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Research article

Insights into the responses of Akabare chili landraces to drought, heat, and their combined stress during pre-flowering and fruiting stages

Damodar Poudyal [a,*], Bal Krishna Joshi [b], Kishor Chandra Dahal [a]

[a] Postgraduate Program, Institute of Agriculture and Animal Science, Tribhuvan University, Kirtipur-10, 44618, Kathmandu, Nepal
[b] National Agriculture Genetic Resources Center, Nepal Agricultural Research Council, 44700, Khumaltar, Lalitpur, Nepal

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ABSTRACT

Drought, heat, and their combined stress have increasingly become common phenomena in horticulture, significantly reducing chili production worldwide. The current study aimed to phenotype Akabare chili landraces (*Capsicum* spp.) in climate chambers subjected to drought and heat treatments during their early generative stage, focusing on PSII efficacy (F_v/F_m), net photosynthetic rate (P_N), stomatal conductance (g_s), leaf cooling, and biomass production. Six landraces were examined under heat and control conditions at 40/32 °C for 4 days and at 30/22 °C under drought and control conditions followed by a 5-day recovery under control conditions (30/22 °C, irrigated). Two landraces with higher (>0.77) and two with lower (<0.763) F_v/F_m during the stress treatments were later evaluated in the field under 55-day-long drought stress at the fruiting stage. In both treatments, stress-tolerant landraces maintained high F_v/F_m, P_N, and better leaf cooling leading to improved biomass compared to the sensitive landraces. Agro-morpho-physiological responses of the tolerant and sensitive landraces during the early generative stage echoed those during the fruiting stage in the field. A climate chamber experiment revealed a 13.9 % decrease in total biomass under heat stress, a further 21.5 % reduction under drought stress, and a substantial 38.7 % decline under combine stress. In field conditions, drought stress reduced total biomass by 28.1 % and total fruit dry weight by 26.2 %. Tolerant landraces showed higher F_v/F_m, demonstrated better wilting scores, displayed a higher chlorophyll content index (CCI), and accumulated more biomass. This study validated lab-based results through field trials and identified two landraces, C44 and DKT77, as potential stress-tolerant genotypes. It recommends F_v/F_m, P_N, and CCI as physiological markers for the early detection of stress tolerance.

1. Introduction

Over the years spanning from 1850 to 1900 to 2011–2020, there has been a significant global increase in average land surface temperature (1.59 °C), and projections (2 °C by 2050) indicate a continuation of this warming trend [1]. The changing climate, marked by rising temperatures and shifting rainfall patterns, poses a substantial threat to horticulture, particularly in vulnerable regions like

* Corresponding author.
E-mail addresses: damodarpoudyal@gmail.com (D. Poudyal), joshibalak@yahoo.com (B. Krishna Joshi), kishor.dahal@iaas.tu.edu.np (K. Chandra Dahal).

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the Hindu Kush Himalayas, including Nepal [2]. Niche-specific landraces in Nepal are facing challenges in adapting to and maintaining quality amid the changing climate. Akabare chili (*Capsicum* spp.), a prevalent chili landrace in Nepal's hilly regions, is popular for its spicy and aromatic fruits [3].

Akabare chili is cultivated from March to December, predominantly relying on rainfed conditions. This crop has shown significant roles in household economies, with a notable benefit-cost ratio of approximately 2, as reported by Poudyal et al. [4]. Fresh fruits of Akabare chili contain about 87 % moisture, 85 mg/100 g Vitamin C, and 14.8 mg/g chlorophyll content [5]. Detail nutritional analysis of Akabare chili landraces is lacking but chili landraces are a good source of capsaicin (7.91–9.94 mg/g), pro-vitamin A (0.6–1.01 mg/g) [6]. Typical fragrance and the myth of no after-burn are special values for the Akabare chili consumers. The morphological diversity and inherent genetics of Akabare chili, as discussed by Poudyal et al. [3], have the potential to enhance plant resilience to abiotic stresses such as drought and heat [7]. Despite an expansion in cultivated land and production, Akabare chili experienced a notable 16.5 % decline in productivity in 2021 compared to 2020 [8], likely attributed to shifts in precipitation and temperature [9].

Major yield-attributing physiological traits of plants are the efficacy or integrity of photosystem II (F_v/F_m) [10], net photosynthetic rate (P_N), stomatal conductance (g_s), leaf chlorophyll content, etc. [11]. Chili, a common crop of the tropics and sub-tropics, is frequently exposed to soil moisture stress and elevated temperature at various stages of its growth, either separately or in tandem, resulting in diminished yield [12], more likely through the alteration of critical physiological processes. Drought stress influences root-to-shoot biomass ratio (RS), net photosynthesis, evaporating cooling, and biomass gain [12]. Likewise, elevated temperature stress negatively affects chili production by diminishing stomatal regulation and carbon gain [13]. Drought and heat stress hinder the overall plant performance of *Capsicum* species by limiting cell development and growth, ultimately resulting in a decrease in shoot and root dry weights [14].

High-temperature stress often coincides with and triggers low soil moisture stress in field conditions [15]. This implies the importance of considering both stresses during phenotypic studies of crops, as highlighted by Lamaoui et al. [16]. Under combined stress conditions, plants may exhibit different responses in terms of physiology, biochemistry, genomics, and related factors compared to a single stressor [17,18]. Prior investigations predominantly examined the response of the crop to individual stimuli with limited exploration of the combined effects of drought and heat on the growth and development of crops [19,20]. Investigating responses to stimuli during both the stress treatment and recovery phase holds promise for uncovering the underlying physiological processes, particularly the plants' ability to restore physiological processes [17].

Drought and heat tolerance in plants are highly dependent on the growth stage. Conducting studies at various growth and developmental stages of crop plants is instrumental in comprehending their responses [21]. Among various growth stages, the reproductive phase, particularly anthesis, is widely recognized as the most sensitive to stresses like drought and heat [22,23]. Understanding the connections between the effects of drought and heat stress at pre-flowering and fruiting stages of crop development both in controlled conditions and field trials is particularly meaningful [21]. Even though, field-level plant phenotyping is challenging, it provides a robust framework for a more nuanced and powerful interpretation of results [24,25]. This approach offers a robust framework for a more nuanced and powerful interpretation of results [26].

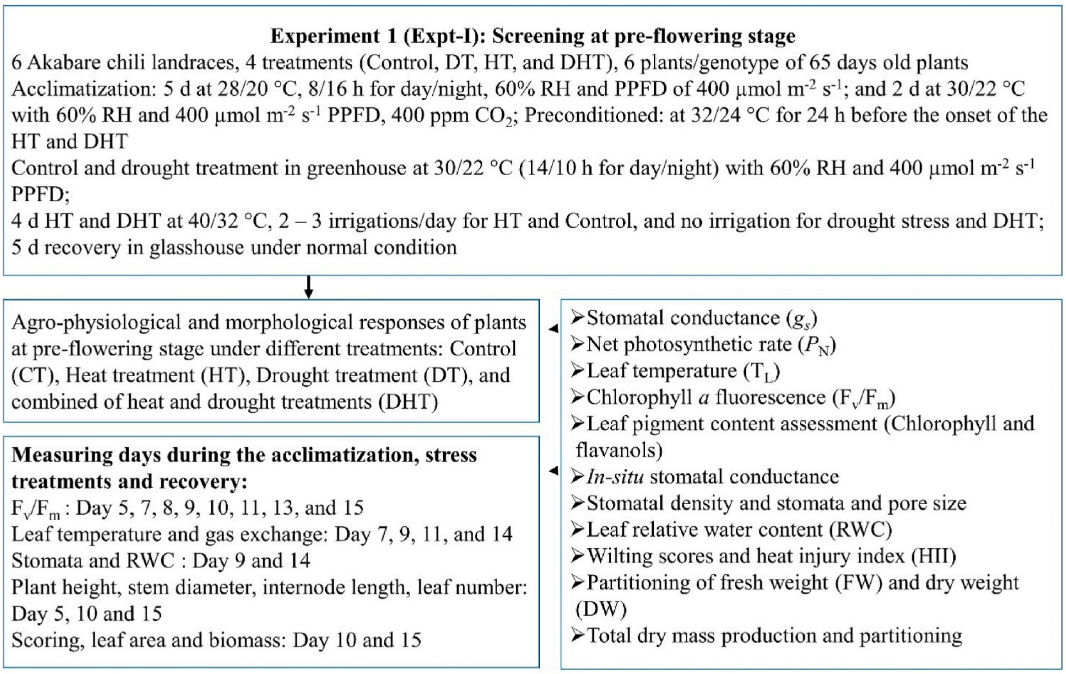

Fig. 1. Workflow of experiment one under controlled conditions in 2021.

