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Leveraging High-Throughput Phenomics and Morpho-Physiological Traits for Selecting Drought-Tolerant Pigeonpea [*Cajanus cajan* (L.) Millspaugh] Genotypes

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Abstract

Drought stress is a significant global environmental challenge that threatens food security, affecting ~ 66% of arable land and resulting in economic losses estimated at USD 37 billion, with further increases anticipated due to climate change. *Cajanus cajan* (pigeonpea), a widely cultivated grain legume in the semi-arid tropics, often experiences 40–50% yield reduction under drought. Understanding the genetic basis of drought tolerance is critical for selecting diverse parental lines and designing effective crop improvement programs. In this study, 47 pigeonpea genotypes were evaluated under well-watered (WW; 60–70% field capacity) and drought-stressed (DS; 18–20% field capacity) conditions, with drought imposed by withholding irrigation from 80 to 120 days after sowing, corresponding to the flowering to pod-setting stage, in a controlled greenhouse. Drought stress significantly reduced most morpho-physiological traits (2.8–68.8%), while vapour pressure deficit and proline accumulation increased by 1–1.5-fold. High-throughput phenomic traits, including digital area (projected shoot area), convex hull area (CHA), calliper length (CL), and near-infrared reflectance (NIR), effectively distinguished drought-tolerant from sensitive genotypes. Nine genotypes, *viz.*, ICPX140196-B-1, ICPX140203-B-1, ICPX140213-B-3, ICPX140203-B-1-5, ICPX140203-B-2, GRG-152, ICPX140205-B-4, ICPX140217-B-1, and ICPX140188-B-3, were identified as drought-tolerant, exhibiting superior photosynthetic efficiency (Net CO_2 assimilation rate), favourable tissue water status, and minimal grain yield reduction (< 5%). Notably, leaf temperature was negatively associated with grain yield. Moreover, photosynthetic parameters showed significant associations with grain yield under stress and exhibited high genetic variability (10–46%) with moderate heritability, highlighting their utility as selection indices. These findings demonstrate the potential of integrating high-throughput phenomics with conventional morpho-physiological traits for the rapid and precise identification of drought-tolerant pigeonpea genotypes, providing insights for introgression breeding and genetic enhancement programs aimed at improving drought tolerance.

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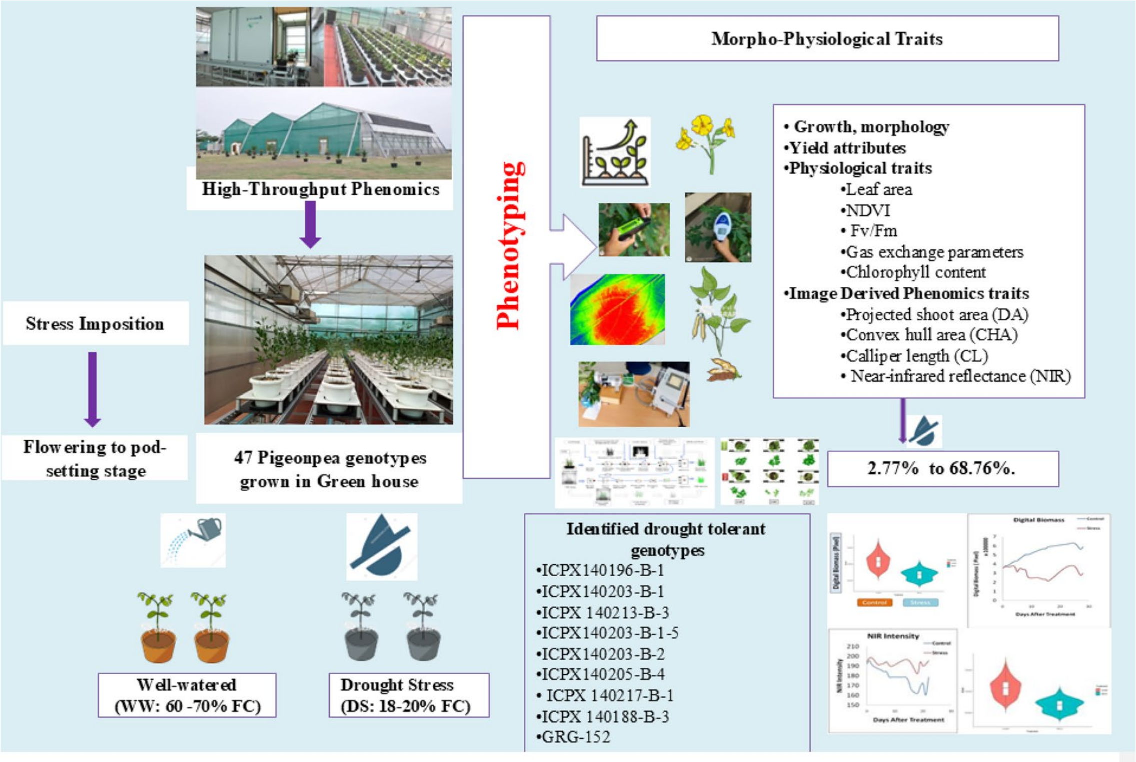
Graphical Abstract



Keywords Abiotic stress · Genotype selection · Phenotyping · Pigeonpea · Pulses · Sensors

Introduction

Climate change significantly impacts agricultural productivity by exposing crops to multiple abiotic stresses, including drought, salinity, and high temperatures, thereby threatening global food security (Abdelaal et al. 2020; Farouk et al. 2024). Drought, in particular, is intensified by shifts in the hydrological cycle and the increasing frequency of extreme weather events, leading to prolonged dry spells (IPCC 2021). Currently, ~ 66% of the world's arable land is affected by drought, a trend expected to worsen with ongoing climate change (Ullah et al. 2022). Consequently, global crop yields have declined by nearly 50% due to water deficits, while a rising population, projected to exceed 9 billion by 2050, further intensifies pressure on food systems (Lamaoui et al. 2018; Bonaccorsi et al. 2020). This escalating demand challenges progress toward achieving Sustainable Development Goal 2: Zero Hunger (Sporchia et al. 2024).

Among crops critical for semi-arid regions, *Cajanus cajan* (L.) Millspaugh, also known as pigeonpea, is a major legume often exposed to drought-induced yield losses. Water deficit during key growth stages, particularly the reproductive phase, reduces photosynthesis, disrupts water relations, and limits biomass accumulation, resulting in yield reductions of 40–55%, compared to 11–15% during vegetative stress (Nam et al. 2001; Lopez et al. 1996). Despite cultivation across over 5 million hectares, yielding 4.5 million tons of dry grain, global pigeonpea productivity remains low, at ~814 kg ha^{-1} (FAOSTAT, 2023). These losses threaten the livelihoods of millions who rely on pigeonpea as a primary source of protein, contributing up to 25% of daily dietary protein in rural India and Sub-Saharan Africa (Kimaro et al. 2020; Tapal et al. 2019).

Addressing these yield limitations requires identifying resilient varieties with high nutritional and economic value. Pigeonpea is consumed as green pods or seeds, while dry seeds are incorporated into staple legume dishes, such as dhal in India (Tapal et al. 2019). Production has increased in South and Central America since 2010, and interest from European countries has also grown due to its amino acid profile and protein quality (Mula et al. 2010; Locali-Pereira et al. 2023). However, despite the development of high-yielding varieties, average yields remain suboptimal due to genetic, agronomic, and environmental constraints, with drought remaining a major limiting factor (Kimaro et al. 2020).

Drought stress triggers multiple morpho-physiological, biochemical, and molecular responses, including increased reactive oxygen species (ROS) production, lipid peroxidation, membrane damage, and impaired photosynthesis and gas exchange, ultimately reducing growth and yields (Kar 2011; Farooq et al. 2014; Shelake et al. 2022). It also restricts stomatal conductance, leaf area, stem elongation, and root growth, thereby affecting water-use efficiency (Ali et al. 2022). Consequently, developing drought-tolerant pigeonpea cultivars is critical for ensuring food and nutritional security. Identifying suitable donor genotypes is a crucial step in breeding programs aimed at enhancing drought resilience.

The success of such programs depends on genetic variability, which underpins the selection and improvement of desirable traits (Ouma et al. 2024). Conventional phenotyping methods, while informative, are labour-intensive, destructive, and time-consuming. High-throughput phenotyping (HTP) offers a rapid, non-destructive method for evaluating multiple genotypes simultaneously, facilitating the early detection of physiological responses to drought (Kim et al. 2020; Moustakas et al. 2022). While HTP has been successfully applied to crops such as mungbean and chickpea (Rane et al. 2021; Pappula-Reddy et al. 2024), its use in pigeonpea remains limited.

In this context, therefore, we hypothesise that drought stress during critical growth stages, particularly the reproductive phase, significantly impacts pigeonpea growth, morpho-physiology, and yield, and that high-throughput phenotyping can effectively identify tolerant genotypes. To test this hypothesis, the present study was undertaken with two main objectives: (i) to identify drought-tolerant pigeonpea genotypes by integrating high-throughput phenomics with key morpho-physiological and yield traits, and (ii) to determine the most informative traits that explain genotypic variation in drought tolerance and support sustained productivity under water-limited conditions.

Materials and Methods

Plant Materials The study evaluated 47 pigeonpea genotypes obtained from the University of Agricultural Sciences, Raichur (UAS, Raichur). These genotypes represent a diverse collection from multiple agroecological zones across India, including germplasm accessions from the Indian National Gene Bank and stable advanced breeding lines from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, and UAS, Raichur. Detailed information on the genotypes is provided in Table 1.

Plant Growth Conditions The experiment was conducted in a controlled greenhouse (Allice Biotechnology®) at the ICAR–National Institute of Abiotic Stress Management, Baramati, India (18° 09' 30.62" N, 74° 30' 03.08" E; 570 m above sea level) during the 2023–24 wet season. Greenhouse conditions were maintained at 32/24°C (day/night), with relative humidity of 60–70% and photosynthetically active radiation (PAR) between 450 and 750 μmol m^{-2} s^{-1}.

A factorial experimental design was employed with two factors: Factor 1 – Water availability, including well-watered (WW) and drought-stress (DS) treatments; Factor 2 – Genotypes. The experiment was arranged in a completely randomised block design (CRBD) with three biological replicates per treatment. Each genotype was sown in plastic pots (25 cm diameter × 20 cm height) filled with 17 kg of a soil mixture of medium black soil and farmyard manure (FYM) in a 50:1 (v/v) ratio. Five seeds were initially sown per pot at a depth of 17–20 mm, and after one week, seedlings were thinned to retain a single healthy plant per pot. Fertilisers were applied at a recommended rate of 25:50:25 kg ha^{-1} in the form of urea (46% N), di-ammonium phosphate (DAP, 18% N and 46% P_2O_5), and muriate of potash (MOP, 60% K_2O), adjusted according to the soil volume. For each biological replicate, three technical replicates were maintained to ensure accurate measurements and minimise experimental variability.

Stress Imposition

Genotypes in the control treatment (well-watered, WW) were irrigated as needed to maintain soil moisture at 60–70% of field capacity (FC; 28–32% soil moisture). In contrast, the drought-stress (DS) treatment was imposed by withholding water from 80 to 120 days after sowing (DAS), covering the flowering to pod-setting stage. During this 40-day stress period, soil moisture was maintained at 17–19%, which is well above the permanent wilting point (< 15%), and monitored weekly using the gravimetric method (Reynolds 1970). After 120 DAS, DS plants were rewatered and allowed to mature (Fig. 1).

