Heat Stress Tolerance in Cowpea

Subjects: Plant Sciences

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Heat stress is often described as a condition of high temperatures that are sufficient to cause permanent damage to plant processes, including shortening the time for photosynthetic contribution to seed production. Heat stress on most plants can impact functions through the direct effects of high tissue temperature or the indirect consequences of the high evaporative demand accompanying hot weather. Understanding the impact of heat stress is crucial for plant breeding because it relates to key adaptive, biochemical, morphological, physiological, and reproductive processes. Despite its ability to thrive in high-temperature environments, cowpea productivity can be hampered by heat stress, particularly when night air temperatures exceed 17 °C. The crop's germplasm pool potentially possesses significant genetic variability that can be harnessed to breed for heat-tolerant varieties.

Keywords: cowpea ; heat stress ; high temperatures ; tolerance ; genetics ; breeding

1. Introduction

Crop productivity worldwide is sensitive to significant changes in temperature and precipitation [1][2][3]. Since 1880, average global temperatures have increased by about 1 °C and are predicted to increase by about 1.5 °C by 2050 and 2–4 °C by 2100 [4][5], necessitating the need to deploy heat-tolerant varieties that can produce greater yields under higher temperatures than the current level [1][2][6]. Theoretically, changes in climatic conditions, such as rising temperature, precipitation, and CO_2 concentrations, could be beneficial by permitting the cultivation of some crops in certain regions [1][7]. However, these changes will negatively affect the productivity of many crops in most geographies, unless adaptation measures are taken [1]. Plants with the C3 photosynthetic pathway, such as cowpea (*Vigna unguiculata* (L.) Walp), should experience increases in photosynthesis with increases in atmospheric CO_2 [8][9]. The crop is a crucial food and nutritional security crop for humans and livestock and serves as a source of income for its value-chain actors, especially in sub-Saharan Africa (SSA) [10]. The utilisation of the crop is expected to rapidly increase in popularity due to its high protein content and relative resilience in harsh conditions compared with some other legumes. Despite the importance of the crop, its yield under farmers' managed conditions is low, owing to a series of biotic and abiotic constraints [10][11].

Heat stress is one of the major abiotic factors limiting cowpea productivity, and it is predicted to be more prevalent with current changing climatic conditions. Improved varieties with resilience in a changing climate and a set of characteristics preferred by value-chain actors are needed in cowpea-producing regions. The crop thrives in relatively high-temperature environments [6][12][13] compared with other legumes. However, during its reproductive phase, cowpea is especially vulnerable to heat stress, resulting in significant yield losses [6][14][15], even though most genotypes have substantially elevated temperature tolerance during the germination and vegetative phases [15][16][17]. A 1 °C increase in night temperature above 16.5 °C between seedling emergence and first flowering has been observed to cause up to a 13.6% decrease in cowpea grain yield [18][19]. If the night temperature exceeds 20 °C, cowpea's pollen viability and anther dehiscence are greatly impaired, which could lead to a significant decrease or complete failure of the pod set [15][20][21]. Heat-susceptible genotypes have exhibited a 12% decrease in first-flush grain yield per degree centigrade increase in average night temperature above 20 °C due to decreases in pod set and harvest index [18]. Similarly, a 4–14% decrease in both pod set and grain yield per degree Celsius increase in night-time temperature above a threshold of 16.5 °C has been observed [19][22]. These yield decreases were attributed to reductions in the proportions of flowers producing pods and the harvest index.

2. Impacts of Heat Stresses and Tolerance Mechanisms in Cowpea

Heat stress is often described as a condition of high temperatures that are sufficient to cause permanent damage to plant processes [3], including shortening the time for photosynthetic contribution to seed production [21][23]. Heat stress on most plants can impact functions through the direct effects of high tissue temperature or the indirect consequences of the high evaporative demand accompanying hot weather [6][23]. Understanding the impact of heat stress is crucial for plant breeding because it relates to key adaptive, biochemical, morphological, physiological, and reproductive processes (**Figure 1**), including molecular changes that adversely affect plant growth, productivity, and ultimately yield [2][3][4]. Identifying the specific plant processes most susceptible to heat stress, whether it damages the photosynthetic source, reproductive sink, or both, is critical because it will determine which selection criterion will most likely enhance a species' heat tolerance [6].

