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Phenotyping Black Gram Genotypes under Water Stress Condition at Flowering Stage for Insights into Genetic Diversity and Stress Tolerance Mechanisms

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ABSTRACT: Climate change poses a significant threat to global food security, emphasizing the need to investigate drought stress and identify genotypes with enhanced stress tolerance. The present study evaluated 40 black gram genotypes by examining key physiological, biochemical and yield traits under water stress and stress-free conditions for resilience over two consecutive *kharif* seasons (2021 and 2022). Results showed that significant effects ($p < 0.01$) of growing seasons, treatments, and their interactions for most of traits, while genotype effects were significant ($p < 0.01$) across all traits. Generally, most morpho-physiological traits exhibited higher values under normal conditions, except for canopy temperature depression (CTD), total soluble sugars, and proline content, which increased under water stress. Cluster analysis grouped the genotypes into five distinct clusters. The study highlights the importance of characterizing key traits for the effective use of germplasm in crop improvement. Several drought tolerant genotypes, including IPU10-21, IPU9-26 and IPU2-43, IPU10-23, IPU10-33, IPU9-13-2 were identified based on their cumulative physiological and biochemical traits as well as yield performance. These genotypes have significant scope in breeding programs aimed at enhancing resilience and ensuring sustainable productivity under climatic change scenario.

Keywords: Biochemical traits, black gram, drought stress, genetic diversity, morphological, physiological

Introduction

Abiotic stresses such as drought, salinity, extreme temperatures, and nutrient deficiencies are major challenges to agricultural productivity, particularly in arid and semi-arid regions. Among these, drought stress is the most damaging, significantly reducing crop yields and posing a serious threat to global food security (Singhal *et al.,* 2023). According to the Food and Agricultural Organization, droughts have accounted for over 34% of global agricultural losses in the last two decades. Arid and semi-arid regions are increasingly prone to "hot droughts," which result in reduced water availability and adversely impacting crop physiological processes during critical growth stages (Yandigeri *et al.,* 2012). In pulse crops, such as black gram, drought stress during flowering and post-flowering stages leads to significantly higher yield losses compared to the vegetative stage (Anitha *et al.,* 2015). Understanding drought's impact across developmental stages is crucial to develop strategies that enhance crop resilience.

Black gram (*Vigna mungo* L.), commonly known as urad bean, is a highly valued legume due to its rich nutritional content, containing approximately 25% protein and 65% carbohydrates. It is a staple food in Indian and South Asian diets, playing a key role in their nutritional intake (Jothimani *et al.,* 2020). In addition to its dietary importance, black gram plays a critical role in sustainable agriculture by improving soil fertility through nitrogen fixation (Dahanayake *et al.,* 2015, Aparna *et al.,* 2020). However, since it is primarily cultivated in rainfed regions, black gram is particularly susceptible to drought stress, with yield losses of 20-30% being common during the flowering stage (Pandiyan *et al.,* 2023). This highlights the urgent need to develop drought-tolerant varieties to stabilize production under water-limited conditions. In response to drought stress, black gram exhibits various morphological, physiological, and biochemical adaptations (Joshi *et al.,* 2024) to cope with stress. These include reduced growth, loss of membrane stability (Baroowa *et al.,* 2016), and decreased chlorophyll levels (Mafakheri *et al.,* 2010). The accumulation of proline, an osmo protectant under water stress helps in stabilizing cellular structures under stress (Tas and Tas, 2007). Despite advances in understanding drought tolerance mechanisms, drought continues to limit black gram production, necessitating further research into adaptive traits to ensure long-term agricultural sustainability.

Given this background, the present study was conducted under both irrigated considered as stress-free and water stress conditions to evaluate black gram genotypes for morphological, physiological, and biochemical traits at the flowering stage. The objective was to identify drought-tolerant genotypes and key traits that could serve as reliable markers for screening germplasm under water stress condition and further use them in genetic enhancement program.

Materials and Methods

Plant materials

The study involved 40 black gram genotypes of diverse origins, categorized into early and medium maturity groups. These genotypes included varieties suited to different regions, as well as advanced breeding lines sourced from different centers of the All India Coordinated Research Program (AICRP) on *Kharif* Pulses, under the Indian Council of Agricultural Research (ICAR).

Experimental design

Field experiments were conducted at the ICAR-Central Research Institute for Dryland Agriculture (CRIDA), Hayathnagar Research Farm, located on the Deccan plateau in southern India (17°27'N latitude, 78°35'E longitude, and approximately 515 meters above sea level). The trials were carried out during the monsoon season (June to September) over two consecutive years (2021 and 2022) to evaluate the responses of black gram under both stress-free and stressed conditions. The experiments were set up in plots measuring 1.8 m^2, each planted with 2 rows of 3 meters in length, with 30 cm spacing between plants. A randomized complete block design (RCBD) with three replications was adopted and fertilizers were applied as per the recommended practices (20:40:20 kg/ha of N:P:K). Stress-free plots, where irrigation was provided as needed and served as stress free, while stressed plots were not irrigated from the flowering stage until visual symptoms of stress appeared. Soil moisture data was recorded using TDR moisture sensor at flowering stage to assess the conditions under both stress-free and water stress condition. All observations were recorded at stress point at flowering stages. At the end of stress treatment plants were re-watered again (drought recovery) till maturity to ensure further growth and economic product.