High Throughput Phenotyping

Image Acquisition Plants were meticulously imaged using the Scanalyzer 3D system (LemnaTec GmbH, Aachen, Germany) to capture both visible and near-infrared (NIR) images. Imaging was carried out at 5-day intervals, a total of seven times during the stress period (80–120 days after

Table 1 List of Pigeonpea genotypes and their source used in the present study

Sr. No	Name	Source of seed material
1	CORG-9701	ZARS Kalaburagi
2	EC-843239	ICAR-NBPGR New Delhi
3	GC-11–39	ZARS Kalaburagi
4	GRG-152	ZARS Kalaburagi
5	GRG-811	ZARS Kalaburagi
6	IC-405218	ICAR-NBPGR New Delhi
7	IC-73882	ICAR-NBPGR New Delhi
8	IC-73898	ICAR-NBPGR New Delhi
9	IC-73926	ICAR-NBPGR New Delhi
10	IC-73952	ICAR-NBPGR New Delhi
11	IC-73959	ICAR-NBPGR New Delhi
12	IC-73961	ICAR-NBPGR New Delhi
13	IC-73969	ICAR-NBPGR New Delhi
14	IC-73975	ICAR-NBPGR New Delhi
15	IC-73993	ICAR-NBPGR New Delhi
16	IC-73995	ICAR-NBPGR New Delhi
17	IC-74013	ICAR-NBPGR New Delhi
18	IC-74058	ICAR-NBPGR New Delhi
19	ICPL-87	ICRISAT Hyderabad
20	ICPX 140188-B-3	ICRISAT Hyderabad & ZARS Kalaburagi
21	ICPX 140196-B-1	ICRISAT Hyderabad & ZARS Kalaburagi
22	ICPX 140203-B-1	ICRISAT Hyderabad & ZARS Kalaburagi
23	ICPX 140203-B-1–5	ICRISAT Hyderabad & ZARS Kalaburagi
24	ICPX 140203-B-2	ICRISAT Hyderabad & ZARS Kalaburagi
25	ICPX 140205-B-4	ICRISAT Hyderabad & ZARS Kalaburagi
26	ICPX 140213-B-3	ICRISAT Hyderabad & ZARS Kalaburagi
27	ICPX 140217-B-1	ICRISAT Hyderabad & ZARS Kalaburagi
28	KRG-33	ZARS Kalaburagi
29	NAM-2085	ICRISAT Hyderabad & ZARS Kalaburagi
30	NAM-2151	ICRISAT Hyderabad & ZARS Kalaburagi
31	NAM-2216	ICRISAT Hyderabad & ZARS Kalaburagi
32	NAM-2217	ICRISAT Hyderabad & ZARS Kalaburagi
33	NAM-2282	ICRISAT Hyderabad & ZARS Kalaburagi
34	NAM-2284	ICRISAT Hyderabad & ZARS Kalaburagi
35	NAM-2292	ICRISAT Hyderabad & ZARS Kalaburagi
36	NAM-2314	ICRISAT Hyderabad & ZARS Kalaburagi
37	NAM-2329	ICRISAT Hyderabad & ZARS Kalaburagi
38	NAM-34	ICRISAT Hyderabad & ZARS Kalaburagi
39	NAM-88	ICRISAT Hyderabad & ZARS Kalaburagi
40	Phule Tur	MPKV, Rahuri
41	PKV Tara	PDKV, Akola
42	PT-12–19-2	VNMKV, Parbhani
43	Rajeshwari	MPKV, Rahuri
44	TDRG-272	ARS Tandur
45	TS-3R	ZARS Kalaburagi
46	Vipula	MPKV, Rahuri
47	WRGE-327 X ICPL-15,028	ICRISAT Hyderabad

sowing). High-resolution five-megapixel colour images were obtained in the visible spectrum (400–700 nm) from top and side perspectives (Supplementary Fig. 1). Side-view images were captured at 0° and 90° angles using CCD cameras (piA2400-17gc; Basler, Ahrensburg, Germany). Multi-view images (top and side views) were captured for each plant using the HTP platform. For all traits derived from these images (e.g., digital area (DA), convex hull area (CHA), calliper length (CL), NIR reflectance), the final value reported is the sum of the measurements obtained from all views. This approach ensures a comprehensive

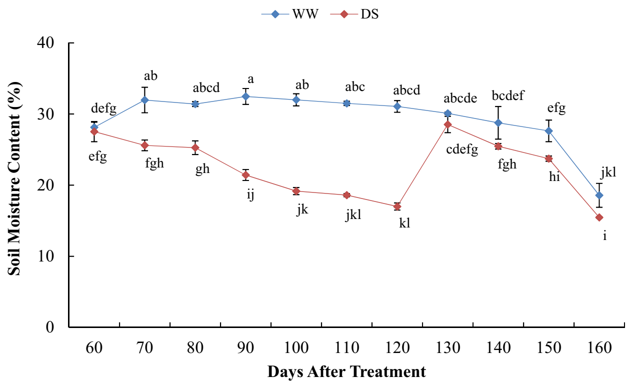


Fig. 1 Soil moisture percentage in pigeonpea under well-watered (WW) and drought-stressed (DS) conditions from 60 days after sowing until harvest, measured gravimetrically. Error bars represent the standard error of the mean (± SE). Significance in soil moisture content (%) under both the conditions – WW and DS, tested at $p = 0.05\%$

integration of plant size, architecture, and canopy characteristics across all captured perspectives.

The NIR-600PGE camera (Allied Vision Technologies GmbH, Stadtroda, Germany) captured the same views for NIR imaging. With a spectral sensitivity range of 900–1700 nm and an optical resolution of 320 × 256 pixels, this camera enabled precise detection of water content in plant shoots via the 1450 nm water absorption band (Seelig et al. 2009).

To address the challenges of grayscale NIR images, segmentation masks from RGB images were overlaid to analyse mean grey values (on an 8-bit scale). Higher values indicated greater reflectance and lower water content, whereas lower values indicated greater absorption and higher water content. This imaging approach offers valuable insights into plant health and water status.

Image Analysis and Data Mining Image analysis was performed using LemnaGrid software (LemnaTec GmbH, Würselen, Germany). To ensure optimal plant segmentation from the background, a region of interest was defined that captured the entire plant while excluding visible imaging hardware, such as the lifter and turner. Segmentation was achieved using a nearest-neighbour colour classification approach, followed by erosion and dilation processes to eliminate noise. All plant-identified segments were then combined into a single object. The extracted image data were subsequently processed and organised into a data table containing various image parameters using the Data Miner software (LemnaTec GmbH, Würselen, Germany). The workflow of image processing was provided in the Fig. 2.

We generated the data on more than 15 different parameters (Supplementary Fig. 2) for each of the plants' three acquired images (top and two side views). However, we present four different parameters that clearly distinguish treatment effects and genotypic responses to water levels.

Morpho-Physiological Trait Measurement

Growth and morphology (measured at harvest/post-harvest): Days to 50% flowering (DFF) was recorded on a whole-plot basis at flowering time. Plant height (PH; cm), number of primary branches (PB), number of secondary branches per plant (SB), pods per plant (PP), seeds per pod (SPP), 100-seed weight (TW) and grain yield per plant (GYPP) were measured at harvest and post-harvest.

Physiological Parameters The following physiological and photosynthetic parameters were measured during the drought stress period, from 115 to 120 DAS.

Leaf area (LA; cm^2 per leaf) was measured non-destructively using a LI-3000 C Portable Leaf Area Meter (LI-COR®) following Kekere and Omoniyi (2016).

The Normalized Difference Vegetation Index (NDVI) was recorded using a GreenSeeker® handheld optical sensor, according to the protocol of Govaerts and Verhulst (2010), and calculated as:

$$NDVI = (NIR - IR)/(NIR + IR)$$

where NIR is near-infrared reflectance, and IR is infrared reflectance.

PS-II efficiency (quantum yield, Qmax = Fv/Fm) was determined using a real-time chlorophyll fluorescence imaging system (FluorCam FC 1000-H®, Photon Systems Instruments).

$$Qmax = Fv/Fm$$

where Fv = Fm-F0. Fv is variable fluorescence, F0 is ground fluorescence, and Fm is maximum fluorescence.

Gas exchange parameters: Leaf temperature (Tleaf; °C), Vapour Pressure Deficit (VPD; Pa/kPa), transpiration rate (E; $\text{mmolm}^{-2}\text{s}^{-1}$), and net CO_2 assimilation rate (A; $\mu\text{mol m}^{-2}\text{s}^{-1}$) were measured between 09:00 and 11:30 AM using a portable gas exchange system (GFS-3000®, WALZ, Germany) following Diao et al. (2024).

Chlorophyll content was assessed using a SPAD-502® chlorophyll meter. Relative water content (RWC; %) was estimated following the Weatherley (1950) method. Membrane stability index (MSI; %) was determined according to Sairam et al. (1997). Proline content ($\mu\text{mol g}^{-1}$ FW) was quantified spectrophotometrically following Bates et al. (1973).

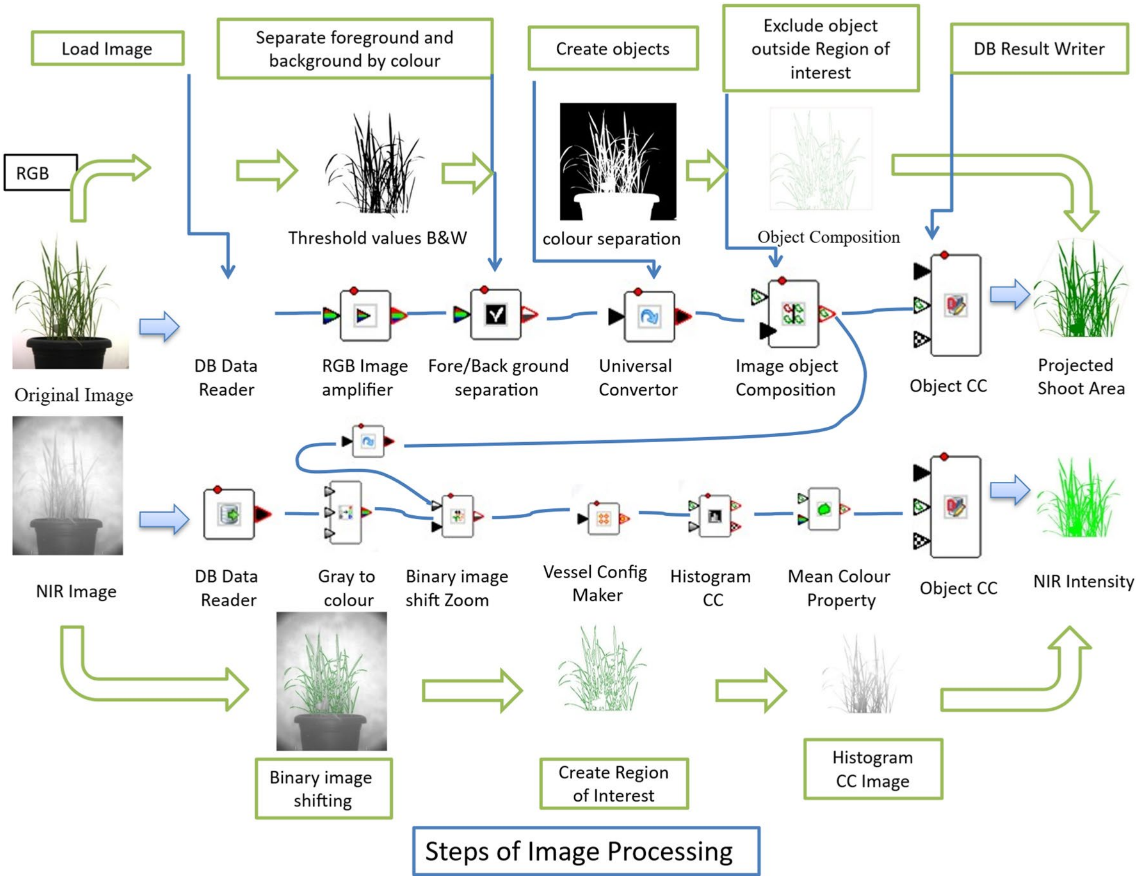


Fig. 2 Steps involved in loading images and generating data using the LemnaGrid software of LemnaTec

Statistical Analysis

Analysis of variance was conducted using SAS 9.3 statistical software (Cary 2011). Variance components for genotypes ($\sigma^2 g$) and error ($\sigma^2 e$) were estimated, and broad-sense heritability ($H^2(b)$) was calculated as:

$$\mathrm{Hb} = \sigma^2\mathrm{G}/(\sigma^2\mathrm{G} + \sigma^2\mathrm{E}).$$

where $\sigma^2 g$ is genotypic variance, and $\sigma^2\mathrm{E}$ is error variance.

Genetic parameters, including genotypic variance (σ^2_G), phenotypic variance (σ^2_P), and error variance (σ^2_E), were determined for each trait. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated according to Burton and De Vane (1953). $H^2(b)$ was also cross-validated using R software (Version 4.4.2) with the variability package.

Furthermore, a correlation analysis was performed between grain yield per plant (GYPP) and other morpho-physiological traits using R (Version 4.4.2). A correlation matrix was computed using the *cor* function and visualised with the corrplot package to illustrate the relationships among traits.

Results

Phenotyping of Pigeonpea Genotypes Under WW and DS Conditions

Analysis of Variance (ANOVA)

The results of the ANOVA revealed significant variances for all the trait studies, for both factors (genotypes and water treatment). Further, the interaction between genotypes and water treatments was also significant (Tables 2 and 3). The results indicate that both genetic and environmental factors influence the expression of genotypes, water treatment, and their interaction in all traits. The study demonstrated that the performance of genotypes varied under prolonged water-stress conditions.

