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Review

Enhancing Cowpea Tolerance to Elevated Temperature: Achievements, Challenges and Future Directions

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Abstract: 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. Progress in improving the crop for heat tolerance has been limited, especially under the hot, short-day environments typical of sub-Saharan Africa. Only a few heat-tolerant varieties have been released, partly due to the limited understanding of heat stress tolerance mechanisms and environmental interaction effects on genotypes, as well as imprecise phenotyping. This review contributes to the literature on cowpea heat stress by highlighting key achievements, challenges, and future directions in breeding heat-tolerant cowpea genotypes and by providing additional information from the recent literature. We opine that the genetic variability for heat tolerance-related traits in cowpea has not been sufficiently exploited in developing varieties adapted to the target production environments. Therefore, attention should be given to assessing the crop's genetic repository by targeting adaptive, morphological, and physiological traits that enhance heat stress tolerance. We propose that breeding programs integrate phenotyping of whole-plant physiological traits and molecular breeding to identify breeder-friendly markers for routine selection. This should be followed by introgression of the heat-tolerant favourable alleles to adapted susceptible varieties using rapid and precise approaches that take advantage of modern genetic and genomic resources such as innovative genetic resources, genomic selection, speed breeding, and genome editing technologies. These tools hold great promise in fast-tracking the development of improved heat-tolerant varieties and incorporating the must-have traits preferred by cowpea farmers and consumers. In view of the likely increase in atmospheric temperature to be occasioned by climate change, there is an urgent need to develop heat-tolerant cowpea varieties to ensure the sustainability of current and future cropping and agri-food systems.

Keywords: heat tolerance; high night temperature; phenotyping; physiological traits; short-day environment

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1. Introduction

Crop productivity worldwide is sensitive to significant changes in temperature and precipitation [1–3]. 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 [15–17]. 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.

High day temperatures (33–36 °C) and soil temperature have been implicated to some extent in reducing pod set in cowpea [16,23], though not as severely as high night temperatures [23]. Day and night temperatures above the tolerable threshold (30/16.5 °C) can occur during the crop's growing season in SSA, either at the start or end of the rains [22]. This potentially reduces the crop's growing window with optimal temperatures for production in the open field and in non-controlled growing conditions [22,24,25]. While some cowpea germplasm has been identified as heat-tolerant under long-day environments, the progress in breeding varieties with heat tolerance has been limited thus far under short-day environments, and known heat-tolerant varieties are limited. This is partly due to limited comprehension of the genetic components of traits that confer heat tolerance under these environments [6,26] and to imprecise phenotyping approaches [13].

Recent progress in genotyping technologies has facilitated the widespread use of high-throughput genotyping in legume research [27,28]. However, accurate phenotyping is the primary bottleneck in uncovering the genetic basis of some complex traits [3,29], which could hamper the pace of breeding programs, especially those with limited budgets and resources. There has been interest in screening cultivated cowpea genetic resources to identify heat-tolerant genotypes [30,31], with limited attempts made to map the quantitative trait loci associated with heat stress tolerance traits [32,33]. Evaluating the available genetic and genomic resources related to heat tolerance can help breeders conduct effective screening and select suitable breeding materials for future climates [24,34]. Hall and co-workers have undertaken a couple of cowpea-focused reviews on heat tolerance in cowpea, with the most recent one by Porch and Hall in 2013 [6,8,14], which examined several aspects of breeding cowpea for heat stress tolerance. Several innovative techniques—including the development of a multiparent advanced generation inter-cross (MAGIC) population, speed breeding, genomic selection, and genome editing tools—have been introduced to expedite the genetic gain in various crops [35–37]. These modern technologies and various interdisciplinary approaches could be adapted to improve cowpea heat tolerance and thus facilitate the delivery of climate-resilient varieties. This review contributes to the existing literature on heat stress, builds on the earlier works, and provides additional information from the recent literature by highlighting the achievements, challenges, and future directions.

2. Achievements in Improving Cowpea Resilience against Heat Stress

This section focuses on the current knowledge and accomplishments in the field of heat stress tolerance in cowpea. This includes the identification of different forms of heat stresses, understanding the mechanisms involved, the screening environments in which assessments have been conducted, approaches used so far, highlighting genotypes identified as heat tolerant, what has been documented on the genetics of heat tolerance traits, and quantitative trait loci mapped to be associated with heat tolerance traits in cowpea.

2.1. 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 [21,38]. 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 [6,38]. 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 [2–4]. 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].

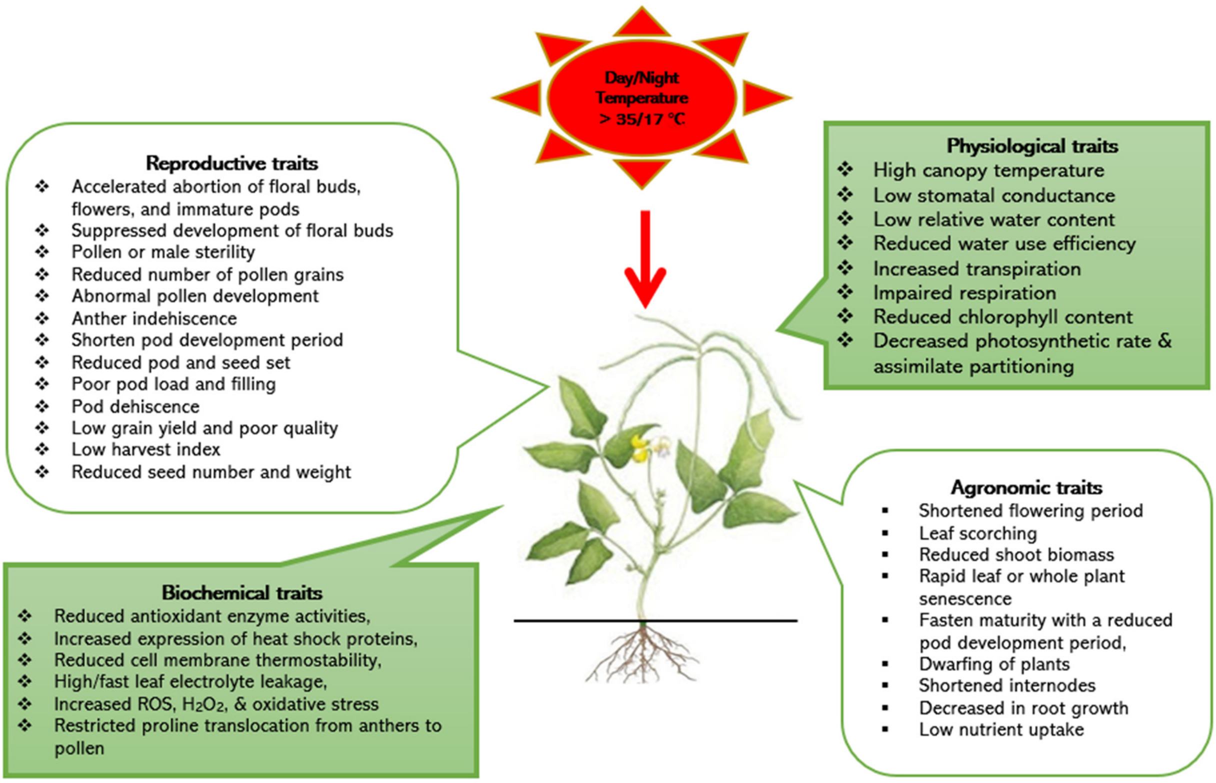

Figure 1. Representative list of traits, processes, and functions impacted by heat stress in cowpea.

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 [39] 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 [15,20,40], resulting in low flower numbers, pod set, and grain yield [9,39,41].