Morphological traits

Phenotypic data were collected from three representative plants of each genotype for several parameters, including plant height (PH, in cm), number of branches (BN), number of clusters (CN), pod length (PL, in cm), number of seeds per pod (SPP), number of pods (PN), pod weight (PW, in gm), hundred seed weight (HSW, in gm), fodder yield (FY, in gm per plant), and seed yield (SY, in gm per plant). Additionally, days to 50% flowering (DH) were recorded on a whole plot basis. These measurements were taken for both stress-free and stressed conditions over the two consecutive years of the study.

Physiological and biochemical traits

Various physiological and biochemical parameters for both stress-free and water stress conditions were recorded. Traits including Fv/Fm and SPAD (Chlorophyll Meter Reading - SCMR) were recorded to assess chlorophyll content. SPAD values were measured using a portable SPAD meter on the fully expanded third leaf from the top, with measurements taken between 10:00 and 11:00 AM. The Normalized Difference Vegetation Index (NDVI) was also utilized to evaluate plant greenness and overall crop health. NDVI reflects a plant's ability to absorb light and reflect infrared light, with healthier plants exhibiting higher NDVI values. Canopy temperature was recorded using handheld infrared thermometer (Yousfi *et al.,* 2019) by targeting the canopy leaves at 45° angle to the horizontal. Canopy temperature depression (CTD) refers to a metric measurement, indicating the difference between air temperature and canopy temperature. This measurement is typically performed in the afternoon under clear, windless conditions, with the most significant genotypic differences in canopy temperature depression (CTD) observed between 2 and 3 p.m. in higher temperature and low relative humidity.

The relative water content (RWC, %), which assesses the plant's water status (Barrs and Weatherley, 1962), and total chlorophyll content (Tchlo, µg/g), which indicates the concentration of chlorophyll (Arnon 1949) was measured in the plant tissues. The membrane stability index (MSI, %) was also measured (Deshmukh *et al.,* 1991) reflecting the integrity of cell membranes under stress conditions (Sai and Chidambaranathan, 2019). Additionally, a biochemical trait, soluble sugars (Kochert, 1978) was estimated to gain insights into the carbohydrate status and overall health of the plants. While proline content (Bates *et al.,* 1973) was assessed as an important indicator of osmotic adjustment and stress tolerance in plants. Together, these traits offer valuable information about the plant's physio-biochemical responses to environmental stressors.

Statistical analysis

The data was statistically analyzed with SAS software (Version 9.3; SAS Institute, Cary, NC). Analysis of variance and least significant difference (LSD) test were applied to compare the genotypes for each trait for individual trial and combined over different water regimes. Variances due to genotypes and error components were used for estimating broad sense heritability (h^2_b) for all traits. The mean data of morpho-physiological and biochemical traits were used for cluster analysis using R software to summarize the variation among genotypes under stress-free and stressed conditions using Ward's method.

Results and Discussion

Analysis of variance under stress-free and water-stress conditions

The combined ANOVA analysis for black gram across two years, under stress-free and water-stress conditions, revealed significant variances in agronomic and physiological traits due to year, treatment, and genotype (Table 1). Physiological parameters including relative water content (RWC), membrane stability index (MSI), SPAD readings, and normalized difference vegetation index (NDVI) were significantly influenced by both treatment and year ($p < 0.01$). Notably, treatment had a strong effect on RWC and MSI, indicating substantial physiological differences between plants under stressed and stress-free conditions. Genotype also had a significant impact on all traits, with pronounced genotype-by-environment interactions, including year × treatment, year × genotype, treatment × genotype, and year × treatment × genotype effects. Significant variances due to treatment, genotype, and genotype × environment interactions across various agronomic, physiological, and biochemical traits highlighted the influence of environmental factors on trait expression over time.

Agronomic traits such as day of flowering (DF), plant height (PH), branch number (BN), and pod number (PN) also showed significant variances due to treatment, year, and genotype and all interaction effects except seeds per pod and hundred seed weight ($p < 0.01$). Significant interactions were observed for year × treatment and treatment × genotype for key yield-related traits, such as seed yield (SY) and total biomass (TB), further highlighting the importance of both environmental conditions and genetic background in determining yield performance under stress conditions.

The physiological and biochemical traits under stress-free and stressed condition was recorded in the second year of experiment. The ANOVA (Table 2) for the second year under stress-free and stressed conditions revealed significant differences in physiological and biochemical traits, including canopy temperature depression (CTD), total chlorophyll content, total soluble sugars (TSS), proline content, and the Fv/Fm ratio. Both treatment and genotype effects were highly significant for CTD, chlorophyll content, TSS, proline content, and Fv/Fm ratio, indicating substantial genetic variability in response to stress. The interaction between treatment and genotype (treatment × genotype) was also significant for CTD, TSS, and proline ($p < 0.01$), suggesting that genotypes responded differently to stress. These results highlight the complexity of genotype responses to abiotic stresses and emphasize the importance of considering both genetic variability and environmental factors in breeding programs aimed at developing drought-tolerant black gram varieties.