Growth, Morphology, and Yield of Pigeonpea Genotypes Under WW and DS Conditions

The study revealed that all pigeonpea genotypes displayed reduced performance under extended water limitation. Figure 3 presents violin plots illustrating the variation in key

Table 2 Analysis of variance for morphological and yield traits of Pigeonpea genotypes under control and stress conditions

Source of variation	df	Mean sum of squares							
		DFF	PH	PB	SB	PP	SPP	TW	GYPP
Factor A (genotypes)	46	337.28*	5425.41*	11.02*	233.99*	39792.33*	0.24*	3.87*	1094.65*
Factor B (WW & DS)	1	4552.08*	16711.31*	59.39*	175.87*	518692.79*	0.90*	66.93*	72.38*
Interaction	46	154.14*	151.47*	9.61*	42.28*	10448.06*	0.16*	1.17*	244.18*
Error	188	25.929	15.35	0.21	0.22	14.31	0.13	0.001	0.002

Table 3 Analysis of variance for physiological traits of Pigeonpea genotypes under control and stress conditions

Source of variation	df	Mean sum of squares									
		NDVI	SPAD	RWC	MSI	proline	Qmax	A	E	VPD	Tleaf
Factor A (genotypes)	46	0.004*	67.13*	252.02*	556.74*	496.78*	0.006*	41.85*	12.45*	27.75*	5.30*
Factor B (WW & DS)	1	0.11*	1806.52*	4947.63	6946.38*	43745.82*	0.17*	8278.18*	2289.23*	3895.21*	986.87*
Interaction	46	0.002*	21.97*	111.21*	249.87*	267.83v	0.002*	64.42*	13.56*	26.48*	6.77*
Error	188	0.00	3.82	26.22	0.11	0.00	0.00	2.54	1.27	1.22	0.11

WW: Well-watered, DS: Drought stress, DFF: days to 50% flowering, PH: Plant Height (cm), PB: Primary branches per plant, SB: Secondary branches per plant, PP: Pods per plant SPP Seeds per pod, TW: Test weight (g), GYPP: Grain yield per plant (g), NDVI: Normalized Difference Vegetation Index, SPAD: Soil Plant Analysis Development, RWC: Relative water content (%), MSI: Membrane Stability Index (%), Qmax: Quantum efficiency of PSII, Tleaf: Leaf Temperature, A: Net Assimilation rate, E:VPD: Vapour Pressure deficit, *-statistical significance at the 5% level

morphophysiological traits across genotypes under WW and DS conditions. A distinct shift in trait-value distributions between the two treatments highlights the significant impact of water availability on genotypic performance and physiological responses. Under DS, most genotypes showed lower trait values compared to the well-watered condition, reflecting the adverse impact of water deficit on plant growth and function.

Interestingly, morphological and yield traits such as days to 50% flowering (DFF), days to maturity (DM), plant height (PH), number of primary branches (PB), number of secondary branches (SB), pods per plant (PP), seeds per pod (SPP), 100-seed weight (TW) and grain yield per plant (GYPP) exhibited greater variability under DS than under WW. Among these, the most considerable reduction under DS was observed for PB (31.97%), followed by PP (25.11%). The smallest reductions were noted for SPP (2.77%) and DFF (7.63%). Other measured reductions included PH (10.95%), SB (10.57%), TW (9.86%) and GYPP (20.68%%).

Exploration of the Genetic Potential of Pigeonpea Genotype Under WW and DS Conditions

Under well-watered (WW) conditions, DFF among the 47 pigeonpea genotypes ranged from 83.00 days (NAAM-88) to 125.00 days (TDRG-272) with a mean of 105.26 days, while under DS conditions it ranged from 80.33 days (GC-11-39) to 115.00 days (ICPX140196-B-1, ICPX140203-B-1 and ICPX140203-B-2) (Table 4). Genotypes IC-73,882, IC-73,969, IC-74,058, NAAM-88 and GRG-152 required more days to flower under stress, whereas most others flowered earlier under DS than WW. Plant height ranged from 98.27 to 237.60 cm under WW and from 88.38 to 189.89 cm under DS, with ICPL-87 being the shortest in both and TDRG-272 (WW) and Vipula (DS) the tallest. The PB and SB both declined under stress: PB under WW ranged from 5 (IC-73969) to 16 (ICPX140203-B-2) and under DS from 3.89 (CORG-9701) to 7.56 (Vipula); SB under WW ranged from 6.13 (IC-73961) to 35.01 (Vipula) and under DS from 4.60 (IC-73969) to 31.13 (Vipula). Pods per plant (PP) dropped under DS compared to WW: under WW, IC-73,975 had the fewest (117.30) and IC-73,882 the most (545.29); under DS, IC-73,993 had the lowest (99.30) and ICPX140213-B-3 the highest (480.55). Notably, genotypes ICPX140203-B-2, IC-73,975, ICPX140203-B-1-5 and ICPX140205-B-4 showed minimal differences in PP between WW and DS, suggesting greater reproductive stability under drought. Seeds per pod (SPP) ranged under WW from 3.67 (IC-73926) to 3.99 (GRG-811) (mean 3.97) and under DS from 3.30 (IC-73898) to 4.33 (GRG-811), with NAM-2085 and GRG-811 consistently highest in both conditions. Test weight (TW) ranged under WW from 7.43 g (GC-11-39) to 12.19 g (KRG-33) and under DS from 6.18 g (GRG-152) to 10.50 g (ICPX140217-B-1); genotypes ICPX140196-B-1, ICPX140205-B-4, IC-74,058, ICPX140203-B-1, GC-11-39, ICPL-87 and PKV Tara showed the least TW reduction under DS. Grain yield per plant (GYPP) under WW ranged from 30.1 g (EC-843239) to 71.56 g (ICPX140203-B-2), while under DS it ranged from 10.01 g (IC-73995) to 69.69 g (ICPX140203-B-1).

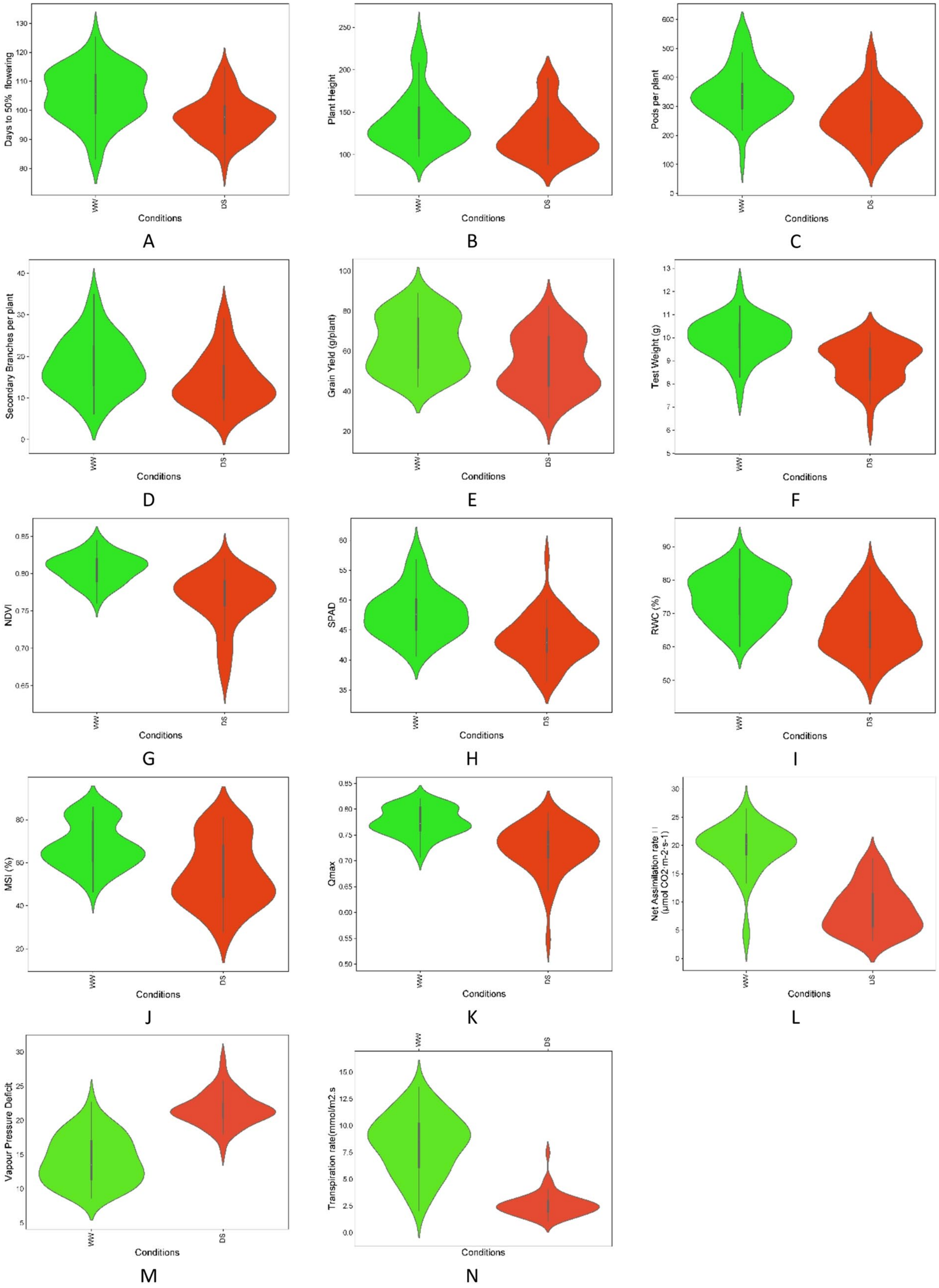

Days to 50% flowering
130
120
110
100
90
80
WW
DS
Conditions
A
Plant Height
250
200
150
100
WW
DS
Conditions
B
Pods per plant
600
500
400
300
200
100
0
WW
DS
Conditions
C
Secondary Branches per plant
40
30
20
10
0
WW
DS
Conditions
D
Grain Yield (g/plant)
100
80
60
40
20
WW
DS
Conditions
E
Test Weight (g)
13
12
11
10
9
8
7
6
5
WW
DS
Conditions
F
NDVI
0.85
0.80
0.75
0.70
0.65
WW
DS
Conditions
G
SPAD
60
55
50
45
40
35
WW
DS
Conditions
H
RWC (%)
90
80
70
60
50
WW
DS
Conditions
I
MSI (%)
80
60
40
20
WW
DS
Conditions
J
Qmax
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
WW
DS
Conditions
K
Net Assimilation rate (µmol CO2 m-2 s-1)
30
25
20
15
10
5
0
WW
DS
Conditions
L
Vapour Pressure Deficit
30
25
20
15
10
5
WW
DS
Conditions
M
Transpiration rate(mmol/m2.s
15.0
12.5
10.0
7.5
5.0
2.5
0.0
WW
DS
Conditions
N

Fig. 3 Violin plot depicting the morpho-physiological trait variation of pigeonpea genotypes under well-watered and drought stress conditions. A: Days to 50% flowering; B: Plant height (cm); C: Pods per plant; D: Secondary Branches per plant; E: Grain Yield (g/plant); F: Test Weight (g); G: NDVI; H: SPAD; I: RWC (%); J: MSI (%); K: Qmax; L: Net Assimilation Rate (μmol/m^{2}s); M: Vapour Pressure Deficit (Pa/kPa); N: Transpiration Rate (mmol/m^{2}s)

Characterisation of Pigeonpea Genotypes for Physiological Traits: Plant-water Relations and Canopy Greenness

Under drought stress (DS), canopy greenness assessed *via* normalized difference vegetation index (NDVI) and SPAD chlorophyll meter readings (SPAD) declined by 5.00% and 10.37%, respectively. Relative water content (RWC) dropped by 11.59% and membrane stability index (MSI) decreased by 16.18%. At the well-watered (WW) condition, genotypes ICPX 140,196-B-1, NAM-2085, NAM-2217 and Vipula exhibited the highest canopy greenness (NDVI ≈ 0.85; SPAD ≈ 58.45) (Table 5). Under WW, ICPX 140,203-B-1 recorded the highest RWC (89.35%) and IC-405,218 the highest MSI (85.75%). Under DS, genotype ICPX 140,203-B-1 maintained the highest NDVI (0.82) and RWC (84.24%). In terms of MSI under WW, IC-73,926 recorded the lowest value (46.49%) and NAM-2217 the highest (86%). Under DS, MSI ranged from 27.85% (NAM-2085) to 81.04% (ICPX 140217-B-1), highlighting broad genotypic variation in physiological responses.

Sink Capacity of a Leaf Through Gas Exchange Parameters

Pigeonpea genotypes exhibited clear differential responses to drought stress in key gas-exchange parameters (Table 5). Under DS, net CO_2 assimilation rate declined by approximately 55.68% and transpiration rate by 68.76%, while canopy temperature increased by 52.40% and proline content rose by 1.5-fold compared to WW conditions. Genotypes, such as IC-73,926, IC-73,975, IC-73,961, ICPL-87, IC-73,995, Phule Tur and TDRG-272, showed large reductions in transpiration under DS, indicating drought sensitivity, whereas genotypes IC-74,058, GRG-811, IC-73,952, ICPX 140,217-B-1 and ICPX 140,196-B-1 exhibited only minimal decreases, suggesting greater drought tolerance. Transpiration rates under WW ranged from 2.07 to 13.61 mmol H_2O m^{-2} s^{-1} (mean 8.29), with GRG-811 lowest and ICPL-87 highest; under DS, rates dropped to 1.08–7.46 (mean 2.59). Vapor pressure deficit (VPD) also changed markedly: under WW it ranged from 8.62 to 22.68 kPa (mean 14.18), whereas under DS it increased to 15.63–28.98 kPa (mean 21.62). Genotypes NAM-2282, NAM-314, NAM-2217, TS-3R and NAM-2216 showed substantial VPD increases under DS, while IC-74,058, ICPX 140,217-B-1, ICPX 140,205-B-4, ICPX 140,196-B-1, IC-74,013 and ICPX 140,203-B-1 showed smaller increases. For assimilation rate, under WW it ranged from 3.49 to 26.52 μmol CO_2 m^{-2} s^{-1} (mean 19.47) with IC-405,218 lowest and ICPX 140,213-B-3 highest; under DS the range was 3.16–17.70 (mean 8.64). Genotypes ICPX 140,188-B-3, ICPX 140,203-B-1, NAM-2282 and CORG-9701 demonstrated minimal decreases in assimilation under DS, thus retaining photosynthetic capacity and making them promising for cultivation in arid and semi-arid regions.