Despite the presence of a huge market in Nepal and South Asia, limited efforts have been made towards crop improvement, leaving many aspects of this wonder spice unexplored [3]. Research on the physiological responses of Akabare chili to drought, heat, and combined stress at different growth stages and growing conditions will be another step in Akabare chili phenomics. Validating disparities in agro-physiological responses between tolerant and sensitive landraces under identical field conditions will substantially support the effectiveness of using F_v/F_m as a phenotyping marker for stress tolerance and strengthen the meaningful connection between screenings in controlled environments and field conditions. The current study had two primary objectives: (i) to improve the understanding of how drought and heat stresses affect Akabare chili development during the pre-flowering and fruiting stages, both individually and in combination, and (ii) to explore the interconnectedness between Akabare chili's ability to withstand drought and heat stress at distinct developmental stages in controlled and field conditions. Our hypothesis posited that intense drought and heat stress will impact the efficiency of PSII, impeding processes related to light harvesting and primary photochemistry, ultimately influencing overall carbon gain. Ultimately, this study will enrich insight into stress tolerance mechanisms in Akabare chili at their different growth stages and spotlight elite and tolerant landraces for future breeding efforts.

2. Materials and methods

2.1. The first experiment: screening at controlled conditions

Plant materials and seedling preparation: The first experiment (Expt-I) employed six Akabare chili landraces as plant material. Notably, three top performers (C44, DKT77, and C45C) and three bottom performers (C64C, BJ77, and PPR77) were specifically chosen based on their F_v/F_m values under stress treatments, as reported in Poudyal et al. [27]. Supplementary Table S1 offers further details on the plant materials.

Seedlings were prepared as described in Poudyal et al. [27]. The transplanted seedlings were grown in greenhouse no. 6 at Agro Food Park (coordinates: 56.198164°N, 10.155551°E, 48 m above sea level), Denmark. Fig. 1 details the growth conditions, treatment levels, day of measurement of particular traits measured, etc.

Expt-I encompassed a total of 144 plants at the pre-flowering stage. Among these, 72 plants were cultivated in the greenhouse, and an additional set of plants was grown in the climate chamber. The growing conditions in both the greenhouse and climate chamber were almost identical, except for variations in temperature levels and soil moisture conditions (Fig. 1).

Seedling hardening and preconditioning: Seedling hardening, preconditioning, and setting of climatic conditions during the treatments inside the climate chambers were done according to Poudyal et al. [27]. Throughout the experiment, the relative air humidity was consistently regulated to keep the vapor pressure deficit (VPD) below 2.0 kPa. Adequate air humidification and recirculation were implemented to ensure a uniform ambient temperature in both the climate chambers and the greenhouse compartments. In the case of plants undergoing drought stress, irrigation was halted 4 h before the onset of thermal stress in the climate chambers, ensuring simultaneous exposure to both heat and drought stress (Fig. 1). This methodology follows the approach outlined by Zhou et al. [28].

Treatments: The experiment employed plants aged 65 days at a stage between first branching to anthesis with 41 ± 12 fully matured leaves and a height of 109.22 ± 19.78 cm for four days, incorporating four treatments:

i. Control (CT): Plants were grown at a day-night temperature of 30/22 °C in the greenhouse. They received regular irrigation with a nutrient solution as mentioned in Poudyal et al. [27] having a pH of 5.8 and an electrical conductivity (EC) of 2.08 mS cm^{-1}.
ii. Drought stress (DT): Day-night temperature was the same as CT in the greenhouse. However, the plants did not receive irrigation, inducing drought conditions.
iii. Heat stress (HT): The plants were well-irrigated with a nutrient solution like CT and exposed to elevated temperatures of 40/32 °C (day/night) in the climate chambers.
iv. Combine of drought and heat stress (DHT): Day-night temperature was the same as HT, and irrigation was withheld during the treatment period in the climate chambers.

Following the 4-day-long stress treatments, a 5-day-long recovery phase was started, where plants were subsequently cultivated under normal conditions (CT). Corresponding to the stress treatments applied, subsequent recovery phases are designated as drought recovery (DR), heat recovery (HR), drought-heat recovery (DHR), and CR signifies control conditions in the recovery phase. The four days of stress treatment are labeled T1 for day one and so forth up to T4, while the five days of recovery are denoted as R1 for recovery day one and so on up to R5.

Experimental setup: The experiment adopted a completely randomized design (CRD), comprising four groups, each consisting of 36 plants representing six landraces. One group was randomly assigned to the CT, another group underwent DT, the third experienced HT, and the last group was exposed to DHT. Normal and drought stress conditions were maintained in the greenhouse compartments, while heat and combined stress were applied in the climate chamber. To harmonize measurements at the start of the photoperiod in both the climate chamber and the greenhouse, a 2-h time difference was implemented between their respective photoperiods. Additionally, an extra 2-h difference was introduced between the two chambers to ensure consistent measurements.

2.2. The second experiment: field evaluation

For the second experiment (Expt-II) conducted in the field in 2022, four Akabare chili landraces were selected. To cover the entire range of performance observed in Expt-I, including F_v/F_m, P_N, g_s, and biomass, Expt-II selected two top-performing landraces (C44 and DKT77) and two bottom-performing landraces (C64C and BJ77) in terms of F_v/F_m, P_N, g_s, and biomass. Further information about the selected landraces is provided in Supplementary Table S1.

On 25 February 2022, seeds from four Akabare chili landraces were planted in plastic trays filled with plug mix (Klasmann-Deilmann GmbH, Geeste, Germany). After 37 days of germination, the seedlings were transplanted into the field, where they were cultivated under a well-irrigated condition through the drip irrigation system. A total of 240 seedlings were grown, with 15 per genotype, inside a rainout shelter (plastic tunnel) situated in Thankot, Kathmandu (coordinates: 27.688235°N, 85.22122°E, altitude: 1470 m above sea level).

In 76 days after transplanting (DAT), 50 % of the plants entered the anthesis stage with the first fully opened flower with 320 ± 9 fully matured leaves, the first branching height of 62.68 ± 7.63 cm, and the plant height of 166.35 ± 8.15 cm. All plants got irrigated twice in 12-h intervals for 2 h through drip irrigation (2 L/h). Subsequently, irrigation was halted, with half of the plants exposed to continuous drought for 55 days, while the remaining half were kept under well-watered conditions. Observations on physiological traits started 30 days after initiation of drought stress at 5-day intervals.

Finally, the experiment harvested five plants at random under both drought stress and normal conditions 134 DAT and measured plant parts, including leaves, stems, fruits, and roots, for fresh and dry weights, along with other parameters such as plant height, height to the first branching, and the number of fruits and leaves. Additionally, the experiment also recorded the dimensions of fruits and seeds.

During land preparation, a combination of N: P: K fertilizer (80:40:40 kg/ha) and 20,000 kg of compost fertilizer was applied and thoroughly mixed into the loamy soil with a pH of 6.8. Moisture seepage in the tunnel was minimized by making a drainage channel around the tunnel, and a border of single-row plants was maintained throughout the experiment. Seedlings were randomly distributed among four blocks, each containing four landraces. Fig. 2 illustrates the detailed workflow of Expt-II.

Throughout the Expt-II from April to August 2022, the average relative humidity (RH%), minimum/maximum temperature, and photosynthetically active radiation (PAR) were obtained at values of 66.7 ± 12.2 %, 20.6 ± 2.3 °C/33.1 ± 1.4 °C, and 416.6 ± 24.5 μmol m^{-2} s^{-1}, respectively (data from NASA Langley Research Center POWER Project funded through the NASA Earth Science/Applied Science Program), detail in Supplementary Table S2.

2.3. Assessment of physiological traits

Leaf chlorophyll fluorescence (F_v/F_m) and photosynthetic gas exchange: Gas exchange and F_v/F_m measurements were conducted in situ on the penultimate leaf between 10:00 a.m. and 2:00 p.m. For F_v/F_m measurements, a MINI-PAM Fluorometer (Heinz

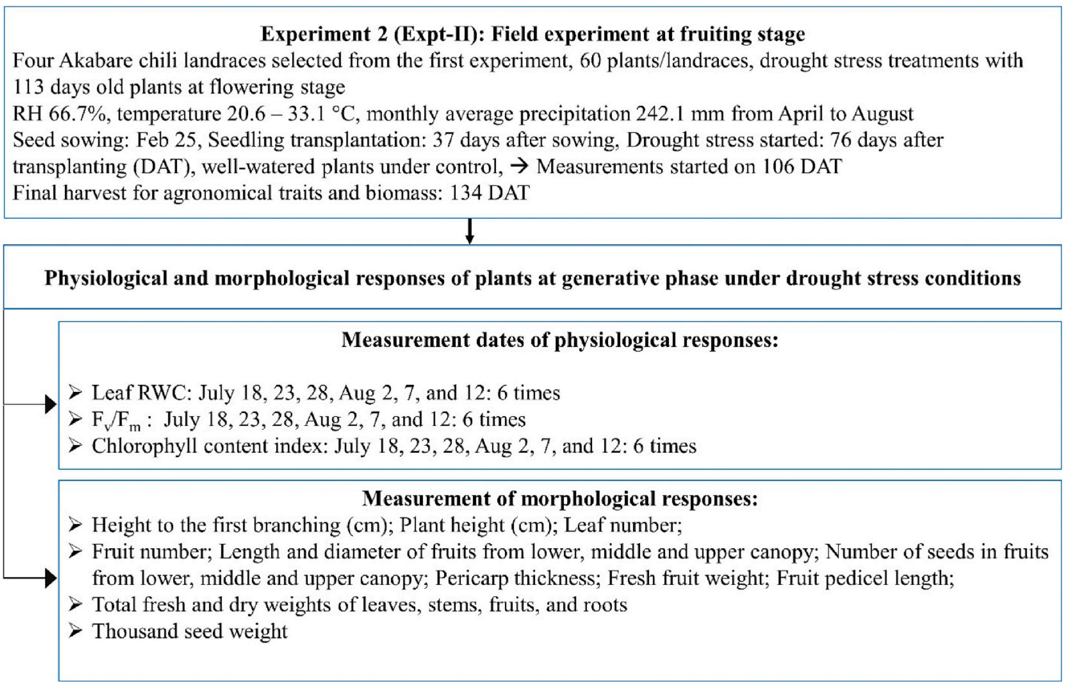

Fig. 2. Workflow of experiment two under field conditions in 2022.

