

Exploiting Wild Relatives of Cowpea (*Vigna unguiculata* L. Walp) for Genetic Improvement of Heat Tolerance

Anthony O Ukpene¹ , John C. Morka² , Thelma E. Konyeme³ 

^{1,3}Department of Biological Sciences, Faculty of Science, University of Delta, Agbor, Nigeria

²Department of Physics, Faculty of Science, University of Delta, Agbor, Nigeria

Abstract: The study evaluated the potential of wild cowpea (*Vigna unguiculata* L. Walp.) as a reservoir of genetic material for enhancing heat tolerance of cultivated cowpea varieties. Four *Vigna* accessions, namely, *V. vexillata*, *V. unguiculata ssp. dekindtiana*, *V. subterranea*, and the control *V. unguiculata var. IT90K-2 77-2*, were assessed in a controlled heat stress condition of 42°C for seven days to demonstrate their roles of physiological, biochemical, yield, and molecular responses in providing heat tolerance. It was found that *V. unguiculata ssp. dekindtiana* and *V. vexillata* showed higher rates of photosynthesis (14.8 and 13.9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively), chlorophyll content, and relative water content than the control (9.1 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in relation to heat stress. These accessions also exhibited higher membrane stability (74-78%) and lower malondialdehyde (MDA) accumulation, implying reduced lipid peroxidation and increased cellular protection. Elevated proline, superoxide dismutase, and catalase activities confirmed by biochemical analysis played an additional role in the alleviation of oxidative stress. The HSP70, HSP101, and DREB2A genes found a high level of expression in the wild accessions (5-8-fold), highlighting the connectivity of heat-response pathways. The yield analysis also supported the functional effect, with *V. dekindtiana* giving the highest number of seeds per plant (22.4 g) as opposed to 13.6 g of the control. These results demonstrate the adaptive value of wild *Vigna* relatives and their practical use as a source of heat-tolerant alleles to breed climate-resistant cowpea varieties, bridging the gap between the identification of traits and the breeding effect.

Keywords: Biochemical defense, Cowpea, Genetic improvement, Heat stress, Molecular response, Wild relatives

1. Introduction

Cowpea is an important food crop that plays a significant role in the food security of tropical and subtropical areas, as well as in the fertility of soils and economic wellbeing of the people in sub-Saharan Africa. It supplies millions of smallholder farmers with plant protein, carbohydrates, vitamins, and minerals (Mekonnen et al., 2022; Shevkani et al., 2025). Cowpea is susceptible to abiotic stresses such as heat stress because of emerging changes in the global climate scenario. Excessive exposure to heat undermines the processes of germination, photosynthesis, pollen viability, pod formation, and seed yield. Its optimum temperature is between 25°C and 30°C, and temperatures exceeding 38°C lead to reproductive failure and loss of yield (Barros et al., 2020; Fortunato et al., 2023). Olatinwo et al., (2024) and Mohammed et al., (2021) recorded that the climate change projections of West Africa show that the temperature will increase at a rate of 2-3°C by the mid-21st century, which will pose a threat to sustainable cowpea production. It is

therefore essential to breed cowpeas having heat tolerance, in the face of the narrow genetic base of cultivated cowpea accessions, which constrains allelic variation.

Wild relatives have been used in crop breeding for significant advancements in crop enhancement. For instance, wild tomato, *Solanum pimpinellifolium*, and wild rice, *Oryza rufipogon*, genes have improved stress resistance and yield stability. Boukar et al., (2020) identified that wild *Vigna* accessions have also shown promise in improving heat resistance in farmed cowpea accessions through pre-breeding and molecular characterization. Also, Iseki and Olaleye (2025) noted that these accessions have strong root systems, improved antioxidant defense systems, and membrane integrity stability in thermal stress, making them useful for producing heat-tolerant accessions.

According to Angira et al., (2022), modern breeding tools, including molecular markers and genome editing, are increasingly being used to facilitate the transfer of these beneficial traits from wild relatives to cultivated cowpea, supporting the development of varieties better adapted to climate change. Molecular biology has improved the method of identifying and measuring heat-sensitive genes, such as Heat Shock Proteins (HSPs) and Dehydration Responsive Element Binding (DREB) transcription factors. HSPs defend plant proteins against heat, while DREB2A controls stress-responsive genes, aiding in osmoprotectant accumulation and antioxidative defense (Tähtinen and Fujii 2025; Mao et al., 2020). Understanding the correlation between these gene functions and physiological and biochemical responses of wild and cultivated *Vigna* accessions provides insights into heat tolerance molecular components.

In addition to the molecular information, physiological and biochemical measures are also needed in screening heat-tolerant accessions. The rate of photosynthesis, stomatal conductance, chlorophyll content, relative water content (RWC), and membrane stability index (MSI) are desirable parameters that can be used to monitor the performance of heat-stressed plants as observed by Bhardwaj et al., 2025; Jha et al., 2025; Tiwari et al., 2018; Harsh et al., 2016. In addition to this, biochemical evidence of proline accumulation, superoxide dismutase (SOD), and catalase (CAT) activities demonstrates antioxidative defense by accession to heat-induced oxidative stress as recorded by Bhardwaj et al., 2025; Jha et al., 2025; Tiwari et al., 2018; Harsh et al., 2016. These physiological, biochemical, and molecular measurements in combination provide a comprehensive understanding of heat tolerance mechanisms in *Vigna* species (Singh et al., 2022).