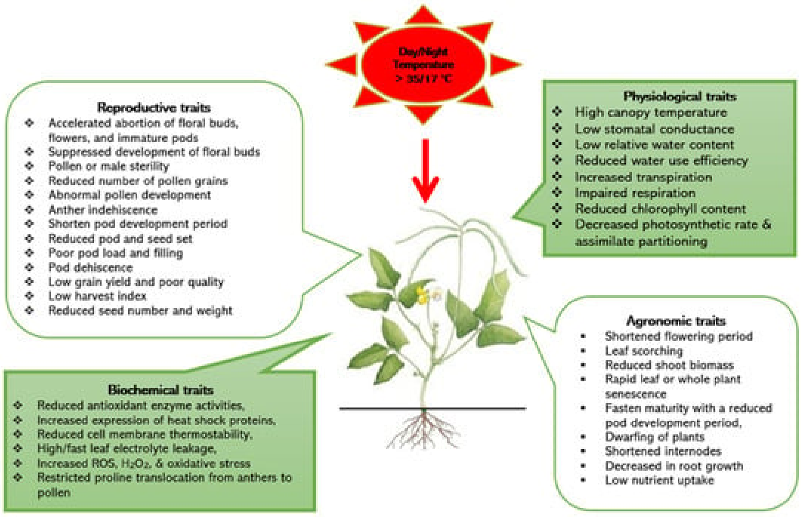

Figure 1. Representative list of traits, processes, and functions impacted by heat stress in cowpea.

The magnitude of high temperature's impact on plants depends on the intensity, length of exposure, and rate of temperature increase because many crop plants possess the ability to adapt to temperature conditions above the threshold for some time [3][6][12]. From seedling to maturity, prolonged exposure to heat stress may increase its severity and trigger different response mechanisms than heat stress imposed at a particular developmental stage. Therefore, applying heat stress throughout the crop's development cycle may be advisable to better understand the physiological and genetic basis of cowpea's response to heat stress. High temperatures could impact seed germination and seedling survival for cowpea, especially when seeds are sown deep into the soil [24] and in soils with high salinity [17]. Even when cowpea seeds can survive up to 50 °C day temperatures with an adequate water supply and still produce substantial vegetative biomass [6], their vegetative growth can be impacted negatively by heat stress [9][15][21]. Elevated night temperatures damage various reproductive processes in cowpea (**Figure 1**), such as floral bud development, pollen viability, anther indehiscence, embryo formation, pod set, and seed development, including abscission and/or suppression of floral buds, peduncles, flowers, and pods [15][20][25], resulting in low flower numbers, pod set, and grain yield [9][24][26].

The stage of the floral development most sensitive to high night temperature occurs 7–9 days before anthesis [15][16]. Similarly, high night-time temperatures reduced the supply of sugars in the peduncles of heat-sensitive genotypes, resulting in poor pod sets [26]. Heat-sensitive genotypes have been observed to experience restricted translocation of proline from anther walls to pollen under heat stress, which damages the reproductive organs [27]. The low pollen viability, anther indehiscence, and poor pod set under high night temperatures have been attributed to premature degeneration of the tapetal layer and a lack of endothecium development [20]. Later reports have demonstrated that complete suppression of the development of the floral buds could occur when there are two or more weeks of consecutive higher night temperatures during the first four weeks following germination [26]. Earlier investigations documented that heat-induced suppression of floral buds occurs only under long days [28]. However, certain genotypes exhibited suppression of floral buds, bud abortions, and retarded peduncle elongation during recent field screening conducted under high day and night temperatures (see **Figure 2**) and short days at the Minjibir location in Nigeria. These genotypes produced very few or no flowers or pods, a behaviour thought to be occurring only under long-day conditions (Saba 2023, IITA, Kano, Nigeria, Personal observation).

