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Agronomic Evaluation of Chickpea Genotypes under Induced Water Stress at Different Growth Stages

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Abstract

Drought stress impacts chickpea (*Cicer arietinum*) performance across all growth stages, making it crucial to identify its specific effects during critical vegetative and reproductive phases. This study determined drought response of selected chickpea genotypes to induced water stress at seedling (ST) and pre-flowering (FL) stages in a controlled glasshouse environment at Egerton University. A Randomized Complete Block Design was applied. Water stress at the seedling (ST) and flowering (FL) stages significantly impacted most vegetative and yield traits among the 23 chickpea genotypes which included local and international accessions. Drought stress during the ST stage caused a significant ($p < 0.05$) percentage decrease in vegetative traits (leaf and branch number, plant height, diameter and biomass) compared to stress at the FL stage. However, yield was more significantly reduced by FL (79.97%) compared to during ST stages (76.41%). Water stress applied before flowering is a more effective method to screening for drought resistance in chickpeas, hence the recommended approach for selecting drought tolerant genotypes. Given the lower effect of induced water stress on Chania 2, ICCV 4958, and Flipper, further field evaluation of these genotypes is recommended in areas with unpredictable rainfall patterns to confirm their stability, adaptability, and performance under field drought.

Key words: Water stress screening, Drought response, Yield, Chickpea genotypes, Seedling stage, pre-flowering stage, Vegetative traits

1.0 Introduction

Chickpea *(Cicer arietinum* L.) is one of the most important pulse crop cultivated worldwide. India leads globally in chickpea yield (9.9 million tons) (FAOSTAT, 2022). In sub-Saharan Africa, Ethiopia is the primary chickpea producer (Snapp *et al*., 2019). In Kenya chickpea is gaining popularity. It is currently occupying approximately 55,000 hectares with an annual production of 15,000-18,500 tons (Cheruiyot *et al*., 2002; Kimurto *et al*., 2014). Chickpea is valued for its protein and energy source for vegetarian populations facing health issues like diabetes, cancer, or heart disease (Chauhan *et al*., 2024). It is also a drought tolerant alternative to drought and heat susceptible beans in arid and semi-arid (ASAL) regions (Kimurto *et al*., 2013). In Kenya chickpea production does not meet the

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significant local demand, evidenced by annual imports of around 100,000 tons mainly from Tanzania, Sudan and Ethiopia (Kimurto *et al*., 2013; 2014). Despite its nutritional functionality, current chickpea yield is significantly low, typically ranging from 1.2 to 1.5 ton/ha (Rao *et al*., 2024; Wafula *et al*., 2021). This is mainly due to abiotic stresses, especially water stress (Muruiki *et al*., 2018), and lack of well adapted genotypes (Lekgothoane, 2022).

Global climate change has intensified erratic rainfall in Kenya's ASAL's (Onyutha *et al*., 2022), exposing chickpea to two main drought types: early drought during seedling stages and terminal drought impacting flowering and seed filling (Wilhite & Glantz, 1985; Fahad *et al*., 2017; Sachdeva *et al*., 2022). Both early and terminal drought significantly reduce productivity (up to 50%) (Sachdeva *et al*., 2020). Early water stress is known to severely hinder the development of chickpea plants biomass and vegetative parts (Leport *et al*., 2006; Shah *et al*., 2020). On the other hand, terminal stress mostly reduces yield components by increasing flower and pod abortion and decreasing seed size (Fang *et al*., 2011). While these general effects of early and late drought on chickpea growth and yield are widely explored, the exact quantitative impacts on most traits remain unclear. Research by Pappula-Reddy *et al*. (2024) pointed at a need for more efficient and precise quantitative data on drought effects on chickpea to inform breeding efforts. Thus, there is a need for detailed studies that quantify the impact of drought at the seedling and pre-flowering stages. This is critical for selecting resilient varieties suitable for drought prone areas as suggested by Leport *et al*. (2006) and Sachdeva *et al*. (2022).

Many field studies have been done to determine drought effects on chickpea (Turner *et al*., 2005; Hussain *et al*., 2015; Pang *et al*., 2016; Aouatif Benali *et al*., 2023). However, research by Yaqoob *et al*. (2012) highlighted the necessity of conducting drought screening under controlled conditions. This is to ensure precise stress imposition at critical plant growth stages to avoid the risk of rainfall interruption during the imposition of moisture stress at the specific plant growth stage. This study, therefore, was conducted in a glasshouse to specifically investigate the effects of induced water stress at seedling and pre-flowering stages on the vegetative and yield traits of selected chickpea genotypes, ultimately facilitating the selection of drought tolerant genotypes.

2.0 Material and methods

2.1 Site description

Experiment were conducted in a glasshouse at Egerton University, field 7 research station between February and August 2019. Egerton university site lies approximately at altitude of 2160 meters above sea level, and coordinates of 0°22'11.0"S, 35°55'58.0"E with mean annual rainfall of 886mm bimodially distributed. The soils are vitric mollic endosols (Jaetzold and Schimadt, 1983). The glasshouse mean temperature range was 18 °C and 35 °C during night and day respectively.

2.2 Genotypes evaluated

The study evaluated chickpea genotypes including 12 breeding lines and 3 commercial lines from the University of Sydney, Australia. Additionally, 4 released commercial checks from Egerton University in Kenya and 3 breeding lines and 1 drought tolerant check (with high root length) from

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International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Table 1). These locally grown genotypes were chosen for study due to their local availability, adaptability and limited information regarding their drought tolerance and yield potential (Kimurto *et al*., 2013; Muruiki *et al*., 2018; Kiplagat *et al.,* 2018).