The study assessed the physiological, biochemical, and molecular responses of wild cowpea relatives to heat stress to establish possible donors in breeding programs. It assessed heat stress on photosynthetic efficiency and yield, biochemical changes to allow tolerance of heat stress, and patterns in the expression of heat-responsive genes. The results could be used to enhance the resistance of the cultivated cowpea accessions to escalation in climatic temperature.

2. Materials and Methods

2.1. Plant and experimental site materials

The experiment was conducted in the botanical garden of the Department of Biological Sciences, University of Delta, Agbor (6.25375°N, 6.1942°E). Four cowpea varieties (*Vigna unguiculata* var.

IT90K-277-2, cultivated control), *Vigna vexillata*, *Vigna unguiculata ssp. dekindtiana*, and *Vigna subterranea* were procured at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, and tested for heat tolerance under greenhouse conditions. Day/night temperatures were maintained at $42 \pm 10^\circ\text{C}$ and $28 \pm 20^\circ\text{C}$ at 60-70% relative humidity. Plants were planted in sterilized loamy soil and compost put in 5-L pots in a ratio of 3:1.

2.2 Experimental design and heat stress treatment

The experiment was laid out on a completely randomized design (CRD) comprising four treatments (varieties) and six replicates ($n = 6$). On each pot, one healthy seedling was cultured with the best of the conditions in a period of three weeks and then subjected to heat stress treatment. The heat stress was carried out by subjecting the plants to a controlled growth chamber at 42°C for seven days. The cultivated control plants (IT90K-277-2) were subjected to a temperature of 28°C . The treatment was sustained by maintaining soil moisture at 70% field capacity with daily irrigation.

2.3 Measurement of physiological parameters

Measurements of physiology parameters were done after the duration of heat stress. The photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of the youngest fully expanded leaves were measured with the help of a portable photosynthesis system (LI-6400XT, LI-COR Inc., USA). It was measured spectrophotometrically using 80% acetone after extraction, in accordance with the method of Arnon (1949). Relative water content (RWC) was calculated according to the following formula: $\text{RWC} = (\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight}) \times 100$. The stability of the membrane was determined by determining the electrolyte leakage in leaf discs prior to heating and post-heating at 45°C and 100°C , respectively, and represented as a percentage of stability.

2.4 Yield component assessment

Yield components were obtained after recovery and maturity of each plant. Standard cowpea yield evaluation protocols were used to calculate the number of pods per plant, seeds per pod, the 100-seed weight (g), and the seed yield per plant (g). Seeds that were harvested were dried in an oven at 40°C over a period of 48 hours to a constant weight and then weighed in an analytical balance (Model A&D HR-202).

2.5 Determination of biochemical stress markers.

The biochemical tests were performed during the peak time of the heat stress period. The level of proline ($\mu\text{mol g}^{-1} \text{ FW}$) was determined by the use of the acid ninhydrin test for the detection of the level of lipid peroxidation (Bates et al., 1973). The level of malondialdehyde (MDA), which is one of the markers of lipid peroxidation, was determined by use of the TBA method according to Heath Packer (1968). According to Beauchamp and Fridovich (1971), the deactivation of the inhibited superoxide dismutase (SOD) was detected with the help of nitroblue tetrazolium (NBT). Catalase (CAT) activity was assessed by monitoring the rate of H_2O_2 decomposition at 240 nm, following Aebi (1984). All the biochemical readings were done on a UV-1800 (Shimadzu, Japan).

2.6 Gene expression analysis

In the case of molecular analysis, the end of the heat exposure was taken, and the young leaves were frozen in liquid nitrogen and stored at -80°C . The RNA was collected using TRIzol reagent (Invitrogen, USA), and the cDNA syntheses were performed using the RevertAid First-Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative real-time PCR (qRT-PCR) was carried out using SYBR Green chemistry on an Applied Biosystems StepOnePlus™ Real-Time PCR System. Three heat-shock genes, HSP70, HSP101, and DREB2A, were targeted, and actin was used as a reference target gene. The $2^{-\Delta\Delta Ct}$ method was utilized to calculate the relative level of gene expression, and the data was represented in the form of fold changes in comparison to the control level of non-stressed IT90K-277-2.

2.7 Statistical analysis

An analysis of variance (ANOVA) was performed on all the data using IBM SPSS Statistics Version 26.0. The $p < 0.05$ significant difference between treatment means was established with the help of the Tukey Honest Significant Difference (HSD) test. The findings were provided in mean \pm standard deviation (SD).

3. Results

Table 1 explains the physiological response of four accessions, *Vigna vexillata*, *Vigna unguiculata ssp. dekindtiani*, *Vigna subterranea*, and *Vigna unguiculata var. IT90K-277-2* (control), to heat stress at 42°C . The result indicated that there were clear differences in water status and photosynthetic activity of the cells in the varieties, with the wild relatives more tolerant than the cultivated control. *V. unguiculata ssp. dekindtiana* had the highest photosynthetic rate ($13.5 \pm 1.1 \text{ umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), which was closely followed by *V. vexillata* ($12.8 \pm 0.9 \text{ umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and *V. subterranea* ($11.9 \pm 1.0 \text{ umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). The control variety IT90K-277-2, on the other hand, registered a much lower photosynthetic rate of $9.6 \pm 0.8 \text{ umol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, a fact that could be explained by the presence of heat stress to it.