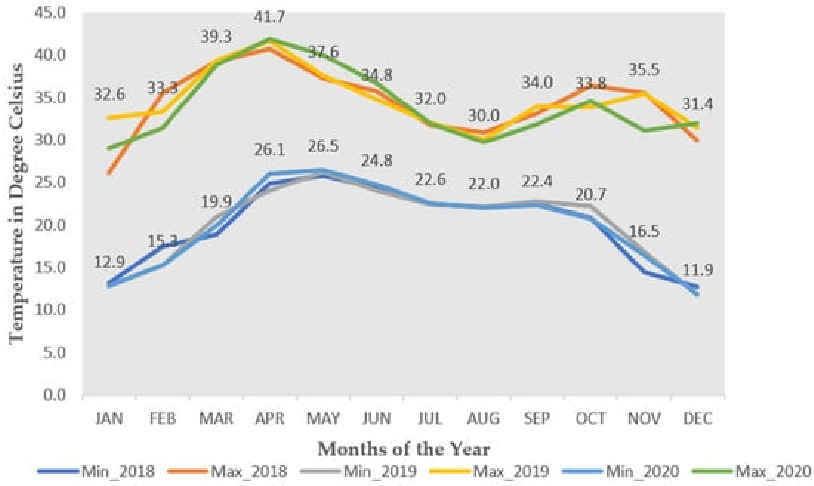

Figure 2. Average monthly minimum and maximum temperature of typical cowpea production zones in Minjibir, Kano State in Northern Nigeria.

Heat stress tends to shorten some genotypes' flowering and maturity time, which has a penalty on the yield. However, genotypes that initiate flowering before the onset of extreme heat may evade the adverse effects of such high temperatures [29]. Rapid leaf senescence and maturity have been observed during pod filling in heat-sensitive genotypes when night temperatures are high [9][25][30], resulting in decreased photosynthetic activity and yield because the plants tend to divert resources to deal with thermal stress, thereby limiting the resources for reproductive development [21][31]. Thus, varieties that display delayed leaf senescence under heat stress conditions may possess more effective tolerance mechanisms [32]. In addition, higher day and night temperatures significantly shorten the pod development period in both heat-sensitive and tolerant genotypes [15][30], which reduces the time for pod filling and assimilates partitioning, ultimately reducing the varieties' grain yield. Heat-susceptible genotypes experienced delayed flowering at night temperatures above 20 °C, likely due to heat-induced floral bud suppression or abortion, whereas heat-tolerant genes were found to enhance early pod production in hot environments by accelerating reproductive development and increasing pod set [18].

Tolerance to heat stress can be attributed to two main factors: avoidance and tolerance. While heat avoidance involves the ability of plant tissues to maintain lower temperatures compared with control plants when exposed to elevated temperatures, heat tolerance refers to the plant's ability to sustain essential functions even when its tissues are exposed to high-temperature conditions [24]. Heat avoidance mechanisms encompass several processes, including transpirational cooling, leaf orientation and movement effects, variances in the reflection of solar radiation, and the shielding of sensitive tissues from sunburn through leaf shading [24]. Three heat tolerance mechanisms have been described in regard to the above impacts of high temperatures on cowpea's reproductive development [29]. These are tolerance at the early floral bud stage that conferred the ability to produce flowers under hot, long-day (≥13 h day^{-1}) conditions [33], which is influenced by phytochrome [28]; tolerance during pollen and anther development that conferred the ability to set pods under high night temperatures [15]; and tolerance during embryo development that conferred the ability to produce large numbers of seeds per pod under high day or night temperatures [16].

3. Variability in Germplasm, Genetics, and Genomic Resources for the Improvement of Heat Stress Tolerance

Successful development of heat-tolerant varieties begins with the identification of sources of favourable alleles [34]. Genotypic differences in cowpea germplasm for heat stress have been established, and studies have identified specific heat-tolerant lines, mostly under hot, long-day conditions (**Table 1**). Examples of the exploration of genetic resources include Patel and Hall (1990) [35], who assessed responses to high temperatures during the reproductive stage in hot fields and growth chambers and developed a genotypic classification system based on observed variations in floral bud emergence duration, abortion, peduncle elongation suppression, flower production, and podding among genotypes under long-day conditions (41/24 °C day/night) [35] and grouped the accessions into eight categories based on these traits, including whether the peduncle had normal or suppressed elongation. The classification system provides insights into heat responses in genotypes that will aid in selecting parents for breeding heat-tolerant genotypes. Similarly, Ehlers and Hall (1996) [36] evaluated African and USA genotypes under various temperatures and photoperiod conditions in the glasshouse, categorizing them into 11 groups based on photoperiod response, juvenility (minimum time taken for the appearance of floral buds under short days), and suppression of floral bud development and pod set under hot, long days. These kinds of classification systems are valuable for breeders and agronomists, as they can help understand the genetic variations related to these traits and aid in selecting appropriate genotypes with desired heat response traits for breeding programs targeting tropical and subtropical production environments [36]. An example of the utility of this kind of finding is the registration of a cowpea genotype "Mouride" in Senegal as a heat-tolerant variety based on its earliness traits, reaching physiological maturity at 65 days, helping it to escape heat stress [37].