Walz GmbH, Effeltrich, Germany) was used. The CIRAS-2, a portable gas analyzer (PP Systems, Amesbury, MA, USA) was used to measure the net photosynthetic rate (P_N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (C*i*, μmol CO_2 mol^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}). The VPD was kept below 2.0 kPa during the measurements. Three leaves per plant, totaling nine leaves per genotype, were selected for F_v/F_m measurements, while at least one leaf per plant, totaling four leaves per genotype, were chosen for photosynthetic gas exchange measurements. The photosynthetic gas exchange measurements were conducted in situ in the climate chambers, twice for stress treatment (T1 and T3) and twice for the recovery phase (R1 and R4) during the Expt-I.

In Expt-II, the OS30p Chlorophyll Fluorometer (Opti-Sciences Inc., Hudson, NH) was used to measure the F_v/F_m.

Leaf cooling: In Expt-I, leaf temperature (T_L) and ambient temperature (T_A) were recorded in situ as described in Poudyal et al. [27]. Leaf temperature depression (LTD) was determined as LTD = (T_L – T_A) and percent leaf cooling as % LC = [(T_A – T_L) ÷ T_A] × 100 %. Nine fully developed leaves per genotype were chosen randomly from the top of the canopy for leaf temperature measurements. No T_L data was available for Expt-II.

Stress scoring: Plants experiencing DT, HT, and DHT conditions underwent morphological symptom assessment. The heat injury index (HII) was evaluated under HT and DHT conditions, while the wilting score (WS) was estimated under DT conditions, following the methodology outlined in Poudyal et al. [27]. Four independent evaluators conducted the scoring before the destructive measurements in Expt-I and Expt-II.

Leaf chlorophyll and relative water content: MPM-100 and CCM-200 plus (Opti Sciences, Inc., Hudson, NH) assessed the leaf chlorophyll content index (CCI) in Expt-I and Expt-II, respectively. The assessment of relative water content (RWC) in leaves followed the method outlined by Smart and Bingham [29]. Both experiments considered a total of nine fully expanded leaves per genotype for the evaluation of RWC and leaf pigment content.

2.4. Assessment of agro-morphological traits

Floral and seed traits: In Expt-I, recording occurred for the total number of floral buds, flowers, and dropped flowers separately from six plants per genotype during the stress treatments and from three plants per genotype during the recovery phase. No new floral buds were observed during the stress treatments. To distinguish the remaining flowers during the stress treatments from those newly developed during the recovery phase, marks were made on old flowers. Unfortunately, Expt-I did not yield any fruit, resulting in missing fruit-associated data.

Genotype-specific observations on flower drops during stress treatments and the subsequent recovery were calculated individually. The flower drop attributed to stress treatment was determined by dividing the number of dropped flowers by the total count of flowers recorded before the initiation of stress treatment, expressed as a percentage. Likewise, the flower drop during the recovery phase was calculated by dividing the number of dropped flowers by the sum of the flowers retained from the stress treatment and those newly developed during the recovery phase, also expressed as a percentage.

In Expt-II, the dimensions of five fruits each from the lower, middle, and upper canopy were recorded and averaged for the final value under both control and drought stress conditions. Additionally, the number of seeds in each fruit, the thickness of the pericarp, and the length of the pedicel were measured. Physiologically matured seeds of each landrace were kept for thousand seed weight (TSW) calculation. The TSW of each landrace was calculated according to the ISTA Rules, Chapter 10 [30] by counting eight replicates of 100 pure seeds at 10 ± 0.35 % seed moisture.

Morphological traits: Expt-I measured some morphological traits like the height to the first branching, plant height, stem diameter, and internode length between 3rd and 4th nodes, leaf number, and flower number for individual landraces in three replications. In addition to these traits, Expt-II also measured fruit number, fruit diameter, fruit length, pedicel length, and fresh fruit weight in five replications.

Fresh and dry weights of plant parts: In Expt-I, three plants per genotype underwent the first harvest at the end of the stress treatment, T4 to assess total biomass production and partitioning under CT, DT, HT, and DHT conditions. Leaf counting and leaf area measurement considered all leaves with an area ≥2 cm^2. A leaf area meter (Model 3100, LI-COR Inc., Nebraska, USA) estimated the total leaf area (LA) of each plant. Marks were placed on the topmost older leaves of remaining plants after the first harvest to distinguish them from the newly developed leaves during the recovery phase. Finally, the remaining three plants per genotype underwent the final harvest at the end of the recovery phase, R5 for biomass measurement.

During Expt-II, five randomly selected plants each from both control and drought stress conditions were harvested at the end of the drought stress treatment. This harvest aimed to assess biomass partitioning, encompassing parameters such as fruit number, leaf number, plant height, branching height, etc. In both experiments, fresh weights of respective plant parts were recorded, and root fresh weight was determined after washing the roots and blotting away surface water. The respective dry weights of plant parts were obtained after oven drying at 70 °C for 72 h and measured in grams.

Calculation of ratios: In Expt-I, the water use efficiency (WUE) was calculated as a ratio between P_N to E, [WUE = P_N/E]. The total root dry weight was divided by the total shoot dry weight to obtain the root-to-shoot ratio (RS). The percentage of dry matter proportion (DMP) was estimated as the proportion of total vegetative dry weights (TVDW) to the total vegetative fresh weights (TVFW) and expressed in percentage, [DMP = (TVDW/TVFW) × 100 %]. The gain amount in the respective dry weights of each landrace during the recovery phase was calculated as, [Gain in recovery = (DW_R – DW_S)], where DW_S represents the dry weight of the plant parts recorded for the stress treatment, and DW_R denotes the dry weight of same part recorded for the recovery phase of respective stress treatment. In this way, the gain amount of RDW, RS, and DMP, for individual landraces were calculated. The decreased F_v/F_m values (DFV) were determined by calculating the difference between the control and stress values of F_v/F_m for the respective landraces, divided by respective control values and expressed as percentages, [DFV = (F_v/F_m-Control – F_v/F_m-Stress)/F_v/F_m-Control × 100 %]. Similarly, the increased F_v/F_m values (IFV) were estimated as the difference between the recovery values and stress values for the respective landraces, divided by respective stress values and expressed as percentages, [IFV = (F_v/F_m-Recovery – F_v/F_m-Stress)/F_v/F_m-Stress × 100 %].

2.5. Data analysis

The analytical approach employed several statistical methods. For the Expt-I data, a three-way ANOVA was accomplished. This allowed the disentanglement of the influence of three crucial factors: genotype, treatment, and stress condition. Similarly, the similar data sets obtained from Expt-II were subjected to a two-way ANOVA with genotype and treatment. When ANOVA indicated significant interactions between these factors, further analysis was performed using pairwise t-tests.

Repeated measures ANOVA was applied to the data sets concerning F_v/F_m, gas exchange measurements, leaf temperature, leaf pigment, and water contents. This facilitated the understanding of how average values evolved over various measurement dates and environments. To gauge variations in landraces and treatments for the assessed traits, comparisons were made using Tukey's HSD as a post hoc test with a significant level of $\alpha = 0.05$. Values for treatment and recovery phase were compared with control. Data were analyzed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) and SigmaPlot for Windows Version 15.0.0.13 (Inpixon HQ, Palo Alto, CA) was used for data visualization. All results are presented with their

