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Evaluation of Kabuli Chickpea Genotypes for Tropical Adaptation in Northern Australia

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Abstract: Chickpea is one of the economically important legume crops adapted for winter season production in tropical climates. This study evaluated the physiological, morphological, and biochemical traits of eight Kabuli chickpea genotypes in an Australian tropical environment. The result revealed significant differences between genotypes for seed emergence, plant height, primary shoots, leaf number, leaf area index, gas-exchange parameters, seed yield, carbon discrimination ($\Delta^{13}C$), and natural abundance for nitrogen fixation. Among the tested genotypes, AVTCPK#6 and AVTCPK#19 exhibited late flowering (60–66 days) and late maturity (105–107 days), and had higher leaf photosynthetic rate (A_{sat}) (28.4–31.2 μmol m^{-2} s^{-1}), lower stomatal conductance (gsw) (516–756 mmol m^{-2} s^{-1}), were associated with reduced transpiration rate (T) (12.3–14.5 mmol m^{-2} s^{-1}), offered greater intrinsic water-use efficiency (iWUE) (2.1–2.3 μmol m^{-2} s^{-1}/mmol m^{-2} s^{-1}), and contributed a higher seed yield (626–746 g/m^2) compared to other genotypes. However, a larger seed test weight (>60 g/100 seed) was observed for AVTCPK#24, AVTCPK#8, and AVTCPK#3. Similarly, a high proportion (45%) of larger seeds (>10–11 mm) was recorded for AVTCPK#24. Furthermore, a higher %Ndfa in AVTCPK#6 (71%) followed by AVTCPK#19 (63%) indicated greater symbiotic nitrogen fixation in high-yielding genotypes. Positive correlation was observed between %Ndfa and seed protein, as well as between seed yield and plant height, primary shoots, leaf count, leaf area index, leaf photosynthesis, stomatal conductance, transpiration rate at pod filling stage, biomass, and harvest index. An inverse correlation between ($\Delta^{13}C$) and iWUE, particularly in AVTCPK#6 and AVTCPK#19, indicates greater heat and drought tolerance, required for high-yielding Kabuli chickpea production in northern Australia.

Keywords: chickpea; water-use efficiency; carbon discrimination; heat tolerance

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1. Introduction

Chickpea is considered the oldest globally grown pulse and is ranked as the second most produced legume crop, cultivated on 14.8 million hectares with an annual production of 18.09 million metric tons harvested in 2022 [1]. Chickpea is classified into Desi and Kabuli types. Desi chickpea is predominately grown (80%) compared to Kabuli (20%) [2]. In Australia, chickpea is grown on 615,750 ha [1], with Kabuli chickpea contributing ~15% of the acreage. The crop is grown across several agricultural regions, with major production concentrated in Western Australia (Ord River Irrigation Area in northern WA), Southern Australia, and Victoria [3]. Although the northern region is a newer expansion of Australian chickpea production, it now contributes over 90% of the chickpea cropping area [4], but only 5% of Kabuli-type chickpea is cultivated in this region. The Australian northern grain region includes southern and central Queensland and northern New South Wales [5].

With the increasing demand for chickpea snacks [6], Kabuli chickpea fetches comparatively higher market prices, up to three times the price in India [7]. In the USA, the

cost of 50 g of Kabuli chickpea seed snacks, comprising 100 seeds, can be as much as 60% higher than Desi chickpea [8]. However, the cost of Kabuli chickpea depends significantly on seed size [9]. Despite its higher market value per kilogram than Desi chickpea, several factors have limited Kabuli's extension and production. The availability of early maturing varieties with greater tolerance to heat and dry conditions has been the main constraining factor for its commercialization and expansion in northern Australia [10].

The widespread use of Desi chickpea has shadowed the cultivation of Kabuli chickpea despite its importance. This is largely due to the relatively fewer production constraints associated with Desi compared to Kabuli. However, Kabuli chickpea has a higher nutritive value in terms of organic matter digestibility (OMD), short-chain fatty acids (SCFA), and metabolizable energy (ME) than Desi chickpea [11]. While the consumption of Desi chickpea is primarily restricted to the Middle East and Southeast Asia, it possesses valuable genetic alleles that can be used to improve Kabuli chickpea, enabling it to adapt to the different tropical environmental conditions of northern Australia [6].

The tropical environment of northern Australia has a warm climate with uncertainty in precipitation throughout the year [12,13]. Crops grown in this environment often face heat stress, ultimately reducing production [14]. Chickpea is generally grown as a winter crop that prefers threshold day and night temperature ranges between 21–29 °C and 15–21 °C [15]. Although chickpea is grown as a winter crop, shorter winter season leads chickpea plants to face high temperatures at the terminal stage [16]. Climate change issues, which predict increasing temperature and dryness, make it important to understand the response of the plant towards high temperature in order to breed heat-tolerant genotypes [15]. Hence, breeding genotypes with heat tolerance and greater yield per unit of available water (water productivity and water-use efficiency) is one of the most important challenges for the tropical adaptation of Kabuli chickpea.

To improve chickpea adaptation in warmer environments, different plant traits and climatic parameters should be monitored. One important impact of such conditions is leaf senescence due to disruption of chlorophyll content, which reduces photosynthetic rate, stomatal conductance, and transpiration rates [17]. The physiological link between C^{13}/C^{12} carbon discrimination ratio ($\Delta^{13}C$) and stomatal conductance, carbon assimilation rate, transpiration, and water-use efficiency (WUE) has been well illustrated [18,19] at the leaf level. This relationship has been utilized in various studies to select a genotype suitable for warmer and drier growing environments.

Furthermore, the constant use of chemical fertilizers and cropping without proper rotational planning has led to a decrease in soil organic matter and N mineralization potential in northern Australia [20]. As an alternative to chemical fertilizers, the use of legume crops in rotation has been reported as favorable in many studies [21,22]. However, the capacity to fix atmospheric nitrogen varies with plant genetic factors and environmental factors [21]. Studies have investigated the effect of genotypes on the nitrogen fixation ability of various crops. For instance, a study conducted by Belane, Asiwe [22] found genetic differences in the nitrogen fixing ability of thirty-two genotypes of cowpea using the ^{15}N abundance technique.

Several studies have investigated the variation in plant responses due to genotypic and environmental differences [23]. Hence, for the successful expansion of Kabuli chickpea in the tropical environment of Northern Australia, it is crucial to evaluate new genotypes for their adaptation to warmer growing conditions. This would enable chickpea growers to adapt new genotypes more easily for achieving desired yield potentials. Currently, some genotypes have been developed and released by Pulse Breeding Australia (PBA) with the aim of improving yield and stress tolerance in new genotypes. For instance, Macarena (a very large-seeded Kabuli chickpea, 9–11 mm), PBA Magnus, Genesis Kalkee and Bumper (large Kabuli chickpea, 8–10 mm), and PBA Monarch, Genesis 114 (medium Kabuli chickpea, 7–9 mm) are recommended for the northern region. However, the choices for Kabuli chickpea remain quite limited.

With rising interest in expanding the cropping area in northern Australia, there is a need to find suitable genotypes that are well adapted to the warmer growing conditions of northern regions. Addressing this issue will also contribute to fulfilling the increasing global demand for chickpea. Notably, Kabuli chickpea has emerged as a crop with comparatively higher protein content and market price compared to Desi chickpea [24]. Screening for Kabuli chickpea genotypes could be industry relevant as it contributes to the identification and selection of desired traits for the release of new high-yielding genotypes suitable for warmer environments in northern Australia. The main objective of this research is to examine the adaptive response (crop phenological, morphological, and physiological traits) of eight Kabuli chickpea genotypes in the tropical environment of northern Australia under strategic irrigation conditions.

2. Materials and Methods

2.1. Trial Location

This experiment was conducted during the dry season of 2023 at the Central Queensland Innovation and Research Precinct (CQIRP), located at 630 Ibis Ave, Rockhampton, QLD 4701, Australia (23.37° S, 150.52° E, altitude 11 masl). Replicated field trials were conducted in garden-raised beds. The map of the experiment site is shown in Figure 1.

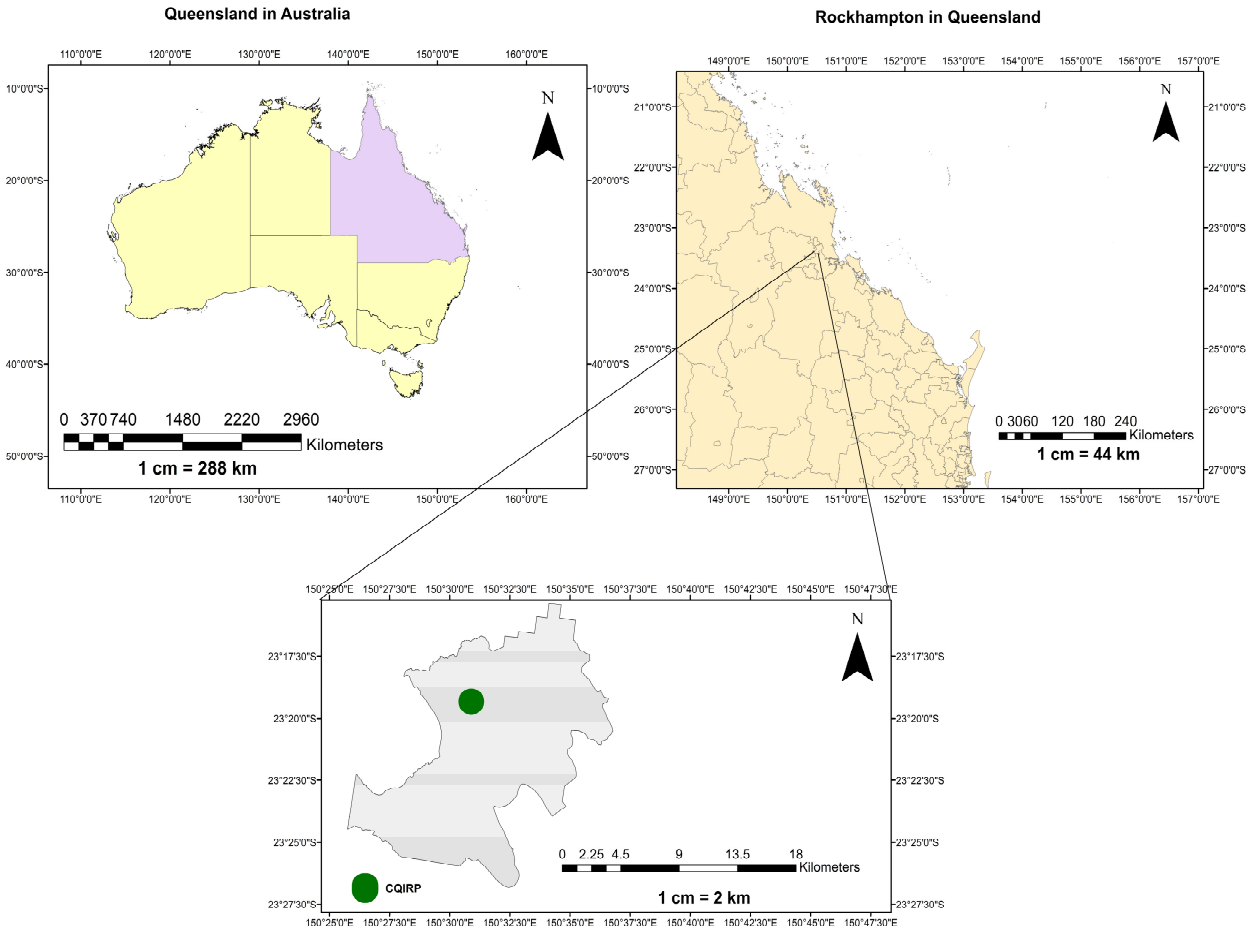

Figure 1. Location of experimental site as determined by GIS mapping using GIS software version ArcMap10.7.

