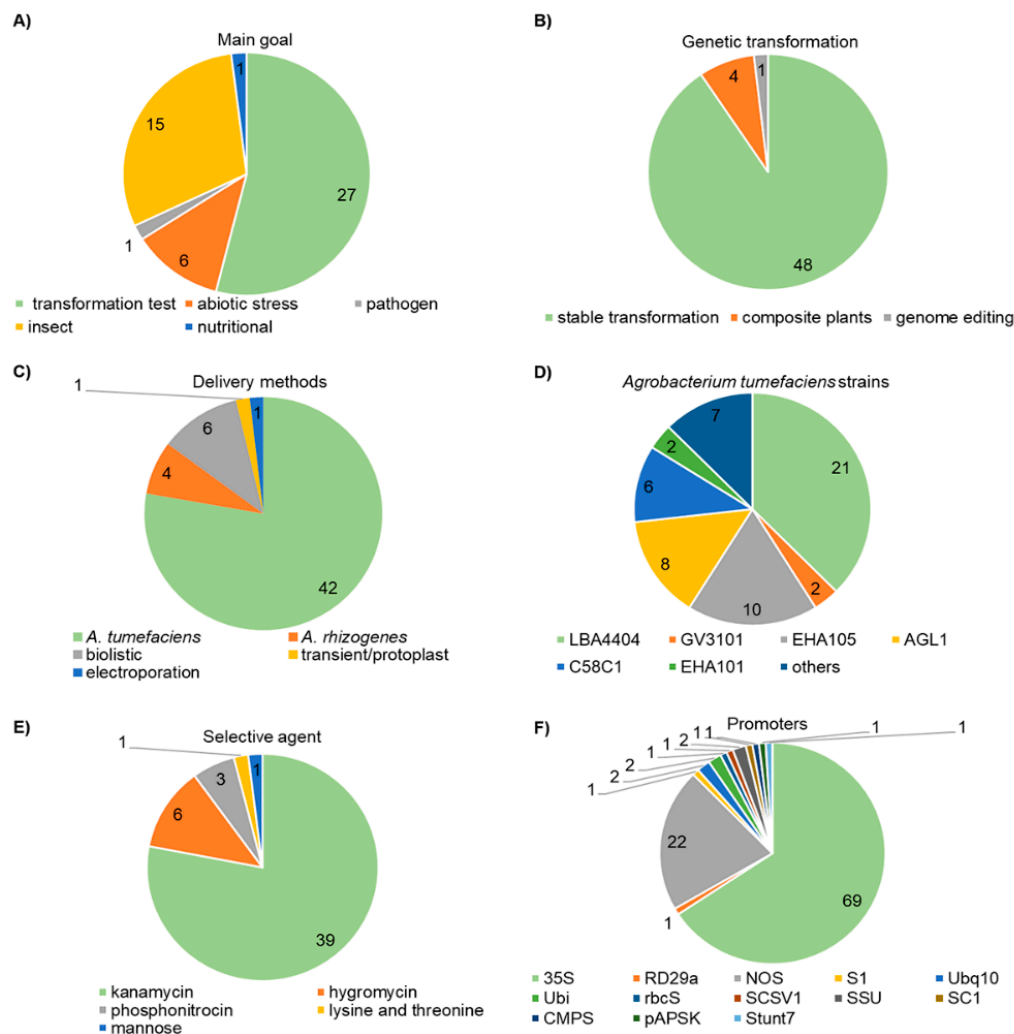


Figure 2. Brief overview of the challenges and focuses given in research involving genetic transformation and genome editing of chickpea (*Cicer arietinum*) worldwide. (A) Main goals of transformation and genome editing; (B) genetic transformation strategy; (C) delivery methods of DNA; (D) *Agrobacterium tumefaciens* strains used in genetic transformation; (E) selective agent used in tissue culture; (F) promoter sequences used in controlling the expression of target genes. The data presented here are information mined in Table 2, ranging from 1991 to 2022 ($n = 49$, published articles).