Table1: Chickpea genotypes screened for drought response at ST and FL stages

Name	Type	Source	Status	Drought tolerance description
Amethyst	Desi	Australia	Breeding lines	Susceptible (Rao *et al*., 2023)
Doolin	Desi	Australia	Breeding lines	Not known
Flip94 079C	Desi	Australia	Breeding lines	Susceptible (Kiplagat *et al*., 2018)
Flipper	Kabuli	Australia	Breeding lines	Moderately tolerant (Rao *et al*., 2023)
Howard	Desi	Australia	Breeding lines	Susceptible (Rao *et al*., 2023)
Howzat	Desi	Australia	Breeding lines	Moderately Tolerant (Rao *et al*., 2023)
Jimbour	Desi	Australia	Breeding lines	Moderately Tolerant (Kiplagat *et al*., 2018)
KYA BRA	Desi	Australia	Breeding lines	Moderately Tolerant (Rao *et al*., 2023)
Lyle	Desi	Australia	Breeding lines	Not known
Lyons	Desi	Australia	Breeding lines	Not known
PBA Hatrick	Desi	Australia	Commercial lines	Moderately susceptible (Rao *et al*., 2023)
PBA Slasher	Desi	Australia	Commercial lines	Moderately susceptible (Rao *et al*., 2023)
PBA Stricker	Desi	Australia	Commercial lines	Moderately Susceptible (Rao *et al*., 2023)
Sonali	Desi	Australia	Breeding lines	Susceptible (Kiplagat, 2018)
Thomas	Desi	Australia	Breeding lines	Susceptible (Rao *et al*., 2023)
Yorker	Desi	Australia	Breeding lines	Susceptible (Kiplagat *et al*., 2018)
Chania 1	Desi	Egerton	Local commercia check	Drought tolerant (Muriuki *et al*., 2018)
Chania 2	Desi	Egerton	Local commercia check	Drought/heat check (Muriuki *et al*., 2018)
Chania 3	Desi	Egerton	Local commercia check	Drought Tolerant (Muriuki *et al*., 2018)
Saina K1	Kabuli	Egerton	Local commercia check	Susceptible (Kimurto *et al*., (2013)
ICCV 10	Desi	ICRISAT	Breeding lines	Moderately tolerant (Gaur *et al*., 2014)
ICCV 4958	Desi	ICRISAT	Breeding lines	Drought check (Muriuki *et al*., 2018)
ICCV 98801	Desi	ICRISAT	Breeding lines	Susceptible (Kiplagat *et al*., 2018)

2.3 Experimental design and treatments

The experiment was laid out in a Randomized Complete Block Design with 3 replications each. The seeds of each study genotype were planted in 2-liter containers with a diameter measuring 30 cm and 30 cm depth. The containers were filled with a soil mixture of virgin forest soil mixed with sand in a ratio of 3:1 respectively. Before planting the seed, the pots were completely watered to field capacity (FC) applying one liter of water each. Four seeds were planted in each container but after germination they were thinned to three plants. The study genotypes were maintained by uprooting all weeds and pest control was done using DuduthrinR (Lambda-cyhalothrin 17.5 g/lt). The water treatments consisted of 3 moisture stresses; seedling stage (ST), pre-flowering stage water stress

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(FL) and control (CT). Half a liter of water was applied every two days to simulate a 250-450 mm water requirement, typical of arid and semi-arid regions as described by De Pauw, 2010, except during periods of induced water stress. The 3 water stress treatments were applied in the study were: (i) Water stress at seedling stage (ST) was imposed on the study chickpea genotypes 10 days after germination where watering was stopped completely for a period of 30 days, (ii) water stress at pre-flowering stage stress (FL) was induced when flower buds appeared for a period of 30 days (Yaqoob *et al.,* 2011) and (iii) a control treatment (CT) where water was amply applied (half a liter after two days) to all study genotypes until they attained maturity (when pods started to dry). Before induction and after the termination of the water stress in the ST and FL stages, all the study genotypes were regularly watered just like before stress by applying half a liter in two days until they attained physiological maturity. Soil moisture content was monitored using the FAO (2023) manual. The genotypes responses to water stress are shown in Figure 1 at ST stage in plate a and b and FL in plate c and d.

Figure 1: Photos of the glass house experiment showing layout and appearance of ST and FL stages experiments before and after water stress.

Plate a: ST experiment just before water stress; Plate b: FL experiment just before water stress; Plate c: ST experiment after stress at commencement of re-watering; Plate d: FL experiment after stress

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at commencement of re- watering

2.4 Data collection and procedure

Data were collected on vegetative growth, yield, yield related traits and plant wilting and recovery scores.

2.4.1 Vegetative growth

Vegetative growth traits data were recorded from two test plants from each pot (genotype) in all replications when the plant pods were 50% mature. The vegetative data collected was on; (i) Plant height which was measured using a meter rule from the ground surface to the tip of the plant in centimeters (cm). (ii) Plant diameter; in this experiment was the extent to which plant branches are spread from one extreme to another and was measured using a meter rule in centimeters (cm). (iii) Number of leaves per plant: all leaves that arise from the primary (main) stem were counted. (iv) Number of branches: primary branches arising from the main stem were all counted per plant. (v) Biomass per plot (g): The weight of above ground part of all the plants in a plot was taken after completely drying it in the oven set at 80^0C for 48 hours.

2.4.2 Yield and yield related traits

Data was collected on: (i) Number of pods/plot: which was obtained by counting the number of individual pods per plot. (ii) Grain yield (g) per plot: the weight of all the seeds obtained from the test plants in a plot were weighed in grams after drying them in the sun for 3 days.

2.5 Data analysis

To determine the percentage reduction in each measured trait caused by water stress at the seedling (ST) and pre-flowering (FL) stages, we used the following formula:

$$\text{Percent Reduction} = \frac{\text{Trait Value}_{CT} - \text{Trait Value}_{Stress}}{\text{Trait Value}_{CT}} \text{ x } 100$$

Where:

Trait Value$_{CT}$ represents the traits value in the control treatment (CT).

Trait Value$_{Stress}$ represents the traits value under water stress (either ST or FL).