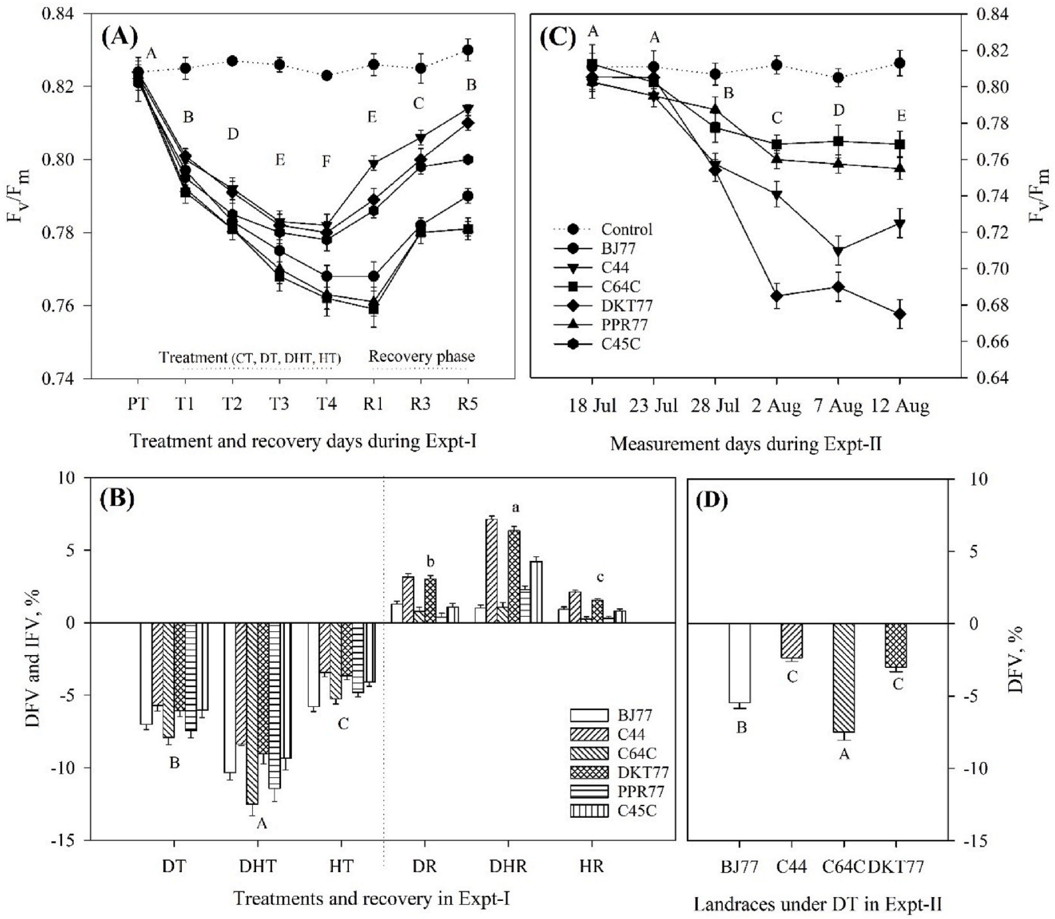

Fig. 3. The efficacy of PSII (F_v/F_m) was measured as chlorophyll fluorescence during the Expt-I and II. In Expt-I, (A) F_v/F_m values averaged for pretreatment (PT), stress treatment days (T1 – T4), and the recovery phase (R1 – R5); and (B) Decreased F_v/F_m values (DFV, difference between control and stress values) and increased F_v/F_m values (IFV, difference between recovery and stress values) in percentages during treatment and recovery phase. Stress treatment started on day T1 (treatment day 1 and so on), four conditions were employed: control (CT), drought (DT), heat (HT), and combined drought and heat stress (DHT), and stress lasted for four days followed by a recovery phase (R1, recovery day 1 and so on) under normal conditions for five days. In Expt-II, (C) F_v/F_m values under control and drought stress conditions; and (D) % DFV of individual landraces under drought stress (DT). Control values in dotted lines are averaged for all landraces for individual treatment and recovery days. Labels above the dashed lines in the X-axis of 3A coincide with treatment and recovery days. Data are mean values ± SD, n = 9, and different letters above the line and bar graphs denote a significant difference ($p < 0.05$). Legend in C is common for A and in B for D.

respective mean ± standard deviation (SD).

3. Results

3.1. F_v/F_m demonstrates sensitivity to combined and drought stress

The average F_v/F_m value (0.825) across the landraces under pre-treatment (PT) was significantly higher than on other measurement days. F_v/F_m values for T1 and R5 were similar but significantly lower than the PT, and higher than on other measurement days. T4 produced significantly the smallest F_v/F_m value (0.773) among all the treatment days (Fig. 3A).

Compared to the average control value of 0.826, stress treatments led to a significant 7.1 % decrease in F_v/F_m in Expt-I. The percentage DFV for DT, DHT, and HT were 6.7 %, 10.2 %, and 4.5 %, respectively (Fig. 3B). Among the stress treatments, the most substantial significant reduction of F_v/F_m (8.6 %) was observed in C64C, followed by PPR (7.9 %) and BJ77 (7.7 %), while the least significant reduction was observed in C44 (5.8 %), followed by DKT77 (6.2 %) and C45C (6.5 %). A higher gain percentage (3.7 %) of F_v/F_m was found under DHR, where C44 showed a higher gain percentage (4.1 %), followed by DKT77 (3.6 %) (Fig. 3B).

In Expt-II, drought stress induced a reduction in F_v/F_m across all landraces. On July 28, there was a significant reduction (12.2 %) in F_v/F_m, which further declined by an additional 18.5 % over the next five days as shown in Fig. 3C. Under continuous drought stress, F_v/F_m gradually decreased with an average of 5.52 % throughout the treatment, with the most pronounced effect observed in C64C and BJ77. Across the six measurement dates, landraces BJ77, C44, C64C, and DKT77 showed average F_v/F_m values of 0.754, 0.783, 0.733, and 0.776, respectively, all significantly lower than the control value (0.81 ± 0.003) (Fig. 3C). The mean values of DFV% were

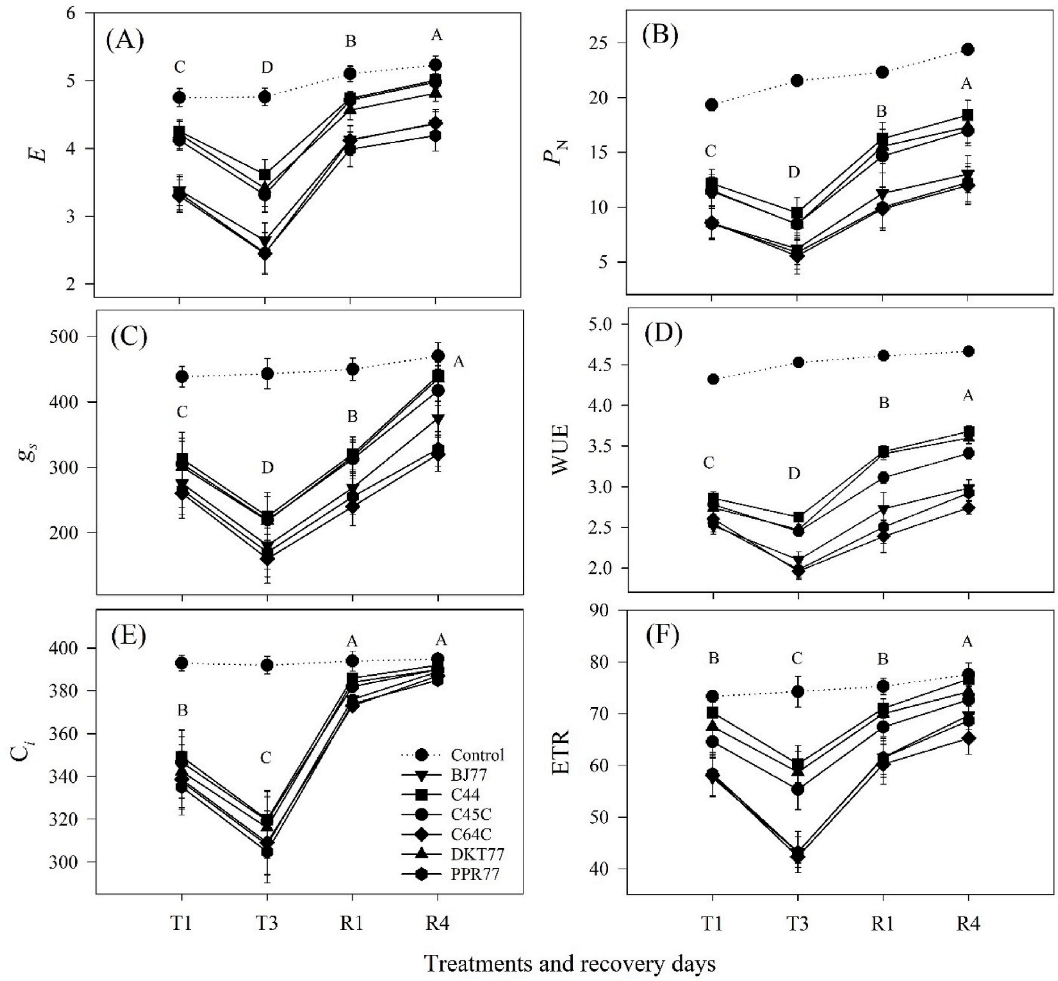

Fig. 4. Gas exchange measurements of six Akabare chili landraces under stress treatment and recovery phase. In Expt-I, stress treatment started on day 1 (T1) and ended on day 4 (T4). Recovery observation started after stress on day 1 (R1) and lasted for five days (R1 to R5). In figure; (A), Transpiration rate (E); (B), Net photosynthetic rate (P_N); (C), stomatal conductance (g_s); (D), Water use efficiency (P_N/E); (E), Intercellular CO_2 concentration (C*i*); and (F), Electron transfer rate (ETR). Data are mean values ± SD (n = 4) and different alphabets indicate differences among treatment days ($P < 0.05$).

significantly higher for C64C (7.5 %), BJ77 (5.6 %), and lower for C44 (2.4 %) and DKT77 (3 %) (Fig. 3D).