Given these data, it becomes increasingly important to choose the appropriate methods to be used for genetic transformation or genome editing of chickpea, strategies for delivering genetic elements into plant cells or tissues, and the best types of tissues or explants to be used for genetic transformation or editing, the most effective in vitro selection agents, as well as the most appropriate and strategic genetic elements to compose the transcription units present in the minimal expression cassettes or in the CRISPR/Cas9 constructs so that the objective can be effectively achieved [120]. Therefore, the genetic transformation of chickpea embryonic axes or organogenic or embryogenic callus mediated by *A. tumefaciens* strains LBA4404, EHA105 or AGL1, in vitro selection with kanamycin, hygromycin or

ammonium-gluphosinate, under cultivation in medium containing MS or Gamborg B5 salts and vitamins, and different hormones for plant regeneration such as thidiazuron, isopentenyl adenine (2ip), and 6-benzylaminopurine (BAP) are recommended. In contrast, several advances still need to be made in chickpea with genome editing, improving genome editing efficiency when used as a basis the transgenesis to anchor the CRISPR/Cas9 system in the plant genome, testing and optimizing viral vectors carrying the full CRISPR/Cas9 system, testing other nucleases such as Cpf1, establish and improve genome editing using DNA-free strategies (non-transgenic) in callus masses, both procedures coupled with a good regeneration protocol of edited plants. Furthermore, it is extremely important and indispensable to invest significant efforts in basic research to find powerful target genes to be regulated or edited to obtain improved agronomic traits with minimal resources. However, there are several candidate genes already characterized in plant species phylogenetically related or close to chickpea, such as *Medicago truncatula* and *Arabidopsis thaliana*, that can be used either in transgenic manner or to be targeted for editing via CRISPR/Cas9 [121].

Table 2. Timeline from 1991 to 2022 of genetic transformation and genome editing of chickpea (*Cicer arietinum*) worldwide.

Chickpea Cultivar	Delivery Method	Selectable Marker Gene	Promoter	Selective Agent	Reporter Protein	Target Gene	Plant Tissue	Improved Trait	TE (%)
ICCV2, ICCV10, ICCV92944, ICCV37, JAKI9218, and JG11 [122]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	35S:: <i>uidA</i> 35S:: <i>nptII</i>	kanamycin	GUS	<i>uidA</i>	embryonic axes	genetic transformation test	4.6 to 8.6
DCP 92-3 [103]	<i>A. tumefaciens</i> strain GV3101	<i>nptII</i>	<i>rd29a::AtDREB1a</i> 35S:: <i>nptII</i>	kanamycin	unused	<i>AtDREB1a</i>	cotyledons with half embryonic axes	tolerance to water deficit	0.1
ICC283 and/or ICC8261 [102]	DNA-free CRISPR/Cas9 NHEJ	unused	Cas9::NLS gRNA	unused	unused	4CL RVE7	protoplast	genome editing test and drought tolerance improvement	non-informed
ICCV89314 [123]	<i>A. tumefaciens</i> strain EHA105	<i>nptII</i>	NOS:: <i>nptII</i> 35S:: <i>uidA</i>	kanamycin	GUS	<i>uidA</i>	plumular meristem	genetic transformation test	44
HatTrick [105]	<i>A. tumefaciens</i> strain AGL1	<i>nptII</i>	35S:: <i>uidA</i> S1:: <i>nptII</i> 35S:: <i>GmFerritin</i> NOS:: <i>CaNas2</i> NOS:: <i>OsNas2</i> 35S:: <i>DsRed</i>	kanamycin	GUS	<i>GmFerritin</i> , <i>AtBAG4</i> , <i>TIBAG</i> , <i>CaNas2</i> , and <i>OsNas2</i>	half-embryos	stress tolerance and grains biofortification	0.66 to 2.1
ICC4958, BDG2 56, ICC17258, ICC1885, ICC8261, and local varieties [124]	<i>A. rhizogenes</i> strain R1000, ARqua1, and MSU440	<i>nptII</i>	35S:: <i>uidA</i> 35S:: <i>nptII</i> <i>Ubiq10::mCherry</i> others	kanamycin	GUS mCherry DsRed GFP	several genes	seedlings	genetic transformation test	50
Annigeri, C235, CPS 1, JG-62, K850, Vijay, and WR-315 [125]	<i>A. rhizogenes</i> strain K599	unused	35S: <i>AtTT2::GFP</i>	unused	GFP	<i>AtTT2</i>	chickpea hairy roots	resistance to pathogen	72.5 to 73.3 23.5 to 61.6
Annigeri 1 [109]	<i>A. tumefaciens</i> strain EHA105	<i>nptII</i>	35S:: <i>cryIIAa</i> NOS:: <i>nptII</i>	kanamycin	unused	<i>cryIIAa</i>	embryonic axes	resistance to insect	6.62 to 16.12
C235 [126]	<i>A. tumefaciens</i> strain EHA105	<i>nptII</i>	35S:: <i>nptII</i> 35S:: <i>uidA</i>	kanamycin	GUS	<i>uidA</i>	apical meristem explants	genetic transformation test	1.2
non-informed [106]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	35S: <i>CaHDZ12</i> 35S: <i>CaWRKY70</i> 35S:: <i>uidA</i> NOS:: <i>nptII</i>	kanamycin	GUS	<i>CaHDZ12</i> <i>CaWRKY70</i>	non-informed	abiotic stress tolerance	non-informed

Table 2. Cont.