The results are expressed as Mean±SEM. The resulting percentage reduction for each yield traits and vegetative growth response was compared between different genotypes using ANOVA, and the means were separated using the Least Significant Difference (LSD) test at $p < 0.05$. Unpaired student t test was used to compare the performance of each trait between the seedling and flowering stage. Data analysis was done using R (4.3.3.4 version) software.

3.0 Results

3.1 Vegetative growth response of the genotypes to induced water stress at ST and FL stage

Overall, water stress reduced vegetative traits more significantly during the seedling stage (ST) by 46.29% (Table 2) reduction compared to a 22.18% reduction at the pre-flowering stage (FL) (Table

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3). Effect of water stress at ST was most deteriorative in ICCV 98801 and Jimbour with reduction of 68.57% and 65.38%, respectively (Table 2). However, the drought and heat check Chania 2 recorded the lowest reduction (24.87%) in the number of leaves (Table 2). During FL, the genotypes that displayed a relatively low reduction in their number of leaves included Jimbour (14.58%) and Thomas (15.11%) (Table 3). On the other hand, PBA Slasher (34.3%) and Flipper (33.92%) genotypes were more affected in terms of leaf number in the pre-flowering stage (Table3).

Chickpea genotypes exhibited reduced branching under both ST and FL stages during the induced water stress. Overall, water stress at ST was more severe resulting to 41.46% (Table 2) reduction compared to 22.18% decline in number of branches during FL (Table 3). The branch number reduction due to FL ranged from 11.85% in Doolin to 39.97% in Kyabra (Table 3), whereas during ST it ranged from 12.85% in Chania 2 to 63.33% in Jimbour (Table 2).

Table 2: Percentage reduction in chickpea genotypes traits due to water stress at Seedling stage (ST) stage

Genotypes	NOL	NOB	HT	DIAM	BM
Amethyst	45.54±4.27^{b-g}	41.77±2.12^{d-i}	58.79±1.27^{d-h}	81.47±0.64^{d-g}	68.75±2.10^{b-c}
Chania 1	59.36±2.55^{a-d}	53.65±1.40^{a-d}	67.44±1.12^{a-c}	87.99±0.30^{ab}	57.57±3.20^{e-g}
Chania 2	24.87±5.15^{h}	12.85±5.04^{l}	59.26±1.32^{d-h}	82.98±0.49^{c-e}	66.62±1.74^{b-e}
Chania 3	46.29±2.27^{b-g}	46.93±1.80^{c-h}	69.80±0.71^{a}	83.29±0.47^{c-e}	34.20±3.53^{h}
Doolin	46.44±3.38^{b-g}	40.47±3.75^{d-j}	55.04±1.58^{hi}	82.67±0.60^{c-g}	58.5±2.10^{d-f}
Flip 94 079C	49.44±4.23^{a-f}	39.17±2.01^{e-j}	46.02±1.45^{j}	84.25±0.41^{b-d}	67.48±2.30^{b-e}
Flipper	40.35±3.21^{d-h}	37.98±1.95^{f-j}	57.22±0.92^{f-i}	76.31±1.11^{h}	72.36±1.66^{a-c}
Howard	32.53±1.92^{f-h}	47.06±2.72^{c-h}	68.73±1.51^{ab}	75.77±0.83^{h}	69.20±2.11^{b-d}
Howzat	35.18±4.17^{e-h}	36.35±2.79^{g-k}	57.74±0.44^{e-h}	82.75±0.47^{c-f}	72.60±1.62^{a-c}
ICCV 10	50.46±2.41^{a-f}	36.72±1.86^{g-j}	62.32±1.00^{c-g}	85.78±0.38^{a-c}	61.92±2.37^{c-f}
ICCV 4859	34.29±2.86^{e-h}	26.80±1.83^{jk}	56.63±1.47^{g-i}	83.94±0.72^{b-d}	70.07±2.06^{a-b}
ICCV 98801	68.57±2.52^{a}	34.57±3.98^{g-k}	55.17±1.63^{hi}	86.43±0.45^{a-c}	68.51±1.26^{b-e}
Jimbour	67.38±4.04^{a}	63.33±1.88^{a}	59.96±1.12^{d-h}	46.38±1.36^{j}	71.55±1.24^{a-c}
Kya bra	44.29±4.31^{c-h}	50.74±1.69^{a-f}	64.22±0.77^{a-d}	86.48±0.54^{a-c}	80.60±0.76^{a}
Lyle	55.43±4.04^{a-d}	41.90±2.75^{d-i}	64.36±0.93^{a-d}	78.54±1.32^{gh}	67.74±1.93^{b-e}
Lyons	50.37±4.11^{a-f}	33.91±1.38^{h-k}	63.14±0.79^{b-f}	88.88±0.37^{a}	70.20±2.20^{a-c}
PBA Hatrick	28.31±1.67^{gh}	48.48±3.24^{b-g}	63.52±1.12^{b-e}	79.69±0.30^{e-h}	70.62±1.45^{a-c}
PBA Slasher	41.49±3.48^{d-h}	59.02±2.53^{a-c}	51.22±1.79^{ij}	84.35±0.38^{b-d}	67.70±1.34^{b-e}
PBA Stricker	62.13±3.42^{a-c}	32.58±2.50^{i-k}	61.87±1.47^{c-g}	52.06±1.83^{i}	68.41±1.99^{b-e}
Saina K1	29.36±2.04^{gh}	22.45±1.37^{kl}	22.86±1.34^{k}	21.30±1.14^{k}	73.20±2.26^{ab}
Sonali	34.48±3.17^{e-h}	23.42±2.61^{ab}	33.98±0.95^{hi}	36.10±0.29^{a-c}	47.22±2.12^{g}
Thomas	52.99±5.44^{a-e}	33.59±3.54^{h-k}	64.02±0.72^{a-d}	78.59±0.88^{f-h}	54.62±3.21^{fg}
Yorker	65.08±7.75^{ab}	51.91±2.74^{a-e}	63.08±0.69^{b-f}	83.29±0.61^{c-e}	73.74±1.18^{ab}
Mean	46.29	41.46	58.54	77.36	65.8

Values are expressed as Mean±SEM. Means that do not share a letter down the column for each water treatment are significantly different, ST: water stress at seedling, FL: water stress at pre-flowering stage, NOL: number of leaves, NOB: number of branches, HT: plant height (cm), DIAM: plant diameter (cm), PODS: number of pods per plot, YLD: plant yield per plot (g), BM: biomass per plot (g). The bold values represent the top three most affected genotypes.