2.2. Growing Environment

This experiment was conducted during the winter months (May–September 2023) in Rockhampton. Weather data, including rainfall and temperatures throughout the experiment, were obtained from the Australian Government Bureau of Meteorology (BOM)

station. The weather station at Rockhampton airport (station number 039083), approximately 15 km from the trial site, was selected for data collection. According to the BOM, Rockhampton's climate is classified as subtropical, with an average annual rainfall of just over 800 mm. In 2023, the total rainfall was 718 mm, slightly below average, with 105.8 mm received during the crop period. The maximum daily temperature during the experiment ranged from 18.4 °C (July) to 35.1 °C (Sep), with an average daily maximum temperature of 26.6 °C. The average daily minimum temperature was 13.7 °C, ranging from 5.4 °C (May) to 20.4 °C (July) (Figure 2).

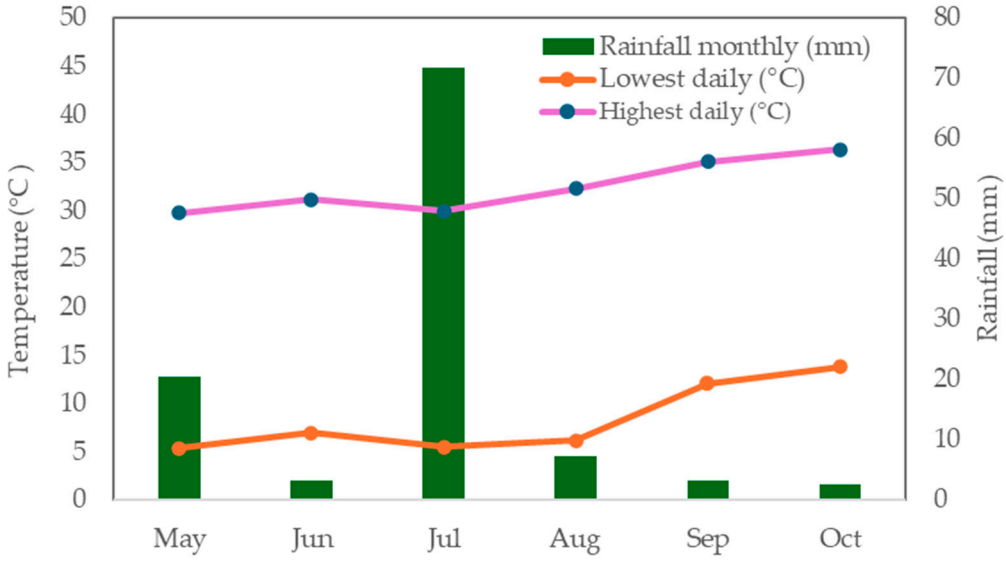

Figure 2. Temperature and rainfall data for the experiment site during the crop period.

2.3. Soil Type

Chickpea seeds were sown in garden-raised beds containing vertosol soil with a pH of 5.83. This soil was sourced from a local rainfed cotton farm in Alton Downs, central QLD Australia. A composite soil sample of 1 kg was collected from the raised beds and analyzed by the Environmental Analysis Laboratory (EAL) at Southern Cross University (SCU), Australia, following their method for Agricultural Soil Analysis Test methods. The soil composition was as follows: total nitrogen 0.1%, ammonium nitrogen 15 mg/kg/N, nitrate nitrogen 5.4 mg/kg/N, phosphorous 68 mg/kg/N, potassium 2.1%, calcium 51%, carbon/nitrogen ratio 15.9, and estimated organic matter 2.64%.

2.4. Trial Plots (Garden-Raised Bed) and Experimental Layout

The garden-raised beds measured 2.1 m in length and 1.1 m in width, with an open base in contact with the ground. A total of 40 raised beds were arranged 1 m apart from each other. Conducting the experiment in the raised beds ensured uniformity of the treatments assigned to each bed. There were no identifiable sources of variation related to bed type, environment, soil properties, slope, or soil moisture. In the beds, seeds were sown in three rows (35 cm apart) with 10 cm spacing within rows, maintaining a density of 26 plants/m^2.

The experiment was laid out in a Randomized Complete Block Design (RCBD); the seeds were planted on 24 May 2023 and maintained as a strategically irrigated crop. The treatment consisted of eight genotypes with five replications. Within each block, the genotypes were assigned randomly to the garden-raised beds using an online research randomizer tool (https://www.randomizer.org/, accessed on 10 April 2024).

2.5. Seed Source, Sowing, and Irrigation

The seeds for the trials were sourced from the Australian seed technology company AgriVentis Technologies Pty Ltd. (Sydney, NSW, Australia) (https://www.agriventistech

nologies.com.au/) (accessed on 2 March 2024). The genotypes used in the experiment were AVTCPK#1, AVTCPK#3, AVTCPK#6, AVTCPK#8, AVTCPK#12, AVTCPK#19, AVTCPK#24, and AVTCPK#25. The seeds were surface sterilized by immersing them in a 1% chlorine (*v*/*v*) solution for 1 h, then washed thoroughly with distilled water three times. They were inoculated (2.5 g inoculant per kg seed) with a peat-based slurry for chickpea seed inoculation (Group N, CC1192 nodulators, using Nodulaid® by BASF, Victoria, Australia).

The garden-raised beds were fitted with drip irrigation (three drip tubes with seven emitters per bed) with tap control to individual beds, ensuring even distribution of water along the bed. The emitters had a nominal flow rate of 2 L/h, resulting in a total discharge rate of 42 L per hour per bed. Prior to sowing, the field capacity of the soil was determined following the protocol mentioned by Imakumbili [25].

Bed moisture content was monitored, and the water required was calculated to ensure appropriate irrigation until crop maturity. Depending on the rainfall and weather conditions, water was applied weekly for 45 min to maintain the required soil moisture between the field capacity and refill point level.

2.6. Crop Management (Weed, Disease, and Pest)

Periodic hand weeding was practiced, keeping the bed free from winter weeds. Common weeds observed during the trial included Common Purslane (Portulaca oleracea), Stinging Nettle (*Urtica chamaedryoides*), and Bermuda Grass (*Cynodon dactylon).* Despite efforts to manage weeds and prevent disease outbreaks, collar root rot, a common fungal disease in chickpeas, affected some beds during the seedling stage. To verify the presence of the disease, plant samples were submitted to UniSQ laboratory in Toowoomba, QLD Australia, for diagnosis. Collar root rot, caused by soil-borne fungus *Sclerotium rolfsii Sacc*, was recorded, typically associated with high soil moisture availability at the seedling stage. The disease was controlled with the application of Mancozeb plus garden fungicide and miticide (Yates, Australia, at a rate of 5 g/L of water). This treatment was applied once weekly at the initial stage of the disease spread, and the frequency was changed to every fortnight as the plants started to recover. Genotype AVTCPK#1 was the most affected, while AVTCPK#8 was the least infected among the tested genotypes.

Regarding insect pests, *Helicoverpa punctigera,* a major pest of chickpeas, was spotted during the flowering and podding stages. Control of this pest was achieved through fortnightly application of Yates, Australia 200 mL/30 L Mavrik (a.i.Tau-fluvalinate 240 g/L), an effective treatment against both chewing and sucking insect pests.

2.7. Data Collection

2.7.1. Phenological Traits

Five plants from the middle row (data row) of each plot were selected for data collection on phenological traits at vegetative growth stages. The following phenological traits were assessed:

- Days to 50% emergence: Counted the number of days until 50% of the seeds had emerged.
- Plant height (cm): Measured from the ground surface to the uppermost node at 30, 45, 60, and 75 days after sowing (DAS) and at harvest.
- Number of primary shoots: Counted the primary shoots of five randomly selected plants from the data row at 30, 45, 60, and 75 DAS and at harvest.
- Number of leaves: Counted the fully expanded leaves at 30, 45, 60, and 75 DAS.
- Canopy light interception: Measured using a Ceptometer LP-80 (AccuPAR LP-80, Decagon Devices, USA). Light intensity readings above and below the canopy taken on clear days between 10 am and 2 pm to assess light interception from the biomass.
- Plant height at position of first pod (cm): Measured the height of the first pod from the base of the plant using a ruler just before harvest.

- Above-ground biomass: After harvest, above-ground biomass from five plant samples per replication was oven-dried at 65 °C. The seed yield was added to calculate the total above-ground biomass.
- Harvest index: Calculated as the ratio of seed yield to the above-ground biomass.

2.7.2. Reproductive Traits

The data on various reproductive traits were collected from visual observations of the plants once ≥50% of the plants in the beds reached the reproductive stage. The following traits were assessed:

- Days to flowering and days to podding: Recorded the number of days from sowing until the plant reached reproductive stage. Days to flowering was noted as the day when the plant started to produce fully open flowers. Days to podding was determined by counting the days from sowing to the initiation of podding.
- Days to maturity: Maturity was defined as the stage when 50% of the pods reached full size and showed signs of harvest readiness, indicated by a color change to yellow. The day to maturity was recorded when ≥50% of the pods had reached this stage.
- Growing degree days (GDDs) for 50% emergence, flowering, podding, and maturity: GDD was calculated to describe the heat units required for plant growth and development at different phenological stages. The metrics account for the specific heat requirements of chickpea, which is sensitive to both low (mean of maximum and minimum daily temperature < 15 °C) and high (maximum temperature > 35 °C) temperatures [26]. The formula used to calculate GDDs is as follows:

$$\text{GDD} = 1/2\ (\text{Tmax} + \text{Tmin}) - \text{Base Temperature}$$

where:

- Tmax = maximum daily temperature
- Tmin = minimum daily temperature
- Base temperature = 5 °C for chickpea crops [27]

This formula helps in understanding the phenological behavior of different chickpea genotypes under specific agroclimatic conditions [26,28,29].

2.7.3. Physiological Traits

The following physiological traits were assessed:

- Leaf gas-exchange parameters: Photosynthetic gas-exchange parameters, such as carbon assimilation rate (A_{sat}), stomatal conductance (gsw), and transpiration rate (T), were recorded from a fully expanded uppermost leaf. Measurements were taken using an open gas-exchange system of an Infrared Gas Analyser (IRGA) with an integrated fluorometer (Li-6800 Multiphase FlashTM Fluorometer, Portable Photosynthesis System, LiCor, Lincoln, NE, USA) with a leaf surface area of 1 cm^2 and an ambient CO_2 concentration of 370 μmol m^{-2} s^{-1}. The measurements were taken from 10 different plants from the data row of each bed at the 50% flowering and podding stages of each genotype. Data were recorded between 9:00 and 11.00 am.
- Chlorophyll content: Leaf chlorophyll content data were taken on the same day as the gas-exchange parameters from 10 plants per plot using a fully expanded topmost leaf. Measurements were obtained using a Konica Minolta SPAD 502 meter (Osaka, Japan).
- Carbon discrimination ($\Delta^{13}C$): Uppermost fully expanded leaf samples were scissor clipped at the end of the reproductive stage from each plant. Leaf samples were oven dried at 60 °C for 48 h and ground into a fine powder using a 1 mm sieve. Approximately 6–7 mg of the resultant powder was loaded into tin capsules and placed in a 96-well sample tray. The samples were analyzed by stable isotope laboratory (SIL) at Griffith University, Australia, for C and N isotopes using isotope ratio mass spectrometry (CF-EA-IRMS) in EA 1108 CHN elemental analyser (Thermo Fisher,

Milan, Italy) coupled to a Delta Plus mass spectrometer (Thermo Fisher, Milan, Italy). The result received was the ratio of ^{13}C to ^{12}C, usually expressed as $\delta^{13}C_{leaf}$, and the ratio of ^{14}N to ^{15}N was expressed as $\delta^{15}N_{leaf}$. Final discrimination for ^{13}C isotope (Δ) by the plants, compared to the atmosphere carbon isotope, was determined using the formula by Kohn [30]. The V-PBD value of air ($\delta^{13}C_{air}$) was assumed to be −8% [31].

$$\Delta^{13}C = (\delta^{13}C_{air} - \delta^{13}C_{leaf})/(1 + \delta^{13}C_{leaf})$$

- The ^{15}N abundance: For this study, wheat leaves were sampled as reference plants, grown in each plot, and used to determine soil N uptake and %Ndfa. The ^{15}N values were determined by isotope ratio mass spectrometry (CF-EA-IRMS) using an EA 1108 CHN elemental analyser (Thermo Fisher, Milan, Italy) coupled to a Delta Plus mass spectrometer (Thermo Fisher, Milan, Italy) in the laboratory of SIL, Griffith University, Australia. The ^{15}N sample values were expressed as $\delta^{15}N_{leaf}$ values (parts per thousand (%) relative to atmospheric N_2).