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 [41]. 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 [42]. 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 [41]. Earlier investigations documented that heat-induced suppression of floral buds occurs only under long days [43]. 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).

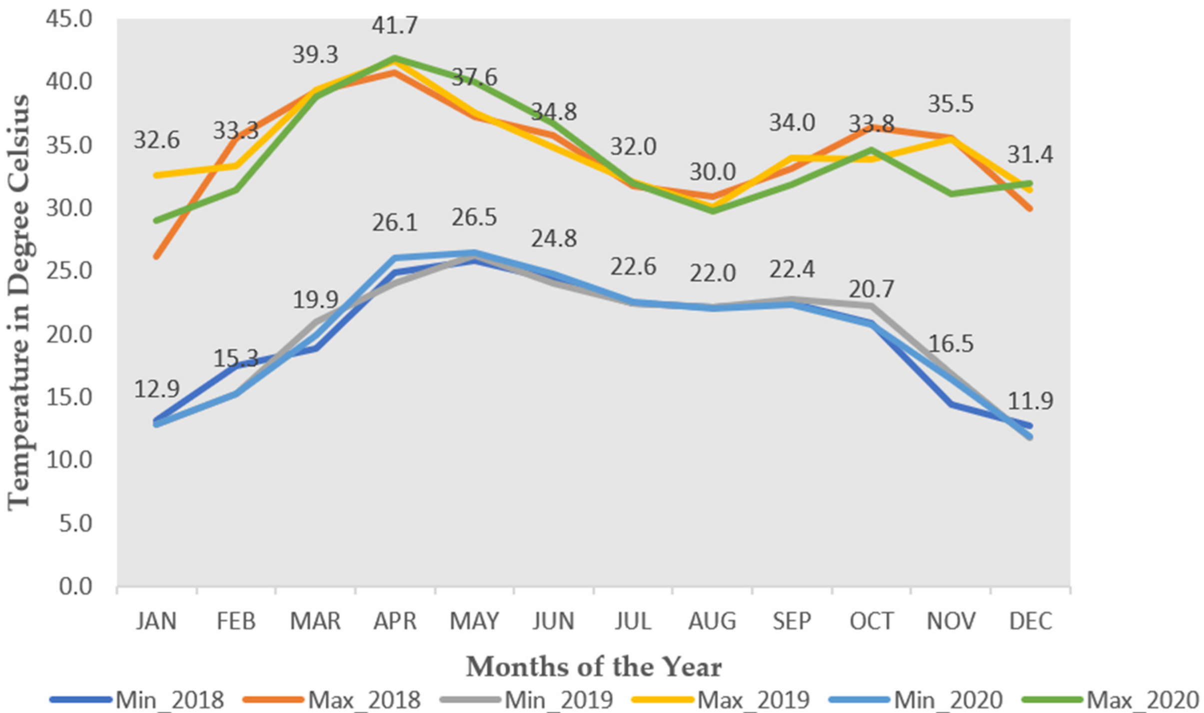

Figure 2. Average monthly minimum and maximum temperature of typical cowpea production zones in Minjibir, Kano State in Northern Nigeria.

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 [44]. Rapid leaf senescence and maturity have been observed during pod filling in heat-sensitive genotypes when night temperatures are high [9,40,45], 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 [21,46]. Thus, varieties that display delayed leaf senescence under heat stress conditions may possess more effective tolerance mechanisms [47]. In addition, higher day and night temperatures significantly shorten the pod development period in both heat-sensitive and tolerant genotypes [15,45], 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 [39]. 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 [39]. Three heat tolerance mechanisms have been described in regard to the above impacts of high temperatures on cowpea's reproductive development [44]. These are tolerance at the early floral bud stage that conferred the ability to produce flowers under hot, long-day ($\geq$13 h day^{-1}) conditions [48], which is influenced by phytochrome [43]; 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].

2.2. 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 [49]. 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) [26], 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) [26] 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) [34] 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 [34]. The details of various classes are provided (Supplementary Table S1). 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 [50].

Table 1. 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	[26]
4	IT93K-452-1, IT98K-1111-1, IT93K-693-2, IT97K-472-12, IT97K-472-25	Pod and grain yield traits	Hot field	[49]
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	[45]
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	[40]
9	IT96D-610	Heat-shock proteins and other stress-protective proteins	Glasshouse and field	[51]
10	Genotype H36	Leaf electrolyte leakage, NPP, PP, PHT, HI, GYD, and SDW	Growth chambers, glasshouse, and hot field	[52]
11	IT97K-472-12, IT97K-472-25, IT97K-819-43, & IT97K-499-38	NF, PS, and GYD	Field	[53]
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	[41]
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	[54]
15	TN88-63, A73-2-1 and TVx 3236	NF, PS, and GYD	Hot field	[55]
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	[56]
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	[31]
19	Prima and TVu4552	NPP	Field	[57]

Table 1. *Cont.*

No.	Tolerant Lines	Key Traits Assessed [1]	Screening Environments	References
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	[34]
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	[58]

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 [59]. Traditional breeding approaches have been used to provide insights into the genetics of cowpea heat tolerance [39,60]. 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 [44]. 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 [39,44].

The inheritance of tolerance to heat stress during floral development is reported to be governed by a single recessive gene that is highly heritable [61], indicating that heat tolerance for flower production can be fixed by selection for abundant flower production in the F_2 generation [8,39]. 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 [57]. 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 [44,57]. 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 [60]. 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 [12,31]. 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 [22,55]. 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 $\times$ E) interaction [32,33,58]. 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 [27,35,62]. 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 [63]. 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 an RIL population [32]. In another study, three QTLs were associated with the seed coat's heat-induced browning in two RIL populations [33]. 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 [58]. 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 genotype-by-environment 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 [64–66] 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 [36,67] and minicore [68], 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 [69], methylation filtration technology enabled the selective cloning of the gene-rich, hypomethylated segment, yielding over 250,000 gene-specific sequence reads [70]. Later, a whole-genome shotgun sequencing of var. IT97K-499-35 resulted in a 323 Mb assembly [27], while improved assembly sizes of 568 Mb and 609 Mb for vars. IT97K-499-35 and IT86D-1010 were achieved [71]. Lonardi et al. (2019) [62] 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-declining terminal stresses, such as cold, drought, heat, and salinity [72]. This wealth of genomic and transcriptomic data presents opportunities to identify stress-resilience genes, potentially uncovering mechanisms to enhance heat stress tolerance [73].

2.3. 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 [26,34]. However, the number of commercial varieties with known heat tolerance is very low [56,74] 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 [8,39,44]. 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 [75,76]; 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 [44]. 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 [44]. 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 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 [8,44]. 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 [77]. 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 [26], 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.

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	[32]
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	[33]
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	[33]
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	[58]
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	[58]
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	[58]

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

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]. We illustrated a comprehensive approach 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).