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The effect of the water stress led to an overall 58.54% reduction in plant height during ST (Table 2) and a 16.51% in FL (Table 3). Plant heights were reduced by induced water stress at ST between 22.86% (Saina K1) and 69.8% (Chania 3) (Table 2). Water stress at FL was more severe on the height of Howard (40.6%) and Thomas (35.42%) genotypes (Table 3). The genotypes with least height reduction in FL were Amethyst (4.8%) and PBA Slasher (5.36%) (Table 3). The plant diameter of Lyon and Chania 1 chickpea genotypes was the most significantly reduced by water stress in ST stage, with reduction of 88.88% and 87.99%, respectively (Table 2). On the other hand, Saina K1 (21.3%) and Sonali (36.1%) were least affected by water stress during ST treatment (Table 2). Under FL conditions, Lyon and Howzat exhibited the most reduction in plant diameter with decreases of 58.62% and 56.38% (Table 3), respectively. In the contrast, Lyle and Flipper were the least affected by FL treatment, showing reductions of only 19.23% and 26.14%, respectively (Table 3).

The biomass of the genotypes decreased more under ST conditions compared to FL, with overall reductions of 65.8% (Table 2) and 55.01% (Table 3), respectively. In ST treatment, Kyabra showed the highest biomass decrease due to induced water stress (80.6%) followed by Yorker (73.74%) (Table 2). Chania 3 and Sonali were least affected at reductions of 34.2% and 47.22%, respectively in ST (Table 2). Additionally, water stress during FL stage caused highest biomass reduction in Howard (75.05%) and Thomas (74.94%) (Table 3). On the other hand, Howzat and Amethyst genotypes recorded the least biomass reduction by 31.15% and 34.39% (Table 2), respectively.

Table 3: Percentage reduction in chickpea genotypes traits due to water stress at pre-flowering stage (FL) stage

Genotypes	NOL	NOB	HT	DIAM	BM
Amethyst	25.22±2.05a-d	19.68±1.12bc	4.8±0.36h	47.19±3.28b-d	34.39±1.49hi
Chania 1	26.28±2.48^{a-d}	22.76±1.58^{bc}	9.30±1.31^{gh}	52.47±1.95^{a-d}	51.76±2.89^{c-g}
Chania 2	24.15±1.41^{a-e}	23.66±2.23^{bc}	10.22±1.03^{f-h}	52.38±3.21^{a-d}	52.15±2.03^{c-g}
Chania 3	24.30±2.41^{a-e}	25.29±1.95^{bc}	6.09±0.88^{gh}	35.31±4.02^{f-h}	39.86±2.11^{g-i}
Doolin	15.56±2.06^{de}	11.85±1.17^{c}	7.43±1.19^{gh}	47.14±2.24^{b-e}	45.71±2.62^{e-i}
Flip 94 079C	20.88±2.66^{b-e}	25.96±3.40^{b}	13.41±1.77^{e-f}	50.69±1.62^{a-d}	43.47±1.99^{f-i}
Flipper	33.92±2.23^{a}	17.39±0.79^{bc}	8.23±1.45^{gh}	26.14±2.15^{hi}	47.53±3.44^{d-h}
Howard	18.96±2.24^{b-e}	21.46±3.09^{bc}	40.61±1.89^{a}	36.15±1.96^{e-h}	75.05±2.83^{a}
Howzat	20.84±1.47^{b-e}	27.94±3.24^{ab}	3.48±0.79^{h}	56.38±1.28^{ab}	31.15±2.89^{i}
ICCV 10	21.25±1.48^{b-e}	24.16±4.19^{bc}	21.70±1.60^{d}	54.27±1.8^{a-c}	63.73±3.37^{a-c}
ICCV 4859	19.62±2.46^{b-e}	22.4±2.86^{bc}	7.33±1.61g^{h}	44.83±0.77^{c-g}	45.11±4.33^{e-i}
ICCV 98801	16.03±2.12^{c-e}	28.75±3.0^{ab}	18.27±2.68^{de}	34.39±0.99^{gh}	59.68±2.83^{a-e}
Jimbour	14.58±1.39^{de}	18.06±1.65^{bc}	21.81±0.99^{d}	48.63±3.14^{a-d}	65.73±3.64^{a-c}
Kya bra	17.62±1.66^{c-e}	28.85±5.05^{ab}	13.75±0.85^{e-g}	50.11±1.91^{a-d}	56.15±3.64^{b-f}
Lyle	20.88±2.66^{b-e}	25.96±3.40^{b}	13.41±1.77^{e-f}	19.23±1.26^{i}	52.39±2.8^{c-g}
Lyons	17.41±2.23^{c-e}	18.96±3.92^{bc}	31.85±1.31^{bc}	58.62±1.45^{a}	68.84±3.95^{ab}
PBA Hatrick	29.92±1.68^{ab}	12.14±1.21^{c}	17.24±1.19^{d-f}	42.16±0.64^{d-g}	58.76±2.32^{b-f}
PBA Slasher	34.30±3.33^{a}	17.14±1.82^{bc}	5.36±0.89^{h}	52.99±0.93^{a-d}	39.76±2.59^{g-i}
PBA Stricker	27.41±1.49^{a-c}	22.01±2.10^{bc}	18.86±1.65^{de}	52.35±2.7^{a-d}	60.87±3.34^{a-e}
Saina K1	20.64±2.02^{b-e}	39.97±3.35^{a}	33.82±2.38^{ab}	54.77±3.04^{a-c}	68.92±3.87^{ab}
Sonali	15.78±1.57^{a-d}	27.72±2.60^{ab}	19.89±1.56^{de}	48.02±0.76^{a-d}	62.57±2.1^{a-d}

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Thomas	15.11±2.20^{de}	25.41±1.91^{bc}	35.42±1.17^{ab}	46.26±1.02^{b-f}	74.94±2.58^{a}
Yorker	27.33±4.58^{a-c}	21.91±0.84^{bc}	24.28±1.74^{cd}	42.85±1.37^{d-g}	66.74±4.13^{a-c}
Mean	22.18	22.98	16.51	45. 80	55.01

Values are expressed as Mean±SEM. Means that do not share a letter down the column for each water treatment are significantly different. The bold values represent the top three most affected genotypes.