Table 1. A representative list of heat-tolerant genotypes, traits assessed, and screening environments from various studies.

No.	Tolerant Lines	Key Traits Assessed [1]	Screening Environments	References
1	Prima	DTF, DTM, NOB, NPB, FP, NPP, PS, PP, and PDW	Growth cabinets	[12]
2	TVu 4552, Prima, PI 204647	DTF, NFA, pollen viability, PCA, SR, ovule viability, NFDA, NFIA, IA, FPSB; NP SPP and other yield components	Hot, long-day field and growth chambers	[15]
3	Prima, TVu 4552, UCR 204, PI 204647, 750-1, IT84D-448, IT84D-449, IT84S-2127, 7964	Days to first macroscopic floral bud, DTF, the extent of floral bud abortion, PDL, and PS	Hot field and growth chambers	[35]
4	IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25	Pod and grain yield traits	Hot field	[34]
5	Epace 10 and Marataoã	Germination, shoot and root length, and seedling dry weight	Germination chamber	[17]
6	TVu4552 and Prima	Flower abscission (%), PP, SDWT, NSPP, and GYD	Field supplemented with thermostats	[30]
7	Itaim	DTF, DTM, physiological and biochemical traits, SDW, RDW, GYD, PDWT, PDL, PL, NPP, and NSP	Growth chambers	[21]
8	Tapaihum	PP, SPD, SDWT, SFW, and SDW	Growth chambers	[25]
9	IT96D-610	Heat-shock proteins and other stress-protective proteins	Glasshouse and field	[38]
10	Genotype H36	Leaf electrolyte leakage, NPP, PP, PHT, HI, GYD, and SDW	Growth chambers, glasshouse, and hot field	[39]
11	IT97K-472-12, IT97K-472-25, IT97K-819-43, & IT97K-499-38	NF, PS, and GYD	Field	[40]
12	Genotype 7964	Phenology, floral, pollen, pod, and other reproductive traits	Greenhouses and growth chambers	[20]
13	NA *	Phenology, floral, pollen, NPD, PDL and PS traits	Growth chambers with supplemented lighting system	[26]
14	Genotypes 518 and 7964	Phenology; flower traits; PS; carbohydrate contents of the peduncle; starch in leaves, stems and peduncles; photosynthesis rate; leaf area; and shoot biomass yield	Growth chambers	[41]
15	TN88-63, A73-2-1 and TVx 3236	NF, PS, and GYD	Hot field	[42]
16	H36, 1393-2-1, H8-8-27, H8-14-12, H14-10-1N, and H35-5-10	PHT, SDW, NPP, SPP, SDWi, and HI	Field	[18]
17	CB27	Flower production, and PP	Hot field	[43]

No.	Tolerant Lines	Key Traits Assessed [1]	Screening Environments	References
18	TVu4552, Prima, H14-10-27, H14-10-23, H8-14-13, H8-8-4, H8-9-3, 518-2, B89-600, TN88-63 etc.	DFF, NPP, NP, and GYD	Greenhouses	[44]
19	Prima and TVu4552	NPP	Field	[45]
20	518-22, Prima, TVu4552, H8-9-3, H8-8-4, H8-14-13, H14-10-23, H14-10-27 etc.	Photoperiod response, DTF, PS, and GYD per plant	Field and glasshouses	[36]
21	IAR-48, GEC, IT98K-277-2, Yacine, and IT98K-1092-1	DTF, Visual heat ratings, SDWTPP, PDWT, and Weight of 100 seeds.	Field and glasshouse	[46]