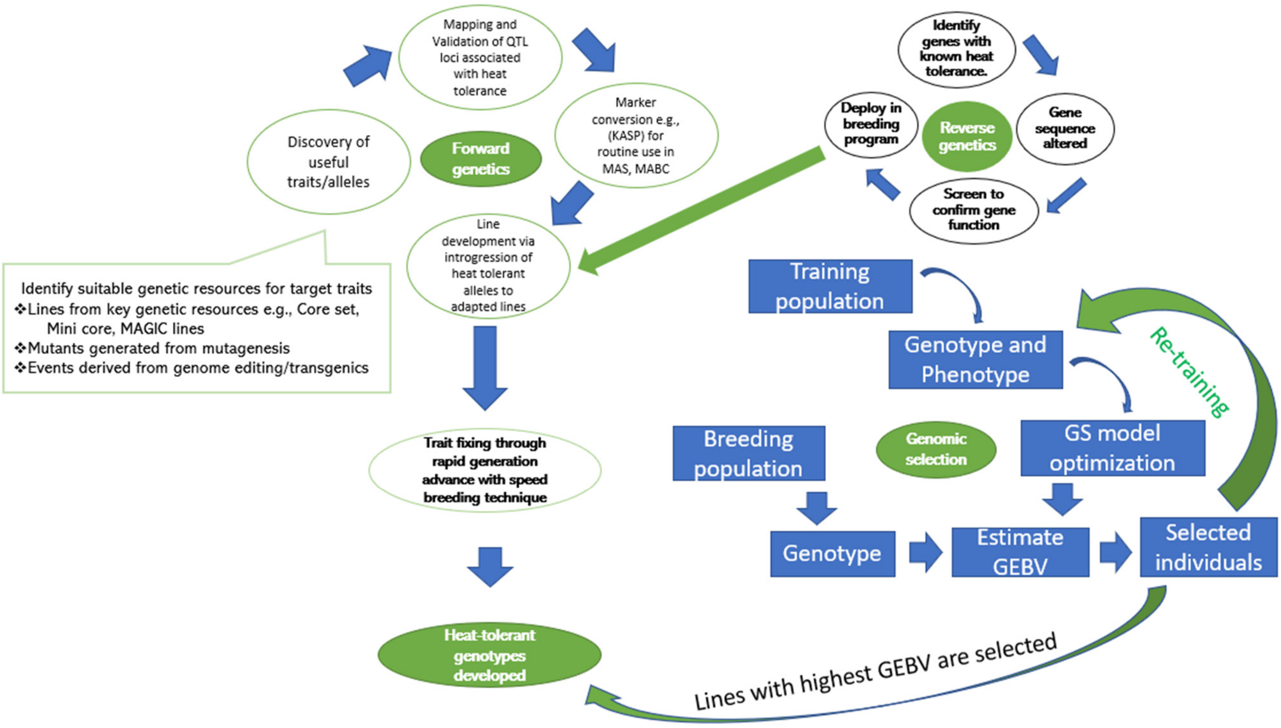

Figure 3. Proposed integrated approaches for genetic improvement of cowpea for heat tolerance. The scheme proposed for discovering heat-tolerant genotypes using forward genetics, reverse genetics, and genomic selection pipelines. GEBV = genomic estimated breeding value, KASP = Kompetitive Allele-Specific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

3. Challenges in Phenotyping Heat Stress Tolerance in Cowpea

The importance of breeding for thermo-tolerance varieties to sustain cowpea production cannot be overemphasized, especially with changing climate conditions. However, not much progress has been achieved so far because of challenges associated with screening for tolerance, the identification of ideal traits, inter-specific crossing barriers, and limited research under hot, short-photoperiod environments. These barriers are discussed below in greater detail.

3.1. Screening for Heat Stress Tolerance in Cowpea

It is important to establish reliable stress screening methods because genetic improvement for tolerance to stress factors often starts with germplasm screening to identify the sources of resistance. In cowpea, both controlled and field environments have been used in various studies to evaluate heat stress tolerance, leading to the identification of some heat-tolerant cowpea lines (Table 1). These include growth chambers [9,15,26], controlled glasshouses/greenhouses [12,21,78], and open fields [16,26,31]. The efficacy of breeding cowpea for reproductive heat tolerance relies on accurately identifying the factors contributing to pod and seed set under heat stress [19,20,41].

Three categories of heat treatment have been applied in assessing cowpea germplasm response under heat stress. The first approach is growing plants in field environments that have similar day temperatures and different night temperatures to determine the effects of increases in temperature. This should be the most reliable method, but unfortunately, only one study is reported to have demonstrated its application [45]. With this system, daytime conditions were maintained constant between plots, whereas night temperatures were varied between plots using thermostats and plastic sheets, so it is likely that the differences in night temperature caused the differences in productivity between the plots. A second approach involves growing a variety under similar management methods in various field environments with contrasting thermal regimes. A significant problem with studies of this type is that the different environments may have differing factors impacting plant performance other than the night temperature [18]. The third approach involves studies using controlled glasshouses or growth chambers with temperature and light control capabilities. Controlled platforms can be considered ideal for investigating mechanisms of heat tolerance because many factors can be kept constant, and such studies are easily repeatable. However, results obtained from controlled environments may not apply to the field due to other uncontrolled factors, such as biotic and abiotic stresses, that occur concurrently in the field [12,15,16]. For the last approach, cowpea plants can be grown in controlled environments in a variety of ways, such as under long ($\geq$13 h day^{-1}) or short days ($\leq$12 h day^{-1}) with moderate night temperatures (e.g., 30/19 °C), and then transferred to high-temperature (36/27 °C) chambers, where they remain for a specified number of days during the reproductive period before returning to a moderate-temperature chamber, while leaving the controls at moderate or high temperatures for the duration of the experiment [12,15,41]. The most commonly applied heat regime is to grow plants under controlled or field conditions, covering vegetative and reproductive stages throughout the crop lifecycle [26], because inducing heat stress during the flowering stage alone may not provide a complete understanding of a plant's response to heat.

Conducting field screening is crucial as it provides a more accurate representation of the environment for the varieties being developed. Some studies have shown that certain genotypes perform well under heat stress in controlled and field environments [16,31,45], with others specifically under field conditions [34,55,79], while certain genotypes expressed heat tolerance under both hot, long- and short-photoperiod conditions [12,31]. When cowpea plants are grown in the field, they are likely to experience multiple stresses and exhibit more complex responses than plants experiencing single stresses. However, most studies on cowpea heat tolerance have not accounted for the interaction between the response to heat and other biotic stresses such as insects and diseases [16,18,41].

Considering the influence of G $\times$ E interactions regarding heat tolerance screening [8,13,44] and enhancing the efficiency of breeding programs, it is important to devise more precise, low-cost, labour-saving, and easy-to-use phenotyping techniques for measuring relevant traits. Efforts have been made in recent years to develop devices for high-throughput phenotyping, such as thermal imaging, fluorescence systems, and hyperspectral reflectance-based high-throughput phenotyping platforms that can enhance the quality of phenotypic data acquisition [80,81]. Despite these efforts, there are difficulties in defining the experimental imposition of heat stress [3] and an absence of standardised protocols for assessing heat tolerance [6], resulting in high variability in heat treatments, which could lead to the identification of heat-tolerant genotypes being compromised, undermining reproducibility and reliability trials. Appropriate trial management and environmental conditions are essential components of phenotyping in addition to technology deployment. In regions like the dry savanna of northern Nigeria and other dry areas, where cowpea diseases are common during the growing season, well-adapted varieties should possess tolerance to both key biotic stresses and heat stress. Field environments like Minjibir in Kano State (Nigeria), where day and night temperatures above 38/25 °C are common during the dry season (Figure 2), provide a suitable environment for screening cowpea for heat tolerance. This is similar to the Imperial Valley in California, where the same range of temperatures occurs during summer, and it has been recommended as a suitable environment for screening cowpea for heat tolerance [15,16].

3.2. Target Traits for Cowpea Heat Stress Tolerance Screening

In addition to identifying appropriate screening environments, it is equally crucial to define the traits to be assessed and determine the stage of plant development at which measurements will be evaluated [14,24]. Most heat tolerance screening in cowpea has primarily focused on floral production, pod set, and yield as the main target traits. Specific traits such as the number of pods per peduncle, pods per plant, seeds per pod, and seed weight have been established as selection criteria for heat tolerance assessments [26,31]. However, research should focus more on potential heat tolerance surrogate traits and understand their mechanisms further. Indirectly assessing heat tolerance through surrogate traits may deepen our understanding of tolerance because these traits may have relatively simpler inheritance vis-à-vis heat tolerance as a whole. Additionally, trade-offs or synergistic effects among these traits may be essential to dissecting the crop's heat tolerance genetic basis. While several traits could be used as heat surrogates for selecting heat-tolerant genotypes, this section reviews those known to contribute to heat tolerance in cowpea and/or related legume species.