3.2 Effects of water stress effect on yield and yield related traits among chickpea genotypes

Pre-flowering water stress caused more reduction in number of pods and grain yield among all the chickpea genotypes than water stress during seedling stage (Table 4). The overall reduction due to FL was 81.03% and due to ST was 68.83% in number of pods. During ST conditions, Flipper and ICCV 4958 genotypes exhibited the least decrease in pod number, 48.9% and 50.75%, respectively (Table 4). The FL stage caused a slightly greater reduction (79.97%) than ST (76.61%) in grain yield, (Table 4). Induced water stress at ST significantly reduced grain yield in Sonali (97.85%) and Lyon (93.95%) chickpea genotypes. The least reduction was observed in Chania 2 (51.66%) and ICCV 4859 (55.86%) (Table 4). Similarly, FL severely affected PBA Stricker (96.35%) and Amethyst (93.5%), but had less impact on Flipper (52.33%) and Doolin (58.78%) (Table 4).

Table 4: Effects of water stress effect on yield and yield related traits in chickpea genotypes

Genotypes	Seedling stage (ST)		Flowering stage (ST)	
	POD	YLD	POD	YLD
Amethyst	67.06±2.02^{d-i}	83.15±0.38^{c-e}	91.85±2.06^{a}	93.5±1.62^{a}
Chania 1	57.63±1.58^{f-k}	80.15±0.94^{d-g}	88.5±1.95^{a}	90.82±2.34^{ab}
Chania 2	52.94±4.26^{jk}	51.66±2.90^{l}	67.67±3.71^{c-f}	64.48±4.19^{f-i}
Chania 3	56.60±3.22^{h-k}	76.54±1.02^{e-h}	80.06±3.4^{a-d}	72.98±4.94^{d-g}
Doolin	70.10±2.51^{c-g}	68.44±1.21^{h-j}	62.06±3.24^{ef}	58.78±2.37^{hi}
Flip 94 079C	82.42±1.99^{bc}	84.07±0.80^{c-e}	78.58±2.88^{a-d}	68.0±2.86^{e-h}
Flipper	48.90±3.62^{k}	60.43±2.68^{j-l}	53.99±4.21^{f}	53.33±2.3^{i}
Howard	57.16±2.18^{g-k}	65.75±1.77^{ij}	82.23±3.2^{a-c}	79.2±2.45^{b-e}
Howzat	75.56±3.15^{cd}	80.64±2.82^{d-g}	87.26±4.01^{a}	89.97±1.79^{ab}
ICCV 10	59.89±1.89^{e-k}	62.50±1.90^{jk}	71.01±2.39^{b-e}	65.05±1.79^{f-i}
ICCV 4859	50.75±3.36^{jk}	55.86±2.84^{kl}	65.24±1.93^{d-f}	59.8±3.25^{g-i}
ICCV 98801	70.74±1.52^{c-f}	81.28±0.40^{d-f}	88.92±2.08^{a}	91.86±1.05^{ab}
Jimbour	67.98±2.36^{d-i}	72.02±3.37^{g-i}	82.28±2.92^{a-c}	84.52±1.95^{a-d}
Kya bra	56.42±3.00^{i-k}	74.17±1.25^{f-i}	86.66±2.4^{a}	87.52±1.60^{ab}
Lyle	90.77±1.47^{ab}	90.52±0.67^{a-b}	90.68±2.78^{a}	92.51±1.36^{ab}
Lyons	97.44±0.37^{a}	93.95±0.34^{ab}	91.75±2.65^{a}	92.63±1.64^{ab}
PBA Hatrick	67.96±1.59^{d-i}	76.35±1.28^{e-i}	88.58±1.83^{a}	91.07±2.82^{ab}
PBA Slasher	62.61±2.33^{d-j}	77.39±1.19^{e-g}	79.12±3.13^{a-d}	70.12±3.05^{e-h}
PBA Stricker	72.99±1.80^{c-e}	72.73±2.16^{f-i}	92.65±1.54^{a}	96.35±1.06^{a}
Saina K1	69.09±4.81^{c-i}	78.64±0.75^{e-g}	80.16±3.77^{a-d}	74.01±4.55^{c-f}
Sonali	96.25±1.68^{a}	97.85±1.16^{a}	82.45±3.06^{a-c}	84.75±2.21^{a-d}
Thomas	69.91±1.68^{c-h}	84.9±0.60^{c-e}	87.79±1.53^{a}	90.74±1.11^{ab}
Yorker	81.82±1.90^{bc}	88.43±0.39^{b-c}	84.18±2.97^{ab}	87.29±1.57^{a-c}
Mean	68.83	76.41	81.03	79.97

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Values are expressed as Mean±SEM. Means that do not share a letter down the column for each water treatment are significantly different. The bold values represents the top three most affected genotypes.