DTF = days to flowering, DTM = days to maturity, FPSB = flowers with pollen on stylar brush, FP = flowers per peduncle, GYD = grain yield, HI = harvest index, IA = indehiscent anthers, NFA = number of flower abscissions, NFDA = number of flowers with only dehiscent anthers, NFIA = flowers with only indehiscent anthers, NOB = number of branches, NP = total number of pods, NPB = number of peduncles per branch, NPD = number of peduncles, PDL = peduncle length, NPP = number of pods per peduncle, PS = pod set (%), PP = number of pods per plant, PDW = pod weight per plant, PCA = percentage of closed anthers, SDW = shoot biomass, SDWi = individual seed weight, SR = stigma receptivity, SPP = number of seeds per pod. [1] Key traits used to assess heat stress tolerance in cowpea from literature. NA * indicate not available.

Understanding the nature and extent of gene actions for target and associated traits is essential to selecting appropriate breeding strategies and parental lines [47]. Traditional breeding approaches have been used to provide insights into the genetics of cowpea heat tolerance [24][48]. The inheritance of heat stress tolerance as a whole is complex. However, such a gross trait can be divided into simply heritable developmental traits conferred by one or two major genes [29]. Examples of such traits in cowpea include the number of flowers produced per plant, pods set per peduncle, seeds per pod, and seed coat browning under high day or night temperatures [24][29].

The inheritance of tolerance to heat stress during floral development is reported to be governed by a single recessive gene that is highly heritable [49], indicating that heat tolerance for flower production can be fixed by selection for abundant flower production in the F_2 generation [8][24]. Similarly, genetic analysis of pod set under hot, long-day field conditions revealed that a single dominant gene governs the trait. However, the narrow-sense (0.26) and realised (0.27) heritabilities associated with the trait were low, probably due to environmental effects [45]. The authors evaluated F_1 and segregating progenies of F_2 and backcross populations derived from crossing heat-tolerant and heat-sensitive genotypes and suggested that incorporating heat tolerance during pod set into other genetic backgrounds will require several cycles of family selection in advanced generations to fix the trait [29][45]. Some vital heat-tolerant accessions produced undesirable brown discolouration of seed coats when grown under hot-air environments, and a single dominant gene controls the genetics of the trait. It is also established that browning of the seed is not linked with normal brown seed coat pigmentation, nor is it linked to heat tolerance during early floral bud development [48]. The authors further established that heat-tolerant lines with no heat-induced seed coat browning can be recovered using parents with seed discolouration properties. On a general note, the effects of high temperatures on cowpea plants are considered additive and quantitative [12]. These authors argued that the susceptibility to heat stress was additive because the impact of high temperatures was cumulative on a sensitive genotype, as it recorded fewer peduncles per branch, fewer flowers per peduncle, and a reduced pod set.

Most earlier studies on the genetics of heat tolerance in cowpea were conducted under long days and mostly under controlled environmental effects, with very few studies demonstrating the effectiveness of heat tolerance genes under short-day controlled environments [12][44]. Cowpea in SSA is more likely to experience high day and night temperatures (>35/> 20 °C) under short days (<13 h day^{-1}) during the growing season (**Figure 2**), particularly at the start and end of the rains or during reproductive development across most growing regions of SSA countries [22][42]. Therefore, more empirical evidence is needed to shed light on the inheritance pattern of heat tolerance and its associated traits, especially deploying molecular markers to identify more precise quantitative trait loci (QTLs) and conducting such studies in major cowpea-producing hot, short-day environments.