3.2.1. Pollen Viability and Anther Dehiscence

Exposure to night temperatures of 30 °C adversely affects pollen number and viability, reducing flowers, pods, and seeds per pod in cowpea [15,21]. Cowpea pollen viability has been estimated using a couple of methods, such as staining and counting to determine the extent to which pollen became stained by aniline blue in lactophenol [15] and fluorescein diacetate staining with a fluorescence microscope [45,46]. Pollen viability, assessed using staining, showed greater than 90% viability at lower night temperatures (33/22 °C) compared with 22% at high night temperatures (33/30 °C), and the viability of the pollen was also distinguishable using its shape, as viable pollens were large, symmetric, and dispersed, whereas inviable ones were small, irregular, and aggregated [16]. Anther indehiscence caused by high night temperatures correlated with decreased pollen viability, with distinguishable characteristics in viable and inviable pollen [16,23]. In vivo pollen germination was four times greater at optimal night temperatures (33/22 °C) compared with high night temperatures (33/30 °C) [15]. Pollen viability was reduced by high night temperatures during a particular stage of development during the last six hours of the night, and high night temperatures did not significantly impact anther dehiscence during the late-night period [82].

Many of the traditional pollen assessment methods were manual, time-consuming, low-throughput, and labour-intensive, making them unsuitable for screening many genotypes in breeding programs [83]. However, advances in high-throughput phenotyping offer automated pollen number and viability analysis. Technologies like Ampha Z30, Z32, and Z40 by Amphasys employ impedance flow cytometry, providing non-destructive and portable options like P20 for use in the field [84,85]. These methods correlate well with classical fluorescein diacetate staining results [86]. While impedance flow cytometry presents promising capabilities for genotypic differences in breeding programs, the high investment cost may limit its use in smaller programs [59]. Advances in automated pollen phenotyping provide opportunities for more efficient screening of genotypes, especially in breeding programs focusing on tolerance to high temperatures.

3.2.2. Stigma Receptivity and Ovule Viability

High night temperatures have been found to have a minimal effect on cowpea's stigma receptivity and ovule viability. For instance, artificial pollination using the stigmas of plants subjected to high night temperatures (33/30 °C) with pollen from plants grown at lower night temperatures (33/22 °C) resulted in an average level of pod set (40%), which is largely normal for artificial pollination [16]. This observation indicates successful fertilisation of at least one ovule and suggests that the plant's female reproductive organ is less vulnerable to heat stress than the male. In addition, in vivo pollen germination demonstrated that high night temperatures did not largely inhibit stigma receptivity [15,16]. The sensitivity of cowpea to high night temperatures during the months of flowering in the tropics may be partially responsible for the wide variations in grain yield observed between tropical and subtropical regions, with lower grain yields of less than 2.5 t/ha in most areas compared with the Central Valley of California, where yields of 3.0–4.5 t/ha have been obtained. When plants of tropical origins are maintained under optimum night temperature conditions, grain yields comparable with those of subtropical conditions have been observed [15,22].

Assessing the receptivity of the stigma and the viability of the ovules is a low-throughput process and may not be very practical in breeding programs that require screening a large number of genotypes before making selections.

3.2.3. Heat Shock Proteins and Reactive Oxygen Species

Plants respond to various stresses by producing specific proteins the stressor induces or that act as the plant's adaptation strategy. These stress-induced proteins can be markers for identifying tolerant genotypes, thus improving selection efficiency [9]. In adapting to heat stress conditions, plants could produce heat shock proteins (HSPs) and reactive oxygen species (ROS) scavenging enzymes, mostly at temperatures above 35 °C. These HSPs function as molecular chaperones whose primary role is to properly fold heat-denatured proteins, making them crucial as a plant adaptive response to heat stress [3,87], whereas ROS-scavenging enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), are induced to counteract oxidative stress, which is essential for plant survival [87].

Cowpea, cultivated in hot and arid regions, is expected to express HSPs and heat-responsive proteins [51]. While earlier reports found no differences in HSP expression between heat-tolerant and susceptible cowpea [8,39,44], recent studies have advanced our understanding. Proteomic analysis revealed HSPs and other stress-protective proteins in response to heat stress in cowpea genotypes, shedding light on potential thermotolerance mechanisms [51]. Specific proteins, including small HSPs and Nodulin 26, have been identified and characterized in cowpea responding to heat stress [88]. Variability in ROS enzyme activities among cowpea varieties under different temperature regimes has also been observed, emphasizing the importance of understanding the molecular mechanisms underlying HSPs and ROS in cowpea for future crop improvement in changing climates [21,51]. Further research is needed to unravel these specific molecular mechanisms and their potential applications in enhancing cowpea resilience to heat stress.

3.2.4. Proline Concentration in Anthers and Pollen Grains

Proline, a crucial free amino acid, is linked to pollen protection during heat stress and plays a vital role in pollen development, membrane function, and pollen tube growth [3,89]. It is a significant organic osmolyte, accumulating in plants as a response to environmental stress [89]. Differences in proline accumulation in the anthers and pollen of heat-tolerant and heat-sensitive cowpea genotypes have been observed under different temperature conditions [42]. In heat-sensitive genotypes, inhibition of proline translocation from anther walls to pollen during floral development was implicated as the cause of heat injury, leading to male sterility and floral abscission under high night temperatures [42]. The degeneration of the tapetal tissue may be responsible for the lack of proline transfer from the anther walls to the pollen [39].

Enhancing heat tolerance through preconditioning or exogenous application of osmoprotectants like proline has been suggested [3]. Genetic engineering has introduced transgenes for proline production for plant species not capable of natural production or accumulation of proline, resulting in increased growth and crop yield under environmental stresses in various plant species [1,2,89]. However, research on proline's function in cowpea under heat stress is limited, primarily because there has been limited research on this topic, emphasising the need for further investigation to enhance understanding and utilize its potential in genetic improvement programs.

3.2.5. Response to Elevated Carbon Dioxide Concentration

Cowpea, similar to other C3 species, is expected to exhibit a positive response to increasing CO_2 concentrations, which could lead to enhanced rates of photosynthesis [40,90], and this has been reported in studies where CO_2 concentrations were elevated from the 1900s level of 350–360 µmol mol^{-1} to the anticipated near-future concentrations above 700 µmol mol^{-1}. For instance, pod yield increased by 45% in cowpea plants grown at

700 μmol mol^{-1} CO_2 compared with those grown at 350 μmol mol^{-1} CO_2 [40,54]. Although these works were conducted in growth chambers under moderate light conditions and optimum conditions, it is reasonable to expect that in sunny field conditions, the grain yield could increase by more than 45% if the temperature and soil conditions are optimum.

In another study, elevated CO_2 levels compensated for the adverse effects of high temperature alone or combined with high UV-B for most vegetative and physiological traits, including plant height, leaf area, net photosynthesis, and dry matter production [9], with heat-tolerant lines exhibiting abundant pod formation when plants were assessed under elevated CO_2 and high temperatures. The heat-tolerant lines had higher sugar content in the peduncles, indicating a superior response to heat stress and CO_2 enrichment. Because heat-tolerant lines significantly increased pod production in response to elevated CO_2, it was inferred that heat-tolerant genes could enhance adaptation to future climatic conditions characterised by higher CO_2 levels and temperatures [40].

