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Interactive effects of drought and weeds on greengram: Impacts on physiological, biochemical and yield traits

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Global agricultural productivity is increasingly threatened by the combined effects of biotic and abiotic stresses, particularly weed interference and drought, which pose significant challenges to achieving Sustainable Development Goal 2 (Zero Hunger). Weeds alone contribute to an estimated global economic loss of USD 32 billion annual. To ensure food security and develop climate-resilient strategies (SDG 13), this study aimed to investigate the interactive effects of weed competition and drought stress on the physiological, biochemical, and yield responses of greengram. An experiment conducted over two consecutive years (2022 and 2023) evaluated the impact of two key weeds, *Echinochloa colona* and *Trianthema portulacastrum*, at the greengram critical competitive phase under both well-watered (WW) and drought-stressed (DS) conditions. The results revealed that *E. colona* exerted significantly greater adverse effects on greengram than *T. portulacastrum*, particularly under drought stress. This was evidenced by reduced relative water content (WW: 65.26%; DS: 62.31%), membrane stability index (WW: 66.58%; DS: 62.54%), and gas exchange parameters, along with markedly increased oxidative stress markers malondialdehyde (WW: 1.60-fold; DS: 2.08-fold), superoxide ion (WW: 4.04-fold; DS: 5.63-fold), and hydrogen peroxide (WW: 1.41-fold; DS: 2.23-fold). The findings underscore the greater competitive impact of *E. colona* on greengram, particularly under water-deficit scenarios, and highlight the urgent need for integrated weed and drought management strategies to safeguard food security and sustainability.

Keywords Climate change, Drought stress, Crop-weed competition, Greengram, *Echinochloa colona*, *Trianthema portulacastrum*

Weed infestation is a major biotic constraint to achieving higher crop yields worldwide[1]. Weed interference not only hinders the growth and development of cultivated crops but also disrupts their uniformity, resulting in uneven crop maturity and complicating harvesting operations[2]. The impact of weeds on agricultural productivity is substantial, with estimated yield losses amounting to 2.7 million tonnes of grain at the national level. In India, the economic burden is even more pronounced, with weeds causing annual losses exceeding USD 11 billion leading to significant yield reductions i.e., 36% in groundnut, 31% in soybean, 30.8% in greengram, 25% in maize, and 18.6% in wheat[3].

Drought is one of the most perilous abiotic stresses, notably limiting plant growth and causing significant yield losses, thereby threatening food security[4]. Climate change is expected to intensify extreme weather events, including droughts, disrupting the hydrological cycle[5]. Currently, 66% of the world's agricultural area is affected by drought, with projections indicating further increases[6]. Drought stress has already reduced global crop yields by an average of 50%[7]. With the global population expected to exceed 9 billion by 2050[8], food demand will rise, straining the food supply chain and hindering progress toward zero hunger (SDG-2)[9,10]. Drought stress triggers a cascade of plant morphological, physiological, biochemical, and molecular alterations in plants[11,12].

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When drought is combined with weed interference, it exacerbates oxidative stress by promoting the synthesis of reactive oxygen species (ROS), leading to cellular damage, lipid peroxidation, and compromised membrane integrity[1,13–15]. These stress-induced changes collectively impair plant growth and development, resulting in notable yield reductions[12,16].

India produces 25% of the world's pulses, yet its share in total food grain output declined from 16.6% in 1950 to 7% in 2014–15[17], with pulses growing 32% less than cereals in the past decade[18], leading to a significant demand-supply gap. *Vigna radiata* (L.) R. Wilczek (greengram) is a key pulse crop around the globe. Greengram is grown on 7.3 million hectares worldwide, mainly in Asia, with an average yield of 721 kg ha^{-1}.[19,20]. Rich in protein, iron, fiber, and antioxidants, greengram is nutritionally valuable[21]. India contributing over half of global production, though yields remain 80% below potential due to abiotic and biotic stresses[22]. Climate change and water scarcity in arid regions further limit productivity[23–25].

In India, weeds are accountable for a 30.8% reduction in greengram yield, amounting to an annual economic loss of approximately USD 161 million[3]. Halving this loss could significantly boost grain production by nearly 100 million metric tons, contributing to global efforts to reduce hunger. To meet the growing demand driven by population increase, pulse production must rise by 2.2% annually, reaching 39 million tonnes by 2050[26,27]. Achieving this target requires a sustainable increase in yield, with estimates suggesting that a 50% rise in productivity is essential to meet global food needs and eliminate hunger and malnutrition[28]. These challenges underscore the urgent need for strategic interventions from researchers and policymakers in agriculture and food security.

Globally, the most problematic weeds in greengram fields include *Dactyloctenium aegyptium*, *Echinochloa colona*, *Cyperus rotundus*[29], and *Trianthema portulacastrum*[30,31]. The magnitude of weed-crop competition is altered by the various of key resources i.e., water, nutrients, and space, along with light and temperature[32–34]. The extent of yield loss caused by weeds depends on species type, density, emergence timing, and time of interference[35]. Losses are most pronounced when weeds and crops emerge simultaneously under limited resource conditions[36,37]. Weed competition intensifies with prolonged interference, leading to significant yield reductions[38,39]. Under drought or water-stressed environments, weeds often outperform crops, acting as "water wasters" due to elevated transpiration rates and lower stomatal resistance[40–42]. In managed cropping systems, competition for soil moisture between weeds and crops further intensifies water stress, depleting soil moisture at the root area[43].

Despite the increasing frequency of drought stress, many studies have focused on the responses of various weeds to water scarcity[44–46], and the physiological responses of greengram to drought have also been well studied[47,48]. However, there remains a lack of comprehensive understanding regarding the combined effects of biotic stress (weeds) and abiotic stress (drought) on the physiological, biochemical, and yield responses of greengram. Gaining deeper insights into these interactive effects is crucial for addressing global challenges related to sustainable development, predominantly SDG 2 (Zero Hunger) and SDG 13 (Climate Action)[10]. We hypothesize that weed interference significantly influences the physiological, biochemical, and yield traits of greengram under drought conditions. Understanding these interactions will be vital for devising effective mitigation strategies. To fill this knowledge gap, a two-year experiment (2022 and 2023) was designed with the following objectives: (i) to measure the physiological and biochemical responses of greengram to weed interference under drought stress, and (ii) to compare the crop's responses to individual and combined biotic and abiotic stresses in terms of physiological, biochemical, and yield parameters.

Material and methods

Experimental site description and treatment details

A two-year experiment was carried out during the summer seasons of 2022 and 2023 at the greenhouse facility of the ICAR-Directorate of Weed Research, Jabalpur, India. The primary objective of the study was to examine the effect of weed competition (*E. colona* and *T. portulacastrum*) on the physiological, biochemical, and yield responses of greengram [(*Vigna radiata* L.) var. Samrat under well-watered (WW) and drought stress (DS) environments. The experiment was set up in the greenhouse with a 12-hour photoperiod, ambient temperature set at 28 ± 2 °C, relative humidity ranging from 60–75%, and light intensity between 500–1000 µmol photons m^{-2} s^{-1} throughout the experiment to ensure uniform growth and minimize external variability. The experimental layout followed a completely randomized design (CRD) with five biological replicates per treatment. The treatments comprised: (i) greengram grown alone (weed-free), (ii) greengram grown with *E. colona*, and (iii) greengram grown with *T. portulacastrum* (Table 1). The experiment was executed using large earthen pots measuring 46 cm in diameter and 42 cm in height, each filled with 58 kg of homogenized soil. Greengram and weed seeds were sown simultaneously. Upon emergence, weed seedlings were thinned to maintain uniform

Treatments	Crop and weed combinations
Control (Well-watered)	Weed-free greengram (**WFG-WW**)
	Greengram + *E. colona* (**GE-WW**)
	Greengram + *T. portulacastrum* (**GT-WW**)
Drought (Drought stress)	Weed-free greengram (**WFG-DS**)
	Greengram + *E. colona* (**GE-DS**)
	Greengram + *T. portulacastrum* (**GT-DS**)

Table 1. Crop-weed combination and treatment details.

densities i.e., 10 plants per pot (equivalent to 50 plants/m^2) for *E. colona*, and 5 plants per pot (25 plants/m^2) for *T. portulacastrum*. Excess seedlings were carefully removed seven days after emergence to ensure consistent competitive pressure across replicates. Standard crop management practices were followed uniformly across all treatments. This included timely irrigation and fertilizer application based on recommended agronomic guidelines to support optimal greengram growth. Manual weeding was carried out every 5–7 days for the weed-free control treatment to remove any emerging weeds and ensure the absence of competition.

Imposition of drought stress and soil moisture content measurement

The experiment was conducted under two distinct irrigation regimes: well-watered (WW) and drought stress (DS). In the WW treatment, pots were irrigated regularly to maintain optimal soil moisture throughout the experimental period, ensuring consistent growth conditions. Conversely, drought stress was imposed in the DS treatment by withholding irrigation starting at 20 days after sowing (DAS) for a duration of 15 days. During this stress period, plants were closely monitored for visible drought symptoms, including chlorosis, leaf drying, and leaf rolling. Rewatering was initiated upon the appearance of these stress indicators to prevent irreversible damage. To assess the extent of soil moisture depletion under WW and DS conditions, soil moisture content was measured before rewatering. Soil samples were collected from each pot using a sampling auger and placed in pre-weighed containers to ensure accurate measurement. Sampling was performed at two soil depths—the top 5 cm (0 to 5 cm) and the bottom 5 cm (5–10 cm) of the soil profile to capture moisture distribution within the pot. For each depth, triplicate samples were taken to enhance precision and reliability. Immediately after collection, the fresh weight of each sample was recorded. The samples were then oven-dried at 105°–110°C and the final dry weight was noted, and soil moisture content was calculated with the formula described by Schmugge et al. (1980):

$$\textbf{Soil moisture content} = [\text{Weight of wet soil (Ww)} - \text{Weight of dry soil (Wd)} \;/\; \text{Weight of wet soil (Ww)}] \text{ X } 100$$

Sampling and assessment of physiological and biochemical parameters

Physiological and biochemical traits were evaluated before rewatering to determine the influence of drought on greengram cultivar.

Estimation of relative water content (RWC) and membrane stability index (MSI)
RWC was measured using the Barrs and Weatherly[49] method, while MSI was assessed following the Sairam[50] protocol.

Estimation of total chlorophyll level
A 0.1 g leaf sample was extracted in 10 mL of 80% acetone, and O.D. was measured at 663 and 645 nm[51].

Assessment of leaf photosynthesis and its related traits
LI-COR 6400 IRGA (between 10:00–12:00 hrs) instrument was used to assess photosynthetic rate (PN), stomatal conductance (gs), and transpiration rate (E) in fully expanded leaf by following the protocol of Leakey et al.[52].