Chickpea Cultivar	Delivery Method	Selectable Marker Gene	Promoter	Selective Agent	Reporter Protein	Target Gene	Plant Tissue	Improved Trait	TE (%)
DCP92-3 [103]	<i>A. tumefaciens</i> strain EHA105	<i>nptII</i>	35S:: <i>cry1Aabc</i> NOS:: <i>nptII</i>	kanamycin hygromycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin kanamycin	unused	<i>cry1Aabc</i>	decoated seeds	resistance to insect	0.076
ICCV89314 [112]	<i>A. tumefaciens</i> strain AGL1	<i>nptII</i>	<i>Ubi::cry1Ac</i> 35S:: <i>cry1Ac</i> <i>rbcS::cry1Ac</i> 35S:: <i>nptII</i> 35S:: <i>uidA</i>		GUS	<i>cry1Ac</i>	non-informed	resistance to insect	0.8 to 1.72
ICCV-2 [127]	<i>A. tumefaciens</i> strain C58C1	<i>hptII</i>	35S:: <i>hptII</i> 35S:: <i>uidA</i>		GUS	<i>uidA</i>	cotyledonary node	genetic transformation test	2.3
C235 and HC1 [113]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	35S:: <i>cry1Ac</i> NOS:: <i>nptII</i>		unused	<i>cry1Ac</i>	soaking sterilized seeds	resistance to insect	13.4 to 41
C235, BG 256, P362, and P372 [128]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	35S:: <i>uidA</i> NOS:: <i>nptII</i>		GUS	<i>uidA</i>	immature cotyledon	genetic transformation test	1.6 to 2.08
Bch-4 and Bch-5 [129]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	NOS:: <i>nptII</i> 35S:: <i>uidA</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	non-informed
C235 [130]	<i>A. tumefaciens</i> strain EHA105	unused	35S:: <i>cryIAa3</i>		unused	<i>cryIAa3</i>	soaking sterilized seeds	resistance to insect	non-informed
two kabuli and two desi [131]	biolistic	<i>nptII</i>	NOS:: <i>nptII</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	non confirmed
P-362 [111]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	35S:: <i>uidA</i> 35S:: <i>cry1Ac</i>		GUS	<i>cry1Ac</i>	callus derived from mature embryonic axes	resistance to insect	3.6
Jimbour [132]	<i>A. tumefaciens</i> strain AGL1	<i>PAT/bar nptIII</i>	35S:: <i>uidA</i> 35S:: <i>PAT/bar</i> <i>SCSV1::nptIII</i> <i>SSU::cry1Ac</i>		GUS	<i>uidA nptIII</i>	embryonic axes	genetic transformation test	0.37 to 4.3
Annigerig [107]	<i>A. tumefaciens</i> strain LBA4404	<i>hptII</i>	35S:: <i>hptII</i> 35S:: <i>P5CS</i> 35S:: <i>uidA</i>		GUS	<i>P5CS</i>	cotyledonary nodes	salt tolerance improvement	non-informed
Semsen and ICCV 89314 [114]	<i>A. tumefaciens</i> strain AGL1	<i>nptII</i>	<i>SSU::cry2Aa</i> <i>SCI::nptIII</i>		unused	<i>cry2Aa</i>	embryonic axes with half of the cotyledon stems, epicotyls, and embryonal axes	resistance to insect	0.3
Chaffa, PG12, ICC37, and ICC32 [115]	biolistic and <i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	NOS:: <i>nptII</i> 2x35S:AMV:: <i>cry1Ac::uidA</i>		GUS	<i>cry1Ac</i>	cotyledonary node-derived calli and embryo axes	resistance to caterpillar	5 to 16
Pusa-256, KWR-108, Pusa-1003, and non-informed local lines [133]	<i>A. tumefaciens</i> strain EHA105, AGL1, and LBA4404	<i>hptII</i>	35S:: <i>uidA</i> 35S:: <i>hptII</i>		GUS	<i>uidA</i>	cotyledonary node-derived calli and embryo axes	resistance to insect	0.11 to 25.5
C235 [134]	<i>A. tumefaciens</i> strain C58C1	<i>nptII</i>	35S:: <i>P5CSF129A</i> 35S:: <i>nptIII::uidA</i>	GUS	<i>P5CSF129A</i>	axillary meristem	drought tolerance improvement	70	

Table 2. Cont.