While these ideas can offer valuable insights for developing climate-resilient cowpea varieties, adapting the screening for CO_2 responsiveness in breeding programs, especially those with small resources, could pose several challenges. These include infrastructure requirements, the time-consuming nature of these studies, genotype-specific responses, interactions with other environmental factors, and difficulties in scaling up results to field conditions.

3.2.6. Leaf Electrolyte Leakage

Leaf electrolyte leakage, indicative of cell membrane stability, can be used as a critical measure for assessing heat tolerance in cowpea [44,52,91]. This method involves measuring the electrical conductivity of a solution where plant leaf discs are placed, reflecting compromised cell membranes that release essential electrolytes. Elevated electrolyte leakage correlates with reduced membrane stability and is considered a crucial aspect of heat tolerance due to its impact on photosynthetic and respiratory functions [6]. Developing a heat-tolerant variety using conventional selection for high pod set under hot field conditions can take many years [56]. The technique has been employed in cowpea breeding, specifically for reproductive heat tolerance in cowpea [44,52,91]. Studies show a negative correlation between leaf electrolyte leakage and pod set/grain yield in hot, long-day field conditions [91]. Reciprocal crosses confirmed the relationship between slow leaf electrolyte leakage, high pod set, and heat resistance in cowpea [52]. Selecting for slow leaf electrolyte leakage under heat stress in fall and winter, combined with choosing plants with abundant flower production and pod set in summer, proved efficient in breeding heat-resistant cowpea under extremely hot, long-day conditions [52]. Screening for electrolyte leakage at vegetative stages allows for flexible and timely heat tolerance assessments, even outside target periods or environments unsuitable for field screening. While selecting for slow leaf electrolyte leakage aids in early-stage screening, it is complemented by the necessity to assess grain yield at the reproductive stage for a comprehensive evaluation of heat tolerance [6].

3.2.7. Phenology and Yield Traits as Screening Criteria for Cowpea Heat Stress Tolerance

To develop more resilient varieties, phenology and yield traits can serve as reliable screening criteria to identify heat-tolerant genotypes. Several phenology and yield parameters such as days to first flower opening, days to maturity, number of pods per plant, number of seeds per pod, the weight of seeds, shoot and root dry weights, and leaf senescence have been well-studied in cowpea under hot conditions (Table 1) and have proven valuable in selecting for heat tolerance [21,31,58], especially the genotype's ability to produce many flowers and pods under hot conditions [14,57,91]. Likewise, the assessment of total biomass production offers insights into a variety's overall resilience under heat stress because genotypes with higher biomass retention under heat stress conditions may exhibit greater heat tolerance, as they are better equipped to maintain essential growth processes. Furthermore, genotypes with a higher harvest index under heat stress conditions will likely allocate more resources toward seed production, signifying their potential for enhanced tolerance [54]. Therefore, genotypes' ability to produce many flowers, high pod set, delayed leaf senescence, higher seed yield, biomass, and harvest index can serve as invaluable screening criteria. These are good indicators to consider when selecting promising lines for heat tolerance (Table 2; Supplementary Table S2).

The challenge is that most reproductive traits often exhibit low heritability, leading to a relatively slower pace of variety development. In addition, it takes a prolonged duration to develop heat-tolerant varieties when relying on these traits because the assessment of heat tolerance needs to be conducted over multiple generations in hot field nurseries. These nurseries are available only during specific times of the year in certain regions, such as the dry seasons in the savanna areas. Hence, phenology and yield components should not be used as the only selection targets in programs aiming to rapidly deliver improved varieties to meet the prevailing and future challenges of climate change.

3.3. Trait Associations under Heat Stress Conditions

Assessing whether trait associations are positive or negative can provide valuable insights into the feasibility of indirect selection. Studies have shown associations between certain yield traits under heat stress in cowpea that may slow the development of heat-tolerant lines. For example, a field experiment examining the effects of elevated night temperatures revealed a strong negative association between increased flower abscission and reductions in the number of pods per plant [45], which results in reduced pod sets and grain yield. Pollen viability and pod set are positively correlated, which ordinarily offers an opportunity for indirect selection for pod set where suitable hot environments are unavailable to perform pod set evaluation [82]. However, the challenge lies in the fact that pollen viability does not guarantee a successful pod set, requiring that selection based on pollen viability will require evaluation for the pod set in actual performance testing.

Ismail et al. (2000) [92] observed that heat tolerance genes in cowpea enhance early pod production under very hot conditions but not in cooler conditions, as the first-flush grain yield of heat-susceptible lines was negatively correlated with an increase in effective night temperatures above the 20 °C threshold. This further elucidates why grain yields in tropical zones with hotter nights are significantly lower than those in subtropical zones and suggests that heat-tolerant genes would be advantageous in tropical environments where night temperatures above 16 °C are common during the growing seasons (Figure 2).

Seed coat discolouration has been associated with some heat-tolerant genotypes when grown in high night temperatures, such that each increase in night temperature during pod fill resulted in a progressively larger proportion of seeds with brown, shrivelled testas [45]. High night temperatures are associated with accelerated vegetative and reproductive development in cowpea, leading to increased branches, pod weight, and number of reproductive nodes [15,78] and a shortened pod development period that potentially causes embryo abortion and reduced seed size [18]. In some heat-tolerant genotypes, a decrease of up to 25% could occur in the number of seeds per pod under high night temperatures of 30 °C or more, in comparison with their performance under moderate night temperatures of 22 °C [16,45], probably due to limited assimilate supplies to individual pods. Hence, combining heat-tolerance genes with those that enhance vegetative growth is crucial to optimise cowpea yields in tropical regions where night temperatures greater than 20 °C occur during the growing period [34].

3.4. Crossing Barriers between Cultivated Cowpea and Wild Relatives

Wild crop relatives serve as reservoirs of allelic variants that potentially offer valuable diversity that can be harnessed to broaden the genetic base of crops and enhance their resilience and adaptability [93]. Challenges in using heat tolerance in distant wild relatives could arise due to sexual incompatibility and linkage drag [94]. This holds for many crops, including cowpea, where heat tolerance was found in some wild species such as *V. hainiana*, *V. stipulacea*, and *V. riukiuensis* [95]. Still, substantial barriers to interspecific hybridisations between them and cultivated cowpea have made the introgression of the resistance genes difficult. However, cross-compatibility may be observed between some cultivated varieties and their wild relatives [96]. In most cases, successful crosses produce very few seeds, as some embryo and endosperm nuclei degenerate a few days after pollination [97]. To our knowledge, no report of improved cowpea varieties developed using wild relatives as donor genes is available. The underutilization of wild relatives in cowpea variety development may be attributed to other factors in addition to sexual incompatibility issues, such as their small seed sizes, unappealing seed coat colour, and susceptibility to viruses.

Cowpea wild relatives with cross-compatibility with cultivated cowpea exist and possess some valuable traits that could be explored in developing improved varieties, even if crosses are difficult [98]. Recent efforts have led to the identification of some accessions of wild relatives cross-compatible with cultivated cowpea, with superior heat tolerance from section *Catiang* (*V. unguiculata*, *V. dekindtiana*, and *V. baoulensis*), and crosses have been made to elite germplasm (Dr. Ousmane Boukar 2023, IITA Kano, Nigeria, Personal communication). Efforts to identify stress-resilient wild relatives will help address part of the cowpea's global challenges, and advances in emerging technologies like gene editing can help overcome barriers to using wild relatives in cowpea improvement.