Photosystem II Efficiency Under Drought Stress Conditions

Several genotypes maintained high photosystem II efficiency (F_v/F_m) under drought stress, indicating robust tolerance; these included ICPX 140,188-B-3, ICPX 140,203-B-1, NAM-2282, and CORG-9701. Conversely, genotypes NAM-2085, WRG-327, IC-73,961, IC-73,952, IC-73,959, and ICPX 140,213-B-3 exhibited notable declines in F_v/F_m under drought, reflecting reduced photosynthetic efficiency and greater susceptibility to water limitation. Under well-watered (WW) conditions, F_v/F_m ranged from 0.71 to 0.82, while under drought-stress (DS) conditions, it ranged from 0.55 to 0.80 (Table 5).

Estimation of Genetic Parameters of Pigeonpea Genotypes Under WW and DS Conditions

The phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV) across traits under both WW and DS conditions (Table 6). GCV values ranged from 0.61% to 46.43%, while PCV values spanned 3.82% to 49.80%. Low to moderate GCVs (< 10-20%) were observed for traits such as DFF: 8.65% WW, 7.34% DS, SPP: 0.61%, 5.59%, TW: 9.23%, 10.23%, NDVI: 2.15%, 4.70%, SPAD chlorophyll meter reading (SPAD: 8.09%, 8.39%), RWC: 11.20%, 9.66%, MSI: 17.70%, 23.79%, Qmax: 3.41%, 6.01%, and Tleaf: 3.54%, 5.42%. In contrast, higher GCV values (> 20%) were recorded for PH: 23.17%, 22.40%, PB: 27.46%, 12.45%, 42.60%, 41.25%, PP: 28.18%, 33.76%, GYPP: 22.34%, 31.93%, transpiration rate (E: 31.59%, 38.11%), assimilation rate (A: 21.57%, 46.43%), vapour pressure deficit (VPD: 24.06%, 10.95%), and proline content (36.01%, 36.20%).

Heritability in the broad sense (H^2b) ranged from 0.60 to 0.99 under WW and from 0.21 to 0.97 under DS. The lowest heritability was observed in SPP (0.06 under WW; 0.21 under DS). Under DS, TW, MSI and Tleaf exhibited very high heritability estimates (0.94). Traits including PH, PB, PP, GYPP, NDVI, SPAD, Qmax, Tleaf, E, A, VPD and proline consistently showed high heritability under both WW and DS, indicating strong genetic control and positive prospects for selection. When combined with genetic advance as a percentage of the mean, traits such as PH, PB, SB, PP, GYPP, E, A and proline each show high heritability and > 60% genetic advance, and emerge as prime candidates for selection to enhance drought tolerance in pigeonpea.

Correlation Studies

The correlation analysis (Figs. 4 and 5) revealed strong positive relationships between grain yield per plant and several morpho-physiological traits under both WW and DS conditions. Under WW conditions, GYPP correlated highly PH (r=0.81), PB (r=0.88), SB (r=0.77), PP (r=0.85), SPP (r=0.91), TW (r=0.91), NDVI (r=0.92), SPAD (r=0.91), RWC (r=0.94), Qmax (r=0.93), MSI (r=0.94), proline content (r=0.80), transpiration rate (E; r=0.67) and CO_2 assimilation rate (A; r=0.80). On the other hand, GYPP showed a negative, non-significant association with DFF (r = -0.04). Under DS conditions however, the positive associations were generally weaker: GYPP correlated significantly with DFF (r=0.26), PB (r=0.18), PP (r=0.28), SPP (r=0.27), TW (r=0.37), SPAD (r=0.34), RWC (r=0.61), Qmax (r=0.29), MSI (r=0.65), proline (r=0.29), E (r=0.21), VPD (r=0.17) and A (r=0.38). In contrast, PH had a negligible negative correlation with GYPP under DS (r = -0.02), and canopy temperature exhibited a significant negative correlation (r = -0.43).

High Throughput Phenotyping

High-throughput phenotyping (HTP) revealed significant genotype-based variation in shoot architecture and tissue hydration under stress. The projected shoot area (digital area, DA) ranged from 74,094.33 to 131,009.05 megapixels under WW conditions and 50,668.00 to 130,123.28 MP under DS conditions (Fig. 6). Overall, DA declined by 32.28% under DS, with genotype ICPX 140,203-B-1 showing only a 0.68% drop (tolerant) and GC-11-39 suffering a 31.61% decline (sensitive) (Fig. 7). Calliper length (CL) varied from 478.77 to 664.46 MP under WW and 433.75 to 631.09 MP under DS, marking an 11.40% reduction under drought (Fig. 8). Genotype ICPX 140,213-B-3 recorded the highest CL (664.45 MP) under WW, while NAM-2282 had the lowest under DS (478.77 MP) (Fig. 9). Convex hull area (CHA), which represents canopy spread, ranged from 177,220.88 to 281,343.22 MP under WW and from 116,372.44 to 272,093.16 MP under DS, with an overall decrease of 26.31% under DS. Genotype GRG-811 showed the highest CHA at maturity under WW (281,343.22 MP), and ICPX 140,205-B-4 had the highest under DS (272,093.16 MP) (Figs. 10 and 11). Near-infrared (NIR) intensity, used as an indicator of tissue water status, ranged from 171.00 to 181.89 MP under WW and from 172.68 to 189.60 MP under DS (Fig. 12). An increase of 36.64% in NIR intensity under DS compared to WW indicated greater tissue dehydration. Genotype ICPX 140,217-B-1 maintained superior tissue-water status under both conditions, whereas IC-73,882 exhibited the highest tissue dehydration under both WW and DS (Fig. 13).

Discussion

Climate change-driven vulnerabilities pose a significant threat to global food and nutritional security, particularly in developing nations. In this critical global context, legumes like pigeonpea have the potential to serve as resilient solutions to climatic variability, positioning them as climate-smart crops for the future (Bakala et al. 2024). The primary environmental stresses affecting pigeonpea throughout its life cycle include moisture extremes (drought and waterlogging), high temperatures (heat stress), photoperiod sensitivity, and mineral-related deficiencies or toxicities (Megha and Singh 2023).

Drought tolerance is a polygenic trait influenced by the timing, intensity, and duration of stress, as well as the crop growth stage (Araus et al. 2012; Passioura 2012). In water-limited conditions, genetic improvement of drought tolerance characteristics is considered economical and eco-friendly (Blum 2011). Accordingly, understanding genotype (G), environment (E), and genotype × environment (G × E) interactions is key to developing targeted management and breeding strategies. In this context, trait variability and heritability provide the foundation for effective selection. Pigeonpea remains a vital grain legume in arid and semi-arid zones, and therefore, the development of cultivars tolerant to water-limited conditions is more urgent than ever (Pixley et al. 2023).

In our study, we evaluated 47 pigeonpea genotypes under WW and DS conditions using an integrated set of morpho-physiological, yield, and image-derived (high-throughput phenotyping; HTP) traits. The two-way ANOVA indicated significant main effects of genotype, water regime and the interaction (G × W) for many traits. The significant G × W interaction indicates that genotypes responded differently to the water regime: some maintained performance under DS, while others declined sharply, providing scientific justification for identifying specific drought-tolerant genotypes. The present results align with earlier reports by Sarkar et al. (2023) and Pappula-Reddy et al. (2024) on the differential response of pigeonpea genotypes to varying water levels.

Table 4 Per se performance of Pigeonpea genotypes for growth and morphological traits under well-watered and drought stress conditions

Genotypes	DFF		PH		PB		SB		PP		SPP		TW		GYPP	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
EC-843239	109	102	147.6	134.07	11	6.22	11.13	7.6	366.96	295.66	4	4	9.79	7.62	30.1	18.73
IC-405218	112	97	166.94	147.24	7.22	5.89	16.13	11.01	335.62	211.63	4	4	10.55	8.13	67.65	62.53
IC-73882	99	106	159.94	142.74	5.11	5	18.01	14.13	545.29	295.3	4	4	9.83	9	36.36	27.49
IC-73898	115	92	208.35	189.15	8.56	6.11	12.6	10.13	382.29	381.3	4.33	3.33	8.75	8.19	41.93	23.22
IC-73926	114	92	129.16	92.19	8.44	7	23.13	18.6	290.3	238.96	3.67	3.67	11.26	7.93	43.05	31.16
IC-73952	102	102	126.38	100	9	6.22	21.6	13.13	219.29	136.3	4	4	9.75	9.05	49.93	31.37
IC-73959	115	98	128.16	113.37	5.78	7	11.6	8.13	398.29	206.97	4	4	9.21	7.71	33.79	31.07
IC-73961	99	98	120.33	109.79	9.11	7.33	6.13	6.6	423.29	322.63	4	3.67	10.28	8.23	38.88	27.86
IC-73969	98	99	118.44	107.24	5	5.22	19.13	4.6	271.3	219.29	4	3.67	10.01	8.79	43.18	36.63
IC-73975	102	98	116.65	109.04	8.56	7.11	17.6	12.13	117.3	110.62	4	3.67	9.58	8.54	35.69	23.76
IC-73993	112	98	129.74	121.73	10.11	7.33	13.13	10.6	230.62	99.3	4	3.33	9.38	7.66	34.78	26.04
IC-73995	110	98	108.69	94.3	8.22	7.33	11.46	11.6	314.62	163.97	4	3.33	9.53	8.27	36.76	10.01
IC-74013	102	98	109.08	98.82	8.22	6.33	14.13	9.6	306.62	225.9	4	4	9.67	8.63	54.2	29.65
IC-74058	98	102	127.62	109.07	6.22	5.78	12.6	9.13	451.62	238.3	4	3.67	8.35	8.08	45.42	22.1
ICPX 140188-B-3	115	112	121.94	109.27	12.56	5.89	17.01	14.13	353.62	223.3	4	4	10.53	10.25	68.09	63.3
ICPX 140196-B-1	115	115	128.27	119.07	13	6.78	13.01	10.13	435.07	346.86	3.67	4	10.3	10.26	63.23	58.84
ICPX 140203-B-1	115	115	131.94	122.97	13.44	5.33	25.01	22.13	370.96	325.9	4	3.67	10.64	10.26	70.28	66.93
ICPX 140203-B-1–5	114	109	128.6	119.87	12	7	16.01	12.13	347.29	340.3	4	3.33	10.32	9.83	71.56	69.69
ICPX 140203-B-2	115	115	137.6	125.07	16	6.22	15.01	11.13	385.96	380.3	4	4.33	10.37	9.99	68.88	64.37
ICPX 140205-B-4	102	101	132.6	119.02	15	5.78	21.01	17.13	485.96	460.66	4	4	9.49	9.43	67.15	62.15
ICPX 140213-B-3	109	103	152.27	133.38	10.67	7	24.01	20.13	541.29	480.55	4	4	9.66	9.5	61.8	60.2
ICPX 140217-B-1	98	95	140.12	143.38	12	6	25.01	21.13	541.62	359.3	3.67	3.67	10.86	10.5	67.88	63.02
NAM-2085	92	91	126.61	119.37	9.22	5.56	6.13	4.6	342.62	280.3	4.33	4.33	9.77	9.12	55.08	51.55
NAM-2151	110	95	174.88	142.7	7.22	5.78	21.01	18.13	306.62	265.3	4	4	8.96	8.37	41.44	27.96
NAM-2216	102	98	107.6	98.83	9	6.44	10.01	6.13	306.29	225.3	3.67	3.33	10.86	9.51	51.82	46.38
NAM-2217	106	102	116.77	107.82	10	6.78	20.01	15.13	343.29	280.3	4	4	9.71	8.12	60.36	51.88
NAM-2282	103	97	126.94	109.38	10	6.56	15.01	11.13	270.62	170.3	4	4	10.85	9.51	53.73	47.53
NAM-2284	101	92	126.6	114.51	7.56	7.22	11.01	8.13	330.62	120.3	4	4	10.43	9.86	55.56	50.12
NAM-2292	92	92	115.27	107.38	7.44	7	14.01	11.13	342.96	321.97	4	4	10.87	9.08	45.56	25.5
NAM-2314	101	92	116.27	109.17	5.44	5	24.01	21.13	373.96	202.97	4	3.33	10.76	9.86	49.44	36.67
NAM-2329	112	92	106.94	98.82	5.44	5.22	18.01	15.13	252.29	180.3	4	4	10.28	9.11	37.48	30.52
NAM-314	98	88	126.94	114.47	9.22	5.11	14.01	11.13	471.96	243.63	4	4	10.34	9.51	48.63	36.11
NAAM-88	83	88	121.6	109.87	9.11	5.22	10.01	7.13	345.96	295.3	4	4	10.27	9.61	64.63	60.64
Phule Tur	116	102	157.94	148.83	10.89	6.44	28.01	26.13	349.96	314.3	4	4	9.57	8.85	45.08	40.47
PKV Tara	98	97	170.34	160.59	12	5.44	21.01	17.13	283.96	261.3	4	3.67	9.97	9.5	61.01	41.36
PT-12–19-2	110	92	218.71	181.04	7.44	5.78	15.01	11.13	324.29	295.3	4.33	4	10.74	10.08	34.07	24.76
Rajeshwari	102	98	214.77	183.97	8	7.11	24.01	21.13	339.62	228.97	4	4	9.85	8.22	65.33	57.00
TDRG-272	125	98	237.6	189.5	6.56	5.78	32.01	29.13	321.29	213.63	4	4	10.63	9.49	41	28.05
Vipula	102	98	210.7	189.85	9.11	7.56	35.01	31.13	271.29	235.3	4	4	10.31	9.72	65.56	50.25