In Expt-I, three Akabare chili landraces (C64C, BJ77, and PPR77) exhibiting average F_v/F_m values < 0.763 were categorized as stress-sensitive, whereas three landraces (C44, DKT77, and C45C) with higher F_v/F_m values > 0.77 were considered stress-tolerant. Similarly, in Expt-II, two landraces (C64C and BJ77) with an average F_v/F_m < 0.755 were classified as stress-sensitive, while two landraces (C44 and DKT77) with an average F_v/F_m > 0.765 were grouped as stress-tolerant.

3.2. Photosynthetic gas exchange

Treatment day 3 (T3) significantly registered the lowest values of *E* at 2.98 ± 0.03, while R5 demonstrated the highest values at 4.62 ± 0.05 compared to other treatment and recovery days (Fig. 4A). Stress conditions led to a 32.3 % reduction in *E*, whereas during the recovery phase, the reduction narrowed down to 9.3 %, as depicted in Fig. 4A.

Stress treatments reduced P_N by 60.1 % compared to control values (21.91 ± 4.73), while such reduction narrowed down to 36.3 % during the recovery phase (Fig. 4B). The lowest P_N was observed on T3 (7.33 ± 0.11), and the highest on R4 (15 ± 2.11) among the four measurement days. Stress-tolerant landraces exhibited significantly higher P_N values, with 8.83 ± 0.81 and 17.61 ± 1.13 for T3 and R4, respectively, compared to stress-sensitive landraces. Specifically, C64C, PPR77, and BJ77 landraces were observed to be more sensitive to stress treatments for P_N, experiencing a significant 67.1 % reduction compared to the control (Fig. 4B).

Stomatal conductance (g_s) exhibited a pattern similar to *E* and P_N, with an overall reduction of about 46.5 % to the control values (450.4 ± 18.81) due to stress treatments (Fig. 4C). The lowest g_s values were significantly observed on T3 (195.62 ± 39.73), while the average values for R4 were almost near to the control values, showing a small reduction (14.3 %) (Fig. 4C).

Across the stress treatments, there was a recorded 36.1 % reduction in WUE compared to the control values (4.42 ± 0.22), with T3 exhibiting the largest reduction (2.44 ± 0.02). Recovery conditions increased WUE significantly compared to stress conditions. Stress-tolerant landraces showed higher values (3.02 ± 0.01) of WUE values than sensitive landraces (2.52 ± 0.08) across treatments and recovery days (Fig. 4D).

Stress treatments reduced C*i*, largely on T3 (20.5 %), followed by T1 (13.2 %) compared to the control. A small difference between control and recovery values was observed (2.4 %) on R4. Mean values of C*i* on R1 and R4 were significantly higher than those on T1 and T3 (Fig. 4E). C*i* for the stress-tolerant group (359.79 ± 8.81) was significantly higher than values for the stress-sensitive group (351.43 ± 21.81).

Results for electron transport rate (ETR) revealed similar mean values for T1 and R1 (63.4 ± 0.47). The highest values (71.2 ± 11.81) were observed for R4 and the lowest values (50.52 ± 7.81) for T3. Stress conditions reduced the ETR by 24.6 % compared to control. Stress-sensitive landraces showed significantly lower values (50.2 ± 5.69) of ETR compared to the stress-tolerant group with values of 63.5 ± 2.32 (Fig. 4F). A contrast in ETR values was observed between the stress-tolerant and stress-sensitive groups during the measurement days, with respective values of 67.38 ± 5.01 and 57.44 ± 8.74.

3.3. Leaf water, pigment assessment, scoring, and leaf cooling

In Expt-I, the stress values recorded for RWC were markedly reduced by 27 % compared to the control values of 83.7 ± 3.1 %. DHT

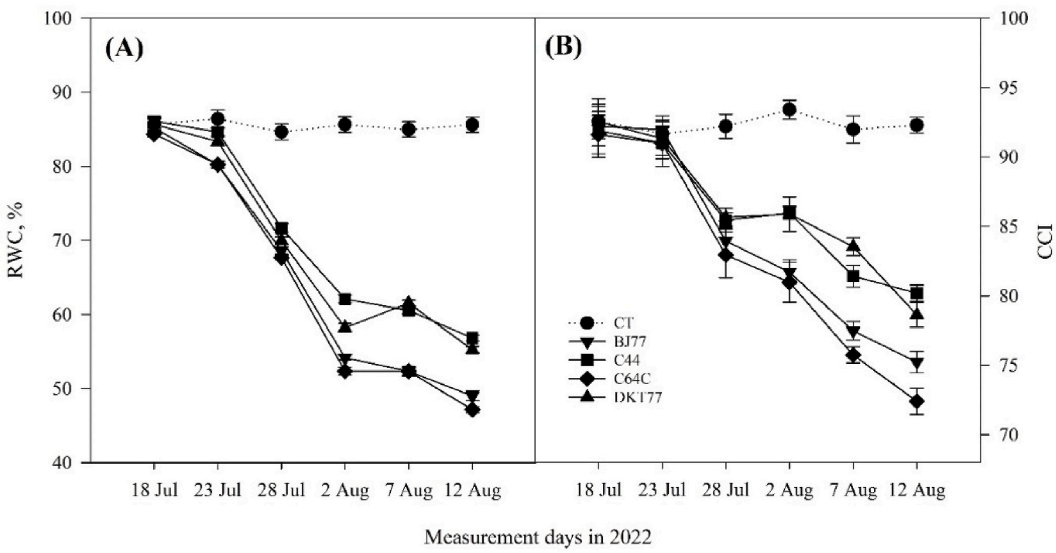

Fig. 5. Leaf relative water content (RWC) percentages and chlorophyll content index (CCI) measured in Expt-II. (A), RWC, %, and (B) CCI of four Akabare chili landraces were measured under drought stress and control conditions (dotted lines) during Expt-II in 2022. Control values (CT) of RWC and CCI for all four landraces were averaged for the day and presented accordingly. Data are mean values ± SD, n = 9, $p < 0.05$.

induced a significant decrease in RWC by 44.8 %, followed by DT, which resulted in a reduction of 33.4 %, while HT caused a slight decrease of 2.3 % in RWC compared to the control values. Under various stress conditions, stress-sensitive landraces showed an additional reduction in RWC by 13.9 % compared to tolerant landraces, which maintained RWC at 67.2 ± 3.36 % (Supplementary Table S3).

Sub-optimal growing conditions led to an 8.5 % reduction in CCI compared to normal conditions (1.3 ± 0.3.). Across the landraces, DHT resulted in a notable 13.7 % reduction in CCI, DT exhibited a 5.3 % decrease, and HT showed a 5.6 % decline compared to the control. Notably, sensitive landraces displayed a 5.5 % lower CCI compared to tolerant landraces (data not shown).

In Expt-II, the application of DT led to a reduction in leaf RWC, ranging from 2 % on the second measurement day of drought stress to a more substantial decrease of 30.4 % on the last measurement day (12 Aug). Sensitive landraces showed a higher reduction of 34.6 % compared to the reduction observed in tolerant landraces, which was 26.3 % (Fig. 5A).

Drought stress caused a progressive decline in CCI, resulting in a 9.2 % reduction. This reduction commenced at 1 % on the second measurement day and increased to 13.7 % on the final measurement day (August 12), in comparison to the control values (92.4 ± 0.55). A notable reduction (10 ± 0.25 %) in CCI was observed for C64C and BJ77, whereas a comparatively lesser reduction (6.7 ± 0.55 %) in CCI was noted for landraces C44 and DKT77 when compared to the control values (Fig. 5B).

In Expt-I, stress-tolerant landraces with higher F_v/F_m values demonstrated significantly lower HII and WS values, as detailed in Supplementary Table S3. For instance, under HT, C44 exhibited an HII of 1.5, and under DHT, it showed an HII of 3.3, which were significantly lower than C64C (2.5 under HT and 4.5 under DHT) under the same conditions, respectively. Results from the field trial revealed significantly lower WS values (2.1 ± 0.05) for C44 and DKT77, which was 30.8 % lower than the WS values observed for the stress-sensitive landraces (Supplementary Table S3).

In Expt-I, all landraces exhibited the same leaf temperature reduction and percent leaf cooling under normal growing conditions (Supplementary Fig. S1). The higher the LTD means cooler the leaves. However, the values of LTD decreased as the stress treatment intensified, reaching the lowest reduction (−2.28 °C) on T3, followed by R1 (−2.3 °C). The landraces C44 and DKT77 consistently showed significantly cooler leaves with LTD of −3.29 °C and −2.94 °C, respectively, throughout the stress treatments (Supplementary Fig. S1A). Similar to LTD, higher leaf cooling was observed for control (10.6 %), followed by HT (8.7 %), DT (7.7 %), and the least LC for DHT (5.4 %). Leaves started cooling gradually during the recovery phase. Among the stress recovery conditions, a significantly higher cooling percentage (9.2 %) was found under recovery from heat stress (Supplementary Fig. S1B).