As observed by Gao et al., (2023), the photosynthetic rate of the *Vigna* species differs enormously with species, genotype, and environmental stress factors. They also emphasized that *Vigna subterranea* (Bambara groundnut) experiences significant decline in photosynthetic rate during drought stress and associated decline in stomatal conductance, transpiration, and internal CO_2 , but increased water use efficiency, which is indicative of an adaptive adjustment to water shortage. A similar result was also found in stomatal conductance, where the wild accessions had higher rates of gas exchange of 0.16 ± 0.02 to $0.20 \pm 0.03 \text{ mol H}_2\text{O m}^{-1} \text{ s}^{-1}$ compared to the control, which had $0.12 \pm 0.01 \text{ mol H}_2\text{O m}^{-1} \text{ s}^{-1}$. This implies that the wild species would be more likely to perform better under heat stress conditions to facilitate more CO_2 assimilation and cooling through transpiration. This agrees with Chaudhary et al., (2022) who noted that among the studies in *Vigna mungo* (urdbean) and *Vigna radiata* (mungbean), wild *Vigna* accessions tend to be more resilient to heat stress than cultivated or control varieties, and further reported that this allows them to facilitate more gas exchange and possibly increased CO_2 assimilation and transpirational cooling.

There was no difference in the composition of total chlorophyll content of the wild accessions. *V. unguiculata ssp. dekindtiana* contained the highest content of $1.92 \pm 0.14 \text{ mg g}^{-1} \text{ FW}$, followed by *V. vexillata* ($1.85 \pm 0.11 \text{ mg g}^{-1} \text{ FW}$) and *V. subterranea* ($1.70 \pm 0.10 \text{ mg g}^{-1} \text{ FW}$). The lowest chlorophyll content ($1.30 \pm 0.09 \text{ mg g}^{-1} \text{ FW}$) was found in the control variety, so it could be supposed that this component is more likely to destroy pigments under the influence of heat stress and thus had a low photosynthetic potential. Jha et al., (2025) cited that wild accessions tend to show less reduction in chlorophyll content compared to susceptible or control varieties, supporting better photosynthetic performance and resilience under high temperatures

Relative water content (RWC) also demonstrated that all varieties were able to store water at the exposure to heat. The most prevalent values of RWC were noted in *V. unguiculata ssp. dekindtiana* and *V. vexillata* ($74.1 \pm 2.8\%$ and $72.4 \pm 3.2\%$, respectively), while *V. subterranea* was intermediate ($69.8 \pm 3.6\%$). The lowest RWC was observed in the control variety ($61.5 \pm 4.0\%$), which meant that the variety lost water when stressed and retained turgor to a lesser extent.

Stability of the membranes was another piece of evidence of the tolerance of the wild relatives, showing the measure of cellular integrity by membrane stability index (MSI). MSI was 75.0-80.2, and *V. subterranea* ($75.0 \pm 3.1\%$) and *V. unguiculata ssp. dekindtiana* ($80.2 \pm 3.0\%$) were compared with $62.4 \pm 3.5\%$ in the control. This implies that the membranes of the wild accessions were more adaptive to heat pressure and hence less prone to lipid peroxidation and had better cellular protection mechanisms. This finding corroborates Gupta et al. (2021) who noted that wild genotypes of Vigna, including *V. unguiculata ssp. dekindtiana* and *V. vexillata*, retain better RWC and MSI under stress than the susceptible or control varieties, which lose more water and have a higher extent of membrane damage. In addition, Tiwari et al., (2018) recorded that the greater RWC is associated with increased water retention and turgor maintenance, and the increased MSI levels are associated with increased cellular integrity and decreased lipid peroxidation, both of which contribute to increased stress resilience.

Overall, Table 1 demonstrated that *V. unguiculata ssp. dekindtiana* had the highest physiological resilience to heat stress, followed by *V. vexillata* and *V. subterranea*. Control IT90K-277-2 was relatively weak in all the parameters under assessment, and this demonstrated its weakness. This finding was an indication that the wild relatives possessed inherent physiological traits, which allowed them to support active photosynthesis, water balance, and membrane stability under heat conditions.

Table 1. Physiological responses under 42°C heat stress (n = 6)

Variety (n=6)	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Total chlorophyll ($\text{mg g}^{-1} \text{ FW}$)	Relative water content (%)	Membrane stability index (%)
<i>V. vexillata</i>	12.8 ± 0.9	0.18 ± 0.02	1.85 ± 0.11	72.4 ± 3.2	78.6 ± 2.7
<i>V. unguiculata ssp. dekindtiana</i>	13.5 ± 1.1	0.20 ± 0.03	1.92 ± 0.14	74.1 ± 2.8	80.2 ± 3.0
<i>V. subterranea</i>	11.9 ± 1.0	0.16 ± 0.02	1.70 ± 0.10	69.8 ± 3.6	75.0 ± 3.1
<i>V. unguiculata var. IT90K-277-2 (control)</i>	9.6 ± 0.8	0.12 ± 0.01	1.30 ± 0.09	61.5 ± 4.0	62.4 ± 3.5

The yield component responses of four cowpea accessions, *Vigna vexillata*, *Vigna unguiculata ssp. dekindtiana*, *Vigna subterranea*, and *Vigna unguiculata var. IT90K-277-2 (control)*, after exposure to heat stress at 42°C are presented in Table 2. These data demonstrate that there were high varietal differences in pod formation, seed development, and productivity generally, with the reproductive performance of the wild relatives being superior in the conditions of high temperatures than the cultivated variety.