Table 4 (continued)

Genotypes	DFF		PH		PB		SB		PP		SPP		TW		GYPP	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
WRG-327	120.33	106.33	160.1	149.7	6.56	5.33	30.01	26.13	361.29	269.3	4	4	11.38	9.89	55.3	41.13
KRG-33	110.33	92	158.02	149.05	8.67	6.33	20.01	15.13	460.29	330.3	4	3.67	12.19	10.01	52.58	43.02
CORG-9701	93.33	88	153.35	136.22	10	3.89	15.13	13.6	294.29	230.3	4	4	8.28	7.97	45.43	31.51
ICPL-87	92.33	88.33	98.27	88.38	9	6.11	6.13	9.01	372.29	349.31	4	4	9.57	9.24	55.34	43.04
TS-3R	105.67	97.67	146.94	137.41	10	5.22	17.01	13.13	285.62	251.3	4	3.67	10.93	9.6	60.23	51.72
GRG-811	110	98.33	106.94	96.57	7.33	5.22	24.6	18.13	316.15	247.67	4.33	4.33	9.04	8.03	65.18	53.51
GRG-152	83.33	88	132.94	92.93	7.44	7	26.13	16.6	262.85	180.3	4	3.33	9.17	6.18	57.64	34.64
GC-11–39	109	80.33	107.6	90.38	7.33	6.11	18.13	9.6	262.62	169.66	3.67	3.67	7.43	7.11	61.16	34.94
Mean	105.26	97.79	140.66	125.27	9.05	6.15	17.87	14.06	346.98	260.13	3.99	3.84	10.01	8.97	52.32	41.50
Min.	83	80.33	98.27	88.38	5	3.89	6.13	4.6	117.3	99.3	3.67	3.33	7.43	6.18	30.1	10.01
Max	125	115	237.6	189.85	16	7.56	35.01	31.13	545.29	480.55	4.33	4.33	12.19	10.5	71.56	69.69
CD@5%	1.15	11.63	8.58	0.12	0.67	0.81	0.01NS	0.01NS	8.28	7.01	0.48NS	0.67	0.01	0.01	0.98	1.56

WW: Well-watered, DS: Drought stress, DFF: days to 50% flowering, PH: Plant Height (cm), PB: Primary branches per plant, SB: Secondary branches per plant, PP: Pods per plant SPP Seeds per pod, TW: Test weight (g), GYPP: Grain yield per plant (g), NDVI: Normalized Difference Vegetation Index, SPAD: Soil Plant Analysis Development, RWC: Relative water content (%), MSI: Membrane Stability Index (%), Qmax: Quantum efficiency of PSII, Tleaf: Leaf Temperature, A: Net Assimilation rate, E:VPD: Vapour Pressure deficit

Morphological and Yield Responses of Pigeonpea Genotypes To Drought Stress

Under DS, we observed pronounced reductions in many morphological and yield traits. For example, PB decreased by 31.97%, PP by 25.11%, and GYPP by 20.68%. These results corroborate earlier reports that reductions in pod number and pod-set constitute the key yield penalty under drought in pigeonpea (Deshmukh and Mate 2013; Nam et al. 1994). Interestingly, seeds per pod were least affected, suggesting that for some genotypes the reproductive sink (seed-set) was maintained relatively intact even under stress. The reduction in test weight likely reflects the formation of smaller, shrunken seeds under DS conditions (Ali et al. 2022). The primary physiological reasons include a limitation of photosynthate supply during the flowering-to-pod phase, elevated ABA, and reduced activity of enzymes such as acid invertase (Liu et al. 2025). Notably, some genotypes, ICPX140188-B-3, ICPX140203-B-1, ICPX140203-B-1-5, ICPX140203-B-2, ICPX140213-B-3 and ICPX140217-B-1 sustained grain yield reductions of less than 10% under DS. These genotypes combined good pod/seed numbers and superior test weight under stress, suggesting stronger partitioning of biomass into the economic sink and inherent drought-adaptive traits (Vanaja et al. 2015; Sadia et al. 2025).

Physiological-trait Modulation Under Drought

The physiological parameters varied significantly by genotype under DS—traits like NDVI and quantum yield (Qmax). In comparison, showed lower reductions (≈ 5% and 6.49%, respectively) under DS than many other parameters, while net CO_2 assimilation rate dropped by 55.68% and transpiration by 68.76%. Proline mode increased 1.5-fold, and vapour-pressure deficit (VPD) rose 52.40%. The significant drop in assimilation and transpiration is consistent with limitations in stomatal conductance, CO_2 entry, and impaired PS II performance (Singh and Singh 2011; Haghpanah et al. 2024). Relative water content (RWC) and membrane stability index (MSI) are reliable indicators of plant-water status and cell membrane integrity under drought, respectively (Farooq et al. 2019; Mubarik et al. 2021). In our study, RWC declined by 11.29% and MSI by 51.43% under DS, with tolerant genotypes showing minimal reductions (< 5%) in these traits. This suggests better ROS scavenging and membrane protection in those lines (Basu and Kumar 2021; Murali et al. 2025).

Table 5 *Perse* perfromace of Pigeonpea genotypes for physiological parameters under well-watered and drought stress conditions

Genotype	NDVI		SPAD		RWC		MSI		Qmax		Tleaf		E		VPD		A		Proline	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
EC-843239	0.81	0.8	50.9	41.3	63.41	57.88	69.37	64.42	0.76	0.74	29.69	31.85	7.28	2.4	15.36	18.5	20.08	6.53	10.92	33.43
IC-405218	0.81	0.79	48.53	47.7	80.16	65.97	85.42	71.49	0.82	0.78	30.16	31.38	10.29	3.24	15.36	18.6	11.71	3.49	10.81	49.06
IC-73882	0.81	0.75	46	36.65	65.98	60.55	83.06	74.42	0.8	0.74	28.18	29.32	9.67	1.73	11.77	21.22	20.84	4.14	14.25	68.33
IC-73898	0.81	0.79	44.4	42.25	65.79	56.85	67.45	42.23	0.75	0.74	26.59	32.41	6.2	2.03	14.8	18.24	21.95	7.68	11.01	24.2
IC-73926	0.8	0.73	47.65	45.35	66.91	59.21	46.49	29.45	0.77	0.74	28.79	31.46	12.44	1.41	9.94	21.54	23.24	5.59	9.65	24.03
IC-73952	0.78	0.78	50.54	48.7	65.45	59.84	66.27	49.02	0.78	0.71	27.41	32.03	3.27	2.23	17.17	21.12	20.23	4.66	12.38	38.53
IC-73959	0.78	0.77	48.37	39.2	69.36	58.39	57.39	46.03	0.77	0.7	29.27	32.47	8.19	2.36	12.66	23.85	23.05	3.95	9.98	25.3
IC-73961	0.82	0.81	40.6	37.15	74.13	72.31	60.43	39.36	0.76	0.68	26.61	31.55	11.65	1.42	10.52	21.47	20.41	3.16	9.45	14.7
IC-73969	0.8	0.79	42.35	36.55	71.35	66.03	58.15	40.55	0.75	0.73	27.39	31.32	9.76	1.95	12.37	19.6	21.77	3.71	12.62	37.67
IC-73975	0.83	0.79	46.1	42.05	74.27	59.74	66.33	46.11	0.78	0.77	26.87	31.92	12.47	1.76	10.67	22.21	22.78	4.33	14.82	28.43
IC-73993	0.81	0.79	49.32	47.1	64.56	61.1	60.05	48.54	0.77	0.76	27.63	31.91	9.48	2.26	13.02	22.01	21.96	9.7	10.65	25.3
IC-73995	0.8	0.76	47.65	44.45	72.41	61.72	64.01	42.46	0.73	0.71	27.69	31.24	12.05	1.97	12.44	22.17	21.61	7.6	27.98	35.2
IC-74013	0.83	0.78	48.4	44.95	73.99	60.19	59.25	57.4	0.77	0.71	27.86	31.07	3.39	1.46	18.89	21.7	13.4	11.09	23.98	34.63
IC-74058	0.82	0.8	42.13	40.75	61.68	50.39	51.47	39.18	0.77	0.55	29.72	32.49	3.55	2.93	19.13	19.33	20.3	10.38	13.05	38.03
ICPX 140188-B-3	0.81	0.79	49	44.45	84.07	79.69	82.02	79.84	0.8	0.8	27.69	28.58	5.78	1.53	16.22	23.9	15.08	14.91	15.82	49.73
ICPX 140196-B-1	0.85	0.8	58.45	57.1	80.79	75.49	72.1	60.16	0.81	0.8	27.54	28.36	4.36	2.26	18.26	21.02	18.52	16.22	19.37	51.68
ICPX 140203-B-1	0.83	0.8	45.2	44.45	89.35	84.24	83.55	79.25	0.81	0.8	27.44	28.52	5.81	2.11	19.95	23.7	22.86	16.54	34.87	55.93
ICPX 140203-B-1–5	0.82	0.82	44.55	42.25	82.92	78.12	82.45	74.16	0.81	0.8	27.55	28.37	5.52	2.57	18.64	21.78	14.57	12.97	31.87	57.33
ICPX 140203-B-2	0.79	0.77	50.3	47.61	83.54	78.04	84.47	79.84	0.81	0.8	27.01	28.42	7.84	3.03	13.63	23.6	20.69	17.7	21.77	59.48
ICPX 140205-B-4	0.82	0.79	45.25	43.8	71.08	69.65	81.08	72.1	0.81	0.8	28.46	31.82	7.09	4.06	17.64	21.66	18.04	16.91	21.57	59.28
ICPX 140213-B-3	0.83	0.77	45.35	42.1	80.15	68.81	69.09	42.07	0.82	0.8	26.52	32.86	8.77	2.36	12.33	26.52	21.95	14.26	21.47	60.04
ICPX 140217-B-1	0.82	0.77	54	50.13	85.43	78.49	83.87	81.05	0.8	0.8	27.53	28.46	6.72	4.78	15.36	20.2	15.63	8.65	18.92	65.07
NAM-2085	0.85	0.82	50.45	44.65	81.25	68.97	66.04	27.85	0.76	0.62	28.8	29.47	8.66	2.3	12.62	20.39	15.37	6.07	21.22	55.17
NAM-2151	0.83	0.82	56.8	44.8	69.37	66.15	66.03	28.06	0.77	0.71	27.86	30.14	9.3	4.79	9.92	21.36	20.55	9.85	19.82	33.13
NAM-2216	0.8	0.66	45.3	40.55	72.87	59.16	65.47	37.48	0.75	0.68	28.05	35.05	11.53	3	14.17	28.98	22.69	6.66	19.37	33.93
NAM-2217	0.85	0.74	54.8	48.85	78.97	70.28	86	81.04	0.78	0.72	27.71	28.18	11.91	2.85	13.26	25.73	19.9	5.74	14.22	37.33
NAM-2282	0.79	0.69	44.85	41.65	79.67	61.08	55.38	52.17	0.78	0.76	29.47	33.62	8.83	2.83	19.24	24.67	20.29	5.8	17.22	31.83
NAM-2284	0.77	0.67	44.65	42.85	78.67	70.43	57	44.05	0.77	0.73	30.03	32.48	5.14	2.75	22.68	21.18	12.9	7.05	10.62	45.53
NAM-2292	0.81	0.71	42.8	36.45	72.65	66.5	63.18	59.25	0.76	0.71	28.99	32.18	9.23	2.6	17.65	20.3	16.4	6.02	10.57	38.38
NAM-2314	0.78	0.7	42.3	38.25	69.78	65	61.27	44.39	0.76	0.7	28.25	32.35	7.29	2.48	16.93	24.53	20.71	4.8	10.52	35.38
NAM-2329	0.81	0.79	46	38.7	71.65	55.58	65.46	46.3	0.77	0.73	27.02	32.33	10.13	2.5	12.49	22.04	20.42	6.24	16.12	20.48
NAM-314	0.82	0.78	44.55	43.6	77.14	69.25	66.21	51.46	0.76	0.73	27.82	34.34	5.13	2.66	17.04	24.82	17.44	10.26	19.02	36.08
NAAM-88	0.82	0.81	46.1	42.9	82.56	77.46	83.41	76.31	0.79	0.75	27.34	28.7	9.9	3.46	13.44	21.02	19.05	9.26	12.27	37.53
Phule Tur	0.82	0.78	49.85	42.3	65.27	51.47	63.03	47.09	0.78	0.77	27.78	28.81	11.05	1.14	8.62	21.06	18.94	4.22	15.57	47.98
PKV Tara	0.76	0.7	56.05	42	77.95	60.23	62.12	59.42	0.71	0.64	27.43	29.12	8.66	1.49	9.29	22.15	21.07	4.45	18.87	91.03
PT-12–19-2	0.79	0.78	50.1	44.25	76.12	70.16	70.34	46.2	0.75	0.71	27.84	28.7	10.59	2.62	9.26	20.44	18.4	10.17	19.92	27.92
Rajeshwari	0.82	0.77	41.15	40.8	70.47	61.78	54.34	46.48	0.74	0.7	28.6	29.32	6.06	7.46	13.5	19.48	18.34	12.68	11.52	28.83
TDRG-272	0.84	0.8	48.7	47.1	76.25	61.93	54.31	47.15	0.77	0.72	28.14	28.99	10.4	1.59	10.05	21.73	21.52	14.26	11.62	22.48
Vipula	0.85	0.77	52.35	49.13	79.27	71.28	80.33	75.49	0.76	0.73	27.6	28.84	5.5	2.09	15.85	20.98	19.2	6.08	20.32	57.53