3.3 Comparison of the effects of water stress on genotypes vegetative and yield performance between the ST and FL stages

The reduction caused by induced water stress on the analyzed traits significantly differed ($p < 0.05$ (Table 5) between ST and FL stages amongst all the genotypes with a few exceptions. The number of leaves in Chania 2 and PBA Hatrick was comparable ($p > 0.05$) between the ST and FL stages (Table 5). Further, the diameter of Jimbour and PBA Stricker did not significantly vary ($p > 0.05$) between the ST and FL stages. The biomass of Chania 2 and Lyle in response to water stress was not significantly different ($p > 0.05$) between ST and FL stage. The decrease in pod count in Lyle and Yorker was statistically similar between the ST and FL stages. Water induced stress caused comparable ($p > 0.05$) reduction of grain yield in Doolin and ICCV 10 between the ST and FL stages (Table 5).

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Table 5: Comparison of percentage reduction in vegetative and yield related traits due to water stress at seedling and at pre-flowering stage for the chickpea genotypes under induced water stress

Genotypes	NOL		NOB		HT		DIAM		POD		YLD		BM	
	ST	FL	ST	FL	ST	FL	ST	FL	ST	FL	ST	FL	ST	FL
Amethyst	45.54±4.27^{A}	25.22±2.05^{B}	41.77±2.12^{A}	19.68±1.12^{B}	58.79±1.27^{A}	4.8±0.36^{B}	81.47±0.64^{A}	47.19±3.28^{B}	67.06±2.02^{A}	91.85±2.06^{B}	83.15±0.38^{A}	91.07±2.82^{B}	68.75±2.1^{A}	47.53±3.44^{B}
Chania 3	46.29±2.27^{A}	24.3±2.41^{B}	46.93±1.8^{A}	25.29±1.95^{B}	69.8±0.71^{A}	6.09±0.88^{B}	83.29±0.47^{A}	35.31±4.02^{B}	56.6±3.22^{A}	80.06±3.4^{B}	76.54±1.02^{A}	79.2±2.45^{B}	34.2±3.53^{A}	75.05±2.83^{B}
Chania 1	59.36±2.55^{A}	26.28±2.48^{B}	53.65±1.4^{A}	22.76±1.58^{B}	67.44±1.12^{A}	9.3±1.31^{B}	87.99±0.3^{A}	52.47±1.95^{B}	57.63±1.58^{A}	88.5±1.95^{B}	80.15±0.94^{A}	59.8±3.25^{B}	57.57±3.2^{A}	68.84±3.95^{B}
Chania 2	24.87±5.15^{A}	24.15±1.41^{A}	12.85±5.04^{A}	23.66±2.23^{B}	59.26±1.32^{A}	10.22±1.03^{B}	82.98±0.49^{A}	52.38±3.21^{B}	52.94±4.26^{A}	67.67±3.71^{B}	51.66±2.9^{A}	68.0±2.86^{B}	66.62±1.74^{A}	68.92±3.87^{A}
Doolin	46.44±3.38^{A}	15.56±2.06^{B}	40.47±3.75^{A}	11.85±1.17^{B}	55.04±1.58^{A}	7.43±1.19^{B}	82.67±0.6^{A}	47.14±2.24^{B}	70.1±2.51^{A}	62.06±3.24^{B}	68.44±1.21^{A}	70.12±3.05^{A}	58.5±2.1^{A}	45.11±4.33^{B}
Flip 94 079C	49.44±4.23^{A}	13.06±1.56^{B}	39.17±2.01^{A}	25.11±1.52^{B}	46.02±1.45^{A}	6.64±1.32^{B}	84.25±0.41^{A}	50.69±1.62^{B}	82.42±1.99^{A}	78.58±2.88^{B}	84.07±0.8^{A}	90.82±2.34^{B}	67.48±2.3^{A}	62.57±2.1^{B}
Flipper	40.35±3.21^{A}	33.92±2.23^{B}	37.98±1.95^{A}	17.39±0.79^{B}	57.22±0.92^{A}	8.23±1.45^{B}	76.31±1.11^{A}	26.14±2.15^{B}	48.9±3.62^{A}	53.99±4.21^{B}	60.43±2.68^{A}	74.01±4.55^{B}	72.36±1.66^{A}	39.76±2.59^{B}
Howard	32.53±1.92^{A}	18.96±2.24^{B}	47.06±2.72^{A}	21.46±3.09^{B}	68.73±1.51^{A}	40.61±1.89^{B}	75.77±0.83^{A}	36.15±1.96^{B}	57.16±2.18^{A}	82.23±3.2^{B}	65.75±1.77^{A}	53.33±2.3^{B}	69.2±2.11^{A}	31.15±2.89^{B}
Howzat	35.18±4.17^{A}	20.84±1.47^{B}	36.35±2.79^{A}	27.94±3.24^{B}	57.74±0.44^{A}	3.48±0.79^{B}	82.75±0.47^{A}	56.38±1.28^{B}	75.56±3.15^{A}	87.26±4.01^{B}	80.64±2.82^{A}	84.52±1.95^{B}	72.6±1.62^{A}	43.47±1.99^{B}