3.5. Limited Research on the Genetic Architecture of Heat Stress Tolerance in Cowpea

Earlier research indicated the existence of genetic variability in cowpea's tolerance to heat stress to a certain extent [26,31,34]. Success in introgressing favourable alleles into farmers' preferred varieties greatly relies upon the nature of gene action and heritability traits. The few studies on the genetics of the reproductive heat-tolerant traits in cowpea indicated low realized heritability under field conditions [57,60]; most of these reports were conducted under long photoperiod and controlled environments, with only a small number demonstrating the efficacy of heat tolerance genes in short-day controlled environments [12,31]. This may be due to the influence of environmental variables, and considering the complex genetic inheritance and strong influence of G $\times$ E interactions under field conditions, the precise measurement of the heat stress response remains challenging [13,44,73]. The impact of heat stress is additive and is governed by quantitative loci [12].

Thus, breeding for heat tolerance in cowpea is still in its early stage for short-day tropical environments and warrants more attention, given the prevailing changing climate conditions. The scarcity of existing heat breeding programs, especially in sub-Saharan Africa is largely due to a lack of ideal screening environments and limited funding. There is a need for more in-depth research to dissect the genetic architecture of heat stress in cowpea under short-day environments where the crop is grown substantially, especially to identify genomic regions, the markers tightly associated with them, and potential candidate genes that control the trait and their effects. To accelerate such progress in developing heat-resilient varieties in the near future, there is a need for emphasis on precise screening, including under natural field conditions, identifying and characterising genetic resources with heat tolerance to facilitate the process of transferring heat tolerance alleles and deepen the comprehension of the genetic factors influencing heat resilience.

4. Future Directions for Assessing Heat Stress Tolerance in Cowpea

With the challenges associated with heat tolerance assessments, strategies that should be pursued to enhance the phenotyping process more effectively and improve the genetic makeup of cowpea for heat stress tolerance are proposed. These include identifying easy-to-phenotype physiological traits, exploiting key genetic resources, genomic tools, and new emerging technologies to increase the precision of heat stress screening and to fast-track the development of new varieties that will be climate resilient. These are discussed below.

4.1. Physiological Traits to Improve Cowpea for Heat Stress Environments

Limited progress has been achieved in identifying genotypic variations in physiological traits in cowpea under heat stress [39], highlighting the need for focused efforts in future research. Physiological traits could significantly support conventional breeding techniques in addressing the challenges of adapting crops to changing climate scenarios [99,100] but have not been largely adopted in most cowpea heat stress assessments. Research on physiological traits can focus on achieving short- and long-term plant breeding impacts. The short-term goals include (i) characterisation of parental materials for use in strategic crossing, (ii) early generation selection using high-throughput techniques, and (iii) identification of useful physiological traits among genetic resources. In the long term, it can improve the understanding of environmental adaptations and the physiological or genetic basis of tolerance mechanisms [81]. Physiological traits (PTs) that can be evaluated at the whole plant and field level are crucial to exploiting the variability for stress tolerance in breeding programs [100]. Examples of whole-plant PTs that can be used to select potential parents include canopy temperature, stomatal conductance, chlorophyll content and fluorescence, and spectral reflectance indices. The effectiveness of some of these procedures has not been adequately demonstrated in most cowpea programs [39,100]. The following PT traits are discussed.

4.1.1. Canopy Temperature

The canopy's temperature (CT) results from the interactions between energy absorption and dissipation mechanisms occurring within the canopy, which is a measure of the evaporative cooling from the canopy surface, and it is correlated with several physiological parameters like stomatal conductance, water status, roots, and yield components [80]. It has been shown to explain approximately 60% of the yield variation in wheat lines under drought stress, and this could be deployed as a selection tool in drought- and heat-stressed environments [101]. Under stress conditions like drought and heat, tolerant lines are expected to have cooler CTs. In cowpea, water deficit significantly increased the CT, indicating that those genotypes close their stomata to avoid dehydration, thereby reducing the ability of the leaves to dissipate the absorbed energy [102]. CT can be measured using infrared thermometers and can be deployed as a high-throughput trait to identify heat-tolerant genotypes because the measurement is quick, simple, inexpensive, and integrative as it involves scoring many leaves at once, thus reducing the error associated with leaf-to-leaf variation. The main downside is that the measurement is quite sensitive to the time of day, phenology, and environmental conditions such as wind and radiation, which could influence the readings. It is recommended to record measurements under low wind and clear sky conditions [80].

4.1.2. Stomatal Conductance

Stomatal conductance (*gs*) can determine plants' transpiration and evaporative cooling under drought or heat stress, because *gs* and gas exchange are regulated by soil water availability [90]. There is a strong relationship between *gs* and CT because *gs* directly affects transpirational cooling [80]. Stomatal conductance can be monitored instantaneously or over a growth period using different methodologies, such as the use of leaf porometers or infrared gas analysers. Hand-held porometers, being a point measurement, are not quick or integrative, and are generally slow, making them unsuitable for large-scale screening. Therefore, under uniform canopy conditions, a single CT measurement provides a faster and more accurate estimation of the rate of transpiration and *gs* of the whole plot [81]. Deeper-rooted genotypes exhibit increased transpiration due to more open stomata and good ground cover, accessing more soil water. Conversely, shallow-rooted genotypes are more sensitive to stomatal closure in response to soil drying, having lower transpiration and enhanced heat tolerance than those with more open stomata [39].

In cowpea, significant variation for *gs* under high-temperature regimes has been observed, with some genotypes experiencing greater photosynthetic activity, which may be due to an increase in *gs* that resulted in widening the opening of the stomata [21]. There are few reports concerning *gs* in cowpea under high-temperature conditions in the recent literature. Hence, some results for *gs* for drought stress are illustrated here to demonstrate the utility of *gs* for heat stress research. Cowpea genotypes demonstrated varying stomatal responses to soil moisture levels during the vegetative stage. The *gs* was restricted under moderate and severe drought, with some genotypes exhibiting higher *gs* under stress. Stomatal conductance, biomass, and grain yield were positively correlated under severe drought stress. Improving cowpea for efficient *gs* can be an important priority for boosting productivity [103].

4.1.3. Chlorophyll Content, Fluorescence, and Net Photosynthesis

Photosynthesis, a crucial process for the growth and development of green plants, is particularly vulnerable to various environmental stress factors, and plants' ability to maintain normal photosynthetic activities under heat would improve stress response. Heat-induced reductions in photosynthesis can influence seedling survival or reduce yields through effects on vegetative or reproductive development [39]. Empirical evidence has shown that heat stress in cowpea is most damaging to economic yield by reducing pod and seed set, which is a clear case of the reproductive sink suffering more damage [44]. The extent to which irreversible damage occurs to the photosynthetic systems in hot crop production environments can be evaluated by measuring chlorophyll fluorescence [39].

Some earlier reports indicated that selecting for high chlorophyll content per unit leaf area was not advantageous, as a cowpea mutant with lower chlorophyll content exhibited similar quantum efficiencies, leaf photosynthesis rates, and grain yield as its parent when grown under high day and night temperatures [104,105]. More recent studies on cowpea under various temperature regimes revealed significant differences in the chlorophyll content, with the highest rates observed in the lowest temperature chamber [21]. An increased photosynthetic rate was observed for cowpea under elevated CO_2 levels with high temperatures Singh et al. (2010) [9]. This contrasts with the findings from Ahmed et al. (1993) [54], where higher day/night temperatures (33/30 °C) alone or with elevated CO_2 resulted in decreased photosynthesis compared with control temperatures (33/22 °C) [54]. The differences in reports may be due to varying temperature treatments, as Ahmed et al. (1993) varied night temperatures only, while Singh et al. (2010) [9] varied both day and night temperatures. Also, more recently, it was observed that photosynthesis could be inhibited due to chlorophyll loss and reduced carbon fixation and assimilation [21] as the photosynthesis of cowpea varied under the high-temperature regimes, with the photosynthetic activity of some varieties not affected. These authors argued that the higher photosynthetic activity experienced by some varieties may be due to increased *gs* and transpiration [21].