Estimation of oxidative stress indicators

Malondialdehyde (MDA) content
Lipid peroxidation was assessed via MDA estimation[53], and absorbance was measured at 532 and 600 nm.

Superoxide anions ($O_2 \cdot^-$)
Superoxide anion levels were assessed using the Wu et al.[54] method with a sodium nitrate standard, recorded absorbance at 530 nm.

Hydrogen peroxide (H_2O_2) content
H_2O_2 levels were measured at 415 nm and assessed using a standard H_2O_2 curve[55].

Antioxidant enzyme assay and nitrate reductase activity

Enzyme extraction
Fresh leaf samples (0.5 g) were extracted in 50 mM phosphate buffer (pH 7.0) with EDTA, PVPP, and PMSF, then centrifuged at 12,000×g for 20 min at 4°C. The supernatant was used for enzymatic analysis.

Superoxide dismutase (SOD)
Superoxide dismutase activity was assessed by its inhibition of nitroblue tetrazolium (NBT) photoreduction at 560 nm. One unit of SOD was specified as the amount required to hinder NBT reduction by 50%, specified as units mg^{-1} protein min^{-1}[56].

Catalase (CAT)
Catalase activity was assessed using Aebi[57] method by tracking the decline in O.D. at 240 nm due to H_2O_2 consumption (extinction coefficient of 45.2 mM^{-1} cm^{-1}).

Peroxidase (POD)
Peroxidase activity was assessed following Kar and Mishra[58] method by tracking purpurogallin formation at 420 nm, specified as absorbance units mg^{-1} protein min^{-1}.

Nitrate reductase activity

One gram of leaf tissue was extracted in 250 mM Tris-HCl buffer (pH 8.5) containing 10 mM cysteine, 1 mM EDTA, 20 µM FAD, 1 mM DTT, and 10% glycerol. The extract was centrifuged at 10,000 × g for 30 min at 4°C. The assay mixture (1.5 mL) included 10 mM KNO_3, 0.065 M HEPES (pH 7.0), 0.5 mM NADH, 0.04 mM phosphate buffer (pH 7.2), and enzyme extract. The reaction was initiated by NADH and terminated after 15 min by adding 1 mL of 1 N HCl with 1% sulfanilamide, followed by 1 mL of 0.02% NEDD. Absorbance was measured at 540 nm after 10 min. One enzyme unit (U) was defined as the quantity forming 1 nmol of product[59].

Non-enzymatic antioxidants

The non-enzymatic antioxidant defense under drought stress was evaluated by measuring proline, total phenolics (TPC) and total soluble sugars (TSS) as follows:

Proline content

Proline was estimated following the protocol of Bates et al.[60], which was homogenizing 0.5 g leaf samples in 3% sulfosalicylic acid. The filtrate was used for analysis, with absorbance noted at 520 nm.

Total phenolic content (TPC)

Total phenolic content was measured following Singleton and Rossi[61] method by recording extract absorbance at 765 nm.

Total soluble sugars (TSS) and starch

Total soluble sugars Following McCready et al.[62], TSS content was measured by extracting 0.1 g leaf samples in 80% ethanol, centrifuging, and recording the absorbance at 630 nm. Values were specified as mg g^{-1} FW using glucose as a standard.

Starch Leaf starch content was estimated following the method of McCready et al.[62]. A 0.1 g leaf sample was extracted with 80% ethanol and centrifuged. The resulting pellet was dried and treated with perchloric acid to extract starch, followed by a second centrifugation. The supernatant was then assessed using anthrone reagent.

Yield and yield attributes and weed parameters

Five plants per treatment were carefully uprooted at harvest, and the yield and yield attributes were recorded. Weed indices, including plant fresh biomass, dry biomass, root length, root volume, and leaf area to assess weed growth and competition.

Data analysis

Analysis of variance (ANOVA) was employed to evaluate the main and interaction effects of multiple independent factors on physiological parameters, in accordance with the experimental design. To identify statistically significant differences among treatment means, Tukey's Honestly Significant Difference (HSD) test was applied as a post-hoc analysis, effectively controlling for Type I error and enabling accurate pairwise comparisons across treatments and weed management strategies. All ANOVA and post-hoc analyses were performed using SPSS software (Version 16.0; SPSS Inc., Chicago, IL, USA), with significance determined at the 5% and 1% levels. Correlation and principal component analyses (PCA) were conducted using GRAPES software. Heatmap analysis performed using SR-Plot to visualize patterns of variation across treatments and traits by clustering similar responses.

Results

Soil moisture content (SMC)

SMC was remarkably affected by weed presence and drought stress. In weed-free greengram, SMC was 27.63% (top 5 cm) and 32.43% (below 5 cm) under well-watered (WW) environment but declined to 8.29% and 10.02%, respectively, under drought stress (DS). The presence of *T. portulacastrum* further reduced SMC to 26.78% (top) and 27.49% (below) under WW, with a sharper decline under DS to 7.73% and 8.79%. Weed *E. colona* interference led to a greater reduction, with SMC dropping to 23.96% (top) and 27.44% (below) under WW and plummeting to 6.51% and 7.89% under DS. The most severe moisture depletion occurred with *E. colona* under DS, highlighting its strong competitive impact on greengram (Figs. 1 and 2).

RWC, MSI and total chlorophyll content

Analysis of variance (ANOVA) has shown that weed interference (*T. portulacastrum* and *E. colona*) under drought stress had a significant effect ($P< 0.001$) greengram physiological parameters, including RWC, MSI and total chlorophyll content (Fig. 3 a,b,c). However, the interaction was not significant for MSI. Weed interference significantly lowered the greengram RWC, MSI and total chlorophyll content compared weed-free greengram under both WW and DS. A significant reduction in greengram RWC (WW: 65.26%; DS: 62.31%), MSI (WW: 66.58%; DS: 62.54%) and total chlorophyll content (WW: 1.89 mg/g FW; DS: 1.76 mg/g FW) has been noticed in the presence of *E. colona.* Whereas *T. portulacastrum* interference has moderate effect of greengram RWC (WW: 76.38%; DS: 71.05%), MSI (WW: 69.52%; DS: 65.73%) and total chlorophyll content (WW:2.96 mg/g FW; DS:2.47 mg/g FW). A significant reduction in RWC, MSI and total chlorophyll content in DS suggests that greengram experienced extreme DS by both the weeds. However, the degree of decrease was higher with *E. colona* as compared *T. portulacastrum.*

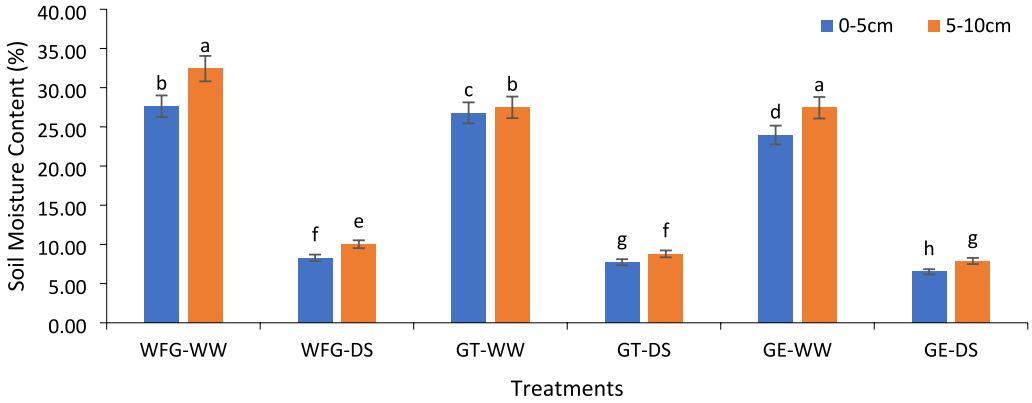


Fig. 1. Soil moisture content of greengram pots influenced by *T. portulacastrum* and *E. colona* under well-watered (WW) and drought stress (DS) environments. *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona*. The data shown above denote the Mean ± SE (n = 5). Distinct alphabets on error bars depicts significant variations (p = 0.05) as per tukey test.

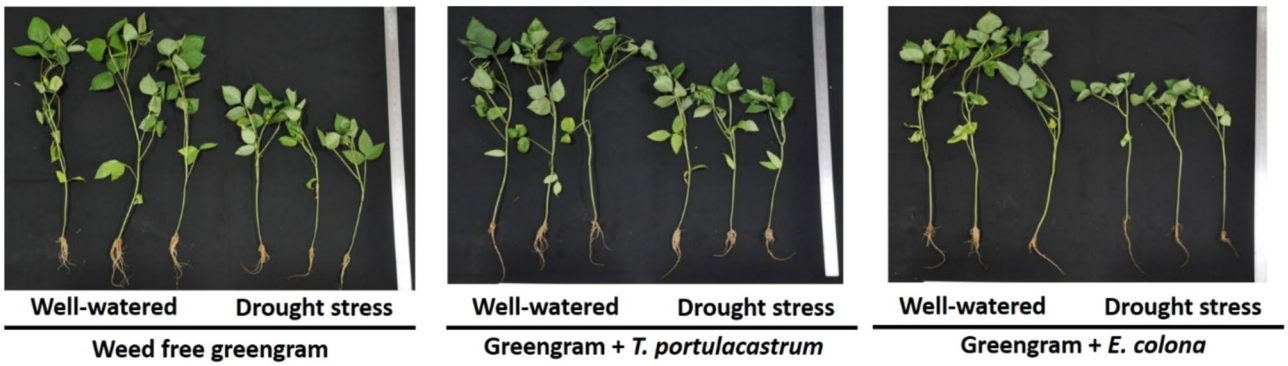


Fig. 2. Impact of weed interference on greengram growth and morphology.