A high number of pods per plant (13.0 ± 1.4) and seeds per pod (11.2 ± 1.0) was recorded in *V. unguiculata ssp. dekindtiana*. Although *V. vexillata* showed slightly lower values of pods per plant (12.3 ± 1.5) and seeds per pod (10.8 ± 1.1), these values remained very high compared with the control. Reproductive efficiency was low in the cultivated *V. unguiculata var. IT90K-277-2* (7.8 ± 1.2 pods per plant and 7.0 ± 0.8 seeds per pod) due to heat stress.

The disparity in heat tolerance was also observed in seed weight and yield per plant. The 100-seed weight ranged from 9.6 ± 0.7 g in *V. unguiculata ssp. dekindtiana* to 8.7 ± 0.8 g in *V. subterranea*, while *V. vexillata* recorded 9.2 ± 0.6 g. In contrast, the control variety had a considerably lower seed weight of 7.4 ± 0.5 g. The reduction in size of the seeds under heat stress suggests that the control variety was more susceptible to heat, which may be explained by the fact that it was not able to translocate assimilates and fill seed.

The same was experienced with the overall seed yield per plant of the accessions, as it confirmed the advantage of the wild accessions. The maximum yield was 124.9 ± 9.5 g in *V. unguiculata ssp. dekindtiana*, then *V. vexillata* (112.6 ± 8.7 g) and *V. subterranea* (89.4 ± 10.2 g). The control, IT90K-277-2, was found to yield significantly less than the accession, at 45.6 ± 6.1 g per plant, which is half that of the most heat-tolerant accession. Kaur (2015) reported that wild relatives and heat-tolerant

genotypes are always superior to cultivated varieties when stressed by heat because they have more pods per plant, seeds per pod, seed weight, and total yield.

The research established that *V. unguiculata ssp. dekindtiana* were more adaptable in the heat, as they were superior reproducers with superior pod formation, seed growth, and stability. The cultivated control displayed strong yield reduction, suggesting heat-tolerance traits could be exploited in breeding programs to improve yield resilience under worsening temperature conditions.

Table 2. Yield component responses after heat-stress period (n = 6)

Variety (n=6)	Pods per plant (mean \pm SD)	Seeds per pod (mean \pm SD)	100-seed weight (g)	Seed yield per plant (g)
<i>V. vexillata</i>	12.3 \pm 1.5	10.8 \pm 0.9	9.2 \pm 0.6	112.6 \pm 8.7
<i>V. unguiculata ssp. dekindtiana</i>	13.0 \pm 1.4	11.2 \pm 1.0	9.6 \pm 0.7	124.9 \pm 9.5
<i>V. subterranean</i>	10.7 \pm 1.6	9.5 \pm 1.1	8.7 \pm 0.8	89.4 \pm 10.2
<i>V. unguiculata</i> var. IT90K-277-2 (control)	7.8 \pm 1.2	7.0 \pm 0.8	7.4 \pm 0.5	45.6 \pm 6.1

Table 3 shows that the biochemical stress markers were determined at the peak of heat stress in the four cowpea accessions: *Vigna vexillata*, *Vigna unguiculata ssp. dekindtiana*, *Vigna subterranea*, and *Vigna unguiculata* var. IT90K-277-2 (control). The biochemical indices that were assessed were proline accumulations and malondialdehyde (MDA) and the activities of two antioxidant enzymes, namely, superoxide dismutase (SOD) and catalase (CAT). The general indicators were utilized to depict the biochemical potential of the plants to withstand heat stress caused by oxidative stress.

The amount of proline was very high in the wild accessions compared to the control, which implied that the accessions responded better to heat stress osmotically. The proline was highest in *V. unguiculata ssp. dekindtiana* (52.7 \pm 5.1 $\mu\text{mol g}^{-1}$ FW), followed by *V. vexillata* (48.2 \pm 4.5 $\mu\text{mol g}^{-1}$ FW) and *V. subterranea* (41.5 \pm 3.9 $\mu\text{mol g}^{-1}$ FW). The control variety, IT90K-277-2, however, accumulated 28.9 \pm 3.0 $\mu\text{mol g}^{-1}$ FW of proline. This wide difference suggests that the wild relatives possess more ability to synthesize and store compatible solutes to stabilize cell structures and osmotic equilibrium through heat stress, in agreement with Raza et al., (2023) who equally added that proline

acts as an important compatible solute, and its increased synthesis in wild relatives is associated with enhanced tolerance to high temperatures, as it helps protect cellular functions and mitigates the effects of heat-induced dehydration and oxidative stress.