To calculate the %Ndfa, the $\delta^{15}N_{leaf}$ values of wheat samples were compared with the $\delta^{15}N_{leaf}$ values of each chickpea genotype following the equation below. For the B value, −1.65% was adopted following the method by Peoples, Bergersen [32].

$$\%Ndfa = 100 \times (X - Y)/(X - B)$$

where,

X = $\delta^{15}N_{leaf}$ values of non-N-fixing wheat samples,
Y = $\delta^{15}N_{leaf}$ values of N-fixing chickpea samples, and
B = $\delta^{15}N_{leaf}$ values of test legume deriving all its N nutrition from N_2 fixation (−1.65%).

- Nodule scoring: Five sample plants were scored for the nodule count following the method of nodule scoring system for pulse legumes by the Centre for Rhizobium Studies (CRS) at Murdoch University, Australia. Sample plants were carefully dug at harvest; excess soil was removed and washed carefully. The nodules were scored according to the size and distribution of nodules on the root system.

2.7.4. Harvesting and Yield Traits

The plants were harvested manually after full maturity. First, the outer two guard rows were removed. The number of plants in the middle data row was recorded, and the plants were harvested to collect the harvest data. The harvesting date varied for different genotypes. Pods were threshed manually and seed oven-dried at 30 °C to remove moisture. Above-ground biomass was oven dried at 65 °C to measure the oven-dry biomass and total above-ground biomass from each plot. The number of pods, number of seeds per pod, dry biomass, and above-ground biomass were measured and expressed as dry weight per square meter. The number of pods with double seeds was also recorded. Seeds were sieved to measure the seed diameter, expressed as the percentage of seeds with a diameter of 10–11 mm. Seeds were randomly selected and counted using a seed counter (Waver IC-VA, Aidex, Toyomae-Cho, Japan) and weighed on an electronic weighing balance to measure seed test weight.

2.7.5. Seed Protein Content (%)

The seeds harvested after maturity were freeze-dried for a week until completely moisture-free. Subsequently, seeds were ground into a fine powder using a grinder (Breville the coffee and spice TM, BCG200, NSW, Australia). The resulting dried powder was utilized to determine carbon and nitrogen content. A carbon nitrogen analyzer (Leco Trumac N Nitrogen/protein Elemental Analyzer, USA) was used to measure carbon% and nitrogen%. Crude protein (CP) content was calculated using the following equation [33]:

$$CP\ (\%) = Nitrogen\ (\%) \times 6.25$$

Freeze-dried chickpea powder samples (n = 40) were weighed in the range of 0.1500–0.1515 g on a ceramic boat, which was then loaded into the LECO analyzer and combusted inside the furnace at 1100 °C [34].

2.8. Data Analysis

Data were subjected to one-way analysis of variance (ANOVA) for one factor analysis (genotypes) using R software version 4.2.1. The Shapiro–Wilk test was performed to check the normality of the data before conducting ANOVA. The least significant difference (LSD) was used to record the differences among the treatments, with a significance level of $\alpha < 0.05$. Correlation coefficients for treatments were determined using Pearson's method. Regression analysis was carried out to evaluate the relationship between root nodulation and seed yield using Microsoft Excel 2022. Principal component analysis (PCA) and hierarchical clustering analysis using dendrogram were performed and presented using R software version 4.2.1, where the data were scaled before computing.

3. Results

3.1. Vegetative Traits

3.1.1. Days to 50% Emergence

The days for 50% emergence ranged from 7.6 to 12.8 days, or 111 to 188 accumulated heat units, presented as growing degree days (GDDs), among the genotypes (Table 1). The genotype AVTCPK#6 recorded early emergence (8 days/112 GDDs), whereas AVTCPK#24 and AVTCPK#25 recorded significantly late emergences (13 days/189 GDDs). The detailed emergence data for the eight chickpea genotypes are presented in Table 1.

Table 1. Days to 50% emergence along with the cumulative growing degree days (GDDs) of the eight chickpea genotypes are presented in parenthesis.

Genotypes	50% Emergence, Days After Sowing—DAS (GDDs)
AVTCPK#1	9.0 ^{cd} (125.0)
AVTCPK#3	8.8 ^{cd} (125.0)
AVTCPK#6	7.6 ^{d} (111.3)
AVTCPK#8	10.8 ^{b} (157.2)
AVTCPK#12	10.0 ^{bc} (141.7)
AVTCPK#19	9.0 ^{cd} (125.0)
AVTCPK#24	12.6 ^{a} (188.7)
AVTCPK#25	12.8 ^{a} (188.7)
Mean	10.1
F-value	12.9
p-value	2.7×10^{-1}
LSD	1.5

Significant codes: Same letters display no significance, while mean with different letters displays a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.1.2. Plant Height

A significant difference in plant height between the genotypes was observed at 30, 45, and 75 DAS and at harvest (Table 2). The plant height at harvest ranged from 49 to 75 cm, with AVTCPK#6 being significantly taller, followed by AVTCPK#19 and AVTCPK#24, while AVTCPK#12 and AVTCPK#1 had the shortest plant height. Although AVTCPK#6 and AVTCPK#19 recorded taller plant height at harvest, at the initial stages (30 and 45 DAS), their plant height was not different than other genotypes (Table 2). All the genotypes had plant heights in the range of 42–50 cm at 60 DAS, except for AVTCPK#24, which

showed a noticeable rise in plant height until 60 DAS. The detailed plant height for the eight genotypes over different growth stages is presented in Table 2.

Table 2. Chickpea plant height during vegetative stage, reproductive stage, and at harvest.

Genotypes	Plant Height 30 DAS (cm)	Plant Height 45 DAS (cm)	Plant Height 60 DAS (cm)	Plant Height 75 DAS (cm)	Plant Height Harvest (cm)
AVTCPK#1	28.4 ± 1.1^{ab}	38.3 ± 3.2^{ab}	48.6 ± 3.6^{a}	50.1 ± 0.6^{de}	50.9 ± 0.9^{de}
AVTCPK#3	24.5 ± 0.8^{cd}	36.7 ± 1.8^{bc}	47.0 ± 2.0^{a}	50.9 ± 1.7^{cde}	52.1 ± 0.3^{de}
AVTCPK#6	19.1 ± 0.7^{f}	23.4 ± 0.9^{e}	48.8 ± 4.6^{a}	63.3 ± 1.8^{a}	74.7 ± 3.4^{a}
AVTCPK#8	26.0 ± 0.7^{bc}	35.6 ± 3.2^{bc}	45.6 ± 3.4^{a}	54.5 ± 1.3^{bcd}	53.4 ± 3.6^{cd}
AVTCPK#12	27 ± 1.5^{bc}	36.8 ± 0.8^{bc}	43.4 ± 1.6^{a}	45.7 ± 1.8^{e}	48.6 ± 1.6^{e}
AVTCPK#19	20.3 ± 0.7^{ef}	26.4 ± 1.3^{de}	42.4 ± 1.5^{a}	57.5 ± 1.9^{b}	63.8 ± 1.6^{b}
AVTCPK#24	30.3 ± 1.6^{a}	44.2 ± 1.2^{a}	54.9 ± 1.8^{a}	55.9 ± 0.6^{bc}	57.4 ± 2.1^{c}
AVTCPK#25	22.3 ± 1.4^{de}	32.1 ± 2.7^{cd}	46.3 ± 2.9^{a}	52.4 ± 3.4^{bcd}	53.2 ± 0.9^{cde}
Mean	24.7	34.2	47.1	53.8	57.1
F-value	12.4	10.5	1.8	7.9	23.2
p-value	4.16×10^{-1}	2.12×10^{-6}	0.1	2.76×10^{-5}	4.45×10^{-10}
LSD	3.1	5.9	8.5	5.5	5.1

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.1.3. Primary Shoots

The number of primary shoots varied significantly between genotypes across the growth stages (30–75 DAS). Throughout the growing period, genotypes AVTCPK#6 and AVTCPK#19 recorded significantly more primary shoots compared to the other genotypes (Table 3).

Table 3. Number of primary shoots in eight chickpea genotypes at vegetative and reproductive stages and at harvest.

Genotypes	Number of Primary Shoots at 30 DAS	Number of Primary Shoots at 45 DAS	Number of Primary Shoots at 60 DAS	Number of Primary Shoots at 75 DAS	Number of Primary Shoots at Harvest
AVTCPK#1	2.6 ± 0.2^{ab}	2.7 ± 0.1^{b}	2.9 ± 0.1^{b}	3 ± 0.1^{b}	3.0 ± 0.6^{b}
AVTCPK#3	2.6 ± 0.2^{ab}	2.9 ± 0.1^{b}	3.1 ± 0.1^{b}	3.1 ± 0.1^{b}	3.2 ± 0.1^{b}
AVTCPK#6	3.0 ± 0.3^{a}	4.8 ± 0.8^{a}	7.3 ± 0.6^{a}	9.4 ± 0.9^{a}	9.6 ± 0.8^{a}
AVTCPK#8	2.5 ± 0.2^{ab}	2.9 ± 0.2^{b}	3.0 ± 0.2^{b}	3.2 ± 0.2^{b}	3.4 ± 0.3^{b}
AVTCPK#12	2.4 ± 0.2^{b}	2.8 ± 0.1^{b}	2.8 ± 0.1^{b}	3 ± 0.2^{b}	3.0 ± 0.2^{b}
AVTCPK#19	2.8 ± 0.2^{ab}	4.8 ± 0.2^{a}	7 ± 0.8^{a}	8.3 ± 1.3^{a}	8.7 ± 0.7^{a}
AVTCPK#24	2.7 ± 0.1^{ab}	2.7 ± 0.2^{b}	3.2 ± 0.2^{b}	3.1 ± 0.1^{b}	3.5 ± 0.2^{b}
AVTCPK#25	1.8 ± 0.1^{c}	2.7 ± 0.1^{b}	3.2 ± 0.2^{b}	3.2 ± 0.2^{b}	3.5 ± 0.2^{b}
Mean	2.5	3.3	4.1	4.6	4.7
F-value	3.6	9.7	24.4	23.7	44.3
p-value	0.006	4.6×10^{-6}	2.46×10^{-10}	3.51×10^{-10}	1.71×10^{-13}
LSD	0.5	0.9	1.1	1.6	1.2

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.1.4. Leaf Shape and Leaf Type

Chickpea plants generally have three types of leaf shapes: normal (fern shape), simple (unifoliate), and multipennate (bipinnate). There was a significant variation between genotypes, and two major leaf types were noted among the eight genotypes. Genotypes AVTCPK#6 and AVTCPK#19 had normal (fern-shaped, bipinnate) leaves, while the remaining genotypes showed simple (unifoliate) leaf shapes.