Gaseous exchange parameters

Weed interference under DS has a significant ($P< 0.001$) influence on most of the gas exchange indices, including rate of photosynthesis (P_N), stomatal conductance (*gs*) and rate of transpiration (*E*) (Fig.4. a,b,c). Weed interference remarkably lowered the greengram P_N, *gs* and *E* compared weed-free greengram under both WW and DS. A significant reduction in greengram P_N (WW: 21.30; DS: 11.18), *gs* (WW: 0.44; DS: 0.38) and *E* (WW: 3.11; DS: 2.89) has been noticed in the presence of *E. colona.* Whereas *T. portulacastrum* interference had moderate effect of greengram P_N (WW: 25.30; DS: 14.37), *gs* (WW: 0.53; DS: 0.47) and *E* (WW:5.30; DS:3.41). A significant reduction in P_N, *gs* and *E* in both the conditions (WW and DS) specifies that greengram experienced extensive DS from both the weeds. However, the degree of decrease was higher with *E. colona* than *T. portulacastrum.*

Oxidative stress markers

Weed interference significantly ($P< 0.001$) elevated the greengram oxidative stress markers like MDA levels, superoxide ion levels, and H_2O_2 levels under both WW and DS environments (Fig. 5. a,b,c). A notable enhancement in greengram MDA (WW: 1.60fold; DS: 2.08fold), superoxide ion (WW: 4.04fold; DS: 5.63fold) and H_2O_2 (WW: 1.41fold; DS: 2.23fold) has been noticed in the presence of *E. colona.* Whereas *T. portulacastrum* interference had moderate effect of greengram MDA (WW: 86%; DS: 1.11fold), superoxide ion (WW: 1.78fold; DS: 2.70fold) and H_2O_2 (WW: 21.04fold; DS: 97.08fold). A significant upsurge in MDA, superoxide ion and H_2O_2 in both the environments (WW and DS) indicated that greengram experienced intense DS by both the weeds. However, the degree of enhancement was more with *E. colona* as compared *T. portulacastrum.*

Enzymatic, non-enzymatic antioxidants and nitrate reductase activity

SOD, CAT and POD and non-enzymatic antioxidants like proline and total phenolic content exhibited significant differences ($P< 0.001$) due to weed interference under both WW and DS conditions. Weed interference significantly enhanced the antioxidant enzymes under both WW and DS environments (Table 2). A notable enhancement in greengram SOD (WW: 2.18fold; DS: 4.33fold), CAT (WW: 1.16fold; DS: 1.51fold) and POD (WW: 2.53fold; DS: 5.11fold) has been noticed in the presence of *E. colona.* In contrast, *T. portulacastrum*

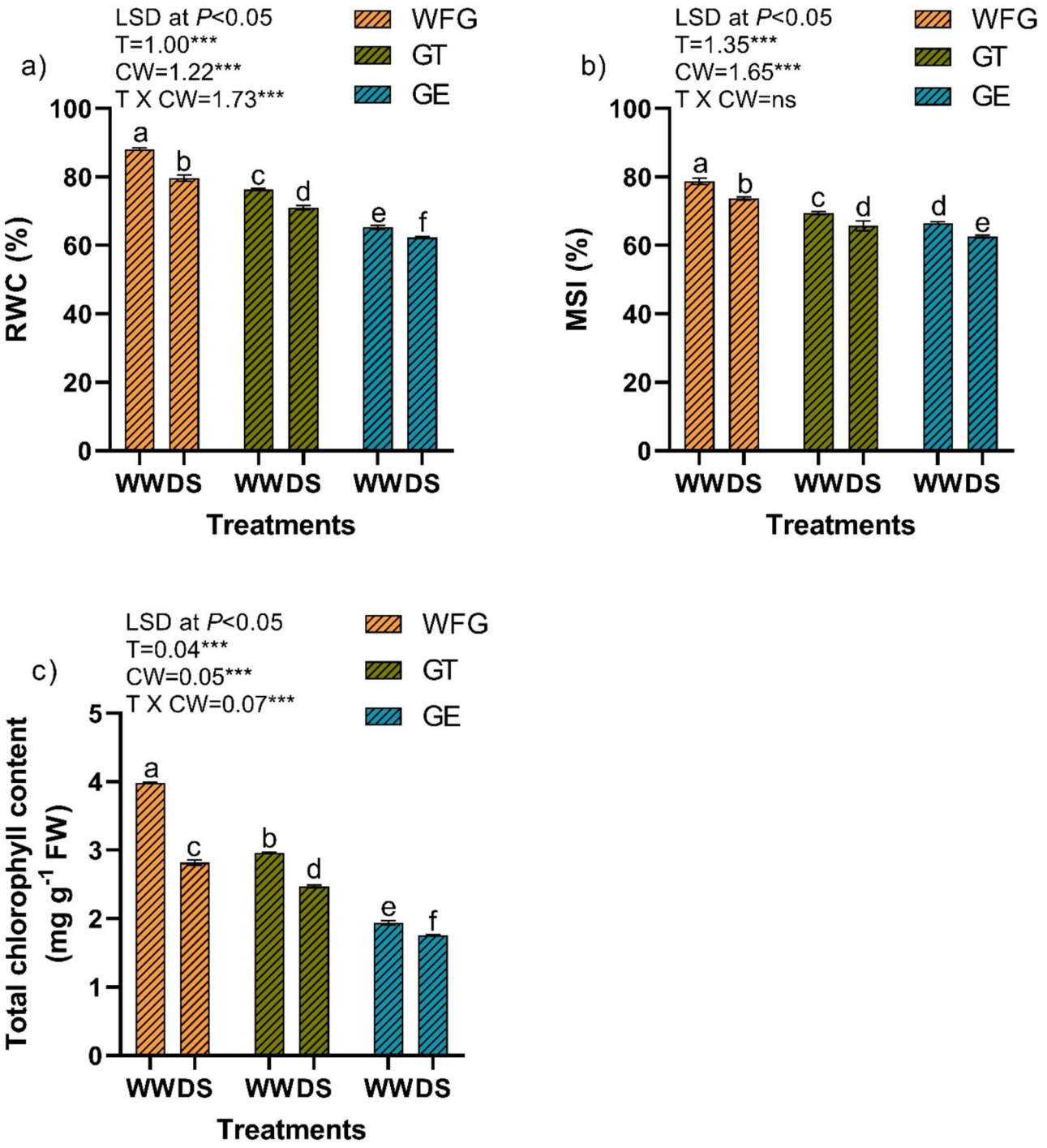


Fig. 3. Effect of weed interference (*T. portulacastrum* and *E. colona*) on greengram RWC (**a**), MSI (**b**), and total chlorophyll content (**c**) under control drought stress. Data are expressed as Mean ± SE (n = 5). *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona*. Distinct alphabets on error bars depicts significant variations (p = 0.05). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns represent non-significance. CW: crop-weed combinations, T: treatment, CW × T: interaction effect.

interference had moderate effect of greengram SOD (WW: 94.26%; DS: 1.35fold), CAT (WW: 88.80%; DS: 1.03fold) and POD (WW: 1.05fold; DS: 1.68fold).

In a similar trend weed interference also enhanced the non-enzymatic antioxidants in both WW and DS environments (Table 2). A remarkable enhancement in greengram proline (WW: 60.41%; DS: 72.64%) and TPC (WW: 66.64%; DS: 1.11fold) has been noticed in the presence of *E. colona*. Whereas *T. portulacastrum*

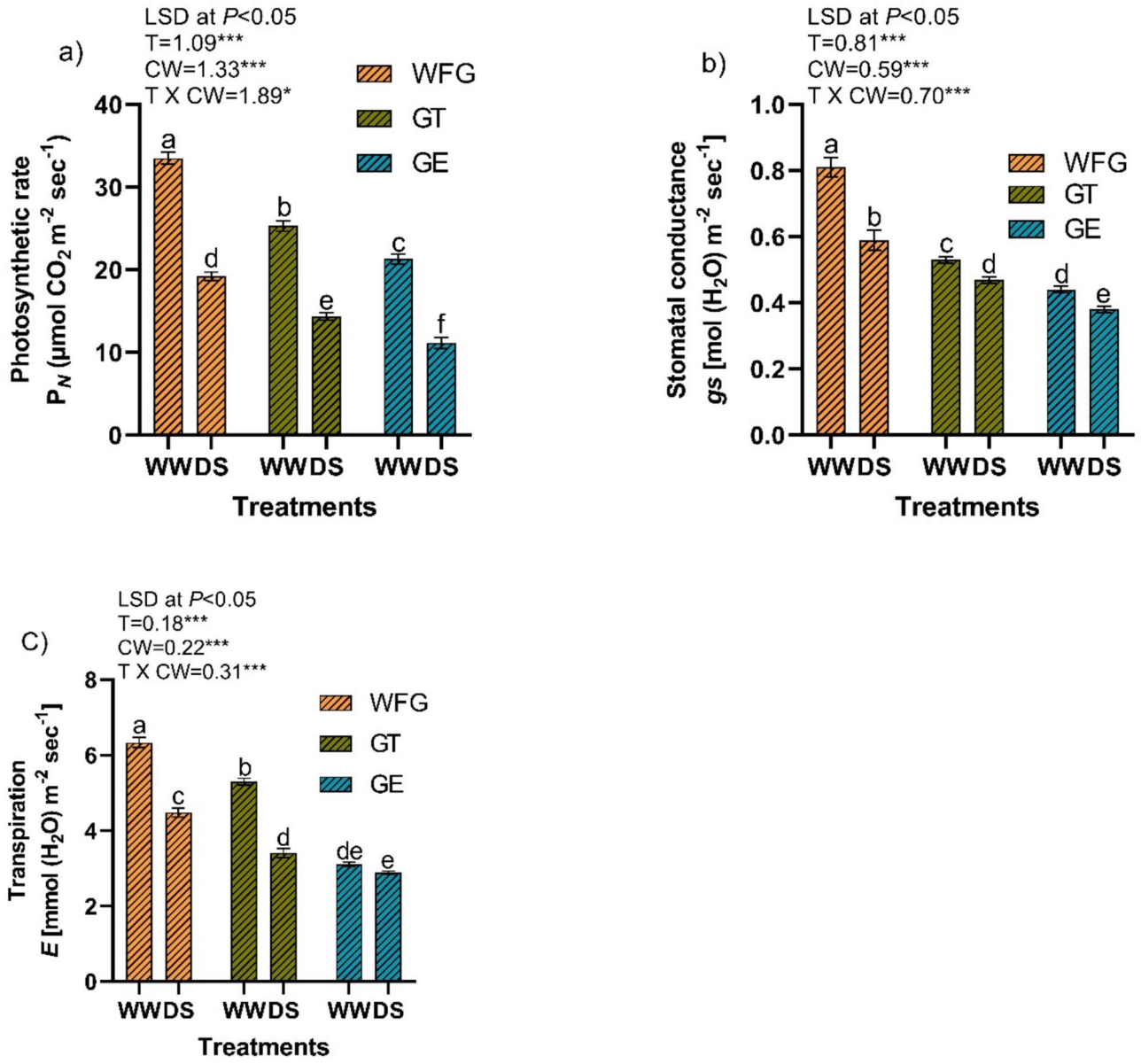


Fig. 4. Effect of weed interference (*T. portulacastrum* and *E. colona*) on greengram rate of photosynthesis (**a**), stomatal conductance (**b**), transpiration (**c**) under control drought stress. Data are expressed as Mean ± SE (n = 5). *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona*. Distinct alphabets on error bars depicts significant variations (p = 0.05). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns represent non-significance. CW: crop-weed combinations, T: treatment, CW × T: interaction effect.

interference had moderate effect of greengram proline (WW: 26.86%; DS: 44.89%) and TPC (WW: 26.04%; DS: 60.86%).