Mendelian genetics has not satisfactorily addressed the complex inheritance of heat tolerance in cowpea, as pieces of evidence suggest multiple genes or QTLs likely govern it and may be influenced by genotype-by-environment (G × E) interaction [46][50][51]. Considerable genomic resources, including mapping populations, genotyping platforms, markers associated with various traits, and reference genome information, have been developed for cowpea that can be deployed by breeding programs to develop varieties with higher genetic gains [52][53][54]. Marker-assisted breeding for heat tolerance will require reliable QTL information, but few studies have identified QTLs associated with heat tolerance in cowpea, most of which have not been validated. QTLs are associated with heat tolerance in other important legume crops [55]. Recent results present evidence of QTLs controlling heat stress tolerance traits in cowpea (**Table 2**). For instance, pod set under heat stress was inherited quantitatively, with about five QTLs in a biparental RIL population [50]. In another study, three QTLs were associated with the seed coat's heat-induced browning in two RIL populations [51]. More recent work used another RIL population, contrasting for heat tolerance, and reported a few more QTLs associated with visual ratings of heat tolerance, seed weight per plant, and number of pods per plant under high-temperature conditions [46]. These QTL studies were based on bi-parental populations, which have limitations regarding recombination events and mapping resolution. In addition, most of these mapping studies were limited to controlled or single environments and did not investigate possible G x E interactions. However, association mapping, which is regarded as having higher mapping resolutions, has been used to identify significant markers associated with various traits related to stress tolerance [56][57][58] and could be deployed for more refined mapping of QTLs. In addition, genome-wide association studies could generate more reliable information on cowpea's genetic architecture of heat tolerance traits, including using the innovative cowpea MAGIC RIL population [59][60] and minicore [61], which can deliver higher mapping resolutions. This approach has yet to be explored for breeding heat tolerance in cowpea. Efforts to understand the cowpea genome have made substantial progress. Initially estimated at 620 Mb through flow cytometry [62], methylation filtration technology enabled the selective cloning of the gene-rich, hypomethylated segment, yielding over 250,000 gene-specific sequence reads [63]. Later, a whole-genome shotgun sequencing of var. IT97K-499-35 resulted in a 323 Mb assembly [52], while improved assembly sizes of 568 Mb and 609 Mb for vars. IT97K-499-35 and IT86D-1010 were achieved [64]. Lonardi et al. (2019) [54] released a reference genome (var. IT97K-499-35) with an assembly size of 519.4 Mb. Compared with the reference genome, recent de novo assemblies of six accessions unveiled a pan-genome with 80% core and 20% non-core genes, which will significantly enhance the understanding of the crop's genetic diversity. There is also evidence of specific transcription factors being useful for translational research and molecular breeding of cowpea, as overexpression of two native NAC genes (*VuNAC1* and *VuNAC2*) promoted germinative, vegetative, and reproductive growth and conferred multiple abiotic stress tolerance in a commercial cowpea variety, with such overexpressor lines having remarkable tolerance to major yield-limiting terminal stresses, such as cold, drought, heat, and salinity [65]. This wealth of genomic and transcriptomic data presents opportunities to identify stress-resilience genes, potentially uncovering mechanisms to enhance heat stress tolerance [66].

Table 2. Quantitative trait loci associated with heat-tolerance traits in cowpea.

No	Mapping Population and Size	Parent-1	Parent-2	Marker System	Trait Assessed	Study Environment	Number of QTLs Mapped	Chr	PVE (%)	Reference
1	F_8-RIL with 141 lines	CB27 (Heat tolerant)	IT82E-18 (Heat sensitive)	SNPs	Number of pods and peduncles	Greenhouse and field environments	Five	2, 7, 6, 10, and 3	18.1, 17.1, 16.2, 16, and 11.5	[50]
2	F_{10}-RIL with 113 lines	IT93K-503-1 (*Hbs* positive)	CB46 (*Hbs* negative)	SNPs	Visual inspection of dried seeds for brown discolouration of seed coat	Greenhouse	Two	8 and 3	28.3–77.3, and 9.5–12.3	[51]
3	F_8-RIL with 136 lines	IT84S-2246 (*Hbs* positive)	TVu14676 (*Hbs* negative)	SNPs	Visual inspection of dried seeds for brown discolouration of seed coat	Greenhouse	One	1	6.2–6.8	[51]
4	F_8-RIL with 175 lines	GEC (Heat tolerant)	IT98K-476-8 (Heat susceptible)	SNPs	Heat-tolerance visual ratings	Field and greenhouse environments	Two	1 and 10	7.66 and 10.64	[46]

No	Mapping Population and Size	Parent-1	Parent-2	Marker System	Trait Assessed	Study Environment	Number of QTLs Mapped	Chr	PVE (%)	Reference
5	**F_8-RIL with 175 lines**	**GEC (Heat tolerant)**	**IT98K-476-8 (Heat susceptible)**	**SNPs**	**Seed weight per plant**	**Field and greenhouse environments**	**Two**	**3 and 10**	**17.05 and 11.37**	[46]
6	**F_8-RIL with 175 lines**	**GEC (Heat tolerant)**	**IT98K-476-8 (Heat susceptible)**	**SNPs**	**Number of pods per plant**	**Field and greenhouse environments**	**Three**	**3 and 10**	**22.93, 5.93, and 7.62**	[46]

Chr = chromosome, PVE = phenotypic variance explained, QTLs = quantitative trait loci.