3.4. Assessment of percent flower drop

The stress treatment resulted in a substantial flower drop of 59 %, with an additional 42.5 % of drops occurring after the stress period, as depicted in Fig. 6. Notably, DHT exhibited the highest percentage of flower drop at 94.3 %, while HT shared a similar drop percentage with DT at 41.4 %. Complete flower drop was observed in BJ77, C64C, and PPR77, with 83–93 % of such drop recorded for C44, C45C, and DKT77 under DHT. Across stress treatments, stress-sensitive landraces displayed a 66 ± 4.05 % flower drop, whereas the stress-tolerant group showed a slightly lower percentage (52.1 ± 3.35 %). During the recovery phase, a significantly higher flower

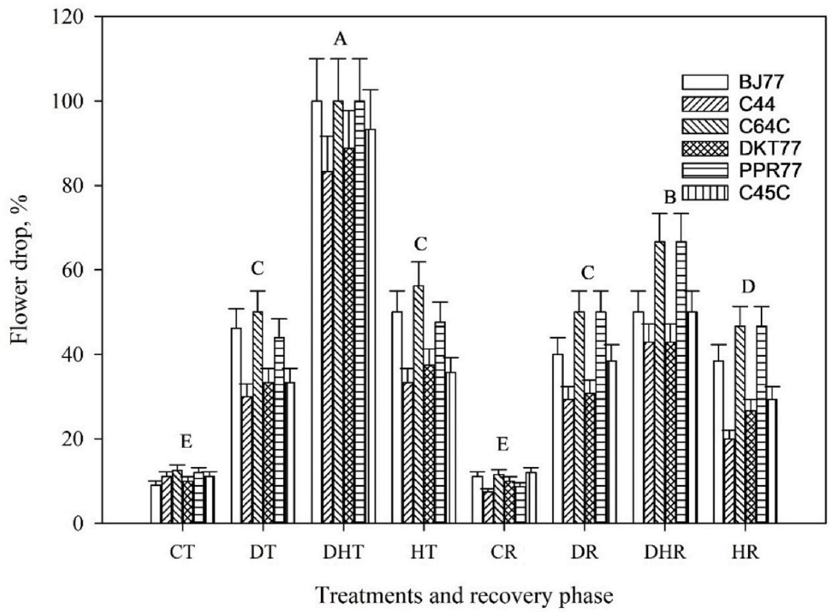

Fig. 6. Percent flower drop during the stress treatments and recovery phase in Expt-I. CT, control treatment; DT, drought stress; HT, heat stress, DHT, the combined stress of drought and heat; R denotes the recovery phase of respective stress treatments and CR, control conditions during the recovery phase. Data are mean values ± SD, $p < 0.05$, n = 6 during stress treatments, and n = 3 during the recovery phase in Expt-I.

drop was observed for DHR, followed by DR compared to HR and CR. In control conditions, only a small percentage (10.5 ± 1.01 %) of flower drops were observed (Fig. 6).

3.5. Biomass production and partitioning

In Exp-I, the imposition of stress conditions resulted in a reduction in the shoot, root, and total dry weights across six landraces, as illustrated in the left column of Fig. 7. Specifically, the total shoot dry weight (ShDW) experienced a decrease of 25.3 % due to stress compared to the control (8.74 ± 1.02 g), as shown in Fig. 7A.

Under different stress conditions, HT led to higher ShDW (7.54 ± 0.97 g), DHT resulted in lower ShDW (5.34 ± 0.97 g), and DT produced intermediate ShDW (6.72 ± 0.76 g). These values were 13.7 %, 38.9 %, and 23.1 % lower than the control, respectively, and the differences were statistically significant. During the recovery phase, there was a significantly higher gain in ShDW for CR (2.58 ±

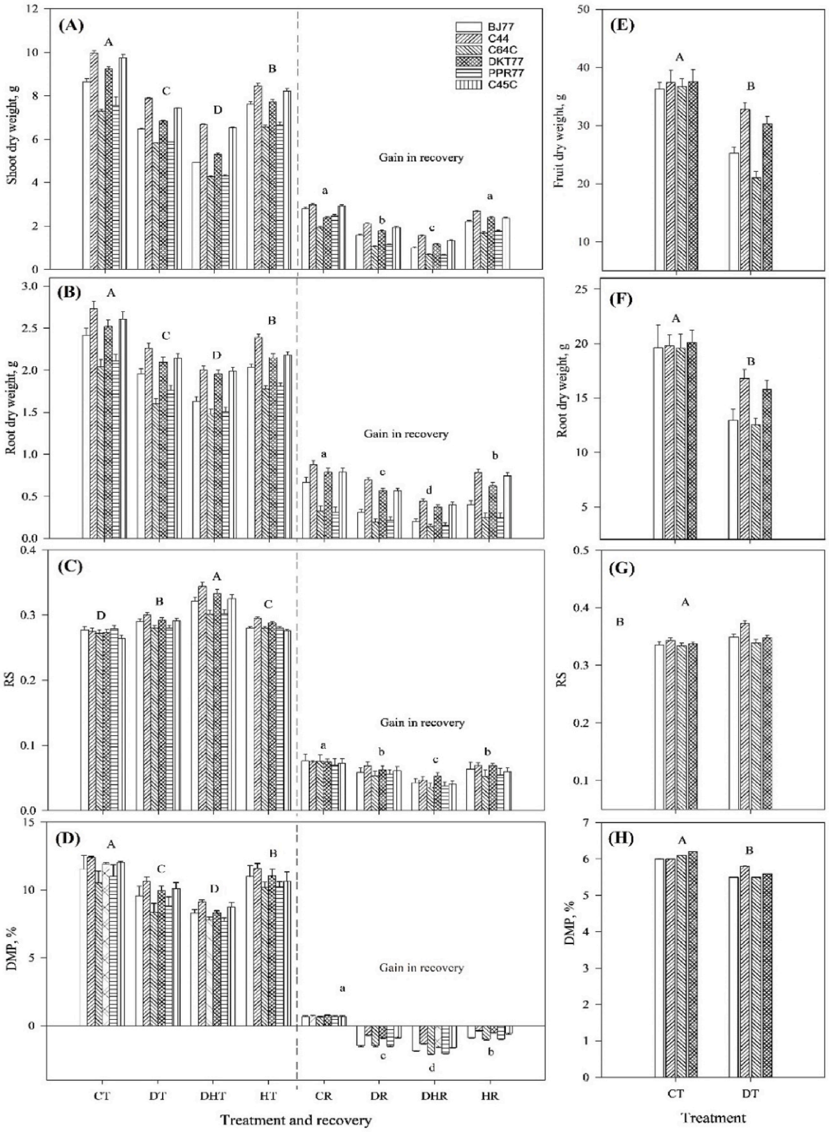

Fig. 7. Dry weights and their ratio for stress treatment conditions and recovery phase in Expt-I and Expt-II. In Expt-I (left column), (A) Shoot dry weight (ShDW), g; (B) Root dry weight, g; (C) RS, Root-to-shoot biomass ratio; (D) DMP, dry matter proportion in percent during the Expt-I. The gain of respective traits during the recovery phase is illustrated on the right to the dotted lines. In Expt-II (right column), (E) Fruit dry weight under CT and DT, (F) Root dry weight (RDW), (G) RS, and (H) DMP percentage during field experiment (Expt-II). In the X-axis, CT & CR indicate control and recovery; DT & DR stand for drought stress and recovery; HT & HR denote heat stress and recovery; and DHT & DHR indicate combined drought and heat stress and recovery conditions. Data are mean values ± SD (n = 3 in Expt-I, n = 5 in Expt-II). Different alphabets denote variations between mean values at $p < 0.05$.

0.37 g) and HR (2.19 ± 0.35 g), while significantly lower gains were observed for DHR (1.06 ± 0.32 g) and DR (1.6 ± 0.39 g). Comparing stress-tolerant and stress-sensitive landraces, the stress-tolerant landraces produced significantly higher ShDW (7.84 ± 1.26 g), and gain portion (2.13 ± 0.54 g) compared to stress-sensitive landraces (6.33 ± 1.23 g and 1.58 ± 0.62 g, respectively).

Under control conditions, the root dry weight (RDW) value was 2.41 ± 0.25 g, and this was significantly reduced by 19.8 % under stress conditions, as illustrated in Fig. 7B. Specifically, DHT caused a substantial reduction in RDW by 26.8 %, DT reduced RDW by 18.2 %, and HT reduced RDW by 14.5 %. In the recovery phase, HR showed the highest gain in RDW with values of 0.51 ± 0.22 g, distinct from DR values (0.42 ± 0.19 g), and DHR exhibited a notably lower gain quantity at 0.29 ± 0.12 g. Comparing stress-tolerant and stress-sensitive landraces, the stress-tolerant group exhibited significant RDW values (2.25 ± 0.24 g), which were 21.6 % higher than those observed for stress-sensitive landraces. Similarly, the significant RDW gain for the stress-tolerant group was 0.64 ± 0.15 g, while it was 0.28 ± 0.1 g for the stress-sensitive group.

Under stress growing conditions, there was an increase in the root-to-shoot ratio (RS) of 8.9 %. Among the different stress conditions, landraces grown under DHT demonstrated the highest RS at 0.32 ± 0.02. In comparison to control values (0.27 ± 0.005), RS increased by 3.6 %, 5.7 %, and 17.4 % under HT, DT, and DHT, respectively. During the recovery phase, the RS gain was highest for CR at 0.074 ± 0.002 and lowest for DHR at 0.04 ± 0.01. The gain in RS was identical for HR and DR, as shown in Fig. 7C.