Nitrate reductase activity showed significant reduction under DS in the presence of weeds (Table 2). In the presence of *T. portulacastrum* recorded reductions of 18.28% and 39.93% in yield under WW and DS respectively, relative to weed-free control. Conversely, *E. colona* interference reduced the yield to 34.70% and 47.39% under WW and DS respectively, relative to weed-free control.

Total soluble sugars and starch

TSS and starch were significantly ($P< 0.001$) influenced by the weed interference under both WW and DS environments (Fig. 6. a). In greengram, in the presence of *T. portulacastrum* recorded notable enhancement of 35.79% and 44.49% in TSS. Similarly, *E. colona* interference enhanced the TSS to 54.73% and 69.45% under WW and DS respectively, compared to weed-free control. Starch showed significant reduction under DS in the presence of weeds (Fig. 6. b). In the presence of *T. portulacastrum* recorded reductions of 11.33% and 33.25% in

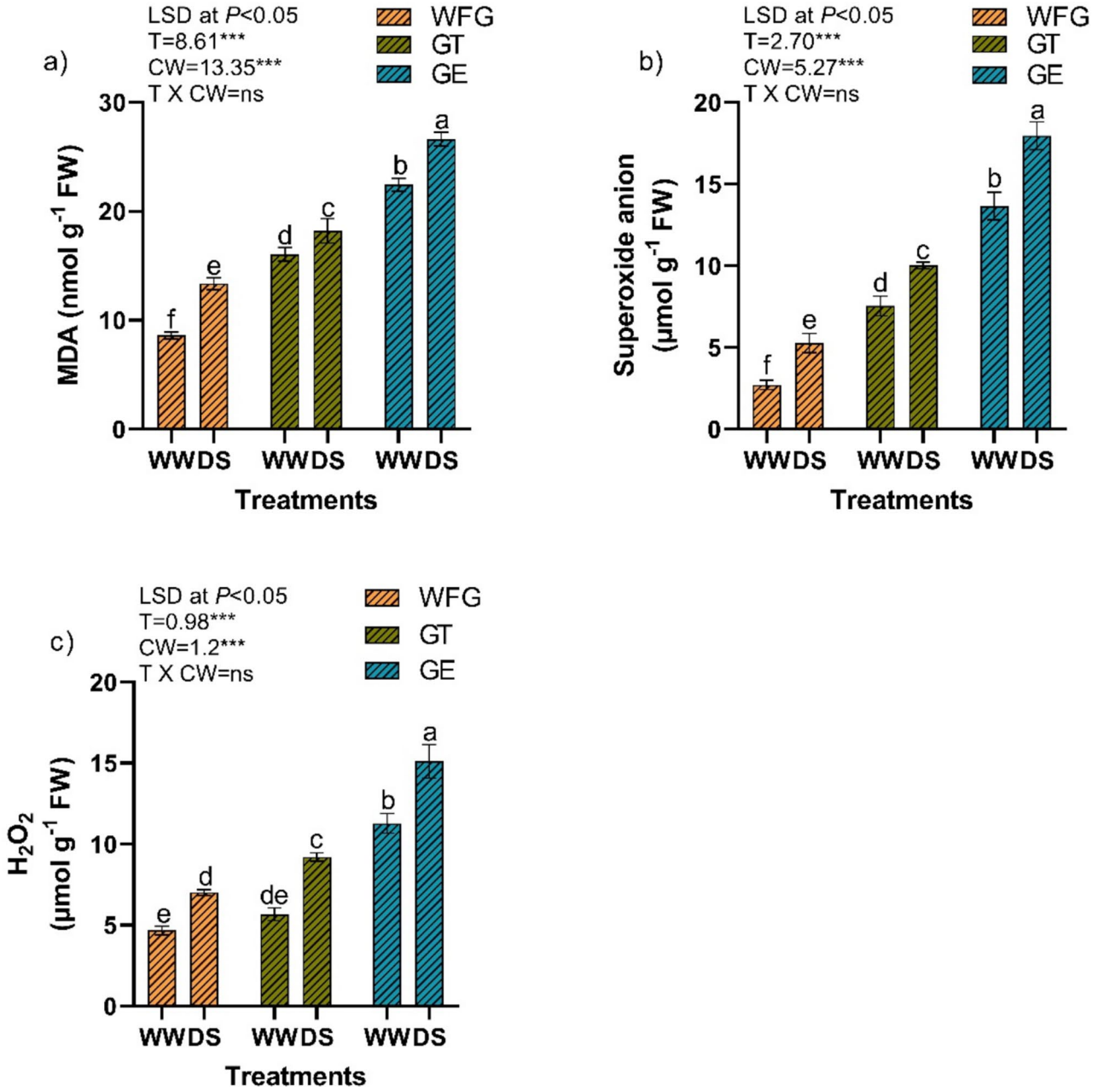


Fig. 5. Effect of weed interference (*T. portulacastrum* and *E. colona*) on greengram MDA levels (**a**), superoxide anion levels (**b**), H_2O_2 levels (**c**) under control drought stress. Data are expressed as Mean ± SE (n = 5). *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona*. Distinct alphabets on error bars depicts significant variations (p = 0.05). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns indicates non-significance. CW: crop-weed combinations, T: treatment, CW × T: interaction effect.

starch under WW and DS respectively, relative to weed-free control. Conversely, *E. colona* interference reduced the starch to 26.31% and 52.25% under WW and DS respectively, relative to weed-free control.

Yield and yield attributes

Weed interference significantly ($P< 0.001$) enhanced the greengram yield and yield attributes under both WW and DS conditions (Table 3). Weed interference significantly influenced the greengram yield and yield attributes like number of pods/plant, pod length, number of seeds/pod, plant dry weight, grain yield/plant and test weight under both WW and DW conditions. A remarkable drop in greengram number of pods/plant (WW: 46.84%; DS: 51.90%), pod length (WW: 9.77%; DS: 19.44%), number of seeds/pod (WW: 14.13%; DS: 21.19%), grain yield/plant (WW: 23.48%; DS: 42.77%) and test weight (WW: 9.74%; DS: 11.65%) has been noticed in the presence of *E. colona*. Whereas *T. portulacastrum* interference had moderate effect of greengram number of pods/plant

Treatment (T)	Crop & Weed combination (CW)	SOD [Umg^{-1} (protein) min^{-1}]	CAT [µmol (H_2O_2) reduced mg^{-1} protein min^{-1}]	POD [Umg^{-1} (protein) min^{-1}]	Nitrate reductase [Umg^{-1} (protein) min^{-1}]	Proline (mg g^{-1} FW)	TPC (mg g^{-1} FW)
WW	WFG	2.67±0.25e	0.128±0.12e	0.24±0.07e	22.33±0.51a	22.86±0.87e	14.37±0.28e
DS	WFG	3.94±0.19de	0.217±0.05d	0.41±0.02de	15.50±0.29c	26.53±0.71d	20.88±0.74c
WW	GT	5.19±0.55cd	0.242±0.16c	0.49±0.04cd	18.25±0.63b	29.00±0.89d	18.12±0.20d
DS	GT	6.29±0.56c	0.261±0.14b	0.64±0.04c	13.42±0.46d	33.12±1.04c	23.12±0.49b
WW	GE	8.50±0.56b	0.277±0.11b	0.85±0.06b	14.58±0.44cd	36.66±1.01b	23.95±0.48b
DS	GE	14.26±1.16a	0.322±0.12a	1.47±0.08a	11.75±0.38e	39.46±0.48a	30.46±0.44a
	T	1.13***	0.01***	0.09***	0.83***	1.53***	0.84***
	CW	1.38***	0.01***	0.12***	1.02***	1.88***	1.03***
	T X CW	1.96**	0.01***	0.17***	1.44**	ns	ns

Table 2. Biochemical response of greengram in the presence of *E. colona* and *T. portulacastrum* under well-watered and drought stress conditions. Data are expressed as Mean ± SE (n = 5). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns indicates non-significance; *WFG* weed-free greengram; *GT* greengram with *T. portulacastrum*; *GE* greengram with *E. colona*; *CW* crop and weeds combinations; *T* treatment; *T X WC*, Interaction between weeds and crop combinations and treatment; *WW* well-watered condition; *DS drought* stress; *SOD* superoxide dismutase; *CAT* catalase; *POD* peroxidase; *Proline* proline content; *TPC* total phenolic content.

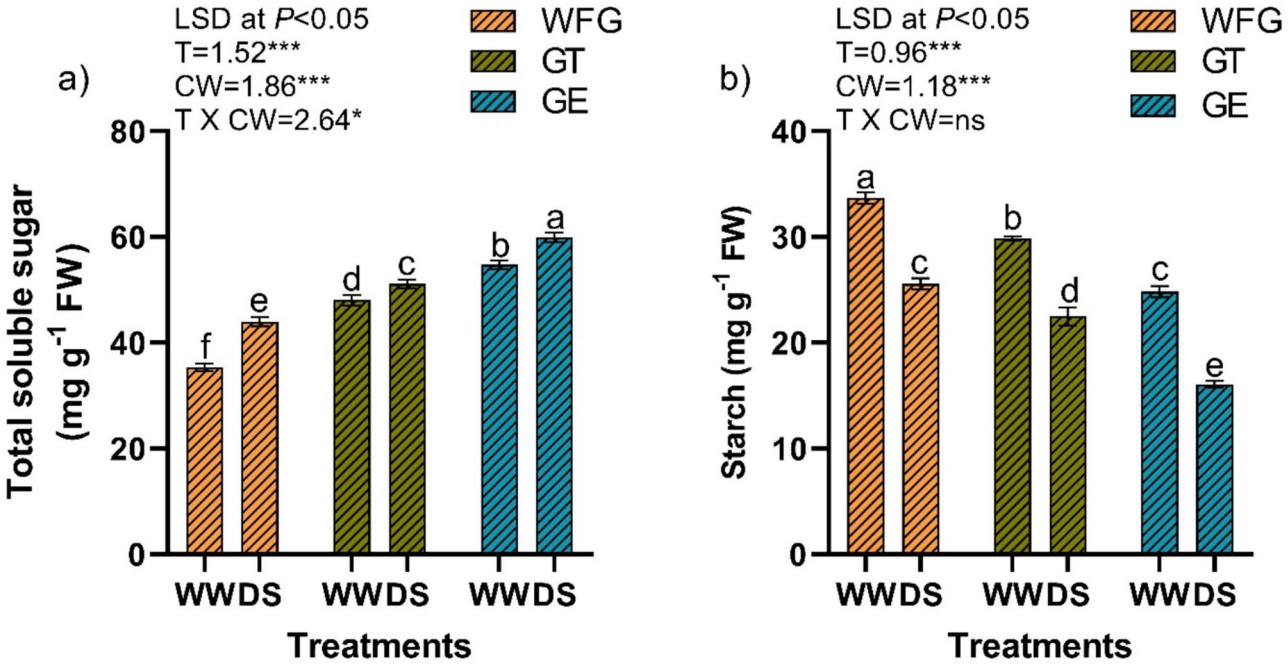


Fig. 6. Effect of weed interference (*T. portulacastrum* and *E. colona*) on greengram total soluble sugars (**a**) and starch (**b**) under control drought stress. Data are expressed as Mean ± SE (n = 5). *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona*. Distinct alphabets on error bars depicts significant variations (p = 0.05). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns indicates non-significance. CW: crop-weed combinations, T: treatment, CW × T: interaction effect.