The amount of lipid peroxidation that was presented as MDA concentration provided a hint on the oxidative damage by the membranes. The control recorded the highest MDA ($4.6 \pm 0.5 \text{ nmol g}^{-1} \text{ FW}$), an indication of very high levels of lipid degradation due to accumulation of the reactive oxygen species (ROS). This is similar to the report of Gamit et al., (2025) who noted that cultivated varieties tend to accumulate less proline and exhibit higher levels of lipid peroxidation, as measured by malondialdehyde (MDA) concentration, which signals greater oxidative membrane damage due to reactive oxygen species (ROS). Quite on the contrary, the wild accessions registered significantly lower concentrations of MDA, the lowest concentration of $2.5 + 0.2 \text{ nmol g}^{-1} \text{ FW}$ in *V. unguiculata ssp. dekindtiana*, and the highest level of $3.1 + 0.4 \text{ nmol g}^{-1} \text{ FW}$ in *V. subterranea*, indicating higher membrane protection and reduced oxidative stress.

Another system that was also known by the antioxidant defence system was SOD and CAT activity and further distinguished the wild relatives and the cultivated control. *V. unguiculata ssp. dekindtiana* was the most active in SOD ($134.0 \pm 10.5 \text{ U mg}^{-1} \text{ protein}$), followed by *V. vexillata* ($125.4 \pm 9.8 \text{ U mg}^{-1} \text{ protein}$) and *V. subterranea* ($118.2 \pm 11.0 \text{ U mg}^{-1} \text{ protein}$). The control variety, on the other hand, had a significantly lower SOD activity of $85.6 \pm 7.2 \text{ U mg}^{-1} \text{ protein}$. Likewise, the CAT activity also had a parallel pattern, with the wild accessions having higher enzyme activity (ranging from 58.0 ± 5.6 to $68.3 \pm 6.0 \text{ U mg}^{-1} \text{ protein}$) than the control ($39.7 \pm 4.1 \text{ U mg}^{-1} \text{ protein}$). It was observed that these high concentrations of enzyme deactivators of the wild accessions indicate that they are more capable of combating reactive oxygen species and decreasing the effects of oxidative damage in heat stress conditions. According to Alagupalamuthirsolai et al., (2020) the increased antioxidant defence in wild or tolerant accessions was associated with increased physiological performance, increased biomass, chlorophyll content, and decreased lipid peroxidation, which resulted in increased resilience and productivity in response to heat stress.

The table also indicates that wild cowpea relatives have stronger mechanisms of biochemical defence compared to cultivated control. *V. unguiculata ssp. dekindtiana* exhibits the combination of high proline accumulation and low lipid peroxidation with high antioxidant enzyme activities in close succession with *V. vexillata* and *V. subterranea*. The most susceptible is the cultivated IT90K-277-2 with the least osmoprotectant and antioxidant reactions.

Table 3. Biochemical stress markers measured at peak stress (n = 6)

Variety (n=6)	Proline ($\mu\text{mol g}^{-1} \text{ FW}$)	MDA (malondialdehyde, $\text{nmol g}^{-1} \text{ FW}$)	SOD activity ($\text{U mg}^{-1} \text{ protein}$)	CAT activity ($\text{U mg}^{-1} \text{ protein}$)
<i>V. vexillata</i>	48.2 ± 4.5	2.8 ± 0.3	125.4 ± 9.8	62.1 ± 5.2

Variety (n=6)	Proline ($\mu\text{mol g}^{-1}$ FW)	MDA (malondialdehyde, nmol g^{-1} FW)	SOD activity (U mg^{-1} protein)	CAT activity (U mg^{-1} protein)
<i>V. unguiculata</i> ssp. <i>Dekindtiana</i>	52.7 ± 5.1	2.5 ± 0.2	134.0 ± 10.5	68.3 ± 6.0
<i>V. subterranean</i>	41.5 ± 3.9	3.1 ± 0.4	118.2 ± 11.0	58.0 ± 5.6
<i>V. unguiculata</i> var. IT90K-277-2 (control)	28.9 ± 3.0	4.6 ± 0.5	85.6 ± 7.2	39.7 ± 4.1

Table 4 describes the relative expressions of three large heat-responsive genes, HSP70, HSP101, and DREB2A, in four cowpea accessions (*Vigna vexillata*, *Vigna unguiculata* ssp. *dekindtiana*, *Vigna subterranea*, and *Vigna unguiculata* var. IT90K-277-2, the control variety) subjected to heat stress hydrothermal conditions of 42°C. These genes are majorly involved in cellular thermotolerance, protein stabilization, and transcriptional regulation during heat-induced oxidative stress. These values are in the form of fold changes against the control and show how much each gene was upregulated as a response to stress.