Chickpea Cultivar	Delivery Method	Selectable Marker Gene	Promoter	Selective Agent	Reporter Protein	Target Gene	Plant Tissue	Improved Trait	TE (%)
ICCV 89314 [116]	<i>A. tumefaciens</i> strain AGL1	<i>nptII</i>	35S::ASAL 35S::uidA 35S::nptII	kanamycin hygromycin kanamycin mannose kanamycin	GUS	ASAL	embryonic axes with half of the cotyledon	resistance to insect	0.066
C235 [134]	<i>A. tumefaciens</i> strain GV3101	<i>pmi</i>	CMPS::pmi		unused	<i>pmi</i>	embryonic axes	genetic transformation test	3
Gökçe, Er, Akçin, Uzunlu, and Küsmen [135]	<i>A. tumefaciens</i> strain C58C1, EHA105, and KYRT1	<i>nptII</i>	NOS::nptII 35S::uidA		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	non-informed
ICC10943 and ICC10386 [136]	sonication and <i>A. tumefaciens</i> strain LBA4404	<i>hptII</i>	35S::CS::uidA 35S::hptII		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	9 to 26
C235, BG 256, Pusa 362, and Pusa 372 [137]	<i>A. tumefaciens</i> strain GV2260, GV3850, LBA4404, and EHA105	<i>nptII</i>	?::uidA ?::uidA		GUS	<i>uidA</i>	cotyledonary nodes	genetic transformation test	1.12
C235 and HC1 [138]	<i>A. tumefaciens</i> strain LBA4404	<i>hptII</i>	35S::hptII Ubi::cry1Ab ?::cry1Ac		unused	<i>cry1Ab</i> and <i>cry1Ac</i>	embryonic axes	resistance to insect	4.92 to 7.7
K850 [119]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	pAPSK::αAII 35S::nptII 35S::uidA		GUS	<i>α-amylase inhibitor</i>	embryonic axes	resistance to insect	0.3
C235 [139]	<i>A. tumefaciens</i> strain C58C1	<i>nptII</i>	NOS::nptII 35S::uidA		unused	<i>nptII</i>	axillary meristem	genetic transformation test	70
C235, BG256, Pusa 362, and Pusa 372 [140]	<i>A. tumefaciens</i> strain LBA4404, EHA105, GV3850, and GV2260	<i>nptII</i>	35S::cry1Ac NOS::nptII 35S::uidA		GUS	<i>cry1Ac</i>	Cotyledonary nodes	resistance to insect	0.32 to 1.12
CDC Yuma [141]	<i>A. tumefaciens</i> strain EHA105	<i>nptII</i>	2x35S::uidA::nptII		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	1.3
P-362, P-1043, and P-1042 [142]	biolistic and <i>A. tumefaciens</i> strain EHA101	<i>PAT/bar</i> , <i>nptII</i> , and desensitized AK gene	35S::PAT/bar 35S::TP::AK 35S::uidA NOS::nptII	kanamycin phosphonitrocin kanamycin lysine and threonine, phosphonitrocin kanamycin	GUS	<i>uidA</i> AK	embryonic axes with half of the cotyledon	genetic transformation test	0.5 to 1.3
H208, ICCL87322, K850, Annigeri, and ICCV5 [143]	<i>A. tumefaciens</i> strain AGL1, C58C1, and LBA4404	<i>PAT/bar</i>	35S::PAT/bar 35S::TP::AK 35S::PGIP		GUS	<i>uidA</i> PGIP	embryonic axes	genetic transformation test	2 to 13.3
Gökçe, Akçin 91, and Izmir 92 [144]	<i>A. rhizogenes</i> strain 15834	<i>nptII</i>	?::nptII		unused	<i>nptII</i>	growing tender shoots	genetic transformation test	5 to 80

Table 2. Cont.