4. Breeding and Selection Methods for Genetic Improvement of Cowpea for Heat Stress Tolerance

Employing conventional breeding techniques, efforts have been undertaken to screen for and identify heat tolerance in cowpea under both controlled and field environments, with a particular focus on the ability of plants to produce abundant flowers and set pods under elevated temperatures, leading to identifying accessions with enhanced heat tolerance (**Table 1**). This approach has been practical under hot, long-day conditions typical of the USA's subtropical areas of southern parts of California [35][36]. However, the number of commercial varieties with known heat tolerance is very low [43][67] due to the long time it takes to develop such varieties using conventional approaches, because heat tolerance has to be incorporated by selecting progeny with the ability to produce flowers and set pods over several generations in hot field nurseries [8]. A pedigree breeding method has been described under subtropical conditions, which proved effective in incorporating heat tolerance during reproductive development [8][24][29]. This involves making simple biparental crosses between parents that exhibited reproductive-stage heat tolerance and desired agronomic traits. In some cases, a backcross approach was applied, where F_1s were crossed with the most productive heat-sensitive parent. The segregating generations (F_2) were screened as single plants in large populations in hot fields under long days based on the plants' ability to produce abundant flowers, a high number of pods per peduncle, adequate seeds per pod, and good seed quality. After that, an additional selection cycle was practiced with F_2-derived families at more advanced generations to incorporate traits that exhibited substantial environmental variation. At this stage, small numbers of progenies are left, and it is possible to evaluate rows of the F_2-derived lines to select the lines with uniformly high pod sets and other desirable characters. Performance tests are then conducted as advanced yield as well as multi-location trials with materials that possess stable heat tolerance and desired agronomic traits. Attempts have been made to use the pedigree approach, with some modifications, to develop heat-tolerant lines for tropical zones [68][69]; two varieties from Ghana were crossed with two heat-tolerant lines from the University of California, Riverside. The segregating populations were then screened in a hot, long-day field nursery in California, and selection was carried out for the ability to produce flowers and set pods. These lines were later tested in northern Ghana for the desired agronomic traits, subjected to multilocation performance tests, and selected, leading to the release of two varieties, though they were not registered as heat-tolerant varieties in Ghana. For tropical environments with short-day conditions, where day and night temperatures are often high above the threshold, a modified approach to the one described for the subtropical climate may be more helpful [29]. The strategy is to use lines of African origin with heat tolerance as parents, and subsequent selection for heat tolerance should be carried out in the African environments. The criteria for selection should focus on the plants' ability to set pods and have large numbers of seeds per pod under high night temperatures as opposed to the abundant flowers and pod sets for subtropical California [29]. In addition to the above, for heat tolerance screening, especially during the rainy season, conscious efforts have to be made to control prevalent pests and diseases that may confound selection for abundant flowering, pod sets, and maintenance of large numbers of seeds per pod, or, selection for heat tolerance should be targeted at off-season nurseries when the populations of these pests are lower [8].

Another similar approach that has been demonstrated in the subtropical zone was crossing between heat-tolerant and adapted desired varieties to develop F_1s and advance them to F_2 generation, then during the summer, test these F_2 progenies in hot, long-day environments or glasshouses with high night temperatures to select plants with the ability to set abundant flowers and pods. In the following fall and winter, two generations of F_3 and F_4 are advanced using single-seed descent or selected for low leaf-electrolyte-leakage as a measure of cell membrane stability, to select heat tolerance during pod set indirectly. During the second summer, replicate families of the F_4 generations are grown in the hot, long-day field nurseries or glasshouses with high night temperatures and in parallel nurseries to screen for desired agronomic traits. In fall and winter, two generations are then advanced in moderate-temperature glasshouses or off-season field nurseries. Finally, the selected F_8 lines are tested for performance in several hot, long-day production environments in the