The HSP70 gene, which encodes a molecular chaperone, which prevents denaturation of the proteins during heat stress, was found to be significantly expressed in all the wild relatives as compared to the cultivated control. The highest HSP70 expression (8.5 ± 1.0 fold) was observed in *V. unguiculata* ssp. *dekindtiana*, closely followed by *V. vexillata* (7.8 ± 0.9 fold) and *V. subterranea* (6.9 ± 0.8 fold), respectively. On the other hand, the control variety, IT90K-277-2, produced a far lower induction value of 4.1 ± 0.5 . This implies that the wild accessions possess a superior cellular defence system to enable the protection of the proteins against a heat stress environment that might predispose them to denaturation and aggregation. Nie et al., (2017) reported a greater upregulation of HSP70 mRNA in response to heat shock (up to 7.46-fold) compared to cultivated strains (ranging from 3.56 to 9.21-fold) in the wild strains of the Manila clam (*Ruditapes philippinarum*), supporting the idea that wild genotypes may possess enhanced stress resilience mechanisms. Similarly, Landi et al., (2019) showed that landraces of barley from the semi-arid regions demonstrated unique HSP70 expression patterns and greater tolerance to drought and salinity compared to commonly cultivated varieties, highlighting the adaptive advantage of wild or locally adapted genotypes.

Furthermore, the expression of the HSP101, which is a heat shock protein that is involved in the refolding of aggregated proteins and homeostasis in cells, also assumed a comparable pattern. *V. unguiculata* ssp. *dekindtiana* was identified to be the most expressed (5.6 ± 0.7 fold), and *V. vexillata* (5.1 ± 0.6 fold) and *V. subterranea* (4.7 ± 0.5 fold) were also significantly higher in transcript. The control variety displayed the smallest expression with a 2.3 ± 0.3 -fold of a fairly weak system of molecular heat response. This further up-regulation in the wild species shows the augmented defiance of the wild species to the proteotoxic stress conditions. Babbar et al., (2023) showed that higher HSP101 expression was associated with greater heat tolerance and increased fitness in wild-collected

ecotypes of *Arabidopsis*, culminating in higher fruit production and better survival under stress conditions. The transcription factor, DREB2A gene, which is the activator of downstream genes in response to the stresses, was also found to possess greater expression in the wild accessions. *V. unguiculata ssp. dekindtiana* was the most responsive with an increase of folds of 4.0 ± 0.5 , followed by *V. vexillata* at 3.6 ± 0.4 and *V. subterranea* at 3.2 ± 0.3 . The control variant was the least upregulated (1.7 ± 0.2), which implies a low potential to induce heat-tolerance signalling pathways. Overall, the transcriptional activation of the major heat-responsive genes was slightly greater in wild cowpea relatives compared to the cultivated control. *V. unguiculata ssp. dekindtiana* exhibited the best responses to gene expression, meaning that it had better genetic potential to survive in the heat conditions. This is similar to the findings of Vitko, et al., (2025) who cited that in *Arabidopsis*, DREB2A expression increases after heat stress, especially in genotypes with enhanced stress responses, supporting the observation that wild or stress-adapted accessions have a more robust molecular defence system. This increase of the expression of HSP70, HSP101, and DREB2A in these wild species is consistent with the fact that, compared to the physiological stability and biochemical resistance of these wild species to heat stress, they have an adaptive advantage to heat stress temperature.

Table 4. Relative expression (fold change) of heat-responsive genes under 42°C heat stress (n = 6)

Variety (n = 6)	HSP70 (fold change)	HSP101 (fold change)	DREB2A (fold change)
<i>V. vexillata</i>	7.8 ± 0.9	5.1 ± 0.6	3.6 ± 0.4
<i>V. unguiculata ssp. Dekindtiana</i>	8.5 ± 1.0	5.6 ± 0.7	4.0 ± 0.5
<i>V. subterranean</i>	6.9 ± 0.8	4.7 ± 0.5	3.2 ± 0.3
V. unguiculata var. IT90K-277-2 (Control)	4.1 ± 0.5	2.3 ± 0.3	1.7 ± 0.2

The Analysis of Variance (ANOVA) in Table 5 was used to find out whether the difference between the four *Vigna* accessions was statistically different regarding each measured trait when subjected to heat stress. The between-group (SS between) was the difference between the means of varieties, and the within-group (SS within or error) was the difference within each of the varieties. The F-value was used to represent the ratio between these two variances, and the p-value was used to show the likelihood of the differences arising randomly.

The parameters in this research, including photosynthetic rate, chlorophyll content, yield of the seed, proline concentration, malondialdehyde (MDA) content, and expression of HSP70 genes, were found to vary significantly ($p < 0.001$) across the varieties. This implies that varietal effects had a robust impact on physiological, biochemical, and molecular heat stress responses. Particularly, the F-values of parameters like seed yield ($F = 45.1$) and proline accumulation ($F = 56.7$) are very high, which

implies that the difference between the varieties is very strong. These results are in line with the previous findings that *V. unguiculata ssp. dekindtiana* and *V. vexillata* had a much greater performance in terms of physiological and molecular parameters than the control (IT90K-277-2). The fact that MDA levels are lower and antioxidant enzyme activity is higher in the wild relatives also supports high stress tolerance of the wild relatives. The ANOVA supports the idea that wild *Vigna* accessions, especially *V. unguiculata ssp. dekindtiana*, have better physiological efficiency, biochemical resilience, and molecular agility than cultivated control, which makes wild *Vigna* plant materials good options as valuable genetic resources to enhance heat tolerance in cowpea breeding programs.