Chickpea Cultivar	Delivery Method	Selectable Marker Gene	Promoter	Selective Agent	Reporter Protein	Target Gene	Plant Tissue	Improved Trait	TE (%)
Semsen [118]	<i>A. tumefaciens</i> strain AGL1	<i>nptII</i>	<i>Stunt7::nptII</i>	kanamycin or phosphonitrocin kanamycin	unused	<i>αAII</i>	embryonic axes with half of the cotyledon	resistance to insect	0.56
C235, BG256, Pusa 362, and Pusa 372 [145]	biolistic and <i>A. tumefaciens</i> strain LBA4404	<i>nptII hptII</i>	<i>NOS::nptII</i> <i>35S::uidA</i> <i>35S::hptII</i>		GUS	<i>uidA</i>	embryonic axes and cotyledonary axes	genetic transformation test	0.05 to 0.8
PG1, PG12, and Chafa [146]	<i>A. tumefaciens</i> strain C58C1, GV2260 and EHA101	<i>PAT/bar nptII</i>	<i>35S::uidA</i> <i>35S::PAT/bar</i> <i>NOS::nptII</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	0.2 to 1.5
6153 and CM72 [147]	biolistic	<i>nptII</i>	<i>35S::uidA</i> <i>NOS::nptII</i>		GUS	<i>uidA</i>	hypocotyl segments	genetic transformation test	non-informed
ICCV1 and ICCV6 [148]	biolistic	<i>nptII</i>	<i>35S::cry1Ac</i> <i>?::nptII</i>		unused	<i>cry1Ac</i>	embryonic axes	resistance to insect	non-informed
Red chickpea, Canitez 87, and MB10 [149]	<i>A. tumefaciens</i> strain LBA4404 and <i>A. rhizogenes</i> strain 9402	<i>nptII</i>	<i>NOS::nptII</i> <i>35S::uidA</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	6.4 to 12.7 5.3 to 10.4
ICCV1 and ICCV6 [150]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	<i>35S::nptII</i> <i>35S::uidA</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	1.16 to 1.96
ICC4918 [151]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	<i>NOS::nptII</i> <i>35S::uidA</i>		GUS	<i>uidA</i>	immature cotyledon	genetic transformation test	non-informed
Italian cultivars [152]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	<i>NOS::nptII</i> <i>35S::uidA</i>		GUS	<i>uidA</i>	embryonic axes	genetic transformation test	4
non-informed [152]	<i>A. tumefaciens</i> strain LBA4404	<i>nptII</i>	<i>NOS::nptII</i> <i>35S::uidA</i>		GUS	<i>nptII</i> and <i>uidA</i>	embryo axes	genetic transformation test	non-informed
Pusa256 [8]	<i>A. tumefaciens</i> strain R1601	<i>nptII</i>	<i>?::nptII</i>	unused	<i>nptII</i>	leaf and stem explants	genetic transformation test	non-informed	

?: information not available; TE-transformation efficiency.

4. Next Steps—Breeding Approaches

Genomic analyses have revealed that most chickpea breeding lines lack some desirable traits that are present in old varieties, called landraces, grown by farmers for hundreds of years. An international team, including researchers in Egypt and Morocco, have sequenced more than 3300 chickpea genomes to examine genomic diversity across various wild and cultivated chickpea strains. This allowed the researchers to trace the history of chickpea domestication and diffusion from its origin in the Fertile Crescent to other parts of Asia and Africa [24]. The team identified 1582 novel genes, including some that might be

helpful in enhancing chickpea resistance to drought, temperature stresses and diseases. The researchers also compared genomic data with crop performance in six locations in India and found favourable sets of DNA variations in landraces that are not present in 80% of cultivated varieties, as well as undesirable mutations responsible for reducing crop yield. The team proposed three breeding approaches that could improve the 100-seed weight—an important yield-related trait—by up to 23%. They involve introducing genomic variations that could improve specific traits in cultivated varieties, improving overall crop performance by choosing the best lines based on genome profiling data, and selecting parent plants that provide a good balance between crop performance and genetic diversity.

ICRISAT's chickpea researchers have developed a breeding protocol that holds the potential to create new varieties of chickpeas in half-time. The Rapid Generation Advancement (RGA) protocol allows the production of six to seven generations of chickpea in a year under controlled greenhouse conditions. Generation time is a game changer for achieving maximum genetic gains in crop plants. Generally, it takes seven to eight years to develop homozygous (identical) lines after hybridization with one crop generation produced per year. Given the growing need for food and nutrition and the mounting pressures of climate change, the demand for improved varieties is more pressing today than ever. RGA in chickpea produces up to seven generations per year and enable speed breeding.