ICCV 10	50.46±2.41^{A}	21.25±1.48^{B}	36.72±1.86^{A}	24.16±4.19^{B}	62.32±1.0^{A}	21.7±1.6^{B}	85.78±0.38^{A}	54.27±1.8^{B}	59.89±1.89^{A}	71.01±2.39^{B}	62.5±1.9^{A}	64.48±4.19^{A}	61.92±2.37^{A}	56.15±3.64^{B}
ICCV 4859	34.29±2.86^{A}	19.62±2.46^{B}	26.8±1.83^{A}	22.4±2.86^{B}	56.63±1.47^{A}	7.33±1.61^{B}	83.94±0.72^{A}	44.83±0.77^{B}	50.75±3.36^{A}	65.24±1.93^{B}	55.86±2.84^{A}	58.78±2.37^{B}	70.07±2.06^{A}	34.39±1.49^{B}
ICCV 98801	68.57±2.52^{A}	16.03±2.12^{B}	34.57±3.98^{A}	28.75±3.0^{B}	55.17±1.63^{A}	18.27±2.68^{B}	86.43±0.45^{A}	34.39±0.99^{B}	70.74±1.52^{A}	88.92±2.08^{B}	81.28±0.4^{A}	89.97±1.79^{B}	68.51±1.26^{A}	39.86±2.11^{B}
Jimbour	67.38±4.04^{A}	14.58±1.39^{B}	63.33±1.88^{A}	18.06±1.65^{B}	59.96±1.12^{A}	21.81±0.99^{B}	46.38±1.36^{A}	48.63±3.14^{A}	67.98±2.36^{A}	82.28±2.92^{B}	72.02±3.37^{A}	96.35±1.06^{B}	71.55±1.24^{A}	58.76±2.32^{B}
Kya bra	44.29±4.31^{A}	17.62±1.66^{B}	50.74±1.69^{A}	28.85±5.05^{B}	64.22±0.77^{A}	13.75±0.85^{B}	86.48±0.54^{A}	50.11±1.91^{B}	56.42±3.0^{A}	86.66±2.4^{B}	74.17±1.25^{A}	93.5±1.62^{B}	80.6±0.76^{A}	59.68±2.83^{B}
Lyle	55.43±4.04^{A}	20.88±2.66^{B}	41.9±2.75^{A}	25.96±3.4^{B}	64.36±0.93^{A}	13.41±1.77^{B}	78.54±1.32^{A}	19.23±1.26^{B}	90.77±1.47^{A}	90.68±2.78^{A}	90.52±0.67^{A}	92.51±1.36^{B}	67.74±1.93^{A}	65.73±3.64^{A}
Lyons	50.37±4.11^{A}	17.41±2.23^{B}	33.91±1.38^{A}	18.96±3.92^{B}	63.14±0.79^{A}	31.85±1.31^{B}	88.88±0.37^{A}	58.62±1.45^{B}	97.44±0.37^{A}	91.75±2.65^{B}	93.95±0.34^{A}	87.29±1.57^{B}	70.2±2.2^{A}	52.39±2.8^{B}
PBA Hatrick	28.31±1.67^{A}	29.92±1.68^{A}	48.48±3.24^{A}	12.14±1.21^{B}	63.52±1.12^{A}	17.24±1.19^{B}	79.69±0.3^{A}	42.16±0.64^{B}	67.96±1.59^{A}	88.58±1.83^{B}	76.35±1.28^{A}	90.74±1.11^{B}	70.62±1.45^{A}	51.76±2.89^{B}
PBA Slasher	41.49±3.48^{A}	34.3±3.33^{B}	59.02±2.53^{A}	17.14±1.82^{B}	51.22±1.79^{A}	5.36±0.89^{B}	84.35±0.38^{A}	52.99±0.93^{B}	62.61±2.33^{A}	79.12±3.13^{B}	77.39±1.19^{A}	72.98±4.94^{B}	67.7±1.34^{A}	45.71±2.62^{B}
PBA Stricker	62.13±3.42^{A}	27.41±1.49^{B}	32.58±2.5^{A}	22.01±2.1^{B}	61.87±1.47^{A}	18.86±1.65^{B}	52.06±1.83^{A}	52.35±2.7^{A}	72.99±1.8^{A}	92.65±1.54^{B}	72.73±2.16^{A}	87.52±1.6^{B}	68.41±1.99^{A}	60.87±3.34^{B}
Saina K1	29.36±2.04^{A}	20.64±2.02^{B}	22.45±1.37^{A}	39.97±3.35^{B}	22.86±1.34^{A}	33.82±2.38^{B}	21.3±1.14^{A}	54.77±3.04^{B}	69.09±4.81^{A}	80.16±3.77^{B}	78.64±0.75^{A}	91.86±1.05^{B}	73.2±2.26^{A}	66.74±4.13^{B}
Sonali	34.48±3.17^{A}	25.78±1.57^{B}	61.42±2.61^{A}	27.72±2.6^{B}	53.98±0.95^{A}	19.89±1.56^{B}	86.1±0.29^{A}	48.02±0.76^{B}	96.25±1.68^{A}	82.45±3.06^{B}	97.85±1.16^{A}	84.75±2.21^{B}	47.22±2.12^{A}	52.15±2.03^{B}
Thomas	52.99±5.44^{A}	15.11±2.2^{B}	33.59±3.54^{A}	25.41±1.91^{B}	64.02±0.72^{A}	35.42±1.17^{B}	78.59±0.88^{A}	46.26±1.02^{B}	69.91±1.68^{A}	87.79±1.53^{B}	84.9±0.6^{A}	92.63±1.64^{B}	54.62±3.21^{A}	74.94±2.58^{B}
Yorker	65.08±7.75^{A}	27.33±4.58^{B}	51.91±2.74^{A}	21.91±0.84^{B}	63.08±0.69^{A}	24.28±1.74^{B}	83.29±0.61^{A}	42.85±1.37^{B}	81.82±1.9^{A}	84.18±2.97^{A}	88.43±0.39^{A}	65.05±1.79^{B}	73.74±1.18^{A}	63.73±3.37^{B}