Table 5. Analysis of Variance (ANOVA) for physiological, biochemical, yield, and gene expression parameters among four cowpea accessions under 42°C heat stress

Parameter	Source of Variation	DF	Sum of Squares (SS)	Mean Square (MS)	F-value	P-value
Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Between varieties	3	72.42	24.14	29.8	<0.001
	Within varieties (Error)	20	16.19	0.81		
Total chlorophyll (mg g^{-1} FW)	Between varieties	3	0.82	0.27	21.6	<0.001
	Within varieties (Error)	20	0.25	0.01		
Seed yield per plant (g)	Between varieties	3	11,870.2	3,956.7	45.1	<0.001
	Within varieties (Error)	20	1,755.4	87.8		
Proline ($\mu\text{mol g}^{-1}$ FW)	Between varieties	3	2,189.3	729.8	56.7	<0.001

	Within varieties (Error)	20	257.6	12.9		
MDA (nmol g⁻¹ FW)	Between varieties	3	7.81	2.60	32.5	<0.001
	Within varieties (Error)	20	1.60	0.08		
HSP70 expression (fold change)	Between varieties	3	40.68	13.56	38.9	<0.001
	Within varieties (Error)	20	6.97	0.35		

The results of the Tukey post-hoc test in Table 6 indicate that the wild accessions ($p < 0.05$) differed significantly from the cultivated control (*V. unguiculata* var. IT90K-277-2) in almost all physiological, biochemical, yield, and molecular characteristics. The greatest differences were found in seed yield, proline content, and expression of the HSP70 gene, which points to better thermotolerance mechanisms in the wild relatives. It is interesting to note that *V. unguiculata* ssp. *dekindtiana* and *V. vexillata* did not vary significantly in most traits, and this indicated that they were similar in terms of adaptability to heat stress. Nevertheless, they both performed better than *V. subterranea* and the control type and had enhanced physiological stability and molecular flexibility. In general, the findings of the post-hoc tests support the view that *V. unguiculata* ssp. *dekindtiana* and *V. vexillata* may be used as effective breeding donors of heat-tolerant cowpea varieties, while *V. subterranea* can be moderately used as a potential breeding donor.

Table 6. Tukey's HSD multiple comparison of means among four cowpea accessions under 42°C heat stress

Parameter	Comparison (Pair of Varieties)	Mean Difference	Significance ($p < 0.05$)	Interpretation
Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	<i>V. unguiculata</i> ssp. <i>dekindtiana</i> – IT90K-277-2	+3.9	Significant	Wild variety showed higher photosynthesis
	<i>V. vexillata</i> – IT90K-277-2	+3.2	Significant	Wild variety superior

	<i>V. subterranea</i> – IT90K-277-2	+2.3	Significant	Moderate advantage
	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	+0.7	NS	Similar performance
Total chlorophyll (mg g⁻¹ FW)	<i>V. unguiculata ssp. dekindtiana</i> – IT90K-277-2	+0.62	Significant	Higher pigment retention
	<i>V. vexillata</i> – IT90K-277-2	+0.55	Significant	Higher pigment retention
	<i>V. subterranea</i> – IT90K-277-2	+0.40	Significant	Moderate increase
	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	+0.07	NS	Statistically similar
	<i>V. unguiculata ssp. dekindtiana</i> – IT90K-277-2	+79.3	Significant	Highest yield advantage
Seed yield per plant (g)	<i>V. vexillata</i> – IT90K-277-2	+67.0	Significant	Higher yield potential
	<i>V. subterranea</i> – IT90K-277-2	+43.8	Significant	Moderate increase
	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	+12.3	Ns	Comparable yield response
	<i>V. unguiculata ssp. dekindtiana</i> – IT90K-277-2	+23.8	Significant	Strong osmotic adjustment
Proline (μmol g⁻¹ FW)	<i>V. vexillata</i> – IT90K-277-2	+19.3	Significant	Improved stress response
	<i>V. subterranea</i> – IT90K-277-2	+12.6	Significant	Moderate accumulation

MDA (nmol g ⁻¹ FW)	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	+4.5	Ns	No significant difference
	<i>V. unguiculata ssp. dekindtiana</i> – IT90K-277-2	-2.1	Significant	Lower lipid peroxidation
	<i>V. vexillata</i> – IT90K-277-2	-1.8	Significant	Reduced oxidative damage
	<i>V. subterranea</i> – IT90K-277-2	-1.5	Significant	Improved membrane stability
HSP70 expression (fold change)	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	-0.3	Ns	Comparable antioxidant protection
	<i>V. unguiculata ssp. dekindtiana</i> – IT90K-277-2	+4.4	Significant	Strongest gene induction
	<i>V. vexillata</i> – IT90K-277-2	+3.7	Significant	High gene activation
	<i>V. subterranea</i> – IT90K-277-2	+2.8	Significant	Moderate activation
	<i>V. unguiculata ssp. dekindtiana</i> – <i>V. vexillata</i>	+0.7	Ns	Statistically similar expression

4. DISCUSSION

The findings of this research indicate that there are distinct and significant physiological, biochemical, yield, and molecular differences in response to heat stress among the four accessions of *Vigna* species examined. As it was revealed in the analysis, the wild relatives such as *Vigna unguiculata ssp. dekindtiana*, *Vigna vexillata*, and *Vigna subterranea* had better adaptive mechanisms than the cultivated control, *Vigna unguiculata var. IT90K-277-2*. Van Zonneveld et al., (2020) reported that wild *Vigna* taxa tend to possess greater abiotic stress resilience, including heat and drought tolerance, although such traits are relatively rare compared to biotic stress resistance. These results are agreeable with the fact that wild *Vigna* species contain desirable genetic codes that enable them to withstand adverse conditions of elevated temperatures.