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third summer. New candidate varieties are chosen from these lines and subjected to further yield testing on experiment stations, followed by yield testing on both experiment stations and farmers' fields [61,62]. Similarly, using the extent of floral development and podding as a selection criterion in a hot, long-day environment in northern India, four varieties of edible vegetable cowpea with substantial heat tolerance have been developed at the Indian Agricultural Research Institute, New Delhi [70]. These varieties exhibited heat tolerance when evaluated in hot, long-day field conditions in California [8]. These approaches have proved effective in hot field environments in the Imperial Valley, California [35], and in northern India with subtropical conditions. It is worth noting that using hot environments as testing sites may have additional disadvantages because selection for other important agronomic traits could be restricted.

While genetic techniques might offer advantages in creating plants capable of withstanding high temperatures, the resultant plants are likely to have lower yields or defects in some agronomic attributes, like height or shorter internodes, compared with closely related heat-sensitive plants [18]. As a result, considerable focus could be placed on other strategies to induce heat tolerance in productive heat-sensitive varieties. A range of techniques has been explored in a few crops like black spruce, tomato, turfgrass, and pearl millet, including applying a diluted solution of inorganic salts, osmoprotectants, growth hormones, and oxidants to plant leaves or treating seeds with these substances before planting, as well as pre-sowing hardening of the seeds at high temperatures [3]. A comprehensive approach is illustrated that could be used to develop heat-tolerant varieties more rapidly and efficiently by combining traditional breeding techniques with the latest advancements in genomics and biotechnology. This process involves the discovery of desirable heat-tolerant genotypes with either morphological, biochemical, physiological, or reproductive heat-tolerance traits by combining classical breeding and genomic techniques. After identifying favourable alleles through mapping and validating heat tolerance loci-associated QTLs, they can be converted into Kompetitive Allele-Specific PCR (KASP) markers. These markers can then be routinely employed in marker-assisted selection (MAS) or marker-assisted backcrossing (MABC) during the development of breeding lines through introgression. A speed-breeding approach can be employed to accelerate generation advancement in fixing heat tolerance traits in adapted varieties to expedite the breeding process. In cases where minor alleles play a role in heat tolerance, genomic selection is proposed and rapid generation cycling is used to develop heat-tolerant varieties swiftly. Furthermore, a reverse genetics approach has been illustrated for identifying useful alleles that will be fixed in elite varieties through rapid generation cycling (**Figure 3**).

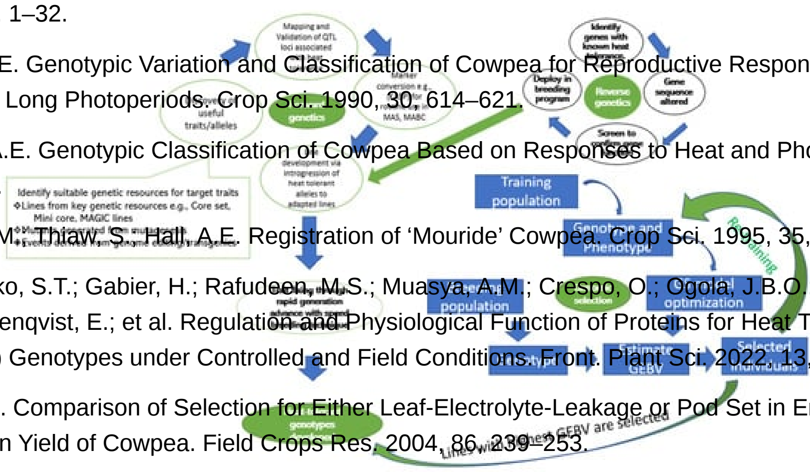

Figure 3. Proposed integrated approaches for genetic improvement of cowpea for heat tolerance. The scheme proposed for discovering heat-tolerant genotypes using forward genetics, reverse genetics, and genomic selection pipelines. GEBV = genomic estimated breeding value, KASP = Kompetitive Allele-Specific PCR, MAS = marker-assisted selection, MABC = marker-assisted backcrossing, MAGIC = multiparent advanced generation inter-cross, QTL = quantitative trait loci.

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