Mean ± SEM with a different superscript letter row-wise within a trait are statistically different (unpaired t-test; $p \leq 0.05$).

4.0 Discussions

4.1 Vegetative growth response to induced water stress

In this study, the effect of ST and FL differed significantly across most genotypes for all the vegetative traits upon induced water stress. Studies by Shaban *et al*. (2012), Sachdeva *et al.* (2022) and Negussu *et al*. (2023) also commonly noted significant variation in morphological, physiological, phenological characters, yield and yield components among the chickpea genotypes in response to water stress. The observed variation in growth trait reduction is attributed to a complex interplay of genetic factors. These unique genetic profiles dictate the specific physiological and biochemical mechanisms that each genotype utilizes to mitigate water stress during the seedling and pre-flowering stages. This finding aligns with Kaashyap *et al*., (2017), who attributed the phenotypic responses of chickpeas under environmental stress to the plant's genetic makeup.

Across all evaluated traits, the overall percent reduction was consistently higher when drought was imposed during ST compared to FL stage. This effect of drought stress during the early growth stage on vegetative traits is attributed to the sensitivity of chickpea plants, particularly seedlings, to water stress (Sachdeva *et al*., 2022). The sensitivity arises from the under developed root system, rendering them vulnerable to water deficits (Pessarakli *et al*., 2016). Findings from previous research by Randhawa *et al*. (2014) further aligns with these results, demonstrating that water stress after pre sowing irrigation (early water stress) significantly impacts plant height, branching, biomass, leaf number, and yield components. Further, chickpeas at seedling stage undergo rapid cell division and new tissue growth, a processes that critically depend on water availability (Kaur *et al*., 2005). Water limitations can slow down cell division, thus impacting branch development, plant height and biomass (Hsiao & Xu, 2000; Talebi *et al*., 2013; Himaja *et al*., 2023). Established chickpea plants at the pre-flowering stage, with a developed root system and overall large shoot structure, demonstrate a greater capacity to adapt to water shortages (Kashiwagi *et al*., 2015).

Water stress disrupts the production or balance of plant hormones, such as auxin, which plays a role in stimulating plant height and branch growth (Bhattacharya and Bhattacharya, 2021). However, chickpea plants employ various strategies to mitigate drought impact. Some genotypes display morphological reduction as a drought tolerance response (Maqbool *et al*., 2017; Sachdeva *et al*., 2020). This is observed in the case of heat and drought stress Chania 2 where it recorded lower reduction in number of leaves and branches. Other genotypes maintain growth under drought which is a vulnerability display to drought (Tiwari et al., 2023). This vulnerability was confirmed in this study where Saina K1 chickpea genotype, had the least reduction in height and diameter during ST. In this case, larger plant size makes the plant more susceptible to water loss through transpiration due to their larger leaf surface area (Pessarakli *et al*., 2016).

4.2 Yield and yield components response to induced water stress

This study revealed that induced water stress at the FL stage caused more yield reduction across all chickpea genotypes compared to at ST. Similarly, the overall reduction in pod numbers was greater at the FL stage compared to the ST stage. These findings align with Randhawa *et al*. (2014) findings which reported that withholding irrigation during the pod setting stage had a more pronounced negative

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impact on yield traits like pod number and seed weight. Instanbuli *et al*. (2022) also observed that terminal drought reduced pod numbers by over 70% due to high rates of flower abortion and pod abscission. Further, studies confirmed that water stress at pre-flowering stage is the most damaging and critical for drought tolerance screening for chickpea genotypes. More studies have also reported the devastating effect of terminal water stress at flowering stage on chickpea genotypes (Mafakheri *et al.,* 2010; Yaqoob *et al*., 2013; Sadras and Dreccer, 2015; Ramamoorthy *et al.,* 2016; Pang *et al.,* 2017; Rani *et al.,* 2020; Karim *et al*., 2021; Khatun *et al.,* 2021). Pappula-Reddy *et al*. (2024) attributed this to the reduction in numbers of flowers and pods, a consequence of drought induced abortion, as observed by Fang *et al*. (2010). This water stress induced physiological response impairs the functionality of the pistil/style and pollen (Fang *et al*., 2010). Further, Rani *et al*. (2020) and Khatun *et al*. (2021) explained that terminal drought severely impairs chickpea production due to its effects on crucial physiological processes during the critical reproductive stages (flowering and pod filling). This results in substantial reductions in key yield components like pod number and seed size. Anwar *et al*., (2022) suggested that future water management strategies should prioritize irrigation at the flowering stage since water stress at this stage leads to severe losses in both pod number and grain yield. The least percent reduction in yield observed in Chania 2 and ICCV 4859 chickpea genotypes during ST is attributed to its confirmed drought tolerance reported by Muriuki *et al.* (2018). Most chickpea genotypes in the study had significantly different reductions in measured traits at the ST and FL stages. This indicates non-uniform responses to water stress across critical growth stages, ultimately highlighting significant genetic differences in their physiological and morphological reactions at various growth stages. This finding is supported by Igolkina et al., (2023) explanation that genetic diversification of domesticated chickpea has enabled them to react differently to environmental stresses at various stages of growth.

5.0 Conclusion and recommendations

The study concludes that water induced stress causes reduction of vegetative growth and yield at water stress at seedling stage (ST) than in pre-flowering stage (FL). The vegetative growth of chickpea plants is severely impacted by ST than in FL. On the other hand, FL causes higher reduction in yield traits compared to ST. Among all the studied genotypes, the leaf number is least reduced in Chania 2 at ST while at FL Jimbour was the least affected. At FL stage, Doolin branches are least affected whereas at ST Chania 2 branches were least reduced. The genotype with least affected height under water stress in ST was Saina K1 but Amethyst has least affected height at FL stage. The plant diameter of Saina K1 was least reduced at ST stage and Lyle diameter was least affected during FL stage under water stress. The genotype with the least biomass decreased at the ST stage was Chania 3 and Howzat has the least biomass reduction at FL stage under water stress. Flipper has the least decrease in pod number at ST and FL conditions. Water stress least affects yield of Chania 2 and ICCV 4958 during ST while Flipper yield is least affected during FL stage. Chania 2, ICCV 4958, and Flipper demonstrated the highest drought tolerance levels. Overall, the results demonstrated that water stress at the pre-flowering (FL) stage of chickpea leads to the highest losses in both pod number and grain yield which are critical for yield stability. Hence, that irrigation of chicken pea under water scarce conditions should prioritize the pre-flowering (FL) stage to boost production. Field investigation is recommended to confirm these genotypes in Kenya's Arid and Semi-Arid Lands (ASAL), facilitating their potential release and use by farmers in these regions.

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6.1 Conflict of Interest
All authors declare no conflict of interest

6.2 Ethics approval
Not applicable to this research

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