The stress-tolerant group exhibited significant RS values at 0.3 ± 0.02, 3.3 % higher than the stress-sensitive group. Additionally, RS gain for the stress-tolerant group was significant at 0.063 ± 0.01, representing an 11.2 % increase compared to the stress-sensitive group.

Stress treatments resulted in a reduction of DMP by 17.3 % compared to control values. Under control conditions, the highest DMP was produced at 11.56 ± 0.64 %. Among the stress conditions, HT exhibited significantly higher DMP at 10.79 ± 0.49 %, followed by DT with 9.57 ± 0.76 %. The lowest DMP was observed for DHT at 8.33 ± 0.5 %. During the recovery phase, DMP gain under DR was revealed at −1.14 ± 0.34 %, significantly higher than DHR (−1.73 ± 0.28 %) and lower than HR (−0.7 ± 0.26 %), as shown in Fig. 7D. Comparing stress-tolerant and stress-sensitive groups, the stress-tolerant group exhibited DMP values of 10.53 ± 1.23 %, which were 9.9 % higher than those observed for the stress-sensitive group. Additionally, the tolerant group showed improved DMP gain (−0.508 ± 0.42 %) under stress recovery compared to the sensitive group (−0.945 ± 0.43 %). Further details regarding the gain or loss percentages of leaf number, stem diameter, plant height, and internodal length are provided in Supplementary Table S4.

In Expt-II, drought stress significantly decreased fruit dry weights (FDW) by 26.2 % compared to the control value of 36.96 ± 0.5 g. Among the landraces, C64C exhibited the lowest FDW value at 21.03 g, marking a 42.7 % reduction compared to the control. Stress-tolerant landraces showed a lesser reduction (15.8 %) with FDW at 31.53 ± 0.33 g, while sensitive landraces exhibited a higher reduction (36.6 %) with FDW at 23.12 ± 0.53 g (Fig. 7E).

Drought stress in the field also reduced RDW by 26.7 %, with stress-sensitive landraces experiencing a prominent 35.1 % reduction. C44 produced the highest RDW at 16.81 g, while the lowest RDW was recorded for C64C at 12.51 g. The stress-tolerant group exhibited 16.31 ± 0.53 g RDW, while the stress-sensitive group exhibited 12.73 ± 0.53 g (Fig. 7F).

Drought stress increased RS values by 4.3 % compared to the control value of 0.34. C44 showed an 8.5 % increment in RS compared to its control value, while it was 1.5 % for C64C (Fig. 7G). The tolerant group showed a 5.9 % higher RS (0.36 ± 0.13) under stress conditions than the control, while sensitive landraces exhibited a 2.7 % higher RS value (0.34 ± 0.22) than the control values. The drought stress reduced DMP by 7.7 %, reaching a value of 5.6 %, where C64C showed the highest reduction (9.5 %), while C44 marked the lowest reduction at 3.4 % compared to the control (Fig. 7H).

During Expt-II, drought stress induced a 20 % reduction in fruits per plant. Notably, C44 and DKT77 exhibited significantly higher fruit numbers (247 ± 12) per plant under drought stress conditions compared to C64C and BJ77 (210 ± 23) (Table 1). The drought stress decreased fruit dimensions (length and diameter) by 13.5 %, thousand seed weights by 9.6 %, and pericarp thickness by 10 %, compared to control values of 2.41 cm^2, 6.78 g, and 2.51 mm, respectively. Drought stress also led to a 13.8 % reduction in the average number of seeds in fruit, decreasing from 58 to 50.

Moreover, drought stress resulted in a substantial 40.2 % reduction in total fruit fresh weight (FFW) under control conditions (1068.65 ± 15.87 g), and a 26.2 % reduction in FDW from the 36.96 g recorded under normal conditions. In fruit parameters, stress-tolerant landraces C44 and DKT77 significantly outperformed C64C and BJ77. The stress-tolerant group produced 693.03 ± 6.54 g FFW, which was 18.1 % higher than the sensitive group (Table 1). Additional details regarding other morphological traits observed for

Table 1
Major yield-attributing traits of four Akabare chili landraces. The tabulated traits were observed under control and drought stress conditions during the field experiment in 2022. The decreased percentage of respective traits under drought stress is calculated for individual landraces. Values are mean ± SEM.

Parameters	Control				Percent decrease under drought stress			
	BJ77	C44	C64C	DKT77	BJ77	C44	C64C	DKT77
Number of fruits per plant	235.2 ± 7.05	242.8 ± 5.89	235.8 ± 4.02	240.8 ± 7.56	19.3	18.53	40.2	14.2
Average fruit length, cm	2.6028 ± 0.07	2.5066 ± 0.04	2.2712 ± 0.08	2.9138 ± 0.03	12.04	11.67	23.01	7.39
Average fruit diameter, cm	2.2712 ± 0.04	2.2356 ± 0.02	2.3092 ± 0.05	2.138 ± 0.03	13.21	12.32	11.57	16.9
Average seeds per fruit	58.1332 ± 0.65	57.4 ± 0.53	58.2668 ± 0.68	58.0666 ± 0.54	13.19	13.01	12.82	13.66
Thousand seed weight, g	6.77 ± 0.01	6.78 ± 0.005	6.79 ± 0.01	6.79 ± 0.007	11.46	8.28	11.99	6.46
Pericarp thickness, mm	2.842 ± 0.19	2.93 ± 0.1	2.13 ± 0.11	2.13 ± 0.11	18.37	5.8	7.98	7.98
Total fruit fresh weight, g	1070.1708 ± 21.95	1067.8712 ± 19.53	1048.298 ± 26.63	1088.246 ± 15.46	40.21	34.65	49.01	36.76
Total fruit dry weight, g	36.26184 ± 1.28	37.352 ± 1.88	36.714 ± 0.46	37.5142 ± 0.76	30.47	12.32	42.71	19.18

the second experiment are provided in the supplementary information (Supplementary Table S5).

4. Discussion

The study successfully investigated six Akabare chili landraces for drought, heat, and combined stresses under controlled and field conditions. Initially selected for their performance in F_v/F_m, P_N, CCI, and biomass at the seedling stage [27], four landraces were further evaluated in field conditions. Comparing findings with previous seedling stage research, the study characterized the selected landraces at their pre-flowering stage in controlled conditions and fruiting stage in the field, focusing on PSII efficacy, photosynthetic performance, and yield potential.

This work adopted F_v/F_m as the primary marker but largely complemented by P_N and CCI for the phenotypic study of Akabare chili landraces. This approach was followed to mitigate potential physiological limitations, possibly arising from the species-specific behavior of F_o [31], and to consider the primary roles of photosynthetic traits in the reduction of carbon uptake [32]. The landraces with the largest and smallest F_v/F_m were categorized into stress-tolerant and stress-sensitive groups, respectively in Expt-I. The distinct departure of F_v/F_m between the tolerant- and sensitive-landraces has corresponded with a clear distinction not only in P_N, and CCI but also in other measured traits like wilting scores, heat injury index, stomata conductance, transpiration rate, leaf relative water content, leaf temperature depression, and total biomass production in both experiments. The landrace C44 consistently maintained the top position in terms of F_v/F_m, P_N, CCI, and total biomass production throughout the experiments, and the landrace C64C remained always in the bottom (Figs. 3–5 and 7). This supports the hypothesis that the adverse effects of drought and heat on the primary function of the photo component of Akabare chili are well reflected in the final biomass accumulation of the plants.

In controlled environments, a 4-day stress period during the pre-flowering stage was found to be sufficient to distinguish between Akabare chili landraces for most of the measured traits under the provided stress level of heat, drought, and their combined stress. In a previous experiment under the same treatment conditions for 7 days, Akabare chili seedlings showed genotype-specific values for F_v/F_m, P_N, CCI, and biomass. In the field experiment, significant differentiation in traits such as F_v/F_m, RWC, and CCI was observed within ten days. In both cases, our observations were in agreement with previous reports [11,33] which discussed the physiological and biochemical characteristics of hot peppers under drought and heat stress conditions. During the field validation trial, drought stress conditions were prolonged for 55 days to observe the fruiting behavior of the landraces in the field. This extension aimed to replicate real field conditions in the Akabare chili growing areas in the mid-hills of Nepal.

Akabare chili landraces exhibited distinct agro-morpho-physiological responses across various growth stages. Tolerant landraces demonstrated enhanced photosynthesis, transpiration, and leaf cooling, indicating improved water use efficiency and increased dry matter production under climate chambers. These results were corroborated by the field trial, where tolerant landraces produced larger fruit biomass. In both conditions, drought, and heat stress affected the functionality of PSII and impaired the carbon assimilation process with depleted levels of leaf chlorophyll index. The combined stress further exacerbated these effects. Upon scrutinizing the findings of the current study and comparing them with a previous investigation [27], a notably higher reduction in F_v/F_m, at 3.83 % and 7.1 %, was observed during the seedling and pre-flowering stages, respectively. This trend was also reflected in parameters such as P_N, g_s, WUE, and RWC, indicating a higher sensitivity of the generative phase to stressful environmental conditions. The observed reductions in physiological responses were consistent with a decrease in biomass production during both stages, echoing findings in tomatoes [23,34]. These outcomes aligned with prior research, where the reproductive phase, particularly the flowering period, was identified as the most sensitive to drought and heat stresses [22,35]. The collective evidence underscores the vulnerability of the generative phase and emphasizes the importance of considering specific growth stages in understanding the impact of stress on plant responses in future studies.