The understanding of the impact of heat stress on cowpea photosynthetic sources is still at its early stage, primarily due to a lack of extensive research on this subject. Our observations from a study measuring net photosynthesis with CI-340 during a recent trial of a large set of cowpea accessions under hot, irrigated conditions in the field showed that heat stress could impact net photosynthesis. The use of a low-cost, high-throughput photosynthesis measurement device like Multispeq [106] that measures several parameters, including photosynthetic active radiation, chlorophyll contents, fluorescence parameters, and a host of other fluorescence base traits could facilitate screening for photosynthesis traits in breeding programs.

4.1.4. Spectral Reflectance Indices

The optical properties of plants impact how the canopy reflects various wavelengths of light, creating a distinct spectral signature that reveals the various components within the crop canopy. To measure this spectral reflectance, field spectrometers and spectro-radiometers covering the visible and near-infrared portions of the electromagnetic spectrum, encompassing those wavelengths, are commonly used for various canopy-related assessments [80]. Analysing the reflected spectra provides a wealth of information about the crop canopy's physiological condition, enabling estimation of parameters like green biomass, photosynthetic area, absorbed photosynthetically active radiation, photosynthetic potential, canopy structure, and grain yield. High-throughput phenotyping (HTP) utilizing spectral reflectance indices has been used to build vegetation indices like the normalized difference vegetation index (NDVI) for assessing plant green biomass [80,107], as demonstrated in wheat, where NDVI showed a positive correlation with grain yield under heat stress [81,101]. Moreover, these measurements can help assess the impacts of nutrient deficiencies and environmental stressors. It is important to highlight that presently, there are limited published data on the application of these indices to cowpea, especially in the context of screening for heat stress. We anticipate these techniques could improve the performance of cowpea under future climate conditions, as demonstrated in wheat and rice [80].

4.2. Exploration of Cowpea Genetic Resources for the Improvement of Heat Stress Tolerance

Genetic improvement of cowpea for hot environments requires understanding the genetic variation for heat stress with the available germplasm resources [34]. The limited availability of known cowpea heat-tolerant genotypes requires researchers to explore more genetic resources for potential sources of heat tolerance and yield traits. Screening for heat tolerance in both cultivated and wild relative cowpea genetic resources in gene banks has been limited, particularly for short-day environments. The International Institute of Tropical Agriculture (IITA) houses over 15,000 accessions in its gene bank from 89 countries, with diverse alleles that can be deployed to develop more adaptive and stress-resilient varieties. These germplasm resources have been assessed based on geographical, agronomical, and botanical descriptors to form a representative core collection of 2062 accessions as diverse as the entire collection [108]. A smaller representative list, named a mini core set and composed of 370 accessions representing the diversity of the main core, has been constituted [10]. In addition, an eight-parent cowpea multiparent advanced generation inter-cross recombinant inbred lines (MAGIC RILs) population has been developed as an essential genetic resource for trait discovery [36,67], with founder parents diverse for several stress factors and important adaptive agro-morphological traits. Exploration of these resources is critical for identifying key adaptive and stress-resilient traits and discovering favourable alleles for emerging threats like drought and heat stresses.

Using adaptive traits and yield components as heat stress tolerance criteria, considerable variance has been reported for cowpea, especially for hot, long-day environments [26,31,34], including the commercial variety *CB27*, released with known heat tolerance [56]. A couple of genotypes have been identified as heat-tolerant lines that could be used as donor parents for further introgression of heat-favourable alleles or tested as candidate varieties (Table 1). Because the development of heat-tolerant cowpea varieties has been slow and the number of released commercial varieties is few, there is an urgent need to explore cowpea germplasm resources such as the cowpea core collection, mini core, MAGIC RILs, landraces, and wild types to identify more potential sources of favourable alleles for heat stress and to increase genetic gain and accelerate the development and subsequent deployment of heat-tolerant varieties.

4.3. Emerging Techniques for Heat-Resilient Improvements

New plant-breeding techniques, such as genomic selection, genome editing, speed breeding, and a host of other tools, hold great promise in fast-tracking the development of improved heat stress-tolerant crops. These techniques can incorporate must-have traits for cowpea farmers and consumers. Some of these approaches have not been thoroughly tested or adopted by most cowpea improvement programs but appear to have some merit from empirical data generated from other crops and/or related legumes [28,109,110]. The following new techniques are reviewed about cowpea.

4.3.1. Genomic Selection to Fast-Track Development of Heat-Tolerant Cowpea Lines

The marker-assisted selection (MAS) and marker-assisted backcross (MABC) approaches require, first, identification of markers tightly linked to a gene or QTL region and validation before their effective deployment in routine selection cycles [111,112]. The MAS approach is more effective for traits controlled by a few major genes than for complex traits controlled by several minor QTLs due to the difficulty of finding the same QTL across multiple environments or different genetic backgrounds [111,112]. MAS comprises selecting individuals based on markers associated with a QTL with significant effects, whereas genomic selection (GS) is a method that does not require QTL mapping or knowledge of trait inheritance [113–115]. It integrates genotype and phenotype data of the training population to generate the genomic estimated breeding values of individuals of the testing population that have not been phenotyped, but genotyped [29,116,117]. With good training populations and phenotype data, GS has the potential to fast-track and shorten the breeding process [114,118] because desired plant types can be selected based on genomic estimated breeding values predicted from the data of training populations [119–121].

Empirical studies in crops have demonstrated a higher genetic gain of GS for various traits, including the quantitatively inherited ones governed by a large number of QTLs [114,118,119]. The GS models have been employed to explore the epistatic interaction in genomic-assisted breeding using various models to understand how the genetic interactions affect the time of flowering, maturity, and seed size in cowpea [121]. Based on these examples, deploying GS could significantly improve heat tolerance in cowpea.

4.3.2. Speed-Breeding Cowpea to Achieve Rapid Generation Advance

Breeding cycles must be shortened to keep up with changing conditions and consumer preferences. The long generation times of most crop plants are partly responsible for the slow genetic improvement rates of key crops [122,123]. A new approach to fast-tracking crop generation, called 'speed breeding (SB),' has been developed, which can shorten generation time significantly [122,124,125] and accelerate breeding progress [109,122,124]. This method can accelerate crop research activities, including regeneration of transformed plants, crossing between parents, and phenotyping of traits using fully enclosed, controlled growth chambers or glasshouses with supplemental lighting systems, optimal temperatures, and high-density planting, allowing rapid generation cycling through single-seed or pod descent with selection for key traits [109,122,124]. Shortening generation times is achieved by reducing the duration from sowing to maturity. This is achieved by growing plants in controlled growth environments with extended supplemental lighting, optimal temperatures, and high-density planting, thus permitting rapid generation cycling through single-seed or pod descent with selection for target traits [122,125,126].