(WW: 20.25%; DS: 39.24%), pod length (WW: 2.58%; DS: 4.50%), number of seeds/pod (WW: 11.15%; DS: 13.38%), grain yield/plant (WW: 15.12%; DS: 16.11%) and test weight (WW: 6.78%; DS: 7.01%).

Impact of drought stress on weed growth and biomass traits

The response of *E. colona* and *T. portulacastrum* to drought stress exhibited significant variation in growth and biomass traits (Table 4; Fig. 7). Both the weeds showed a reduction in indices such as plant fresh weight, dry weight, root length, root volume, and leaf area under DS compared to their respective WW controls. Whereas, the magnitude of reduction was considerably lower in *E. colona*, suggesting a higher degree of drought tolerance than *T. portulacastrum*. Interestingly, root traits such as root length and volume increased under drought stress in both species, with *E. colona* displaying a more pronounced enhancement than *T. portulacastrum*. This

Treatment (T)	Crop & Weed combination (CW)	Plant dry weight (g/plant)	Number of pods/plant	Pod length (cm)	Number of seeds/pod	Grain yield/plant (g)	Test weight (g)
WW	WFG	7.75±0.51a	15.80±0.37a	6.74±0.15a	10.76±0.28a	3.82±0.42	3.45±0.12
DS	WFG	7.25±0.70ab	9.00±0.32cd	6.46±0.12a	9.84±0.26ab	3.48±0.41	3.33±0.04
WW	GT	7.77±0.59a	12.60±0.60b	6.56±0.26a	9.56±0.35abc	3.25±0.21	3.22±0.02
DS	GT	7.51±0.54ab	9.60±0.51c	6.44±0.29a	9.32±0.30bc	3.21±0.48	3.21±0.04
WW	GE	6.92±0.15ab	8.40±0.55cd	6.08±0.36ab	9.24±0.71bc	2.93±0.22	3.11±0.02
DS	GE	5.95±0.23b	7.60±0.51d	5.43±0.17b	8.48±0.45c	2.19±0.07	3.05±0.03
	T	Ns	0.81***	ns	ns	ns	ns
	CW	1.02*	0.99***	0.49**	0.87**	0.69*	0.12*
	T X CW	Ns	1.40***	ns	ns	ns	ns

Table 3. Yield and yield attributes response of greengram in the presence of *E. colona* and *T. portulacastrum* under well-watered and drought stress conditions. Data are mentioned as Mean ± SE (n = 5). LSD at p < 0.05, 0.01, and 0.001 is marked by *, **, and ***, respectively; ns indicates non-significance; *WFG* weed-free greengram; *GT* greengram with *T. portulacastrum*; *GE* greengram with *E. colona*; *CW* crop and weeds combinations; *T* treatment; *T X WC*, Interaction between crop and weeds combinations and treatment; *WW* well-watered condition; *DS drought* stress.

Weed	Treatment	Fresh weight (g)	Dry weight (g)	Leaf area (cm^2)	Root length (cm)	Root volume (cm^3)
T. portulacastrum	WW	$1.57±0.09^c$	$0.15±0.01^c$	$18.49±0.47^c$	$6.67±0.88^d$	$0.10±0.01^d$
	DS	$1.00±0.06^c$	$0.07±0.01^c$	$12.88±0.49^d$	$8.17±0.60^c$	$0.40±0.30^c$
E. colona	WW	$4.77±0.24^a$	$2.19±0.27^a$	$55.24±0.91^a$	$17.33±0.33^b$	$3.17±0.44^b$
	DS	$3.07±0.43^b$	$1.09±0.11^b$	$41.48±0.68^b$	$20.00±0.58^a$	$4.33±0.33^a$

Table 4. Effect of drought stress on weed (*T. portulacastrum* and *E. colona*) growth and biomass traits. Data are mentioned as Mean ± SE (n = 5). *WW* well water condition; *DS* drought stress. Same alphabet in a column is not significantly different at *P* = 0.05 level as per Duncan's test.

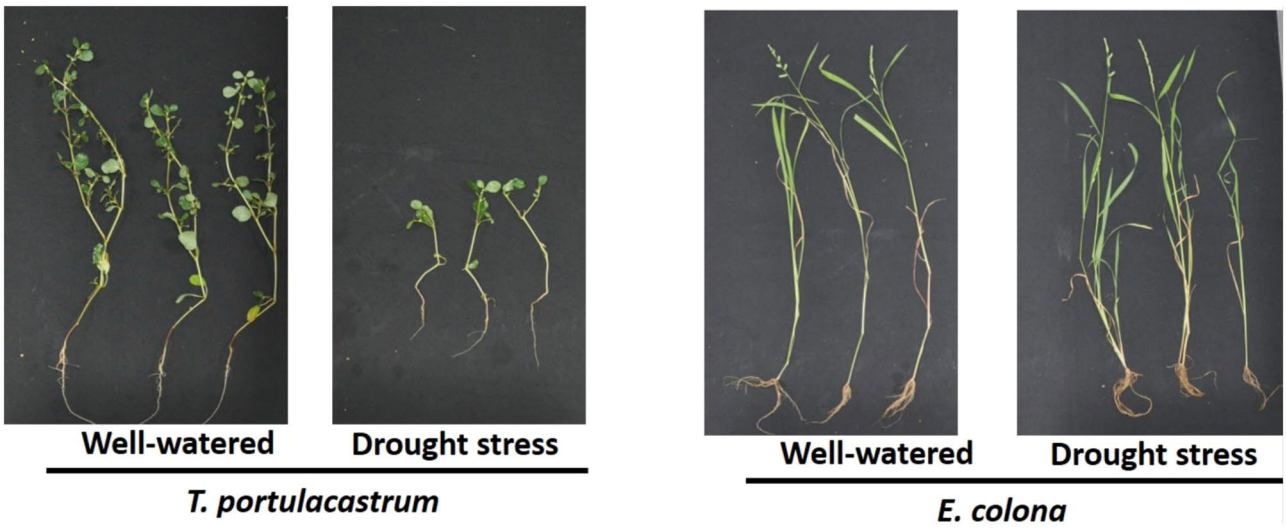


Fig. 7. Impact of drought stress on weed (*T. portulacastrum* and *E. colona*) growth.

increase in root development under stress likely contributed to the better maintenance of aboveground growth in *E. colona*. The relatively lower reduction in growth parameters of *E. colona* under drought stress also appears to influence its competitive ability in crop-weed interactions, particularly with greengram. These findings collectively suggest that *E. colona* possesses more effective adaptive mechanisms to cope with drought stress compared to *T. portulacastrum*.

Assessment of treatment and variable interactions using principal component analysis, correlation analysis and heatmap

Principal component analysis

To evaluate the response of greengram to weed competition from *E. colona* and *T. portulacastrum* under DS, PCA was performed using physio-biochemical and yield-related traits (Fig. 8a, 8b, 8c, and 8 d). The PCA extracted two principal components (PCs), with PC1 and PC2 accounting for 90.1% and 4.8% of the total variance, respectively, thus cumulatively explaining 94.9% of the variation in the dataset. PC1 was primarily influenced by traits such as NPP, Pn, NR, total chlorophyll, MSI, RWC, NSP, starch content, GS, and E. These traits were aligned in the same direction as PC1 in the biplot, indicating a strong positive correlation with it. PC2, on the other hand, captured the variation mainly due to grain yield, plant dry weight, and pod length. The biplot clearly separated treatment groups based on their physiological and biochemical responses. Weed-free greengram under both WW and DS environments (WFG-WW and WFG-DS), along with greengram with *T. portulacastrum* under drought (GT-DS), were positioned on the positive side of PC1, suggesting better physiological performance and tolerance. In contrast, antioxidant and stress indicators such as SOD, POD, H_2O_2, superoxide radicals, MDA, TPC, TSS, and CAT were oriented in the opposite direction of PC1, indicating their negative association with favorable plant performance. Greengram grown with *E. colona* under both control (GE-WW) and drought (GE-DS) conditions, as well as GT-DS, appeared on the negative side of PC1, reflecting the high competitive stress imposed by *E. colona* and, to a lesser extent, *T. portulacastrum*. The vector lengths of the traits in the biplot further illustrated their relative contributions, with plant dry weight and pod length showing significant influence, suggesting these yield components were most affected by weed interference under both moisture regimes.

Pearson correlation

Correlation analysis explored the relationships among various physio-biochemical traits and their association with grain yield in response to weed competition under both WW and DS conditions. The results were visualized using correlograms (Fig. 9), providing a comprehensive overview of the strength and direction of these associations. Under control conditions (Fig. 9. a), traits such as PD, NSP, PL, NPP, starch content, total chlorophyll, RWC, and MSI exhibited strong positive correlations with yield (correlation coefficient, r = 0.75 to 1.0), indicating their crucial role in supporting yield under optimal moisture availability. In contrast, oxidative stress and defense-related parameters MDA, superoxide ion, H_2O_2, TPC, proline, CAT, and SOD showed strong negative correlations with yield (r = –0.75 to –1.0), suggesting that increased oxidative stress negatively impacted productivity. TSS showed a moderate negative correlation with yield (r = –0.25 to –0.5). Under drought stress conditions (Fig. 9. b), several traits maintained their positive association with yield. These included stomatal conductance, transpiration rate, total chlorophyll content, RWC, MSI, starch content, pod length, NSP, TW, and PD, all of which showed strong positive correlations with yield (r = 0.5 to 1.0), highlighting their importance in drought resilience and yield maintenance. On the other hand, traits related to oxidative damage MDA, superoxide ion, H_2O_2, TPC, proline, SOD, and CAT remained strongly negatively correlated with yield (r = –0.75 to –1.0). Additionally, POD showed a moderate negative correlation (r = –0.5 to –0.77), indicating its partial influence on yield under stress. Overall, the correlation analysis emphasizes that physiological stability and reduced oxidative stress are critical for maintaining greengram yield, particularly under drought conditions.