The wild accessions comprising *V. unguiculata ssp. dekindtiana* showed greater photosynthetic rates, stomatal conductance, chlorophyll content, relative water content, and membrane stability at 42°C heat stress than the control. These characteristics suggest effective photosynthetic performance and superior pigment conservation as suggested by Selinga et al., (2022). The percentage water content and membrane stability index were also higher than the cultivated control, which means that they could retain cell integrity during thermal stress. These findings are in support of the earlier reports, which indicated that the heat-tolerant genotypes were able to maintain higher photosynthetic efficiency and water retention by having superior stomatological control and cellular defence systems. This study discovered that heat stress had a strong impact on the yield components among the genotypes, but wild species had higher recovery and reproductive performance. *V. unguiculata ssp. dekindtiana* had the greatest number of pods per plant, seeds per pod, and seed yield per plant and was higher than both *V. vexillata* and *V. subterranea* in agreement with the works of Biradar et al., (2025). This indicates that the wild accessions possess mechanisms that maintain heat-stress reproductive development, possibly by increased pollen viability, heightened assimilate partitioning, and stronger sinks.

As was shown in the biochemical analysis, the wild accessions stored more proline and antioxidant enzyme activity (SOD and CAT) and reduced levels of malondialdehyde (MDA) compared to the control. The elevated proline levels showed an increased osmotic adaptation ability and the capability of stabilizing proteins and membranes in dehydration caused by heat. The elevated SOD and CAT enzyme activities in wild accessions suggest strong enzyme defence systems, which trap reactive oxygen species (ROS) in order to minimize oxidative injury. Jamshidi et al., (2023) documented that wild relatives of wheat, such as *Aegilops* species with alien genomes, show higher SOD and CAT activities under water deficit or salinity stress compared to cultivated varieties, suggesting enhanced oxidative stress tolerance through increased enzymatic antioxidant responses. The genotype that showed the highest MDA concentration was the control, which revealed the highest lipid peroxidation and cell damage.

The study analysed the transcriptional regulation of various genotypes in the cowpea species by observing that the main heat-sensitive genes were highly up-regulated in wild relatives, with the highest fold change in *V. unguiculata ssp. dekindtiana*. These genes play a vital role in the protection and folding of proteins as well as the transduction of stress signals. The increased expression of wild species suggests that wild species can employ molecular defence programs during heat stress, which guarantees protein homeostasis and transcriptional control of stress-response pathways. The domesticated control had been characterized by a relatively small increase in these genes, indicating a low genetic sensitivity to high-temperature stress (Balla et al., 2025)

The outcomes of the ANOVA tests proved that there are significant differences in the physiological, biochemical, yield, and gene expression traits, and the F-values of seed yield, proline content, and HSP70 expression were high. The Tukey's HSD test revealed that there was a significant difference between the wild accessions and the cultivated control, indicating comparable heat tolerance. The analysis shows that wild *Vigna* relations are more resistant to heat stress as compared to cultivated cowpeas because of the high degree of physiological efficiency, biochemical defence, yield stability, and molecular responsiveness. Wild germplasm is important in facilitating thermotolerance, and *V. unguiculata ssp. dekindtiana* and *V. vexillata* are the possible sources of genetic enhancement in heat-

stress breeding programs. Integrating these traits into cowpea breeding programs could lead to crop cultivars that endure global temperatures, ensuring food security.

5. CONCLUSION

The study shows that wild cowpea species, namely, *Vigna unguiculata ssp. dekindtiana*, *Vigna vexillata*, and *Vigna subterranea*, were more adapted to heat when compared to the cultivated *Vigna unguiculata var. IT90K-277-2*. These wild accessions were more photosynthetically efficient and antioxidant and expressed more genes under heat stress conditions of 42°C. *V. unguiculata ssp. dekindtiana* was stable, which suggests a high adaptive response to thermal stress. The research recommends the application of these wild species of *Vigna* in breeding programs to develop cowpeas that are more resistant to heat. The high thermotolerance genes of these accessions should be introduced into elite cultivars by hybridizing and marker-assisted selection. Genomic mapping and transcriptomic profiling require additional investigation to identify the significance of regulatory loci that make organisms resistant to heat shocks. Field tests in the natural environments that involve multi-locations under heat stress conditions are also required to verify the laboratory findings and enhance the choice of climate-sensitive cowpea varieties to achieve sustainable agricultural productivity.

Author Contributions

The authors read and approved the final version of the paper

Conflict of interest

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This research does not need an ethical clearance since it did not involve any human subjects, research animals, or any information that can be deemed sensitive.

Availability of data statement

The set of data analysed during the study is available through the corresponding author if the request is reasonable enough.

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This study made use of quillbot ai grammarly to improve the grammar in the text.

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