The significance of this study lies in its incorporation of combined drought and heat stress alongside individual stressors, offering valuable insights into the physiology of Akabare chili. This targeted approach enables meaningful conclusions about the physiological responses of these landraces to diverse stress conditions and various growth stages, thereby opening avenues for discussions in the context of climate change and chili breeding. Throughout the stress treatments and recovery, the predominant effects of combined drought and heat stress over individual stressors were observed. Such confounded effect of combined stress has also been reported earlier in *Capsicum* [12], *Solanum* [36,37], and *Triticum* [38]. Moreover, the mean values of F_v/F_m, P_N, CCI, and total biomass under drought stress were notably lower than those under heat stress, underscoring the heightened sensitivity of landraces to drought stress, which is consistent with prior observations in chili [39] and tomato [40]. Different gain levels of F_v/F_m (Fig. 3B) and biomass (Fig. 7A, B, C, D) observed during the recovery phase have suggested the disparities in the photo recovery process and stomatal regulation capacities of the landraces as reported in rice [41].

The study observed significant disparities in various physiological traits during the recovery phase, particularly noting that sensitive landraces did not fully recover even after 5 days, indicating chronic photoinhibition. The extended recovery period suggests that recuperation involves complex mechanisms beyond just the repair of the D1 protein in PSII [42]. This variability in post-stress recovery ability among landraces underscores genotype-specific responses, as previously noted in chili peppers [33]. The differences in physiological processes, such as reductions in F_v/F_m and P_N during stress, and their varied recovery among landraces, are largely influenced by genetic factors [38,43–45]. For instance, a slight decrease in F_v/F_m correlated with a substantial decline in P_N, highlighting the sensitivity of these parameters in distinguishing between tolerant and sensitive landraces under stress [23]. Further research into the functional impacts of extreme stress on PSII and the irreparable damage it causes could provide valuable insights for future studies.

The differences in leaf temperature depression values between treatment conditions were significant, showing the differences in leaf cooling percentages. Stress-tolerant landraces have shown higher values for leaf relative water content and lower values of wilting scores and heat injury indices. This has indicated the genotype-specific traits of Akabare chili landraces to drought and heat stress conditions as reported in the case of almond genotypes [46] and discussed by Ref. [47]. A significant negative correlation between net photosynthetic rate, stomatal conductance, wilting scores, HII, g_s, E, leaf cooling, CCI, and other measured responses indicated that the stay-green trait of stress-tolerant landraces contributed to better carbon assimilation through the effective inflow of CO_2 and outflow of water via balancing the source-sink relationships. A strong positive correlation was observed among the measured physiological traits and yield attributing parameters in the present study (data not shown). Current findings are in agreement with the results from chili and almond where drought stress reduced the chlorophyll content and leaf relative water content resulting in necrotic leaves and downregulated the carbon assimilation process [11,46,48]. Higher yield could have been attributed to increased leaf metabolism, magnified by larger leaf area through improved mitochondrial respiration [49]. Further, increased leaf cooling could have served as a mechanism for heat tolerance in tolerant genotypes as its role was reported previously in common beans [50], and tomatoes [23,51].

Results obtained for CCI and RWC under laboratory and field conditions strongly supplemented the understanding of how water and pigment content in the leaves of the landraces vary according to different growth phases and supported the F_v/F_m-centered genotype screening. The reduction in RWC under stress conditions aligns with the previous findings in *Capsicum* [52] and tomato [53]; reduction in CCI was also reported in *Amaranthus*, *Capsicum*, and *Solanum* species [54,55] under stress conditions. A lower RWC and CCI values under stress conditions in this study might have indicated a reduced osmotic regulation in stress-sensitive landraces. Future studies focusing on the recovery physiology of leaf metabolism, including the assessment of leaf water potentials and light-harvesting pigments in Akabare chili, would be of significant interest.

In the current study, 10 % of flowers dropped under normal conditions could be attributed to the species-specific behavior of landraces. A larger percentage of flower drop in the sensitive group conditions could be attributed to their high sensitivity to stressors. Data on the percent fruit set were missing from the Expt-I, but the drought-sensitive landraces exhibited a significant reduction in yield-attributing traits, for instance, total fruit number, fruit fresh and dry weights, total seed numbers, pericarp thickness, etc. The extent of reduction in fruit traits (~22.4 %) in Expt-II could have corresponded to the observed percentage of flower drop in Expt-I. A higher percentage of flower drops could be due to the higher sensitivity of plants to stressors as observed earlier in tomatoes [56,57] and in bell peppers [58], and Akabare chili plants might have compromised the pollination and fertilization processes under stress conditions, as reviewed by Bita and Gerats [59] and Zinn et al. [60]. This study focused solely on the floral drop, but further investigation into the reproductive behavior of Akabare chili is imperative for a comprehensive understanding of its reproductive dynamics.

The current study did not delve into stomatal anatomy due to insufficient newly developed leaves during stress treatments and the recovery phase, however, findings from the previous work [27] could suggest that tolerant landraces such as C44 and DKT77 may possess fewer but more efficient stomata, leading to enhanced leaf cooling. Higher biomass yield of plant parts, including fruits, could have been attributed to the evolutionary and dynamic stomatal functions of stress-tolerant genotypes as discussed by McAdam et al. [61] and Drake et al. [62]. Drastically lower values of transpiration and stomata conductance observed under combined drought and heat stress might be due to the dilemma in the stomata functions, impeding the stomata regulation. For example, drought triggers the stomata closure while heat stress cues oppositely as reported previously on rice [63].

To overcome challenges posed by recurrent drought and heat events, especially pre- and post-flowering, it is highly demanding to improve chili genotypes for enhanced yield potential and stability. In the present study, high throughput plant phenotyping (HTPP) was complemented by morphological envirotyping to dissect the complex nature of drought and heat tolerance of Akabare chili landraces. Through a comprehensive analysis of physiological responses at different growth stages, referring to the results from the previous work [27] and the current findings, this study has proposed two Akabare chili landraces, C44 and DKT77 as a potential candidate for drought and heat stress tolerance breeding. The present study endeavored to optimize available facilities for phenotyping Akabare chili landraces from existing collections. However, it is crucial to acknowledge the potential presence of undiscovered landraces at the farmers' level, beyond the researchers' accessibility. The comparison of Akabare chili landraces with commercial chili cultivars at pre-flowering and fruiting stages was limited owing to the late maturity of the studied landraces compared to the commercial chili cultivar 'Jwala', unlike during the early vegetative stage [27]. Another notable methodological constraint involved the difficulty in implementing combined drought and heat stress during the field experiment, thus limiting the comparison of landraces across all three growth stages. Additionally, the unavailability of necessary equipment at Tribhuvan University, Nepal, posed challenges in measuring photosynthetic gas exchange, leaf temperature, and stomatal anatomy during Expt-II. To enhance the study's robustness and practicality, future research should prioritize minimizing these limitations.

5. Conclusions

This study aimed to improve understanding of how drought and heat stresses affect Akabare chili landraces development during pre-flowering and fruiting stages and to explore the interconnectedness between Akabare chili's ability to withstand these stresses at different developmental stages in controlled and field conditions. By using F_v/F_m, photosynthetic gas exchange measurements, and leaf pigments as selection criteria, the study revealed a strong correlation between F_v/F_m, CCI, and P_N. Tolerant landraces displayed higher values in these parameters, improved leaf cooling, and maintained fruit yield, while sensitive landraces showed significantly lower biomass production during stress treatments and recovery. The findings confirmed that stress-tolerant landraces at the pre-flowering stage exhibit consistent traits at fruiting stage. Drought stress had a predominant effect over heat stress, and combined stress had a very adverse effect. The study recommends screening Akabare chili landraces for stress tolerance using F_v/F_m, CCI, and P_N and identifies landraces C44 and DKT77 as potential candidates for plant breeding programs. This study suggests future research on metabolomics and genomics to improve fruit quality and stress tolerance of Akabare chili landraces.

Data availability statement

Data obtained during the experiments in climate chambers and open field conditions for this study will be made available on request.

CRediT authorship contribution statement

Damodar Poudyal: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bal Krishna Joshi:** Writing – review & editing. **Kishor Chandra Dahal:** Writing – review & editing, Supervision, Methodology, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:All authors have equally contributed to the work entitled "Insights into the responses of Akabare chili landraces to drought, heat, and their combined stress during pre-flowering and fruiting stages." The authors declare that the manuscript as submitted has not been published or accepted for publication, nor is being considered for publication elsewhere, either in whole or substantial part. We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other people who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have considered the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, concerning intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm none of our work or its part has involved either experimental animals or human patients and has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. Authors conform to the legal requirements of Denmark and Nepal where the work was conducted.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36239.

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