Hierarchical cluster analysis and heatmap

In the present study, physio-biochemical parameters under WW and DS environments were aggregated for each treatment and subjected to hierarchical clustering and heatmap analysis, to explore patterns of variation and treatment effects (Fig. 10). Hierarchical clustering grouped the parameters into three distinct clusters (Cluster A, B, and C) based on similarities in their response across treatments (Fig. 10). Cluster A comprised traits such as Pn, NR, starch content, E, total chlorophyll, and NPP. These traits showed elevated levels in weed-free greengram under well-watered conditions (WFG-WW), but were markedly reduced in greengram exposed to *E. colona* under drought stress (GE-DS). Cluster B included PL, PD, grain yield, RWC, TW, Gs, NSP, and MSI. These parameters also exhibited higher values in WFG-WW, indicating better physiological performance and yield potential, while showing a decline under GE-DS conditions. In contrast, Cluster C consisted of stress-related traits such as MDA, superoxide ion, proline, CAT, TSS, SOD, POD, H_2O_2, and TPC. These traits displayed an increasing trend in greengram with *E. colona* under drought stress (GE-DS), suggesting enhanced oxidative stress and defense responses. In WFG-WW, however, these traits remained low, reflecting minimal stress levels. Together, the heatmap and clustering analyses provided a clear distinction among treatments, highlighting the physiological robustness of weed-free greengram under well-watered conditions and the stress vulnerability of greengram coexisting with *E. colona* under drought.

Discussion

Global warming, a consequence of ongoing climate change, has intensified the interplay of multiple stresses in crop production systems. Among these, abiotic factors such as drought, heat waves, and flooding, along with biotic pressures including insect herbivores, pathogens, and weeds, have emerged as critical constraints to productivity[63,64]. The simultaneous occurrence of these stressors has become increasingly common and severe, leading to sub-optimal crop performance by adversely affecting morpho-physiological traits, yield, and overall plant fitness[65]. In light of these challenges, the present study aimed to investigate the interactive effects of weed interference and water availability (drought and well-watered conditions) on the physiological, biochemical, and yield attributes of greengram cultivar. A comprehensive evaluation of physiological, biochemical, and yield attributes was undertaken to better understand the crop's response under combined stress scenarios. Additionally, efforts were made to elucidate the underlying cause-and-effect relationships among these traits,

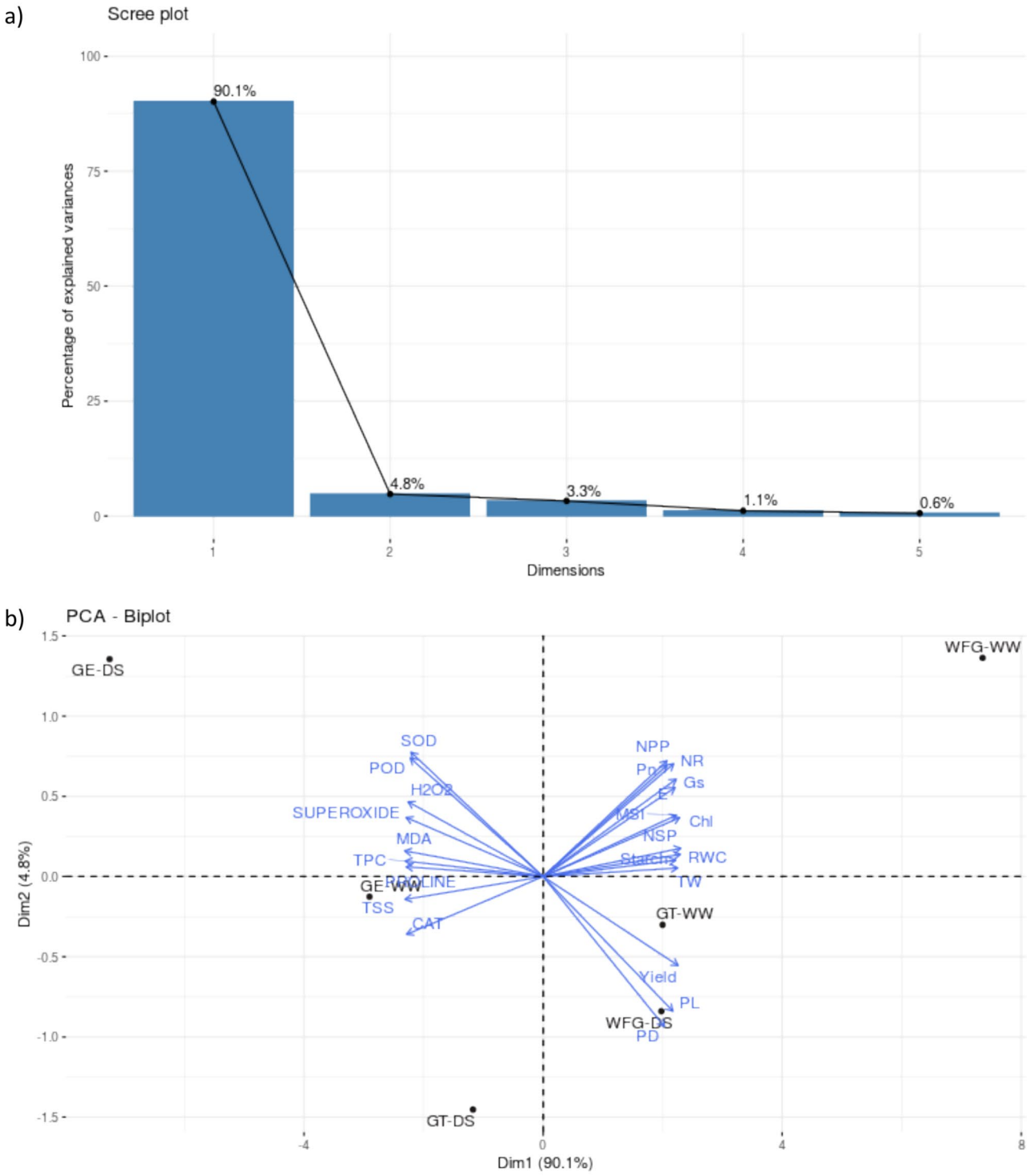
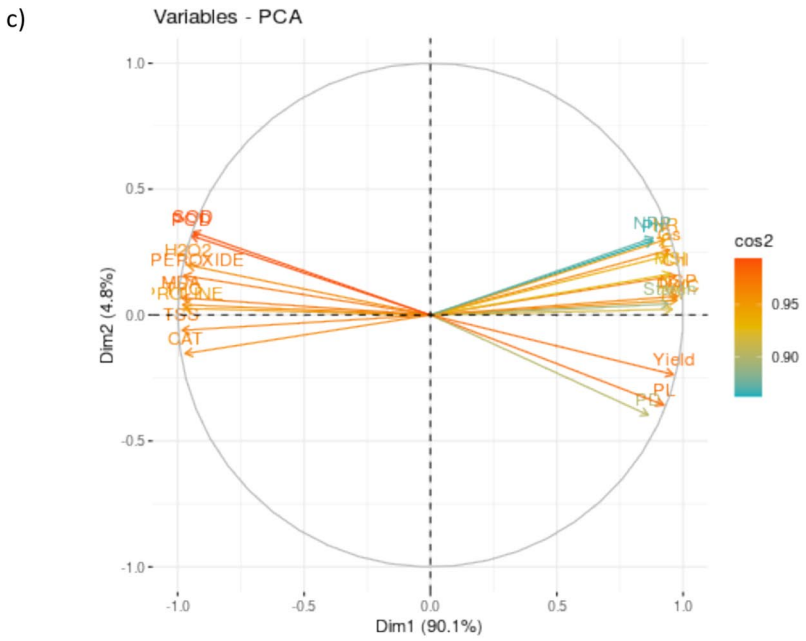


Fig. 8. Principal Component Analysis (PCA) illustrating the association between greengram physiological and biochemical traits under weed interference in well-watered and drought stress conditions. (**a**) Scree plot displaying the percentage of variance described by each PC; (**b**) Biplot representing the interrelationship among physiological and biochemical traits; (**c**) Contribution of each variable to total variability in PC1 and PC2, along with their correlations; (**d**) Correlation plot depicting relationships between variables and principal components. *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona; WW* well water condition; *DS* drought stress.



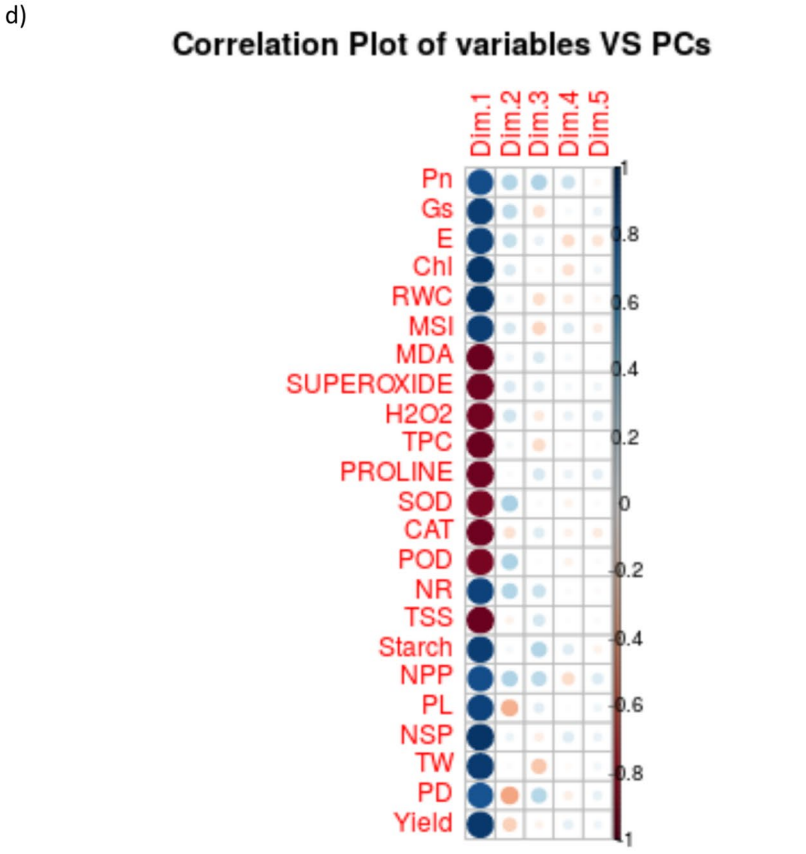


Fig. 8. (continued)

providing valuable insights for developing climate-resilient crop management strategies in alignment with Sustainable Development Goal 13 (SDG-13).