Leaf count per plant varied significantly between genotypes at 30, 45, and 75 DAS. At 75 DAS, AVTCPK#6 and AVTCPK#19 recorded higher leaf counts (204 and 197, respectively), while AVTCPK#1 and AVTCPK#12 recorded lower leaf counts ($\leq$160 leaves per plant). The remaining four genotypes fell in between these ranges (Table 4).

Table 4. Number of leaves at 30, 45, 60, and 75 days after sowing (DAS) in eight chickpea genotypes.

Genotypes	Leaf Count at 30 DAS	Leaf Count at 45 DAS	Leaf Count at 60 DAS	Leaf Count at 75 DAS
AVTCPK#1	24.6 ± 3.8 ^{ab}	60.7 ± 19.7 ^{c}	113.4 ± 27.5 ^{a}	145.5 ± 21.7 ^{d}
AVTCPK#3	27.2 ± 2.3 ^{ab}	79.1 ± 10.1 ^{ab}	144.8 ± 25.7 ^{a}	176.8± 31.3 ^{bc}
AVTCPK#6	27 ± 0.9 ^{ab}	68.0 ± 6.9 ^{bc}	158.6 ± 33.9 ^{a}	204.4 ± 5.1 ^{a}
AVTCPK#8	24.6 ± 2.6 ^{b}	61.8 ± 6.6 ^{c}	156.2 ± 6.9 ^{a}	188.4 ± 13 ^{ab}
AVTCPK#12	26.5 ± 4.8 ^{ab}	63.6 ± 13.9 ^{c}	141.5 ± 13.7 ^{a}	160.2 ± 24.2 ^{cd}
AVTCPK#19	25.8 ± 1.6 ^{ab}	61.7 ± 2.7 ^{c}	156.9 ± 27.5 ^{a}	196.9 ± 9.4 ^{ab}
AVTCPK#24	27.9 ± 2.6 ^{a}	83.2 ± 6.5 ^{a}	150.9 ± 39.7 ^{a}	189.0 ± 16.7 ^{ab}
AVTCPK#25	16.7 ± 1.7 ^{c}	56.1 ± 7.9 ^{c}	144.0 ± 4.3 ^{a}	193.4 ± 21.4 ^{ab}
Mean	25.1	66.8	145.8	181.8
F-value	9.3	4.701	1.61	5.5
p-value	6.15×10^{-6}	0.001	0.17	0.0005
LSD	3.4	12.7	33.3	24.6

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.1.5. Leaf Area Index

The crop leaf area index (LAI) increased steadily over the crop period. There was no significant difference in leaf area index among genotypes at 30, 45, and 60 DAS. However, LAI differed significantly between genotypes, with AVTCPK#6 recording the highest LAI (5.9), followed by AVTCPK#19 (5.3). All other genotypes had lower LAI, ranging from 2.6 (AVTCPK#1) to 3.9 (AVTCPK#25) (Table 5).

Table 5. Leaf area index at 30, 45, 60, and 75 days after sowing (DAS).

Genotypes	LAI 30 DAS	LAI 45 DAS	LAI 60 DAS	LAI 75 DAS
AVTCPK#1	1.2 ± 0.1 ^{a}	1.7 ± 0.1 ^{a}	2.3 ± 0.1 ^{a}	2.6 ± 0.2 ^{c}
AVTCPK#3	1.3 ± 0.1 ^{a}	1.8 ± 0.1 ^{a}	2.4 ± 0.1 ^{a}	3.5 ± 0.7 ^{bc}
AVTCPK#6	1.3 ± 0.1 ^{a}	1.9 ± 0.2 ^{a}	3.2 ± 0.5 ^{a}	5.9 ± 0.4 ^{a}
AVTCPK#8	1.2 ± 0.1 ^{a}	1.8 ± 0.2 ^{a}	2.4 ± 0.4 ^{a}	3.7 ± 0.4 ^{bc}
AVTCPK#12	1.2 ± 0.1 ^{a}	1.6 ± 0.1 ^{a}	2.2 ± 0.2 ^{a}	3.4 ± 0.5 ^{cd}
AVTCPK#19	1.4 ± 0.2 ^{a}	1.9 ± 0.3 ^{a}	3.0 ± 0.5 ^{a}	5.3 ± 0.2 ^{a}
AVTCPK#24	1.5 ± 0.2 ^{a}	1.7 ± 0.1 ^{a}	2.6 ± 0.1 ^{a}	3.9 ± 0.2 ^{b}
AVTCPK#25	1.1 ± 0.1 ^{a}	1.3 ± 0.1 ^{a}	2.0 ± 0.2 ^{a}	3.5 ± 0.3 ^{bc}

Table 5. *Cont.*

Genotypes	LAI 30 DAS	LAI 45 DAS	LAI 60 DAS	LAI 75 DAS
Mean	1.3	1.7	2.5	3.9
F-value	1.1	1.3	1.5	8.7
p-value	0.4	0.3	0.2	1.2×10^{-5}
LSD	0.4	0.5	0.9	1.1

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.2. Reproductive Traits

Days to flowering, podding, and maturity varied significantly between genotypes, ranging from 35 to 66 days (532–953 GDDs). Genotypes AVTCPK#1, AVTCPK#3, and AVTCPK#12 were early flowering (35 DAS), AVTCPK#8, AVTCPK#24, and AVTCPK#25 were mid-flowering (39–40 DAS), whereas AVTCPK#6 and AVTCPK#19 were late flowering.

Likewise, the days to podding also varied significantly, ranging from 46 to 74 DAS. The GDDs required for flowering, podding, and maturity were the highest for genotype AVTCPK#19, followed by AVTCPK#6. Genotypes AVTCPK#1 and AVTCPK#3 had almost similar GDD requirements, with AVTCPK#12 requiring the lowest GDDs. The GDD requirements for flowering, podding, and maturity were comparatively similar for genotypes AVTCPK#8, AVTCPK#24, and AVTCPK#25 (Table 6).

Table 6. Days to flowering, podding, and maturity, along with the cumulative growth degree days (GDDs) of the eight chickpea genotypes, are presented in brackets.

Genotypes	Flowering (DAS)	Podding (DAS)	Maturity (DAS)
AVTCPK#1	35.6 (532.4) ^{c}	46.6 (677.1) ^{c}	91.6 (1307.9) ^{c}
AVTCPK#3	35.6 (532.4) ^{c}	46 (677.1) ^{c}	91.6 (1307.9) ^{c}
AVTCPK#6	60.4 (869.9) ^{b}	71 (1008.7) ^{a}	105.2 (1542.5) ^{a}
AVTCPK#8	37.8 (553.8) ^{c}	48.4 (703.2) ^{bc}	96.8 (1389.2) ^{b}
AVTCPK#12	35.8 (532.4) ^{c}	47.2 (688.8) ^{bc}	91 (1292.8) ^{c}
AVTCPK#19	66.2 (952.5) ^{a}	74 (1052.6) ^{a}	107.8 (1582.1) ^{a}
AVTCPK#24	39.2 (578.7) ^{c}	50 (718.5) ^{b}	93 (1324.8) ^{c}
AVTCPK#25	39.8 (578.7) ^{c}	53 (760.5) ^{bc}	95 (1357.4) ^{bc}
Mean (DAS)	43.3	54.3	96.3
F-value	71.3	27.6	16.5
p-value	3.72×10^{-16}	5.75×10^{-11}	2.05×10^{-8}
LSD	4.3	6.4	4.7

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.3. Physiological Traits

3.3.1. Chlorophyll Content (SPAD Unit)

The leaf chlorophyll content varied significantly between genotypes across all growth stages, except at 30 DAS, with values ranging from 49 to 56. During the early growth stage, up to 60 DAS, AVTCPK#24 had the highest chlorophyll content, followed by AVTCPK#8 and AVTCPK#25. However, at 75 DAS, AVTCPK#6 showed the highest chlorophyll content, followed by AVTCPK#19. Genotype AVTCPK#1 consistently showed the least chlorophyll content throughout the growth period (Figure 3).

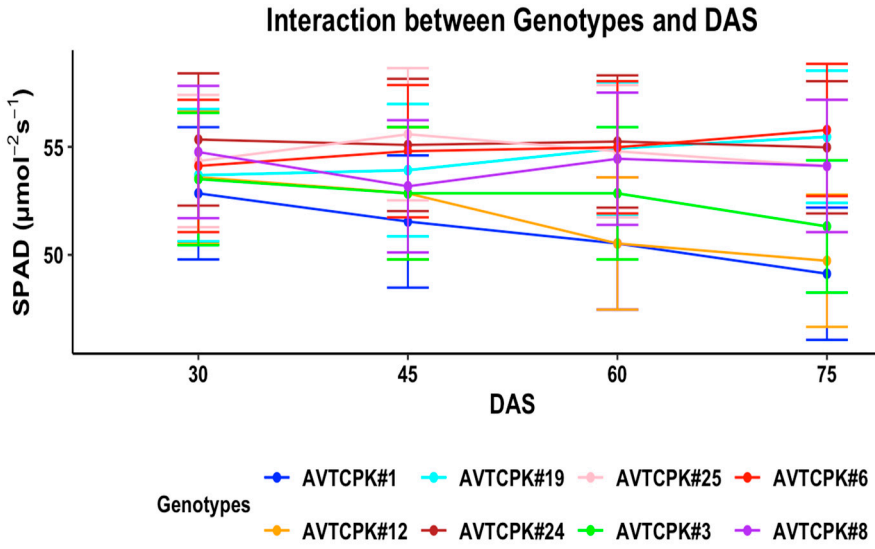

Figure 3. Mean of total chlorophyll (SPAD unit) content at vegetative reproductive stage of eight genotypes. Each vertical bar represents the 'LSD' (least significant difference).

3.3.2. Gas-Exchange Parameters

The leaf photosynthetic rate at flowering ranged from 27.7 to 29.9 µmol m^{-2} s^{-1} but did not differ significantly between genotypes (Table 7). In contrast, iWUE varied significantly, with AVTCPK#6 and AVTCPK#19 recording significantly higher values compared to other genotypes. The photosynthetic rate at pod fill ranged from 27.6 to 31.1 µmol m^{-2} s^{-1}, with significant differences observed among genotypes: AVTCPK#25 had the highest rate, while AVTCPK#1, AVTCP#8, and AVTCP#12 had the lowest (Table 7).

Table 7. The mean of CO_2 assimilation rate (Asat) (µmol m^{-2} s^{-1}), stomatal conductance (gsw) (mmol m^{-2} s^{-1}), transpiration rate (T) (mmol m^{-2} s^{-1}), and intrinsic water-use efficiency (iWUE) (µmol m^{-2} sec^{-1}/mmol m^{-2} s^{-1}) at flowering and podding stages, along with the carbon isotope discrimination rate ($\Delta^{13}C$) of eight Kabuli chickpea genotypes.