Table 5 (continued)

Genotype	NDVI		SPAD		RWC		MSI		Qmax		Tleaf		E		VPD		A		Proline	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
WRG-327	0.79	0.79	44.8	42.3	83.93	64.75	63.22	50.38	0.78	0.67	27.65	28.67	8.18	3.65	9.94	19.92	18.36	9.4	11.77	35.63
KRG-33	0.82	0.77	50.21	47.15	69.91	59.03	77.39	61.34	0.8	0.75	27.93	29.71	8.58	2.55	15.58	20.39	5.24	8.93	12.32	26.88
CORG-9701	0.82	0.78	49.3	44.4	60.13	53.43	55.06	39.55	0.72	0.7	26.41	33.5	8.89	2.66	11.78	21.8	17.03	8.67	11.38	51.33
ICPL-87	0.83	0.82	48.85	46.1	78.74	70.61	64.49	56.31	0.8	0.75	26.99	29.74	13.61	3.22	9.93	23.82	20.92	11.58	19.82	36.93
TS-3R	0.84	0.76	52.5	42.1	80.94	76.08	81.67	77.03	0.8	0.74	27.71	29.11	9.17	3.19	15.86	26.91	23.34	14.3	18.87	42.13
GRG-811	0.79	0.75	45.2	42.65	79.93	72.2	69.49	61.49	0.82	0.79	25.63	33.82	2.07	1.08	19.95	25.39	24.45	3.54	15.81	43.3
GRG-152	0.78	0.77	48.55	40.4	83.97	58.34	61.48	53.24	0.8	0.76	27.2	30.78	8.04	3.46	10.45	23.89	17.73	6.73	9.55	45.86
GC-11–39	0.8	0.75	46.45	43.3	73.38	66.34	69.01	63.11	0.74	0.65	28.84	31.41	10.58	1.89	11	20.91	20.62	11.82	10.27	28.53
Mean	0.8	0.8	47.8	43.5	74.8	65.7	68.0	55.1	0.8	0.7	27.9	30.8	8.3	2.6	14.2	21.6	19.5	8.9	16.1	41.0
Min.	0.8	0.7	40.6	36.5	60.1	50.4	46.5	27.9	0.7	0.6	25.6	28.2	2.1	1.1	8.6	15.6	3.5	3.2	9.5	14.7
Max.	0.9	0.8	58.5	57.1	89.4	84.2	86.0	81.1	0.8	0.8	30.2	35.1	13.6	7.5	22.7	29.0	26.5	17.7	34.9	91.0
CD@5%	0.0	0.0	3.9	1.5	10.4	1.9	0.6	0.5	0.0	0.0	0.7	0.4	0.8	1.4	2.2	1.3	3.1	1.9	0.1	0.2

WW: Well-watered, DS: Drought stress, DFF: days to 50% flowering, PH: Plant Height (cm), PB: Primary branches per plant, SB: Secondary branches per plant, PP: Pods per plant SPP Seeds per pod, TW: Test weight (g), GYPP: Grain yield per plant (g), NDVI: Normalized Difference Vegetation Index, SPAD: Soil Plant Analysis Development, RWC: Relative water content (%), MSI: Membrane Stability Index (%), Qmax: Quantum efficiency of PSII, Tleaf: Leaf Temperature, A: Net Assimilation rate, E:VPD: Vapour Pressure deficit

High-throughput Phenotyping

High-throughput image-based phenotyping enables the precise, scalable, and rapid assessment of physiological changes during drought stress across plant lifecycles (Berger et al. 2010; Cabrera-Bosquet et al. 2012). This technology generates large-scale, multidimensional data for mapping genotype-phenotype associations and integrating them with QTL mapping (Houle et al. 2010; Vadez et al. 2021). Automated, non-destructive phenotyping captures subtle genotypic variations in response timing, surpassing endpoint analyses. Traits such as projected shoot area, calliper length, and NIR reflectance provide deeper insights into drought responses (Maes et al. 2019). In the present study, in addition to conventional traits, we employed non-destructive, high-throughput phenomics to identify surrogate traits and genotypes that could be used in the breeding program to enhance drought tolerance in pigeonpea. We have derived traits, such as digital area (projected shoot area), calliper length, convex hull area, and NIR reflectance, from the same plant at regular intervals using image analysis. Therefore, the use of HTTP enables rapid, non-destructive measurement of plant traits within a short time. Similarly, HTTP has been applied to various crops, including maize, oak, safflower, chickpea, and mung bean, to evaluate drought responses in earlier studies (Wu et al. 2021; Joshi et al. 2021; Pappula-Reddy et al. 2024; Rane et al. 2021). While controlled-environment phenotyping is valuable, space limitations and controlled weather parameters necessitate field-based approaches for large-scale trait evaluation (Yang et al. 2020) to overcome the drawbacks of a controlled phenotyping facility. Expanding high-throughput phenotyping to field conditions facilitates large-scale germplasm screening, accelerating breeding for drought resilience (Jangra et al. 2021).

High-throughput phenomics (HTP) data provided valuable insights into the superior performance of the tolerant group under drought stress. Image-derived traits such as digital area (DA), convex hull area (CHA), calliper length (CL), and near-infrared (NIR) reflectance captured subtle differences in canopy development, leaf expansion, and plant architecture that were not fully evident from traditional morpho-physiological measurements. The tolerant genotypes, viz., ICPX140196-B-1, ICPX140203-B-1, ICPX140213-B-3, ICPX140203-B-1–5, ICPX140203-B-2, GRG-152, ICPX140205-B-4, ICPX140217-B-1, and ICPX140188-B-3, consistently maintained higher DA and CHA values, reflecting sustained canopy growth, while CL and NIR reflectance indicated better water status and leaf turgor. This tolerance is primarily attributed to maintaining a favourable internal water balance under drought stress. The HTP platform enables repeated, whole-plant measurements, capturing traits more comprehensively than

Table 6 Genetic variability parameters for morpho-physiological traits under well-watered and drought stress conditions of Pigeonpea genotypes

Characters	GCV%		PCV%		h^2_{bs}		GA		GAM	
	WW	DS	WW	DS	WW	DS	WW	DS	WW	DS
DFF	8.65	7.34	8.71	10.98	0.99	0.54	18.84	12.09	17.84	12.43
PH	23.17	22.40	23.47	22.57	0.97	0.87	66.27	57.80	47.12	46.14
PB	27.46	12.45	27.85	14.91	0.97	0.69	5.04	1.31	55.79	21.42
SB	42.60	41.25	42.74	41.59	0.99	0.81	13.12	14.77	87.76	89.36
PP	28.18	33.76	28.22	33.91	0.97	0.91	19.10	17.97	57.97	69.54
SPP	0.61	5.59	7.60	12.14	0.06	0.21	0.04	0.20	0.10	5.30
TW	9.23	10.23	9.29	10.34	0.99	0.97	1.89	1.89	19.01	21.07
GYPP	22.34	31.93	22.36	31.95	0.97	0.94	0.43	0.50	45.97	65.71
NDVI	2.15	4.70	2.78	4.88	0.60	0.92	0.02	0.07	3.43	9.34
SPAD	8.09	8.39	9.45	8.66	0.71	0.93	6.72	7.11	14.14	16.74
RWC	11.20	9.66	14.27	10.96	0.61	0.77	13.09	11.20	18.10	17.52
MSI	17.70	23.79	17.78	24.59	0.99	0.97	22.46	25.20	36.60	49.00
Qmax	3.41	6.01	3.86	7.16	0.77	0.70	0.04	0.07	6.20	10.38
CT	3.54	5.42	3.82	5.48	0.85	0.97	1.89	3.50	6.75	11.04
E	31.59	38.11	35.42	49.80	0.79	0.58	4.81	1.56	58.05	60.07
A	21.57	46.43	23.74	48.40	0.82	0.92	7.86	7.92	40.38	91.74
VPD	24.06	10.95	25.82	11.60	0.86	0.89	6.54	4.60	46.17	21.30
Proline	36.01	36.20	36.65	37.65	0.95	0.91	12.12	30.57	75.40	74.58

WW: Well-watered, DS: Drought stress, DFF: days to 50% flowering, PH: Plant Height (cm), PB: Primary branches per plant, SB: Secondary branches per plant, PP: Pods per plant SPP Seeds per pod, TW: Test weight (g), GYPP: Grain yield per plant (g), NDVI: Normalized Difference Vegetation Index, SPAD: Soil Plant Analysis Development, RWC: Relative water content (%), MSI: Membrane Stability Index (%), Qmax: Quantum efficiency of PSII, Tleaf: Leaf Temperature, A: Net Assimilation rate, E:VPD: Vapour Pressure deficit

conventional destructive sampling. Although more than a dozen image-based traits were derived, digital biomass, CL, CHA, and NIR intensity most clearly distinguished between well-watered and drought-stress treatments, enabling precise identification of tolerant and sensitive genotypes. These findings align with previous work in chickpea, where similar HTP-derived traits were effective for phenotyping (Pappula-Reddy et al. 2024).

Breeding Implications

The combination of traits exhibiting high heritability, viz., PH, PB, SB, PP, GYPP, SPAD, NDVI, Qmax, E, A, and proline content, together with a significant genotype × water-treatment (G×W) interaction, provides strong potential for selection in pigeonpea breeding programs. Given that yield is a complex trait controlled by many genes and often shows lower heritability, indirect selection *via* these component traits and surrogate image-based traits can accelerate genetic gain (Furbank et al. 2019). The genotypes identified in this study, including ICPX140196-B-1, ICPX140203-B-1, ICPX140213-B-3, ICPX140203-B-1–5, ICPX140203-B-2, GRG-152, ICPX140205-B-4, ICPX140217-B-1, and ICPX140188-B-3, demonstrated minimal yield reduction under drought stress and the least reduction in key physiological traits, thus representing promising candidates for use as parental lines in drought-resilience breeding pipelines (Fig. 14). Future work should include validation of root system architectonics, multi-environment trials, and incorporation of HTP platforms in field conditions to translate these findings into scalable cultivar development.

Conclusion

In conclusion, the pigeonpea genotypes evaluated in this study responded differently to well-watered (WW) and drought-stressed (DS) conditions. While most traits declined under DS compared to WW conditions, the nine genotypes listed above maintained significantly better growth, morphological attributes, and physiological health under stress. These findings underscore the presence of inherently drought-adaptive phenotypes that can be harnessed in breeding efforts. By integrating traits with high heritability, leveraging image-based phenotyping and understanding genotype × water regime interactions, this study provides a robust framework for developing pigeonpea cultivars better adapted to moisture-limited and fluctuating environments, a critical step toward enhancing food and nutritional security in the face of climate change.