The present study clearly demonstrates that key physiological traits of greengram, such as RWC, MSI, and total chlorophyll content, were significantly compromised by weed interference under both WW and DS environments. Notably, the reductions were more severe in the presence of *E. colona* in contrast to *T. portulacastrum*, indicating a stronger competitive influence of *E. colona* on greengram performance. RWC is a reliable marker of plant water status and metabolic activity under stress, as it reflects the balance between water

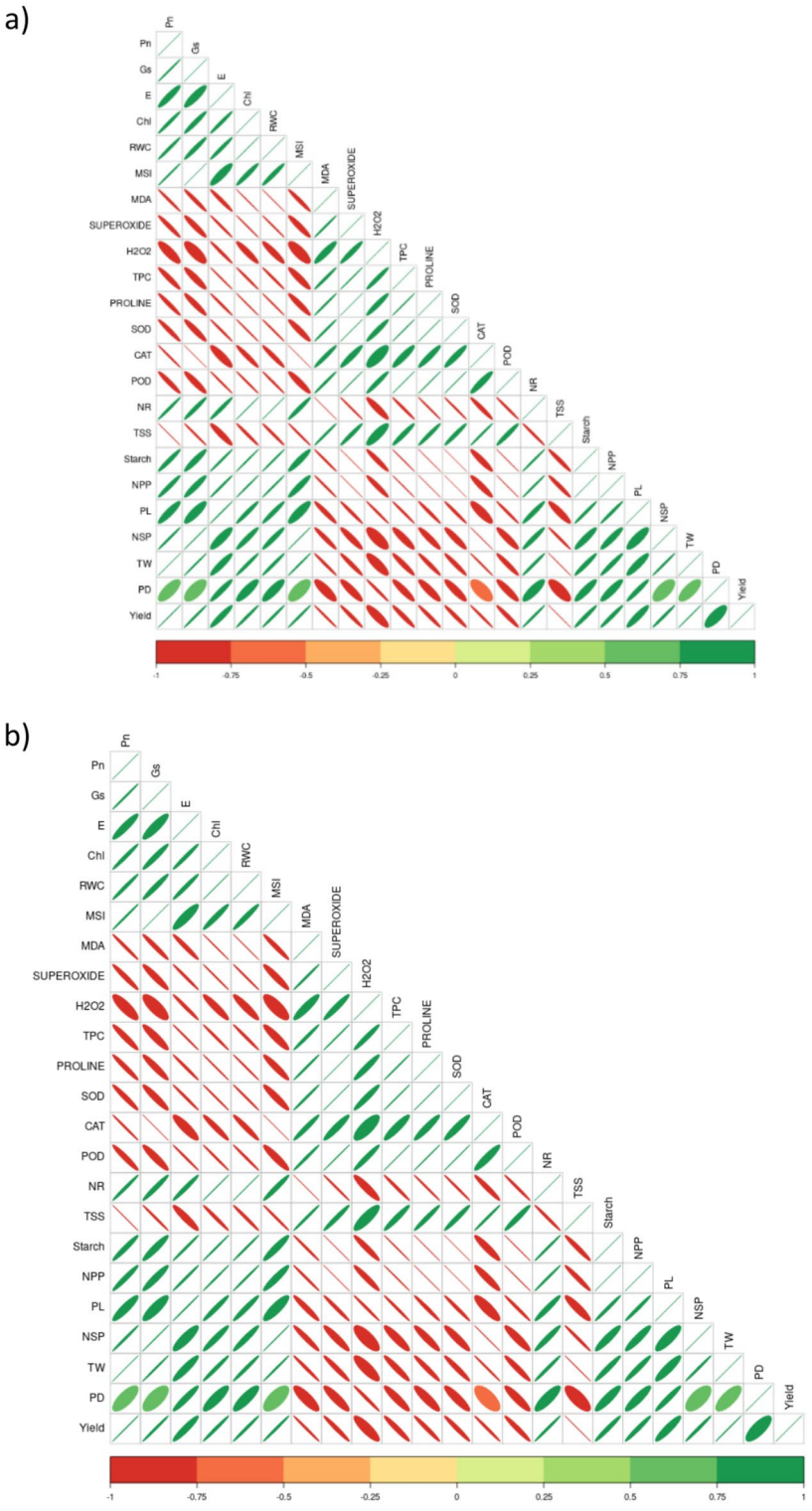


Fig. 9. The correlation coefficient between changes in physiological and biochemical parameters under well-watered (**a**) and drought stress condition (**b**). *Pn* rate of photosynthesis; *Gs* stomatal conductance; *E* rate of transpiration; *Chl* total chlorophyll content; *RWC* relative water content, *MSI* membrane stability index; *MDA* malondialdehyde content; *Superoxide* superoxide ion levels, H_2O_2 hydrogen peroxide levels, *TPC* total phenolic content; and *TSS* total soluble sugar levels; *Yield* grain yield.

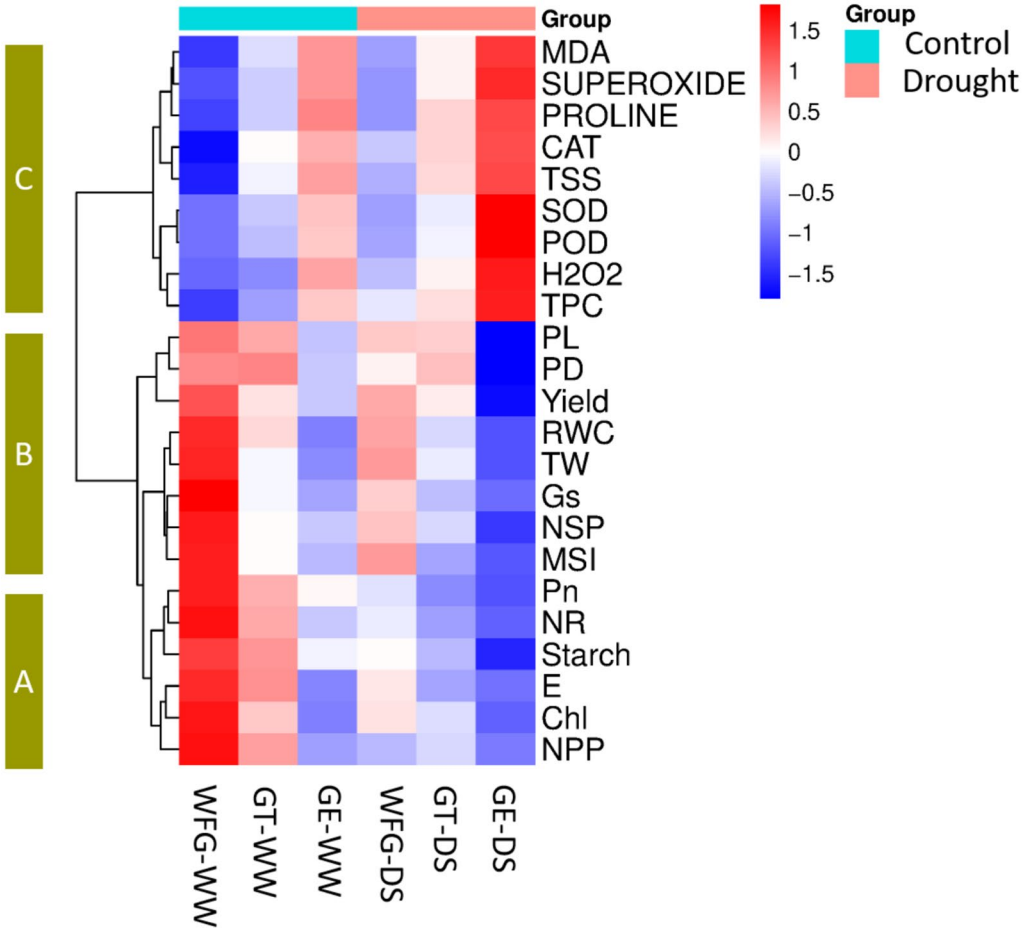


Fig. 10. A heatmap with clustering visually represents the interactions among all investigated components and treatments, displaying the scaled average values of the examined greengram traits. *WFG* weed-free greengram; *GT* greengram+ *T. portulacastrum*; *GE* greengram+ *E. colona; WW* well water condition; *DS* drought stress. *Pn* rate of photosynthesis; *Gs* stomatal conductance; *E* rate of transpiration; *Chl* total chlorophyll content; *RWC* relative water content, *MSI* membrane stability index; *MDA* malondialdehyde content; *Superoxide* superoxide ion levels, H_2O_2 hydrogen peroxide levels, *TPC* total phenolic content; and *TSS* total soluble sugar levels; *Yield* grain yield.

uptake and transpiration[66,67]. The marked reduction in RWC under DS in this study is consistent with earlier reports in greengram and other crops[68]. A concurrent decline in MSI suggests enhanced cellular membrane damage, compromising plant resilience under combined drought and weed stress.

Photosynthetic pigments, particularly chlorophyll, also declined significantly under DS conditions, especially in the presence of weed *E. colona*. Drought-induced chlorophyll degradation is known to result from impaired synthesis, increased chlorophyllase activity, and oxidative damage to thylakoid membranes[69–72]. These impairments reduce light absorption efficiency, hinder photoprotection, and ultimately limit photosynthate production, thereby restricting plant growth and productivity[73,74]. The more pronounced decline in RWC and MSI under *E. colona* interference suggests that this weed exerts greater competitive pressure on greengram, likely due to its aggressive growth and higher resource utilization efficiency under stress. These findings are in agreement with similar observations in rice, where the presence of *Alternanthera paronychioides* and *E. colona* led to significant reductions in RWC, MSI, and chlorophyll content under drought compared to well-watered conditions[1]. DS significantly reduced chlorophyll, RWC, and MSI in greengram, with more severe declines under *E. colona* interference. This suggests that *E. colona* intensifies drought effects through stronger resource competition, highlighting the need for integrated management strategies under water-limited conditions.

Photosynthesis (PS) is a critical physiological process directly contributing to biomass accumulation and crop yield. Under water-deficient conditions, reduced RWC triggers stomatal closure as a water-conserving strategy, which consequently limits CO_2 influx, lowers transpiration rates, and restricts gaseous exchange and electron transport. These changes impair the functioning of both Photosystem I and II (PSI and PSII), suppress rubisco activity, and disrupt ATP synthesis through inhibition of the electron transport chain[75,76]. In the present study, such negative impacts of drought were further intensified by weed interference, as reflected in significant reductions in gas exchange indices i.e., net photosynthetic rate (PN), stomatal conductance (gs), and transpiration rate (E) under both WW and DS environments. Notably, the decline in photosynthetic efficiency

under drought is not solely a consequence of stomatal limitations but also stems from metabolic dysfunctions within the photosynthetic apparatus[77]. Similar patterns were reported in rice by Sreekanth et al.[1], where weed competition under both WW and DS conditions led to pronounced reductions in photosynthetic traits. DS induced reductions in photosynthesis were further aggravated by weed interference, reflecting combined stomatal and metabolic limitations. Effective management of both stresses is crucial to maintain photosynthetic efficiency and crop productivity.