Genotypes	At Flowering Stage				At Podding Stage				$\Delta^{13}C$
	A_{sat}	gsw	T	iWUE	A_{sat}	gsw	T	iWUE	
AVTCPK#1	27.7 ^{a}	672.3 ^{a}	14.6 ^{a}	1.9 ^{b}	27.7 ^{d}	525.2 ^{a}	13.3 ^{a}	2.1 ^{a}	21.9 ^{abc}
AVTCPK#3	29.6 ^{a}	680.9 ^{a}	14.9 ^{a}	1.9 ^{b}	29.8 ^{abc}	625.8 ^{a}	14.2 ^{a}	2.1 ^{a}	21.5 ^{c}
AVTCPK#6	28.5 ^{a}	516.1 ^{b}	12.3 ^{b}	2.3 ^{a}	31.2 ^{ab}	756.2 ^{a}	14.6 ^{a}	2.1 ^{a}	18.9 ^{d}
AVTCPK#8	29.1 ^{a}	743.4 ^{a}	15.5 ^{a}	1.9 ^{b}	29.3 ^{bcd}	633.4 ^{a}	14.3 ^{a}	2.1 ^{a}	22.4 ^{ab}
AVTCPK#12	27.9 ^{a}	675.7 ^{a}	14.6 ^{a}	1.9 ^{b}	28.1 ^{cd}	568.4 ^{a}	13.5 ^{a}	2.1 ^{a}	21.9 ^{bc}
AVTCPK#19	29.6 ^{a}	535.9 ^{b}	12.9 ^{b}	2.3 ^{a}	30.9 ^{ab}	649.9 ^{a}	14.7 ^{a}	2.1 ^{a}	19.1 ^{d}
AVTCPK#24	29.9 ^{a}	735.7 ^{a}	15.5 ^{a}	1.9 ^{b}	30.8 ^{ab}	614.5 ^{a}	14.1 ^{a}	2.2 ^{a}	21.6 ^{c}
AVTCPK#25	29.5 ^{a}	781.3 ^{a}	15.5 ^{a}	1.9 ^{b}	31.4 ^{a}	676.8 ^{a}	15.1 ^{a}	2.1 ^{a}	22.4 ^{a}
Mean	28.9	667.7	14.5	2.0	29.9	631.3	14.2	2.1	21.2
F-value	1.3	6.3	7.8	5.7	4.8	1.5	1.0	0.3	81.2
p-value	0.28	0.0001	3.03×10^{-5}	0.0003	0.001	0.19	0.5	0.9	$<2 \times 10^{-16}$
LSD	2.1	109.9	1.3	0.2	1.9	162.2	1.8	0.3	0.4

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

Stomatal conductance ranged from 516 to 756 mmol m^{-2} s^{-1}, showing significant differences only at flowering, with AVTCPK#6 and AVTCPK#19 showing reduced stomatal conductance at that stage, which increased and peaked during pod filling.

Carbon isotope discrimination ranged from 18.9 to 22.4, significantly varying between genotypes. The highest discrimination (low water use efficiency) was recorded for AVTCPK#25 (22.4) and AVTCPK#8 (22.4), followed by AVTCPK#1 (21.9), while AVTCPK#6 (18.9) and AVTCPK#19 (19.1) had the lowest discrimination (high water use efficiency). Detailed results of carbon discrimination for the chickpea genotypes are presented in Table 7.

3.4. Yield and Yield-Attributing Traits

A significant difference in dry biomass, AGB, and harvest index (HI) was observed among the eight genotypes. The dry biomass yield ranged 308–596 g/m^2, AGB ranged from 563 to 1342 g/m^2, and HI ranged from 0.45 to 0.54. Genotypes AVTCPK#6 and AVTCPK#19 recorded significantly higher biomass yields compared to the other genotypes. The AGB and HI were higher for AVTCPK#6, while AVTCPK#1 had the lowest values among all genotypes (Table 8).

The seed yield also varied significantly between the genotypes, with AVTCPK#6 yielding the highest, followed by AVTCPK#19 and AVTCPK#24, while the lowest yield was recorded for AVTCPK#1.

The yield-attributing characters, viz., the number of pods, double seeds, 100-seed weight, and the proportion of larger seeds (diameter 10–11 mm), varied significantly between genotypes. Genotypes AVTCPK#6 and AVTCPK#19 had a higher number of pods per plant, a greater proportion of double-seeded pods, and a higher number of seeds per pod. In contrast, the test weight (100-seed weight) and proportion of larger seeds were highest (46%) for AVTCPK#24, followed by AVTCPK#3, AVTCPK#8, and AVTCPK#6, while AVTCPK#19 had the lowest values (Table 8).

Seed protein content varied (16–19.9%) significantly between genotypes, ranging from 16.0 to 19.9%. Genotype AVTCPK#6 had the highest protein content (19.9%), followed by AVTCPK#19 (19.2%), while the lowest protein content was recorded for AVTCPK#1 (16.0%) (Table 8).

Table 8. Yield and yield-attributing traits, including dry biomass, above-ground biomass, harvest index, number of pods, double-seeded pods, total seed number, seed yield, test weight, and protein content, of eight chickpea genotypes.

Genotypes	Dry Biomass (g/m^2)	AGB (g/m^2)	HI	Pod Number/m^2	Double-Seed Pods (%)	Total Number of Seeds/m^2	Seed Yield (g/m^2)	Test Weight (g)	Seed Size (10–11 mm) %	Seed Protein Content (%)
AVTCPK#1	308 ± 19.2^{c}	563 ± 34.2^{f}	0.45^{c}	570 ± 60.0^{c}	3.7^{b}	445 ± 24.8^{e}	255 ± 17.4^{e}	55.5 ± 1.6^{c}	23.1^{cd}	16 ± 0.9^{d}
AVTCPK#3	414 ± 37.5^{b}	811 ± 68.4^{de}	0.48^{bc}	945 ± 91.0^{ab}	4.2^{b}	766 ± 61.2^{cd}	397 ± 42.5^{de}	60.2 ± 0.7^{ab}	42.4^{ab}	17.8 ± 0.6^{bc}
AVTCPK#6	596 ± 21.8^{a}	1342 ± 79.1^{a}	0.55^{a}	1285 ± 112^{a}	12.2^{a}	1197 ± 88.3^{a}	746 ± 61.4^{a}	59.0 ± 0.3^{ab}	40.4^{ab}	19.9 ± 0.8^{a}
AVTCPK#8	447 ± 23.4^{b}	909 ± 71.7^{cd}	0.50^{abc}	1025 ± 194^{a}	5.1^{b}	814 ± 105^{c}	463 ± 54.7^{cd}	60.1 ± 2.1^{ab}	43.0^{ab}	17.6 ± 1.3^{bcd}
AVTCPK#12	314 ± 28.8^{c}	627 ± 46.3^{ef}	0.50^{abc}	650 ± 125^{bc}	2.4^{b}	519 ± 42.6^{de}	313 ± 25.8^{e}	58.0 ± 1.1^{abc}	26.3^{cd}	17.4 ± 1.0^{bcd}
AVTCPK#19	582 ± 18.4^{a}	1208 ± 89.6^{ab}	0.51^{ab}	1128 ± 177^{a}	16.3^{a}	1102 ± 132^{ab}	626 ± 73.9^{ab}	54.7 ± 1.2^{c}	17.6^{d}	19.2 ± 0.7^{ab}
AVTCPK#24	465 ± 31.3^{b}	1019 ± 55.8^{bc}	0.54^{ab}	963 ± 88.2^{ab}	3.0^{b}	923 ± 73.5^{bc}	554 ± 41.5^{bc}	60.8 ± 1.1^{a}	45.6^{a}	17.8 ± 1.5^{bc}
AVTCPK#25	449 ± 26.8^{b}	996 ± 82.4^{cd}	0.54^{ab}	1058 ± 151^{a}	2.8^{b}	923 ± 73.5^{abc}	547 ± 69.2^{bc}	57.1 ± 1.4^{bc}	31.5^{bc}	17.1 ± 1.1^{cd}
Mean	446.8	934.5	0.5	953.1	6.2	938.7	838.8	57.5	33.8	17.9
F-value	17.1	15.7	2.8	3.7	5.2	9.0	10.6	4.8	5.7	9.5
p-value	1.46×10^{-8}	3.63×10^{-8}	0.025	0.0055	0.0007	8.37×10^{-6}	1.87×10^{-6}	0.001	0.00038	0.000005
LSD	74.5	196.2	0.1	361.4	6.6	253.1	146.1	4.1	12.9	1.2

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.5. Nitrogen Assimilation (^{15}N)

3.5.1. Nodules Scoring

Nodule scores ranged from 2.4 to 8.4 and varied significantly between genotypes. Genotypes AVTCPK#6 and AVTCPK#19 had significantly higher nodule scores, whereas AVTCPK#1 had the lowest compared to other genotypes (Table 9).

Table 9. Nodule scoring, natural abundances of the rare stable isotope of nitrogen ($\delta^{15}N_{leaf}$), and proportion of N derived from atmospheric N_2 fixation (%Ndfa) of Kabuli chickpea genotypes measured at harvest.

Genotypes	Nodules Score	$\delta^{15}N_{leaf}$	%Ndfa
AVTCPK#1	2.4 ^{f}	2.7 ± 0.1 ^{a}	30.1 ± 4.6 ^{d}
AVTCPK#3	3.7 ^{de}	2.5 ± 0.3 ^{ab}	32.2 ± 3.2 ^{d}
AVTCPK#6	8.4 ^{a}	−0.8 ± 0.3 ^{e}	70.9 ± 8.7 ^{a}
AVTCPK#8	5.0 ^{bc}	1.9 ± 0.2 ^{bc}	42.8 ± 2.3 ^{cd}
AVTCPK#12	3 ^{ef}	2.2 ± 0.9 ^{abc}	30.4 ± 1.6 ^{d}
AVTCPK#19	8.3 ^{a}	−0.2 ± 0.2 ^{d}	62.9 ± 4.4 ^{ab}
AVTCPK#24	5.5 ^{b}	1.9 ± 0.2 ^{c}	53.6 ± 2.01 ^{bc}
AVTCPK#25	4.2 ^{cd}	2.1 ± 0.2 ^{bc}	32.5 ± 2.6 ^{d}
Mean	5.1	1.6	44.4
F-value	52.0	48.1	12.9
p-value	2.22×10^{-14}	6.1×10^{-14}	2.87×10^{-7}
LSD	0.9	0.5	13.0
CV	13.9	26.9	22.7

Significant codes: Same letters display no significance, while different letters display a significant effect. 'LSD' indicates Least Significant Difference. Values are presented as average ± s.e.m.

3.5.2. Natural Abundances of the Rare Stable Isotope of Nitrogen ($\delta^{15}N_{leaf}$)

Genotype values indicate their dependence on soil N versus atmospheric nitrogen for nutrition. Generally, lower $\delta^{15}N$ values in legume crops indicate greater N_2 fixation ability. The $\delta^{15}N_{leaf}$ values ranged from −0.79 to 2.69 and varied significantly between genotypes. The lowest natural abundances of the rare stable isotope of nitrogen were observed in genotype AVTCPK#6, followed by genotype AVTCPK#19 (Table 9), indicating greater nitrogen fixation. Higher $\delta^{15}N_{leaf}$ values were observed in genotypes AVTCPK#1, AVTCPK#12, AVTCPK#24, and AVTCPK#25.

3.5.3. Proportion of N Derived from Atmospheric N_2 Fixation (%Ndfa)

Estimation of the proportion of N derived from atmospheric fixation revealed significant differences among the eight genotypes. The highest %Ndfa (71%) was recorded for AVTCPK#6, while the lowest (30%) was recorded for AVTCPK#1.

3.6. Regression Analysis

Regression analysis indicated that seed yield is positively related to Ndfa % (R^2 = 0.45) (Figure 4).

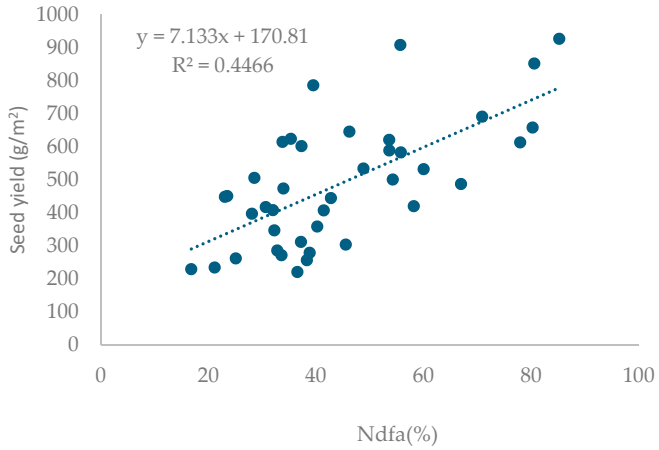

Figure 4. Relationship between proportion of N derived from atmospheric N_2 fixation (%Ndfa) and seed yield.