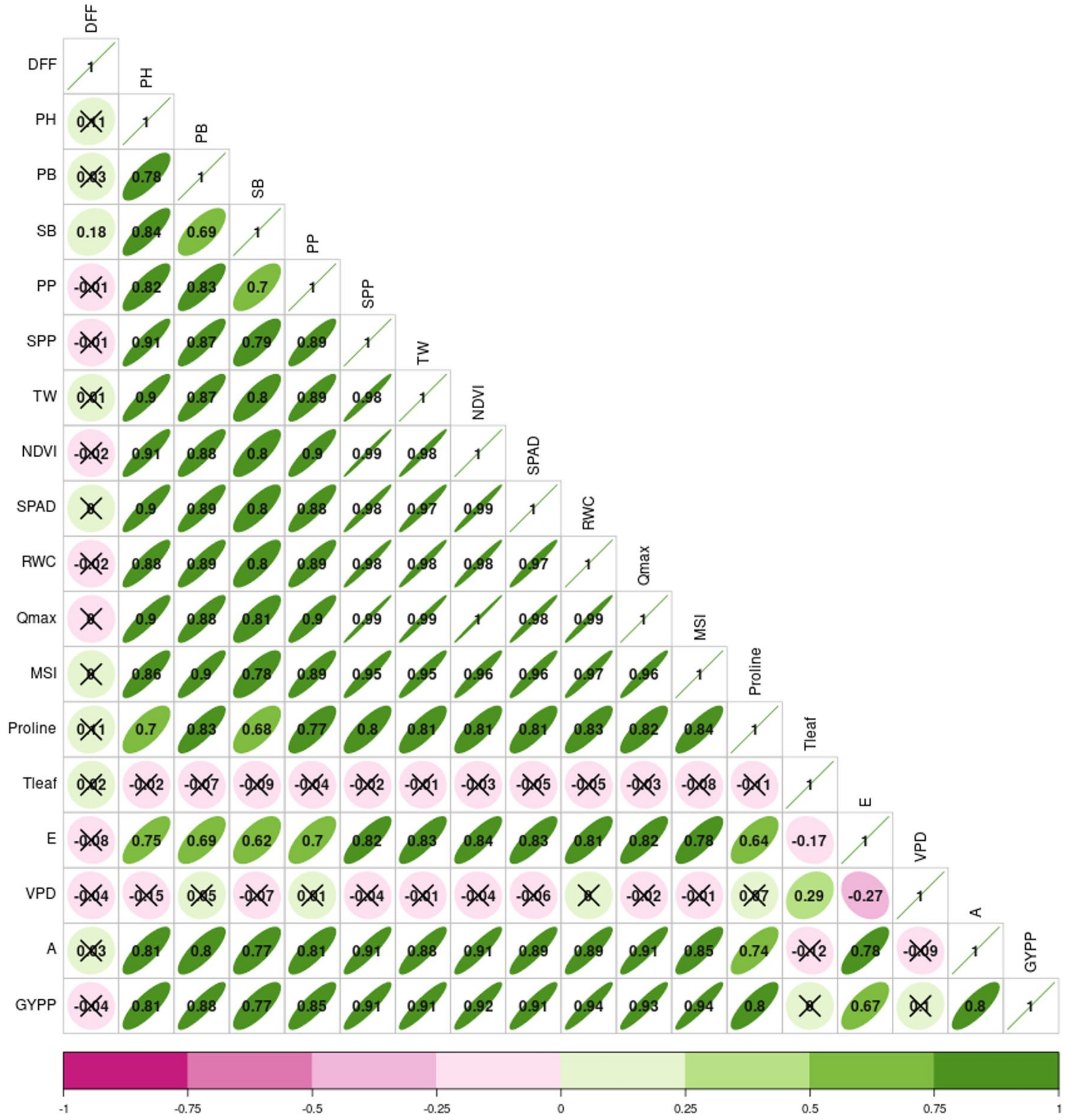


Fig. 4 Correlation coefficient between grain yield and morpho-physiological traits of pigeonpea genotypes under well-watered conditions

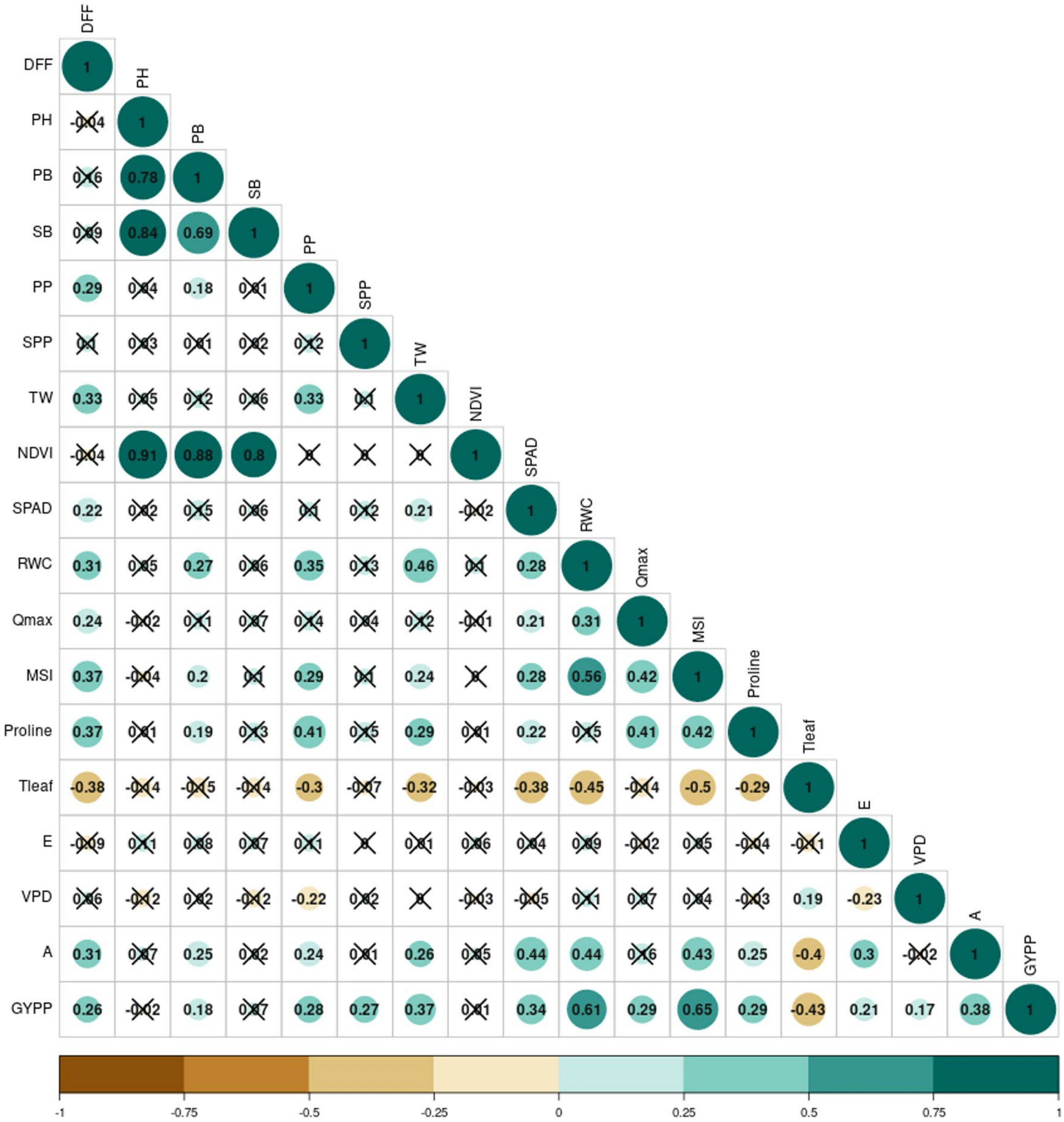
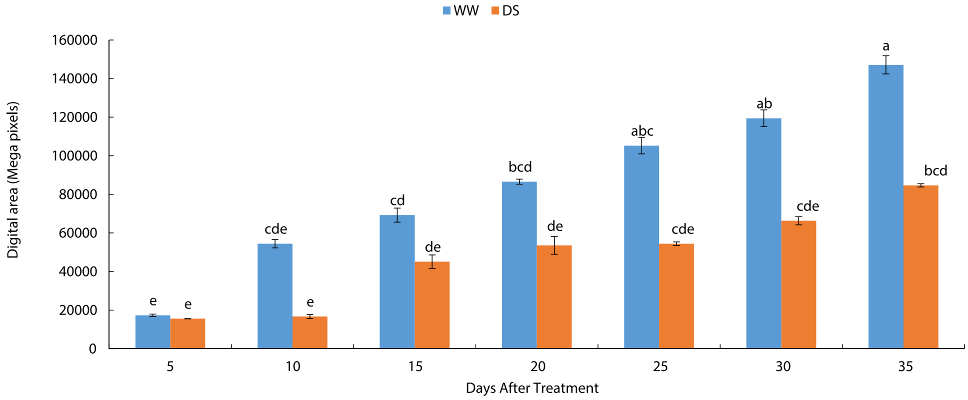


Fig. 5 Correlation coefficient between grain yield and morpho-physiological traits of pigeonpea genotypes under drought stress conditions

Fig. 6 Effect of well-watered and drought stress conditions on digital area over time in pigeonpea genotypes. Significance in DA under both the conditions, WW and DS, tested at $p = 0.05\%$



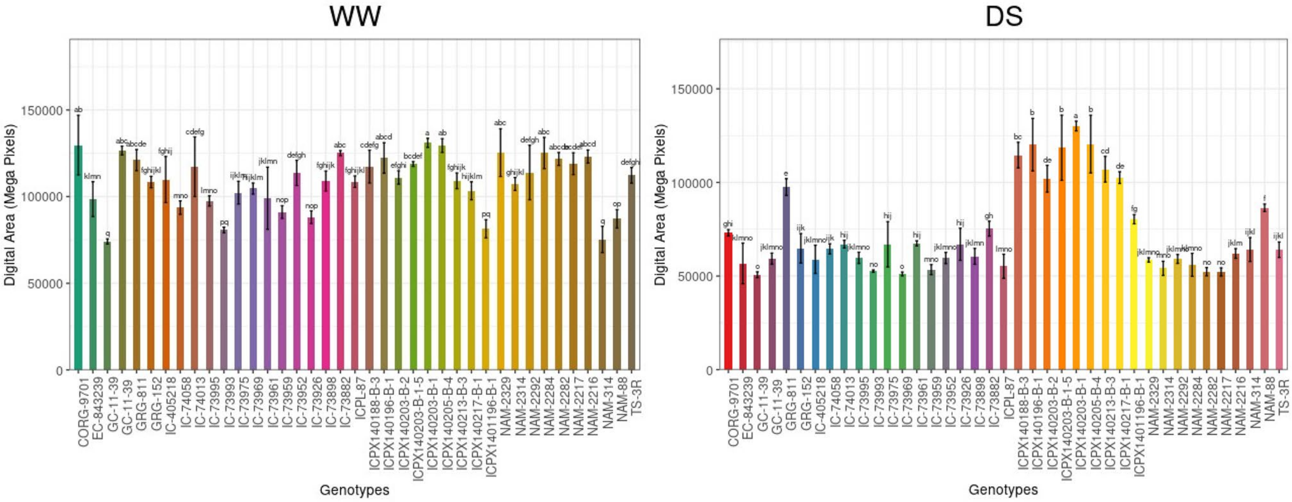
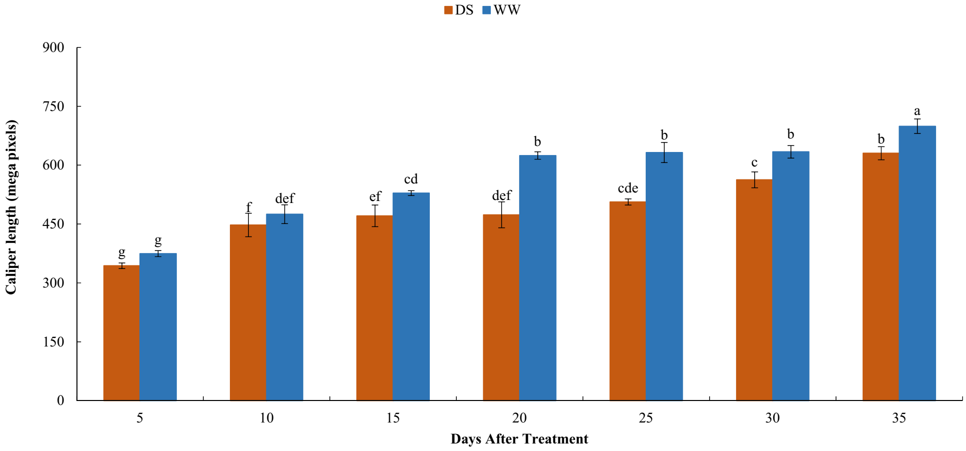


Fig. 7 Variation in digital plant area of pigeonpea genotypes under WW and DS conditions. Significance in DA under both the conditions, WW and DS, was tested at $p = 0.05\%$

Fig. 8 Effect of well-watered and drought stress conditions on caliper length over time in pigeonpea genotypes. Significance in caliper length under both the conditions, WW and DS, was tested at $p = 0.05\%$



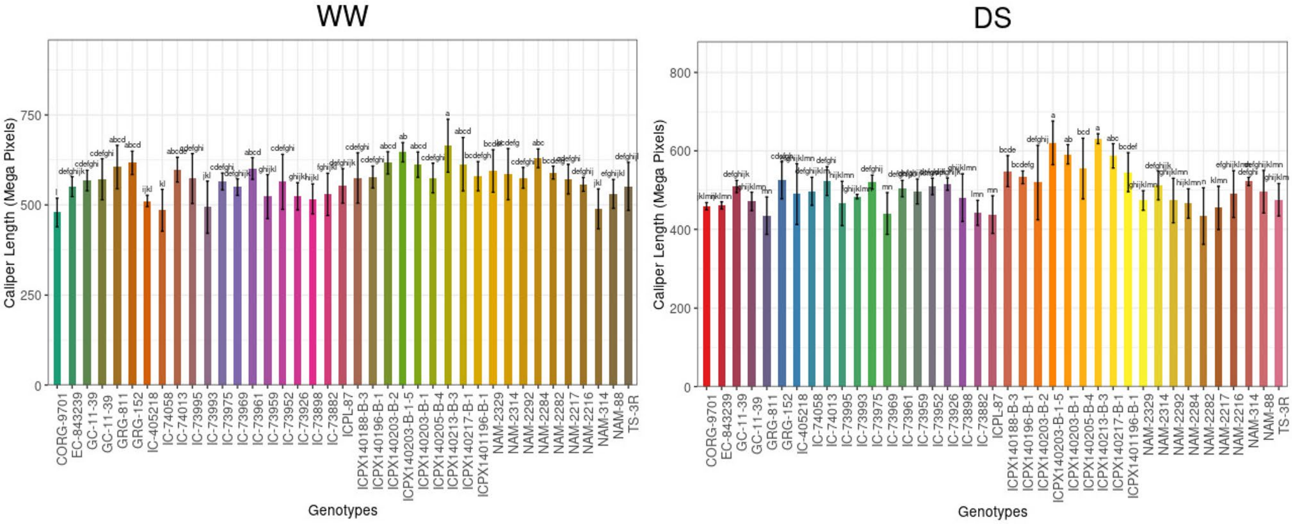


Fig. 9 Calliper length variation in pigeonpea genotypes under well-watered and drought stress conditions. Significance in caliper length under both the conditions, WW and DS, was tested at $p = 0.05\%$