Drought stress remarkably compromises cellular integrity by destabilizing membranes and inducing cell wall injury[78]. Membranes serve as the primary targets of environmental stress, where the accumulation of ROS initiates lipid peroxidation, leading to membrane disintegration, cellular leakage, and ultimately cell death[79,79,80]. The onset of drought triggers a cascade of oxidative stress responses, including elevated ROS generation, lipid peroxidation, and membrane damage, which collectively disturb the plant's physiological and biochemical balance[14,15]. If unmitigated, this oxidative stress can severely impair plant functionality and survival[81]. In the present study, oxidative damage in greengram was evident under both WW and DS conditions when subjected to weed interference. The extent of ROS accumulation, inferred through elevated levels of MDA, a well-established indicator of lipid peroxidation and oxidative injury[11,82] was significantly greater in greengram exposed to *E. colona* compared to *T. portulacastrum*. This specifies that *E. colona* imposed an exaggerated level of oxidative stress, likely due to its aggressive resource competition and interference potential. DS, compounded by weed interference, significantly intensified oxidative damage in greengram, as indicated by elevated MDA levels. The greater oxidative stress under *E. colona* competition highlights its stronger interference potential and underscores the need for targeted weed management to mitigate cellular injury and maintain crop resilience under drought conditions.

Plants employ a complex antioxidant machinery to alleviate oxidative stress caused by drought and other environmental challenges. This system comprises both enzymatic (e.g., SOD, CAT, POD) and non-enzymatic components (e.g., proline, total phenolic content), which work collectively to scavenge ROS and protect cellular structures[83]. However, weed interference has been shown to modulate the activity and expression of antioxidant enzymes, thereby influencing the plant's ability to cope with stress[84–86]. For instance, a reduction in antioxidant activity was reported in soybean under competition with *Cyperus rotundus*[87], suggesting a suppression of ROS detoxification pathways. In contrast, the present study observed a significant enhancement in both enzymatic and non-enzymatic antioxidant defenses in greengram subjected to *T. portulacastrum* and *E. colona* interference under both WW and DS environments. The activities of SOD, CAT, and POD, as well as the accumulation of proline and total phenolic compounds (TPC), increased markedly in response to weed competition, particularly under *E. colona* interference. This elevated antioxidant response may represent a stress adaptation mechanism to counteract the elevated ROS levels generated under combined drought and weed-induced stress. Similar responses were reported by Alderfasi et al.[88], where DS induced significant increases in SOD, CAT, APX, and PPO activities.

Furthermore, nitrate reductase activity was notably reduced in greengram under *E. colona* competition, aligning with previous findings that drought stress suppresses nitrogen assimilation enzymes, as observed in *Triticum aestivum*[89] and *Brassica juncea*[90]. The reduced activity of nitrate reductase under *E. colona* competition may be attributed to a greater depletion of nitrogen resources or interference with nitrogen uptake and assimilation. These results are steady with observations by Sreekanth et al.[1], who reported enhanced antioxidant activity and lower oxidative damage markers in weed-free rice and under mild competition from *A. paronychioides*, whereas competition with *E. colona* resulted in greater oxidative stress. Collectively, the present study highlights that weed competition, particularly from *E. colona*, exacerbates oxidative damage and triggers stronger antioxidant responses in greengram, underscoring the importance of timely and targeted weed management strategies to alleviate oxidative stress and improve crop resilience under drought-prone conditions.

Soluble sugars play a dynamic role in optimizing physiological developments, including photosynthesis and respiration, and function as key osmoprotectants under stress conditions[91]. Their accumulation is a well-established adaptive response to drought, contributing to membrane stabilization and protection against oxidative stress[76]. The present study corroborates these findings by demonstrating a notable upsurge in TSS in greengram under weed interference across both WW and DS conditions. This accumulation likely serves to maintain redox balance and osmotic adjustment, aiding in cellular protection and homeostasis under stress[92,93]. Similar increases in TSS under water deficit have been reported in various legumes such as faba bean[94], greengram[48], and urdbean[95], suggesting that soluble sugar buildup is a conserved stress mitigation mechanism among leguminous crops. Additionally, this sugar accumulation is thought to scavenge ROS, thereby mitigating membrane lipid peroxidation and maintaining physiological stability[96]. Consistent with these observations, elevated TSS levels were also reported in rice under weed competition, particularly in the presence of *E. colona* and *A. paronychioides*, under both moisture regimes[1].

Interestingly, in contrast to the increased TSS levels, starch content was remarkably lowered in greengram in the presence of both *T. portulacastrum* and *E. colona*, with a more substantial drop observed under *E. colona* interference. This starch depletion may reflect the diversion of photosynthates towards the synthesis of osmoprotectants and stress-response metabolites, rather than storage compounds, under competitive and drought stress environments. These findings suggest a metabolic trade-off where plants prioritize immediate survival over energy storage, further emphasizing the complex physiological adjustments required to cope with simultaneous abiotic and biotic stresses.

The extent of yield reduction observed in greengram under weed interference highlights the multifaceted stress imposed by both biotic (weed competition) and abiotic (drought) factors. The intensified oxidative stress under *E. colona* competition, coupled with compromised physiological integrity, suggests that the weed exerts a more aggressive competitive pressure compared to *T. portulacastrum*. This is likely due to its rapid growth habit, high biomass accumulation, and efficient resource capture, which severely limit the availability of light,

water, and nutrients to the crop, especially under water-limited conditions. Furthermore, the observed reduction in key physiological parameters such as RWC, MSI, chlorophyll content, and photosynthetic rate directly contributes to impaired assimilate production and translocation, leading to diminished reproductive output. Such physiological impairment under weed stress has been reported in several studies, where weed-induced suppression of photosynthesis and water status translated into reduced pod formation, seed filling, and grain yield[97,98].

The more severe yield decline under *E. colona* interference also suggests its potential allelopathic effects or stronger root competition, which can further exacerbate stress responses under drought conditions. This is in line with earlier findings in rice and other cereals, where aggressive weeds like *Echinochloa* spp. not only reduced yield but also altered plant metabolism and stress signaling pathways[1]. Moreover, the interactive effects of drought and weed stress appear to have a synergistic impact on greengram productivity. Under DS conditions, the limited soil moisture amplifies the competitive advantage of weeds by increasing the resource asymmetry, thus compounding the physiological strain on the crop. This dual-stress scenario severely hampers reproductive development, particularly pod setting and seed filling, stages that are highly sensitive to water deficits and oxidative stress.

Overall, these findings reinforce the need for integrated weed management strategies, especially under climate-induced water stress scenarios. Implement early herbicide application (15–20 DAS) to target *E. colona* peak growth, use drought-tolerant greengram cultivars to reduce its competitiveness, optimize planting density for better canopy closure, and incorporate mechanical weeding at 20–30 DAS where herbicide use is constrained. The development and implementation of timely and species-specific weed control measures are critical not only to reduce direct competition but also to diminish the compounding effects of abiotic stresses on crop performance. By mitigating the combined impact of weeds and drought, such strategies can enhance physiological resilience and safeguard yield stability in greengram and other pulse crops.

The results of this study align with the observations of Sreekanth et al.[1], who stated that *E. colona* exerted a more pronounced suppressive effect on rice compared to *T. portulacastrum*. Our results further emphasize that the adverse impact of *E. colona* on greengram becomes more severe under DS conditions than under WW conditions. This intensified competitive effect under DS can be attributed to the significant enhancement in root traits, particularly root length and root volume, in *E. colona* as compared to *T. portulacastrum*. The ability of *E. colona* to expand its root system under moisture-limited environments likely provides a competitive advantage by enabling more efficient water acquisition. Such adaptive traits are hallmarks of phenotypic plasticity, a common characteristic of many weed species that allows them to modify their morphology and physiology in response to environmental stresses[99]. These adaptations enhance weed survival and fitness under stress and contribute to their ability to outcompete crops for limited resources. The superior drought adaptability of *E. colona* observed in this study underscores the critical need for developing weed-specific management approaches, particularly under drought-prone conditions, to reduce its competitive impact and safeguard greengram productivity.

The inherent differences between *Echinochloa colona* (a monocot, C_4 photosynthetic pathway) and *Trianthema portulacastrum* (a dicot, C_4 photosynthetic pathway) may influence their competitive abilities. We note that monocots like *E. colona* generally possess narrow, erect leaves, higher tillering capacity, and rapid vertical growth, enabling efficient light capture and early canopy closure, which can suppress competing plants. In contrast, dicots such as *T. portulacastrum* often have broader leaves and a prostrate growth habit, allowing them to exploit horizontal space and intercept diffuse light near the soil surface. Additionally, differences in root architecture fibrous roots in monocots versus a taproot system in dicots affect soil resource acquisition strategies. These morphological and physiological traits may partly explain the observed variation in competitive ability under our experimental conditions.

Conclusion

The present study demonstrates that the interactive effects of weed interference and water availability significantly influence the physiological, biochemical, and yield-related responses of greengram. The findings clearly indicate that *Echinochloa colona* exerts a more detrimental impact on greengram growth and productivity than *Trianthema portulacastrum*, under both well-watered and drought stress conditions. Weed competition, particularly by *E. colona*, amplified drought-induced oxidative stress, as evidenced by elevated ROS accumulation and lipid peroxidation, while simultaneously disrupting key physiological traits such as gas exchange parameters, relative water content, membrane stability, and chlorophyll content. These alterations collectively contributed to substantial yield reductions, especially under drought stress. Importantly, the study underscores the compounded impact of biotic and abiotic stressors on crop performance and highlights the necessity of integrated weed and water management strategies for improving crop resilience. The enhanced understanding of physiological and biochemical responses under dual stress conditions provides a valuable framework for identifying critical stages of vulnerability and resilience in greengram. Looking ahead, future research should focus on identifying tolerant growth stages and implementing mitigation strategies tailored to varying water regimes. Such insights will be crucial in the context of climate change adaptation (SDG-13) and ensuring food security through sustainable crop production (SDG-2), particularly in arid and semi-arid regions increasingly exposed to erratic rainfall patterns and resource constraints.

Data availability

All data generated or analysed during this study are included in this manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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