3.7. Correlation between Traits

The correlation among the studied parameters is shown in Figure 5 Morphological traits, such as plant height (r = 0.9), number of primary shoots (r = 0.8), leaf number (r = 0.9), and LAI (r = 0.7), showed a strong positive relationship with seed yield. Similarly, all the gas-exchange parameters, which include the carbon assimilation rate (A_{sat}) at the podding stage (r = 0.9), stomatal conductance (r = 0.9), and transpiration (r = 0.8), positively correlated with the seed yield, while $\Delta^{13}C$ showed a strong negative correlation (r = 0.7) with the seed yield. A perfect negative correlation (r = −1.0) was observed between $\Delta^{13}C$ and iWUE. Additionally, all yield-related traits were strongly correlated with the seed yield. Similarly, %Ndfa also showed a strong positive correlation (0.9) with the seed yield.

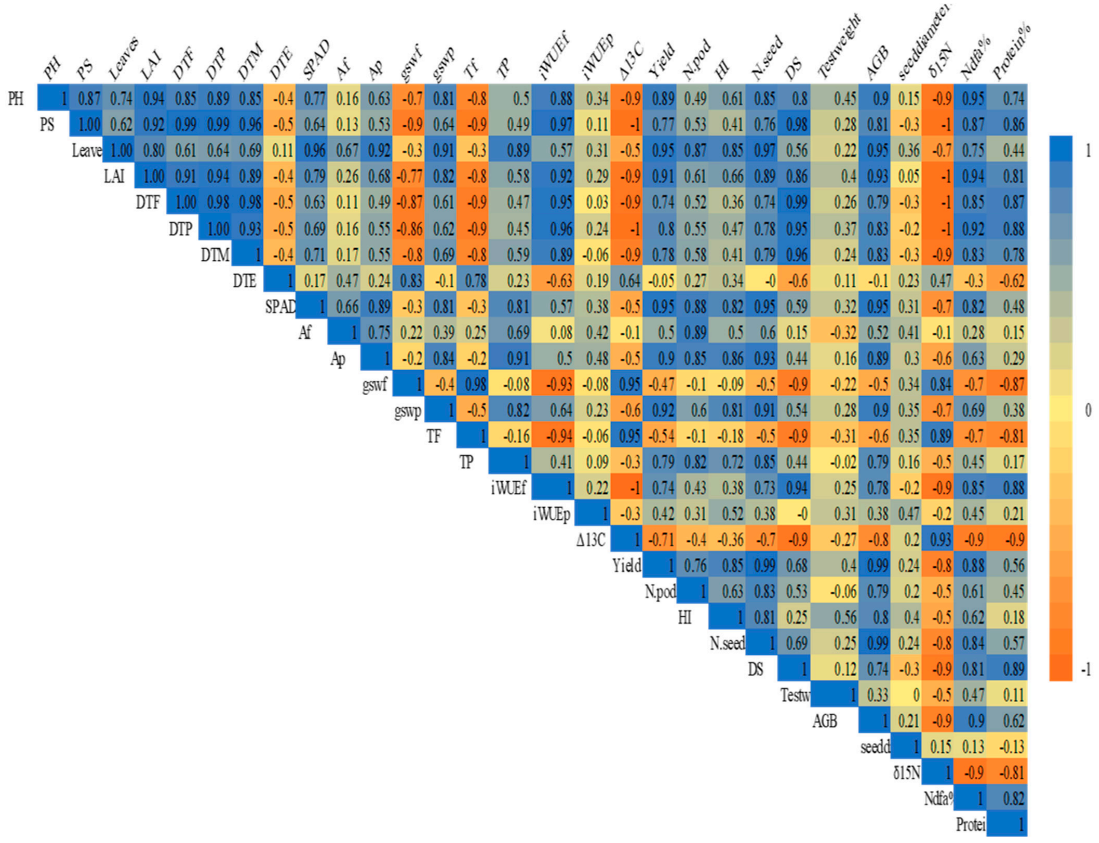

Figure 5. Correlogram showing the relationships between studied traits. Note: Yield (seed yield, g/m^2), PH (plant height), PS (number of primary shoots), Leaves (number of leaves at 75 DAS), LAI75

(Leaf area index at 75 DAS), DTE (days to emergence), DTF (days to flowering), DTP (days to podding), DTM (days to maturity), SPAD (SPAD chlorophyll content at 75 DAS), Af (carbon assimilation rate at flowering, µmol $m^{-2}s^{-1}$), Ap (carbon assimilation rate at podding stage, µmol $m^{-2}s^{-1}$), gswf (stomatal conductance at flowering stage, mmol m^{-2} s^{-1}), gswp (stomatal conductance at podding stage, mmol m^{-2} s^{-1}), Tf (transpiration rate at flowering, mmol m^{-2} s^{-1}), Tp (transpiration rate at podding stage, mmol m^{-2} s^{-1}), iWUEf (intrinsic water-use efficiency at flowering stage, µmol $m^{-2}s^{-1}$/mmol m^{-2} s^{-1}), iWUEp (intrinsic water-use efficiency at podding stage), $\Delta^{13}C$ ($^{13/14}$Carbon discrimination ratio), HI (harvest index), Npod (number of total pods/m2), Nseed (number of seeds/m^2), DS (number of pods with double seed), TW (test weight, g), $\delta^{15}N$, Ndfa% (proportion of N derived from atmosphere), Protein% (seed crude protein%).

3.8. Principal Component Analysis (PCA) and Cluster Analysis (CA)

The PCA analysis was conducted on all recorded parameters to identify genotype grouping and the contribution of the parameters to total data variability in each axis. Eigen values were calculated, revealing that the first four components had positive values, accounting for a cumulative 94.6%. The contributions of two principal components are detailed in Table 10, with individual contributions of 63.4% and 20.2% totaling 83.6%. In PC1, the major contributors were LAI (5.2%), AGB (5%), $\delta^{15}N_{leaf}$ (4.9%), DTP (4.9%), plant height (4.8%), Ndfa% (4.8%), primary shoots (4.8%), seed yield (4.8%), and $\Delta^{13}C$ (4.4%). In contrast, day to emergence (11.3%), Asat at flowering (9.8%), gsw at flowering (7.4%), seed diameter (6.9%), and transpiration at flowering (6.5%) explained the variability for principal component 2, as shown in Table 10.

Table 10. Eigen vectors, Eigen value, and variance for the first two principal components of the studied traits in eight genotypes of Kabuli chickpea.

Variables	PCA1	r^2	PCA2	r^2
Plant height (PH)	0.22	0.05	−0.03	0.00
Primary shoot (PS)	0.22	0.05	−0.13	0.02
Leaves	0.20	0.04	0.21	0.05
LAI	0.23	0.05	−0.02	0.00
Day to flowering	0.21	0.05	−0.14	0.02
Day to podding	0.22	0.05	−0.11	0.01
Day to maturity	0.21	0.05	−0.09	0.01
Day to emergence	−0.08	0.01	0.34	0.11
SPAD	0.20	0.04	0.20	0.04
Carbon assimilation at flowering (Af)	0.08	0.01	0.31	0.10
Carbon assimilation at podding (Ap)	0.18	0.03	0.25	0.06
Stomata conductance at flowering (gswf)	−0.17	0.03	0.27	0.07
Stomata conductance at podding (gswp)	0.19	0.04	0.14	0.02
Transpiration at flowering (Tf)	−0.18	0.03	0.25	0.06
Transpiration at podding (Tp)	0.15	0.02	0.23	0.05
iWUE at flowering (iWUEf)	0.21	0.05	−0.15	0.02
iWUE at podding (iWUEp)	0.07	0.00	0.16	0.02
$\Delta^{13}C$	−0.21	0.04	0.16	0.03
Seed yield(Y)	0.22	0.05	0.13	0.02
Number of pods (N.pod)	0.16	0.03	0.23	0.05
Harvest Index (HI)	0.15	0.02	0.25	0.06

Table 10. *Cont.*

Variables	PCA1	r^2	PCA2	r^2
Number of seeds (N.seed)	0.22	0.05	0.15	0.02
Double seed (DS%)	0.21	0.04	−0.15	0.02
Test weight	0.08	0.01	−0.01	0.00
AGB	0.22	0.05	0.11	0.01
Seed diameter%	0.00	0.00	0.26	0.07
$\delta^{15}N_{leaf}$	−0.22	0.05	0.10	0.01
Ndfa%	0.22	0.05	−0.01	0.00
Protein%	0.18	0.03	−0.17	0.03
Eigenvalue	18.40		5.85	
Variance explained	63.44		20.18	
Cumulative variance	63.44		83.62	

Cluster analysis in PCA was performed using the K-means method, with the number of clusters determined using the Silhouette method, resulting in three clusters. The analysis grouped AVTCPK#1 and AVTCPK#12 in cluster I, AVTCPK#3, AVTCPK#8, AVTCPK#24, and AVTCPK#25 in cluster II, and AVTCPK#6 and AVTCPK#19 in cluster III. Genotypes AVTCPK#6 and AVTCPK#19 had higher contributions to principal component 1, while AVTCPK#1 and AVTCPK#12 had minimal contributions to both principal components. Genotypes AVTCPK#24 and AVTCPK#25 showed higher contributions in PCA 2, as shown in Figure 6. In addition, hierarchical clustering to group the genotypes based on the overall similarity of different traits using dendrogram is shown in Figure 7.

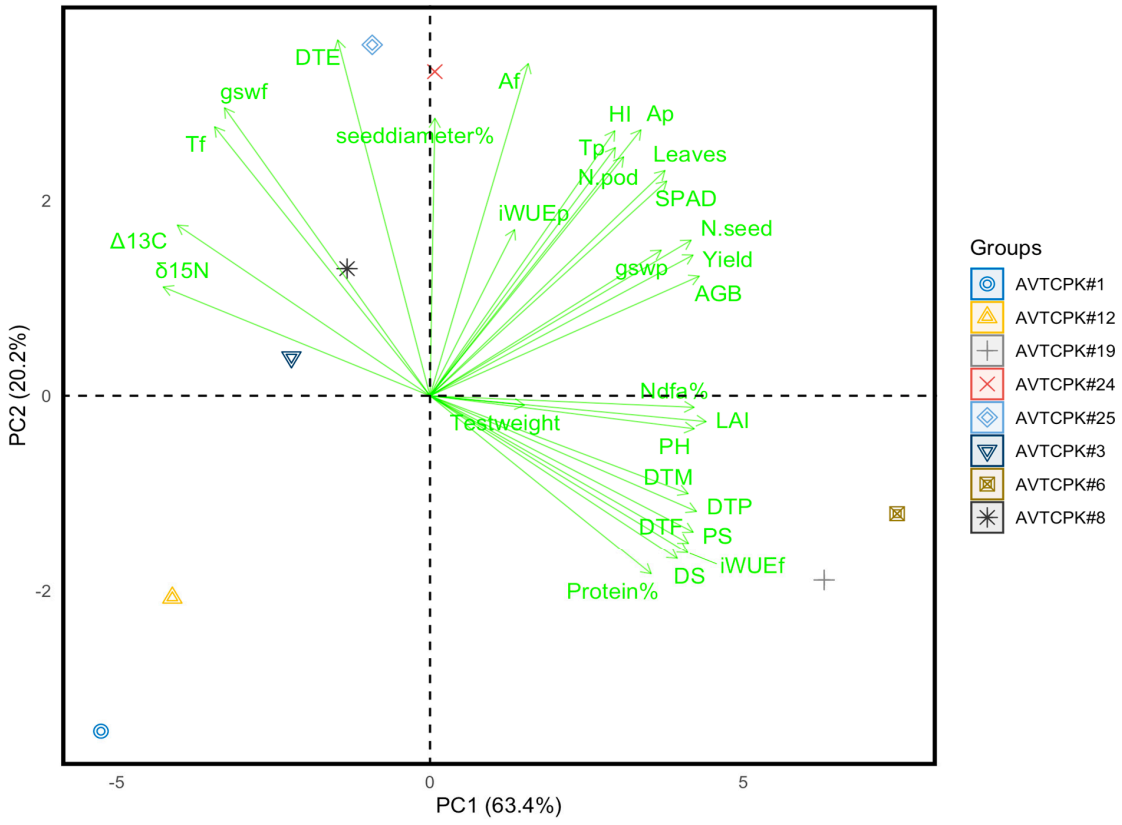

Figure 6. Standard PCA biplot of all traits with their loading vectors and grouping based on cluster analysis. Note: Yield (seed yield, g/m^2), PH (plant height), PS (number of primary shoots), Leaves