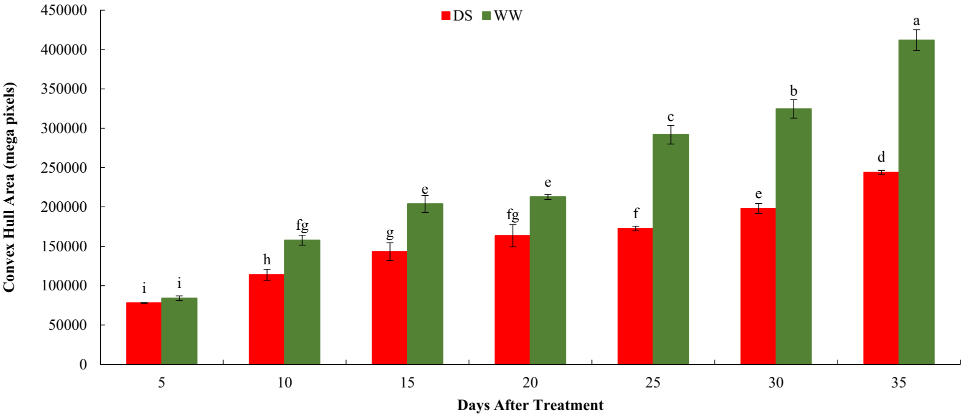


Fig. 10 Effect of well-watered and drought stress conditions on convex hull area (CHA) over time in pigeonpea genotypes. Significance in convex Hull Area length under both the conditions, WW and DS, was tested at $p = 0.05\%$

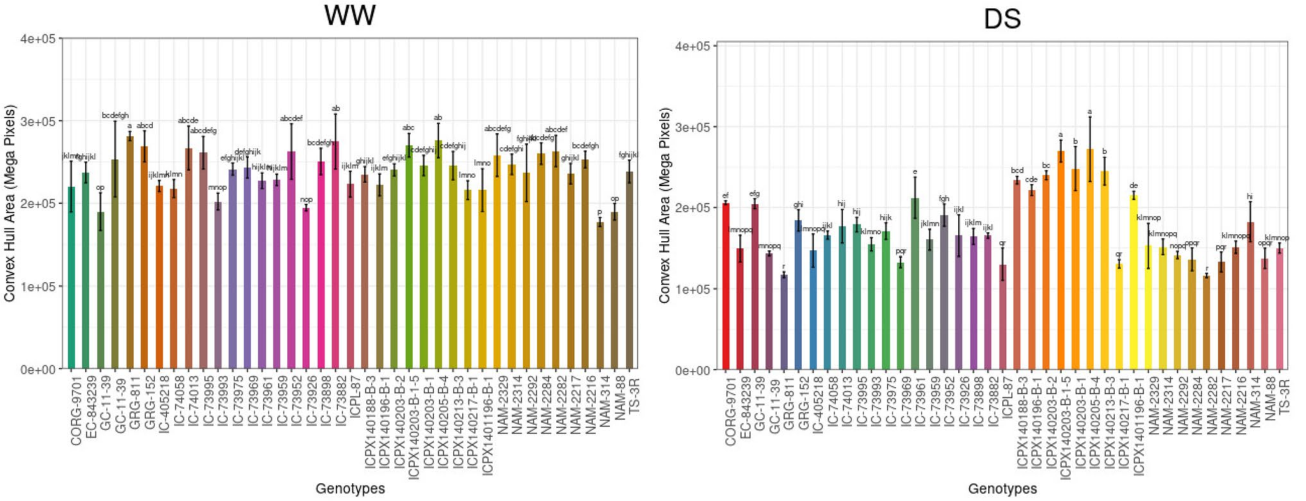


Fig. 11 Variation in convex hull area (CHA) of Pigeonpea genotypes Under Well-Watered and Drought Stress Conditions. Significance in convex Hull Area length under both the conditions, WW and DS, was tested at $p = 0.05\%$

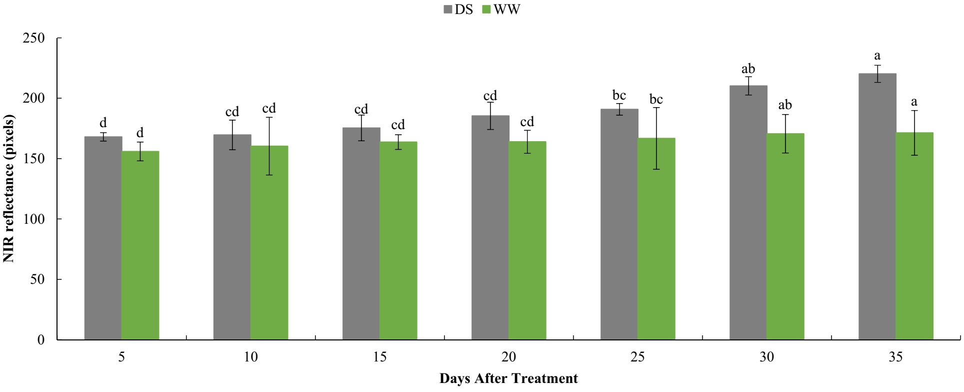


Fig. 12 Effect of WW and DS conditions on tissue water content (NIR reflectance) of pigeonpea genotypes. Significance in NIR reflectance under both the conditions, WW and DS, was tested at $p = 0.05\%$

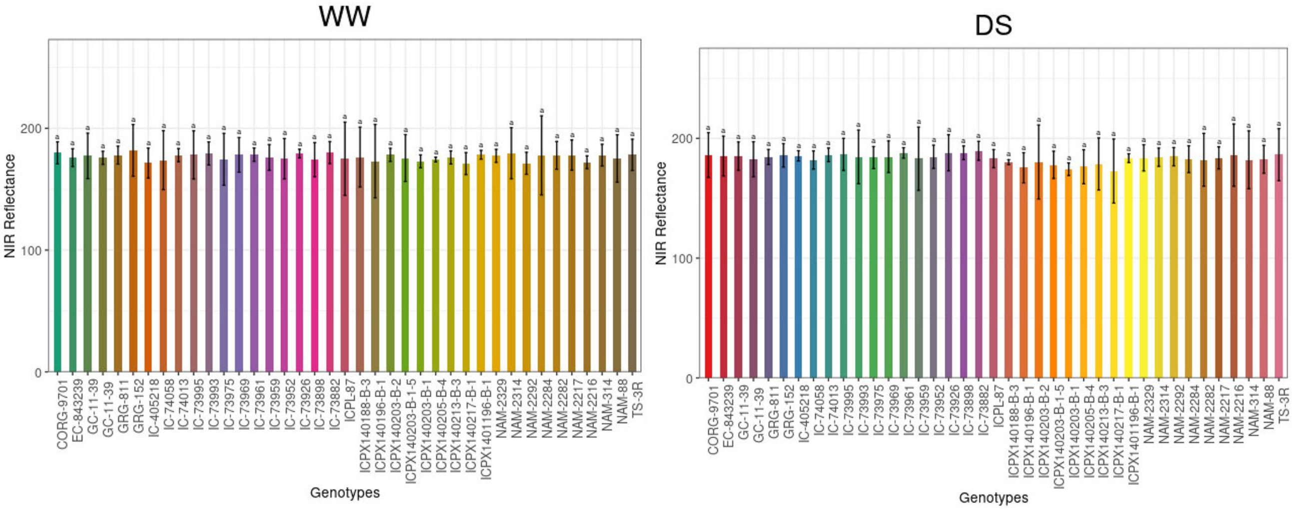
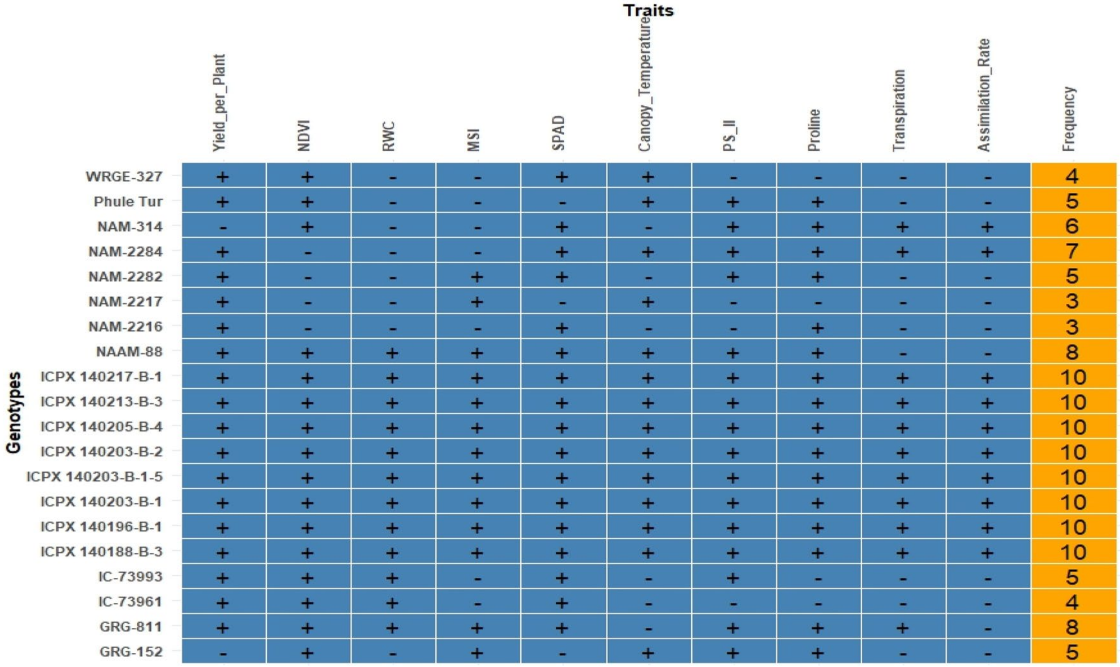


Fig. 13 Variation in tissue water content of pigeonpea genotypes under WW and DS conditions. Significance in NIR reflectance under both the conditions, WW and DS, was tested at $p=0.05$%



	Yield_per_Plant	NDVI	RWC	MSI	SPAD	Canopy_Temperature	PS_II	Proline	Transpiration	Assimilation_Rate	Frequency
WRGE-327	+	+	-	-	+	+	-	-	-	-	4
Phule Tur	+	+	-	-	-	+	+	+	-	-	5
NAM-314	-	+	-	-	+	-	+	+	+	+	6
NAM-2284	+	-	-	-	+	+	+	+	+	+	7
NAM-2282	+	-	-	+	+	-	+	+	-	-	5
NAM-2217	+	-	-	+	-	+	-	-	-	-	3
NAM-2216	+	-	-	-	+	-	-	+	-	-	3
NAAM-88	+	+	+	+	+	+	+	+	-	-	8
ICPX 140217-B-1	+	+	+	+	+	+	+	+	+	+	10
ICPX 140213-B-3	+	+	+	+	+	+	+	+	+	+	10
ICPX 140205-B-4	+	+	+	+	+	+	+	+	+	+	10
ICPX 140203-B-2	+	+	+	+	+	+	+	+	+	+	10
ICPX 140203-B-1-5	+	+	+	+	+	+	+	+	+	+	10
ICPX 140203-B-1	+	+	+	+	+	+	+	+	+	+	10
ICPX 140196-B-1	+	+	+	+	+	+	+	+	+	+	10
ICPX 140188-B-3	+	+	+	+	+	+	+	+	+	+	10
IC-73993	+	+	+	-	+	-	+	-	-	-	5
IC-73961	+	+	+	-	+	-	-	-	-	-	4
GRG-811	+	+	+	+	+	-	+	+	+	-	8
GRG-152	-	+	-	+	-	+	+	+	-	-	5

Fig. 14 Heat map depicting the performance of pigeonpea genotypes for key yield and physiological traits under drought stress, with hierarchical clustering of genotypes and traits based on similarity. In the heat map, "+" indicates positive performance (minimal difference between well-watered (WW) and drought-stressed (DS) conditions), while "-" indicates poor performance (significant difference between WW and DS)

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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