Over 350 improved varieties of chickpea have since been released globally, and about half of these have been released in India, which accounts for about two-thirds of global chickpea production and has the largest national chickpea breeding program in the world. The two international institutes established by the Consultative Group of International Agricultural Research (CGIAR), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (established in 1972) and International Centre for Agricultural Research in the Dry Areas (ICARDA) (established in 1977), have provided a boost to chickpea breeding programmes of national agricultural research systems (NARS) globally through supply of germplasm and improved breeding materials.

The following goals have been undertaken by breeding activities for chickpea that could be related to better drought and heat resistance:

Early maturity—Early maturity is important for spring and autumn-sown rainfed crops in Mediterranean-type environments (e.g., Australia) to avoid terminal drought. The chickpea area under late-sown conditions is increasing in south Asia, particularly in India. In these conditions, early maturity will be the key trait needed to avoid end-of-season drought and high-temperature stress [153].

Drought tolerance—Terminal drought or end-of-season drought is the most important constraint to chickpea production, accounting for 40 to 50% of the yield reduction globally. The development of early maturing varieties has been the most effective strategy for escape from terminal drought. Thus, the need for trait-based selection has been emphasized. Efforts have been made to identify plant traits for drought tolerance and incorporate these traits into well-adapted varieties. Breeding for high root mass is very difficult due to the laborious methods involved in digging and measuring root length and density. Molecular markers closely linked with major quantitative trait loci (QTLs) controlling root traits can facilitate marker-assisted selection (MAS) for root traits. Varshney and his collaborators set out to breed new varieties of chickpeas with drought tolerance and higher yields. They used genetic techniques to breed several traits for drought tolerance. They focused on popular chickpea varieties already grown by farmers.

Low temperature—Freezing (mean daily temperature < -1.5 °C) and chilling temperatures (mean daily temperature between -1.5 to 15 °C) are important constraints to chickpea production in some regions. A pollen selection method was developed in Australia and applied to transfer chilling tolerance from ICCV 88516 to chilling sensitive cultivars, leading to the development and release of chilling tolerant cultivars "Sonali" and "Rupali". These were used successfully to select chilling tolerant progeny from a cross between Amethyst and ICCV 88516 but were ineffective in other crosses [154].

Nutritional quality—Legumes are a source of energy food and healthy foods to eradicate malnutrition from millions of thousands in the developing world especially Bangladesh, Myanmar, India, Pakistan, etc. in Asia and African countries those crops need to be popularized. However, some antinutrient factors like tannin, phytic acid and enzyme inhibitors are also available in legumes. Therefore, legume crops like chickpea, which is a healthy food crop, contain many rich nutrients like iron, zinc, calcium, fibre, proteins, vitamins and carotenoids should be properly investigated. Due to the presence of those nutrients, it is considered medicinal as it possesses anti-diabetic and anti-cholesterol factors. There is a need to search the new gene pools for quality traits through molecular marker selection. There has been negligible input into the improvement of nutritional quality. The protein content of existing cultivars is generally in the range 18–22% but much larger variability (12.4–32.5%) exists in the cultivated and wild species, and this could be exploited to breed higher protein (~25%) varieties. The sulphur-containing amino acids methionine and cystine are the first limiting amino acids. Transgenic technology is being used to enhance the level of sulphur-containing amino acids because the required variation is absent from the primary gene pool. Transgenics developed by introducing a seed-specific chimeric gene encoding sunflower seed albumin (SSA) produced 24 to 94% higher methionine, but 10 to 15% lower cysteine than comparable non-transgenic chickpeas [118].

5. Conclusions

Chickpea, as an economically important crop, is under the influence of global climate change and its yield is severely affected by the rise in temperatures and drought. In the past decade, a significant amount of research deals with the identification of genes that could promote a higher tolerance to drought and high temperatures, ensuring stable productivity in stressed conditions. Different strategies are included in the process of chickpea cultivation to alleviate stress effects, including supplementation with Zn, some new approaches such as the utilisation of priming to the established primed state and higher drought tolerance, as well as some genetic transformation approaches. From the breeding perspective, making the selection from the varieties with a higher tolerance of heat and drought is an enormous task and the testing of large number of varieties has already been undertaken. To ensure that the current trend of early onset of high temperature and drought do not disturb chickpea growth and flowering, breeding strategies should aim for traits related to earlier senescence, forcing podding and seed filling into earlier stages; thus, avoiding drought periods.

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