(number of leaves at 75 DAS), LAI75 (Leaf area index at 75 DAS), DTE (days to emergence), DTF (days to flowering), DTP (days to podding), DTM (days to maturity), SPAD (SPAD chlorophyll content at 75 DAS), Af (carbon assimilation rate at flowering, µmol $m^{-2}s^{-1}$)), Ap (carbon assimilation rate at podding stage, µmol $m^{-2}s^{-1}$), gswf (stomatal conductance at flowering stage, (mmol m^{-2} s^{-1})), gswp (stomatal conductance at podding stage, mmol m^{-2} s^{-1}), Tf (transpiration rate at flowering, mmol m^{-2} s^{-1}), Tp (transpiration rate at podding stage, mmol m^{-2} s^{-1}), iWUEf (intrinsic water-use efficiency at flowering stage, µmol $m^{-2}s^{-1}$/mmol m^{-2} s^{-1}), iWUEp (intrinsic water-use efficiency at podding stage), $\Delta^{13}C$ ($^{13/14}$carbon discrimination ratio), HI (harvest index), Npod (number of total pods/m^2), Nseed (number of seeds/m^2), DS (number of pods with double seed), Test weight (g), $\delta^{15}N$, Ndfa% (proportion of N derived from atmosphere), and Protein % (seed crude protein%).

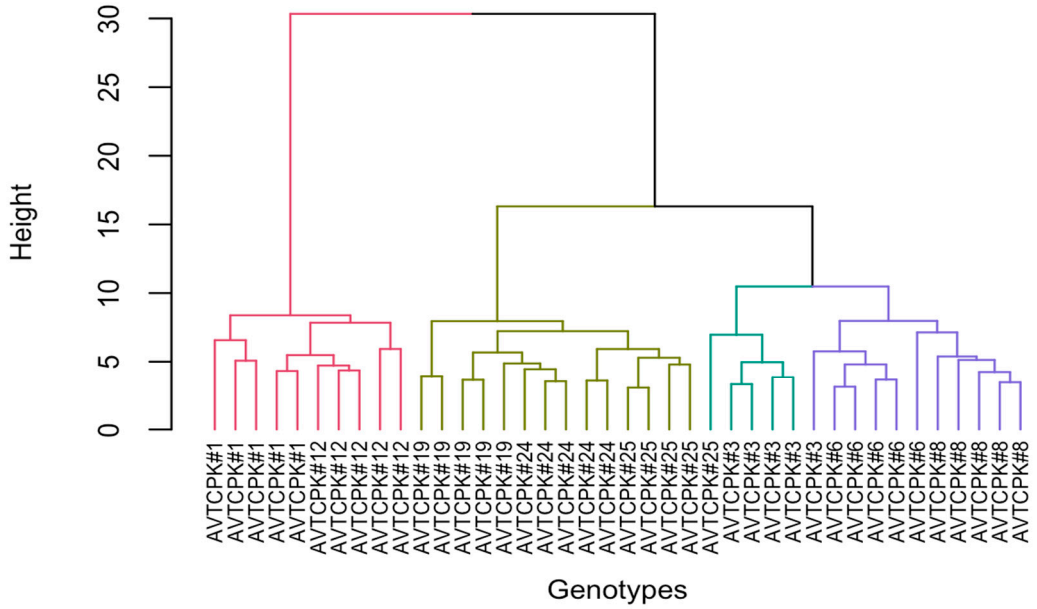

Figure 7. Dendrogram for eight genotypes in K-means method clustering analysis.

4. Discussion

Desi chickpea is the dominant chickpea type grown globally. Kabuli chickpea is traditionally grown in West Asia and the Mediterranean region. Its large seed size attracts popularity with a heavy premium not only for human consumption but also as ruminant feed [11]. Despite their common types, Kabuli chickpea has been reported to have more genetically diverse populations with greater genetic variations than Desi chickpea [6,35]. Beyond morphological and genetic differences, Kabuli chickpea possesses unique features. For instance, Kabuli chickpea is reported to have more primary shoots, better cold tolerance, and higher resistance to iron deficiency compared to the Desi type [36]. Furthermore, the nutritional values in terms of protein, non-fibrous carbohydrates, and soluble sugars for humans as well as ruminants are also higher in contrast to the Desi type [11]. It was previously believed that Kabuli chickpea could not adapt to warmer environments. However, with advancements in crop breeding, the adaptation of Kabuli chickpea has widened to tropical environments [6].

This study found considerable variation in the growth habits of eight Kabuli chickpea genotypes, as revealed by highly significant ($p \leq 0.01$) variations between the genotypes for contrasting phenological, morphological, and physiological traits, as well as yield, yield-attributing, and seed quality traits. The observed variability in the nature and genetic makeup of the evaluated genotypes indicates the potential to use the desirable traits under consideration for further improvement of Kabuli chickpea genotypes for heat tolerance, higher yield, quality, water-use efficiency, and higher nitrogen fixation.

4.1. Variation of Morphological/Phenological Traits

Plant phenology and morphological traits play a crucial role in the strategic adaptation of crops during stress. The days to emergence is an essential parameter that influences various plant traits [37]. A recent study conducted by Walia et al. [38] on chickpea genotypes showed variability in seed germination percentage, indicating that plants with early germination and higher germination rates exhibit early growth, maturity, greater biomass, and seed yield, aligning with our findings. In our study, AVTCPK#6 had 50% emergence in 6–7 days, which is 4–5 days earlier than other genotypes. Furthermore, the emergence of chickpea plays a great role in resource use efficiency and adaptation in rainfed production environments due to early adaptation to available moisture in the field [39].

Plant height is a crucial trait for coping with adverse environmental conditions. In our experiment, the plant height at harvest ranged from 48.6 to 74.7 cm. According to the GRDC [40], the average plant height of Australian Desi cultivars is 50–80 cm, which is similar to the maximum plant height observed in the tested AgriVentis genotypes, AVTCPK#6 and AVTCPK#19, both exhibiting an erect branching habit. However, at the early stage, these two genotypes showed the lowest plant heights (19.1 cm to 74.7 cm) with more primary shoots compared to other genotypes. This helps to cover the ground and minimize surface evaporation and later promotes height for pod formation and yield. Statistical variations were observed among the genotypes (Tables 2 and 3). Bukhari [37] also found significant variation in chickpea plant height. The correlation between plant height at later stages showed a significant relation to seed yield by 89% [37,41], also showing a strong positive correlation between plant height and seed yield.

While primary shoots in chickpea are profuse across all growth stages, the number of shoots in this study was highly significant among the genotypes with higher yields (AVTCPK#6 and AVTCPK#19). Similar genotypic variation in primary shoots was reported by Bukhari [37] in chickpea. The correlation between primary shoots at harvest contributed 77% to seed yield.

The radiation-use efficiency of crops is governed by leaf shape and leaf area index (LAI). Optimum LAI results in greater photosynthetic gains. At early growth stages, larger LAI does not yield significant gains due to greater transpiration loss. However, at advanced growth stages, greater LAI is imperative for higher photosynthetic gains and yield formation [39]. Our results showed that the LAI of AVTCPK#6 and AVTCPK#19 was higher at later stages compared to earlier stages. In contrast, other genotypes had higher LAI at early stages but lower at later stages. The correlation between LAI at 75 DAS and seed yield was 91%. Prior to flowering, both leaf-type genotypes showed slow development in the leaf area until 45 DAS. However, later, the normal (fern shape) genotype exhibited a noticeable increase in LAI, indicating greater biomass production. This aligns with the findings from Abbo et al. [42], who observe a similar trend in five Kabuli chickpea progeny genotypes with simple leaves (G1 and G3) and fern-shaped leaves (G2, G4, and Zehavit).

Abbo et al. [42] noted that at the early stage of plant growth, until 40 DAE, LAI was comparable among genotypes at around 1 m^2 leaf/m^2 soil, but it increased rapidly to around 6.5 m^2 leaf/m^2 soil at 60 DAE. Initially, simple leaf progeny had higher LAI, but as the crop matured, fern-shaped progeny achieved a higher LAI. A significant positive relation (0.9) has been observed between LAI and seed yield (Figure 5).

4.2. Variation of Reproductive Traits

The days of flowering, pod formation, and maturity time are important factors for achieving higher seed yield and are critical stages for the selection of heat-tolerant genotypes [43]. In crops, early flowering and maturity are considered advantageous traits for warmer environments with rainfed chickpea cultivation. In our experiment, genotypes AVTCPK#1, AVTCPK#3, AVTCPK#8, AVTCPK#12, AVTCPK#24, and AVTCPK#25 required almost similar GDDs, while AVTCPK#6 and AVTCPK#19 required comparatively higher GDDs. A similar variation in GDDs among three chickpea genotypes has been reported by Eshan [29] in the recent trial conducted at Ishwari, Bangladesh, in a subtropical climate. The

average cumulative GDD values varied among the three chickpea genotypes from sowing to germination (80.6–132), germination to flower initiation (491.7–950.3), flower initiation to 100% flowering (84.6–230), 100% flowering to physiological maturity (570.2–1146.5), physiological maturity to harvest maturity (72.7–311.7), and in total was 1743.4- 2145.5-degree days at a base temperature (10 °C).

Heat energy significantly influences the photosynthetic ability of a plant [44]. The heat units required for the genotype until maturity ranged from 1307.9 to 1582.2 °C in the eight genotypes. Genotypes AVTCPK#6 and AVTCPK#19 showed higher cumulative GDDs overall until maturity, with longer days to flowering from emergence. Higher GDDs at the vegetative stage enhance crop growth in terms of plant height and biomass. However, GDDs from flowering to the day to podding were lower for AVTCPK#6 (138.9) and AVTCPK#19 (100.2) compared to other genotypes. Shorter GDDs from flowering to podding in these two genotypes (AVTCPK#6 and AVTCPK#19) reduce the flower and pod abortion, resulting in a higher harvest index and leading to higher yield [45].

Although, the growing period of all eight genotypes used in this experiment extended from 96 to 105 days, those flowering later and maturing later had higher yields as compared to others. The correlation among these components had a positive impact on the seed yield, with flowering days, podding, and maturity influencing seed yield by 74.0%, 79.8%, and 78.1%, respectively.

4.3. Variation of Physiological Traits

The SPAD chlorophyll meter measures the relative greenness of leaves, reflecting their chlorophyll content [46,47]. Studies in plants, such as tomato [46] and coffee [47], have reported that SPAD values correlate with the chlorophyll content, indicating that higher SPAD values correspond to higher chlorophyll levels. Furthermore, this study found a significant positive correlation (0.48) between SPAD value and protein content, consistent with the findings from Netto A.T [47]. Significant relation of SPAD chlorophyll content with protein is related to the nitrogen content of leaf. The nitrogen content is the major component of the chlorophyll molecular structure. Higher chlorophyll content indicates higher nitrogen and protein content in plants [46].