It has been successfully applied in several crops, comprising those responsive to long and short day lengths. Up to six generations per year have been achieved for wheat [125] and chickpea [126], five generations in soybean [123,127] and groundnut [128], and four generations of pigeonpea [129]; six to eight generations for cowpea [130] has been recently demonstrated instead of two to three under normal glasshouse conditions or the one or two generations currently possible in the field. In cowpea, the authors showed that obtaining up to eight generations per year was possible for an early-maturing variety [130]. This technique holds great potential to accelerate genetic gains when integrated with other modern crop-breeding technologies, such as high-throughput genotyping, marker-assisted selection, genomic selection, recombinant DNA technology, and genome editing [109,122,124] to achieve more precise and faster results of genetic enhancement [109,122,124] and could be amenable to selection for quantitative traits when combined with marker-assisted breeding [125]. To adequately tap into the full potential of the SB technology of saving time, space, and resources from when the initial crosses are made to the release and commercialization of new varieties, there is a need to understand and proffer solutions to the challenges that could come with the adoption of the technology primarily for small breeding programs with limited budgets. These include access to suitable facilities, adopting significant changes to breeding program design and operations, trained personnel in the protocol, and most importantly, the need for long-term funding [122]. We have yet to be aware of the SB approach being used to fast-track rapid-generation cycling for breeding heat stress tolerance in cowpea breeding programs. Because the crop is highly adaptable to growing in the greenhouse, it can be combined with marker-assisted breeding and high-throughput phenotyping to improve significantly the breeding efficiency for abiotic stresses like heat tolerance.

4.3.3. Applications of Genome Editing in Cowpea

Genome editing (GE) is based on engineered nucleases and cellular DNA repair pathways to make precise, targeted changes to an organism's genome [131]. Various tools and resources are now available to design and deliver edited genome sequences and detect genetic modifications [132,133]. The main tools used to edit genome sequences are zinc finger nucleases, transcription activator-like effector nucleases, and clustered regularly interspaced short palindromic repeat (CRISPR/Cas9) [132,133]. This technology is transgene-free and seems to have broader acceptance compared with transgenic technology because it does not involve introducing foreign genetic materials [134], which raises concerns about biosafety in some societies where people suspect it can be a health risk because of the involvement of foreign genetic material [132]. The most popular GE tool is CRISPR/Cas9, which is a DNA segment containing short repetitions of base sequences that bacteria use as a defence mechanism against viruses [134,135]. It is the most preferred tool for GE because of its reliability, cost-effectiveness, and specificity [132,133]. CRISPR/Cas9 has two main components, a single guided RNA and a Cas9 endonuclease, which identify and edit the sites of interest, respectively [131,134]. CRISPR/Cas9 has been used to enhance several traits such as drought tolerance, tolerance to herbicides, quality of seed flavour, early flowering, improved rhizobia inoculation, nodule development, disease resistance, and yield, among others, in a few essential legume crops like chickpea, soybean, alfalfa, peanut, and *Lotus japonicus* [131,134,135]. To deploy the novel GE technique, two basic requirements are necessary: an efficient protocol for genetic transformation, including its successful regeneration of the desired crop plant, and the availability of the genome sequence, both of which are available for cowpea [62,136,137].

Increasing the yield potential and making it stable across environments is a complex challenge that will benefit from adopting new precision breeding techniques like GE [135]. The CRISPR/Cas9 technology provides novel avenues to stimulate functional genomics analyses of various important biotic and abiotic stress tolerance traits in grain legume crops, especially those with efficient protocols for plant transformation and the regeneration of whole plants, like cowpea [138]. There needs to be more mutation resources and efficient means for gene inactivation to adapt GE for cowpea genetic improvements [138]. To this end, a couple of studies recently demonstrated the potential to use CRISPR/Cas9-mediated GE in cowpea [136,138–140]. Stable transformation systems have previously been developed for cowpea but with low transformation frequency and long regeneration times [136,137]. Hence, these pioneer studies focused on refining transformation protocols and demonstrating the ability of GE tools to inactivate specific genes. For instance, an efficient CRISPR/Cas9 system for symbiosis receptor-like kinase (*SYMRK*) gene inactivation was developed for cowpea using a hairy root transformation mediated by *Agrobacterium rhizogenes* K599 [140]. The *SYMRK* is an indispensable gene for the formation of both nodule and arbuscular mycorrhizal symbiosis, with its homologs identified in cowpea tagged as *VuSYMRK*. The authors used customized guide RNAs targeting the *SYMRK* gene and achieved about 67% mutagenic efficiency in hairy-root-transformed plants. There was complete blockage of nodule formation in the mutants with both alleles disrupted, indicating that CRISPR/Cas9 can potentially manipulate critical agronomic and stress responses in the crop [140]. To further overcome the difficulty of testing CRISPR/Cas9 constructs in stable cowpea transformants, a new study developed a rapid transient leaf assay to test gene expression and editing constructs before stable cowpea transformation [138].

To facilitate the induction and deployment of asexual reproduction in cowpea to manipulate complex traits including hybrid vigour, and to accelerate breeding, meiosis needs to be altered. To achieve the above, the CRISPR/Cas9 technique has been used to identify three cowpea meiosis genes, *SPO11-1*, *REC8*, and *OSD1*, to induce mitosis from meiosis, with the aim of meiosis knock-out for asexual seed induction [139]. The study provided a fast and efficient approach for testing constructs for gene expression and for inducing mutagenesis in genes involved in vegetative and reproductive developmental programs. Also, Che et al. (2021) demonstrated the potential of developing asexual cowpea plants for hybrid development using CRISPR/Cas9 technology, and their results demonstrated that a highly efficient CRISPR/Cas9-mediated editing system can selectively alter cowpea genome DNA sequences [136]. Furthermore, a transient gene expression assay has recently been developed for rapidly testing GE constructs in cowpea by knocking out the cowpea *phytoene desaturase* gene (PDS) [138]. Genome editing efficacy of the CRISPR/Cas9 vector for *VgPDS* with designed sgRNAs in protoplasts was tested using PEG-mediated transformation and in cowpea leaves through agroinfiltration. In both methods, the results revealed several large deletions in the target sequences, making the developed protoplast transformation protocol a versatile tool for testing GE components before initiating plant transformation, thus improving the chance of using active sgRNAs and attaining the desired edits and target phenotype [138]. The above studies have demonstrated the usefulness of GE in cowpea; therefore, an integrated approach involving plant breeding, physiology, genomics, genetic engineering, and gene editing could significantly impact the crop's productivity, mainly by providing invaluable resources for accelerating the development of heat-tolerant varieties for future climates. As our understanding of heat tolerance mechanisms grows through the exploration of genetic architecture and the genes responsible for regulating heat tolerance, the possibility of strategically altering genes presents an invaluable opportunity to expedite the creation of stress-tolerant cowpea varieties.

5. Conclusions

There is an urgent need to develop heat-tolerant cowpea varieties to ensure the sustainability of current and future cropping and agri-food systems. Previous attempts to improve heat tolerance screening and exploit genetic resources in cowpea have shown promising results. Utilising the significant genetic variability in cowpea germplasm resources and adopting new plant breeding techniques and high-throughput phenotyping could fast-track the development of improved heat tolerance in cowpea, which is crucial for changing climate conditions. This article reviews the progress in breeding for heat tolerance in cowpea and highlights critical considerations for effective screening. The article suggests the potential of utilising innovative genetic resources to breed cowpea heat tolerance. High-throughput physiological traits, speed-breeding, and genomic tools such as genomic selection and genome editing are recommended to develop heat-tolerant varieties. It emphasises a holistic approach to cowpea breeding, from pre-breeding activities to variety development, to effectively and efficiently develop heat-tolerant varieties with desirable traits for farmers and consumers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030513/s1, Figure S1: Cowpea global production statistics (A) Grain production from 1990 to 2022. The blue line indicates the area harvested, while the red line shows the volume of the grain produced. The *X*-axis is the years of production, and the *Y*-axis indicates the volume of grain produced, in a million metric tons. (B) Grain production by regions from 1990 to 2022. Table S1: Various classifications of heat-tolerant genotypes in cowpea, based on the response to different temperature conditions and photoperiod behaviour; Table S2: Representative list of heat-tolerant cowpea genotypes, traits assessed, screening environments, and temperature regimes from various studies.

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