AgriVentis genotypes showed SPAD values ranging from 49 to 56 (Figure 3). These variations align with the findings of Kshiwagi K. and Upadhyaya [48], where SPAD readings ranged from 45 to 63 in six genotypes (ICC3077, ICCV2, ICC4958, Annigeri, ICC16374, and ICC15888). The SPAD readings were higher in genotypes during the early stages of plant growth and decreased gradually with maturity and senescence [48]. Genotypes AVTCPK#6 and AVTCPK#19 had lower SPAD values initially but higher at the later stage of growth. Other genotypes consistently had higher chlorophyll content at earlier stages, with a reduction at later stages.

Tsialtas et al. [49] suggested that the differences in stomatal conductance and leaf chlorophyll content contribute significantly to the variations in photosynthetic rates. Similarly, leaf thickness and the photosynthetic apparatus per unit area also affect photosynthetic capacity. From our study, significant variations in CO_2 assimilation rate (A_{sat}) among genotypes were observed only during the podding stage. The rate of leaf net photosynthesis in our study ranged from 27 to 31 µmol $m^{-2}s^{-1}$, which is similar to the values reported by Pang [50] for two chickpea genotypes, DICC8156 and DICC8172, under well-watered conditions. Additionally, Pappula-Reddy [51] reported leaf net photosynthesis in a similar range.

Poursemael et al. [52] evaluated six Iranian chickpea genotypes, reporting stomatal conductance values between 115 and 363 mmol $m^{-2}s^{-1}$, which are slightly lower than those reported in our experiment. In our study, genotypes AVTCPK#6 and AVTCPK#19 showed the least stomatal conductance at flowering stages, accompanied by low carbon assimilation rates and transpiration. However, these genotypes showed higher stomatal conductance at the pod formation stage. Decreased stomatal conductance at the flowering stage can improve yield stability by conserving soil water resources [53], which can be

further used by the plant during an increase in temperature to maintain leaf water status and stomatal conductance [54].

Chickpea transpiration is 72% higher than that of the other beans [53]. A study by Rahbarian et al. [55] on four chickpea genotypes in a growth chamber reported variations in transpiration rate and iWUE across growth stages. Transpiration rates ranged from 3 to 7 mmol m^{-2} s^{-1} at the seedling stage, from 1 to 3 mmol $m^{-2}s^{-1}$ at the flowering stage, and from 2 to 6 mmol m^{-2} s^{-1} at the podding stage. The iWUE values ranged from 5.6 to 30 mmol m^{-2} s^{-1} at the flowering stage and 1.9 to 29 µmol m^{-2} s^{-1} at the podding stage. In our study, the transpiration rate was higher than those reported by Rahbarian et al. [55], likely due to higher stomatal conductance, while iWUE was within but on the lower end of the reported range. Lower transpiration helps conserve leaf water by reducing its loss. Higher transpiration rates can lead to energy loss required for adjusting leaf water potential, affecting the photosynthate sink development. Our results showed that AVTCPK#6 and AVTCPK#19 had lower transpiration losses during flowering compared to other genotypes, and all genotypes exhibited lower transpiration losses at the pod-formation stage than at flowering time. Adjusting stomatal conductance and transpiration is crucial to maintain leaf water potential and protect the plant from leaf damage and senescence in chickpea [43,54]. There is a significant relationship between iWUE and both stomatal regulation and transpiration loss. Higher iWUE, particularly in AVTCPK#6 and AVTCPK#19 during flowering and pod formation stages, supports successful embryo development and seed yield.

The range of differences among the genotypes for the $\Delta^{13}C$ value in this study was 3.45%, similar to the 2.7% range found by Lakshmanan Krishnamurthy et al. [56] in 280 chickpea cultivars at ICRISAT-Patancheru, India. This range also aligns with findings from Kashiwagi et al. [57] in a pot experiment on ten chickpea genotypes that included both Desi and Kabuli in Andhra Pradesh, India.

Table 6 shows the variation in $\Delta^{13}C$ values among genotypes. This variation is consistent with Wallace et al. [58], who reported differences in $\Delta^{13}C$ values among plant species (wheat and lentil) and genotypes grown under the same conditions. Genotypes AVTCPK#6 and AVTCPK#19 had the lowest carbon isotope values, indicating less discrimination between the heavy and lighter isotopes. Carbon isotope discrimination occurs during carbon diffusion from stomata and Rubisco carboxylation, resulting in lower $\Delta^{13}C$ values in dry biomass [19].

A strong negative correlation (−0.8) was observed between the carbon isotope discrimination and above-ground biomass, similar to the earlier findings [56] in field-evaluated chickpea cultivars. Additionally, a strong and significant negative correlation (−0.9) was found between the carbon isotope discrimination and iWUE at the flowering stage, while a non-significant negative correlation (−0.3) was observed at the podding stage. Similar negative correlations have been reported by Raeini-Sarjaz et al. [59] in bush bean.

4.4. Variation in Yield and Yield-Attributing Traits in Genotypes

Introducing a well-adaptive, higher-yielding chickpea genotype to farmers is a proven strategy for increasing productivity. Table 7 shows that the AVTCPK#6 genotype has the highest seed yield, followed closely by AVTCPK#19. This higher yield can be attributed to key traits of these chickpea genotypes, including the number of pods, seeds, and above-ground biomass. The yields from these genotypes are higher than those reported by Graham et al. [60] in NSW, Australia, where three Kabuli chickpea varieties (PBA Royal, Genesis 090, and PBA Magnus) produced grain yields of 2.4, 2.3, and 1.6 ton/ha, respectively, at a plant density of 34.5–37 plants/m^2; the seed test weights were 29.2, 26.1, and 42.6 g/100 seeds, respectively. Significant positive associations were observed between seed yield and yield-attributing characters, such as the number of pods (0.8), number of seeds (0.9), and harvest index (0.9). Similar findings were reported by Kumar et al. [61] in research conducted on sixty-four genotypes of chickpeas at the Food Legume Research Platform-ICARDA, Amlaha, India.

Seed size in Kabuli chickpeas is an important characteristic that determines market prices. A study by Gaur et al. [7] on breeding extra-large seeds in India reported an increasing interest in large-seeded Kabuli genotypes (>50 g 100 seeds); twelve Kabuli chickpea genotypes with two different leaf types (pinnate and simple) exhibited 100-seed masses ranging from 50 to 63.2 g, with ICC17109 showing the highest test weight at 63 g/100 seeds. In our experiment, all the tested genotypes had 100-seed weights exceeding 50 g, with AVTCPK#24, AVTCPK#8, and AVTCPK#3 showing weights greater than 60 g/100 seeds. In contrast, genotype AVTCPK#19 had a comparatively small seed size, with only 17% of seeds in the 10–11 mm range and a lower 100-seed weight, followed by AVTCPK#1 and AVTCPK#12. A positive correlation was observed between the seed yield and both seed test weight (0.4) and seed diameter (0.2). Similar findings were reported for 36 chickpea genotypes in Peshawar, Pakistan [37].

4.5. Proximate Nutrient Composition

High-protein legumes are increasingly sought after by health food markets to assist in maintaining a balanced diet. Therefore, identifying the nutritional characteristics of different genotypes is essential for their effective utilization in breeding programs aimed at improving quality [62]. Significant differences in protein content were observed among the genotypes. Genotype AVTCPK#6 showed the highest protein content, closely followed by AVTCPK#19, while AVTCPK#1 had the lowest. These results align with Chatur [63], which found significant differences in protein content among five Austrian genotypes, with the lowest being 18.1% in Kimberley Large and the highest at 24.5% in Genesis Kalkee. The protein content of the tested genotypes is comparable to chickpea genotypes grown in Syria, Canada, and India, which range from 17.1% to 19.8% [64]. However, Johnson et al. [65] reported a protein content of up to 29.2% in the kernels of five different Desi chickpea varieties grown in Australia, approximately 52% higher than AVTCPK#6. This highlights the variability of protein content among chickpea genotypes, as noted by Frimpong et al. [64], which can also depend on geographical location, environmental factors, and the analysis methods adopted by researchers. A significant positive relationship was observed between the seed protein content percentage and the Ndfa% value (Figure 5).

4.6. Variation in Nitrogen Fixing Ability

The primary challenge to increasing crop yield in the northern Australian tropical environment is associated with depleting nitrogen fertility. Identifying genotypes that can contribute a larger amount of symbiotic N in grain legumes while producing increased grain yield is an important focus for breeders [66]. Evaluating the responses of eight Kabuli chickpea genotypes for symbiotic N performance revealed clear genotypic differences. The data revealed considerable variation in nodule numbers, $\delta^{15}N_{leaf}$, and %Ndfa. In this study, $\delta^{15}N_{leaf}$ varied considerably, ranging from 2.7 in AVTCPK#1 to −0.793 in AVTCPK#6. A lower $\delta^{15}N_{leaf}$ value indicates greater nitrogen fixation [22]. Genotypes with the lowest $\delta^{15}N_{leaf}$ values also exhibited higher %Ndfa, with AVTCPK#6 showing the highest at 72.9%, followed by AVTCPK#19 (62.9%), AVTCPK#24, AVTCPK#8, AVTCPK#25, AVTCPK#3, AVTCPK#12, and AVTCPK#1 (Table 9). Genotypes AVTCPK#6 and AVTCPK#19 showed higher nodule counts and greater nitrogen fixation capabilities. These results are consistent with the findings from Kyei-Boahen, Slinkard [67], who studied $\delta^{15}N_{leaf}$ in Desi and Kabuli chickpeas inoculated with different strains at the flowering stage, reporting $\delta^{15}N_{leaf}$ values ranging from −2.8 to 0.1. Similarly, Belane et al. [22] assessed the symbiotic contribution of 32 cowpea genotypes grown in field conditions at Taung, South Africa, reporting $\delta^{15}N_{leaf}$ values between 66.7% and −21.0%. In this study, Ndfa% and seed yield were significantly correlated (0.88) with yield (Figure 5).

5. Conclusions

This research evaluated morphological, phenological, and physiological traits associated with yield and seed-quality attributes of new Kabuli chickpea genotypes in a tropical

growing environment. The results showed large phenotypic variation among the tested genotypes. Chickpea AVTCPK#6 and AVTCPK#19 were late maturing but showed significantly higher seed yield, linked with greater intrinsic water-use efficiency (iWUE), measured at both the flowering and pod-filling stages. The lower carbon discrimination by leaf tissue that represents the cumulative season-long water-use efficiency was also significantly lower for AVTCPK#6 and AVTCPK#19, suggesting a greater stomatal regulation for carbon assimilation, conferring heat and drought tolerance characteristics to these genotypes, which could be very favorable trait for the selection of genotypes for warmer environments. Furthermore, an inverse relation has been expressed for carbon isotope discrimination with iWUE and seed yield. In terms of seed size, maximum number of seeds with a greater seed test weight (>60 g/100 seed) was observed for AVTCPK#24, AVTCPK#8, and AVTCPK#3. Similarly, larger seeds with diameter >10–11 mm were recorded for AVTCPK#24 (45%). Furthermore, higher %Ndfa in AVTCPK#6, followed by AVTCPK#19, indicated greater atmospheric nitrogen fixation capacity by these genotypes. A positive correlation was observed between %Ndfa and the protein content in seeds, with higher protein (%) found on AVTCPK#6, followed by AVTCPK#19. Similarly, %Ndfa was positively corelated to the seed yield. These findings highlight the genetic variability among the tested genotypes and suggest the use of these high-yielding genotypes for production in tropics and utilizing their desirable traits for breeding Kabuli chickpeas for further improvement of yield, water-use efficiency, and greater N fixation under the tropical production environments.

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