Table 1: Combined ANOVA for morpho-physiological traits in black gram evaluated under stress-free and stressed condition of two years

Source	DF	RWC	MSI	SPAD	NDVI	PH	DH	BN	CN	PL	SPP	PN	PW	SY	TB	HSW	HI
Rep	2	3.2	10.01	2.65	0.001**	21.3**	4.20*	3.10*	0.67	1	1.1*	12.94	0.33	0.68	1.1	0.85**	0.001
Year	1	70.23**	9466.70**	4509.84**	0.34**	754.31**	315.66**	0.37	2547.49**	0.05	31**	8317.34**	2036.1**	764.17**	1932.06**	12.8**	0.624**
Treatment	1	3849.48**	30962.55**	1378.03**	1.77**	594.34**	0.04	132.62**	530.04**	19.3**	37.18**	7567.3**	625.54**	432.02**	857.63**	47.63**	0.38**
Genotype	39	26.26**	358.24**	21.31**	0.011**	56.67**	6.10**	1.58**	11.42**	0.37**	1.06**	187.11**	17.3**	4.68**	48.41**	0.5**	0.011**
Year* Treatment	1	181.67**	3826.46**	5.79	0.05**	64.23**	8.07*	4.74**	157.32**	0.81**	1.88**	78.78**	273.07**	84.57**	153.58**	1.61**	0.026**
Year* Genotype	39	47.81*	328.15**	29.01**	0.011**	52.59**	4.48**	2.28**	13.69**	0.42**	0.81**	116.62**	12.03**	2.26**	28.32**	0.55**	0.011**
Treatment * Genotype	39	9.95**	148.92**	7.92**	0.01**	8.72**	1.53**	1.03**	5.72**	0.19**	0.21	52.45**	6.4**	0.95**	10.01**	0.09	0.003**
Year* Treatment *Genotype	39	7.64**	121.11**	7.60**	0.01**	9.58**	1.95**	0.86	4.49**	0.08**	0.27	46.02**	6.86**	1.11**	11.71**	0.08	0.002**
Error	318	4.41	16.31	1.65	0.001	2.32	0.68	0.56	1.14	0.06	0.22	4.51	0.42	0.35	0.87	0.1	0.001
GM	-	80.29	51.84	44.9	0.7	26.9	35.46	4.87	8.92	4.45	5.62	24.38	8.48	5.69	16.94	4.27	0.33
CV(%)	-	2.62	7.79	2.86	3.21	5.66	2.33	15.33	11.96	5.68	8.34	8.71	7.68	10.46	5.52	7.43	10.19
SEd (Treatment)	-	0.19	0.37	0.12	0.002	0.14	0.08	0.07	0.10	0.02	0.04	0.2	0.6	0.05	0.09	0.03	0.003
SEd (Genotype)	-	0.858	1.65	0.53	0.009	0.62	0.34	0.31	0.44	0.1	0.19	0.87	0.27	0.24	0.38	0.13	0.014
SEd (Treatment* Genotype)	-	1.21	1.65	0.74	0.009	0.62	0.34	0.43	0.62	0.1	0.19	0.87	0.38	0.34	0.54	0.18	0.014

**, **=significant at the 5% and 1% level of significance respectively; where, df =Degrees of freedom, RWC=relative water content (%), MSI=membrane stability index (%), SPAD=chlorophyll meter reading (SCMR), NDVI=normalized difference vegetation index, PH=Plant height(cm), DH=days to 50% flowering, BN=branches number, CN=cluster number, PL=pod length(cm), SPP=number of seeds per pod, PN=number of pods, FY=fodder yield(g/plant), PW=pod weight(g), SY=seed yield(g/plant), TB=total biomass (g/plant), HSW=hundred seed weight (g), HI=harvest index and GM=grand mean of each trait.*

Table 2: Combined ANOVA for biochemical traits in black gram evaluated under stress-free and stressed conditions in second year

Source	DF	CTD	Tchlo	TSS	Proline	Fv/Fm
Rep	2	0.66**	0.12	9.04	0.22	0.01**
Treatment	1	247.74**	73.05**	7539.73**	6429.94**	0.22**
Genotype	39	3.58**	0.43**	68.89**	40.78**	0.004**
Treatment * Genotype	39	1.30**	0.1	12.89**	23.13**	0.001
Error	158	0.1	0.08	1.45	0.46	0.001
GM	-	-3.36	3.61	18.54	12.32	0.62
CV (%)	-	-9.45	7.77	6.5	5.51	5.42
SEd (Treatment)	-	0.041	0.036	0.156	0.088	0.004
SEd (Genotype)	-	0.183	0.162	0.696	0.392	0.019
SEd (Treatment * Genotype)	-	0.259	0.229	0.984	0.555	0.027

**, **= significant at the 5% and 1% level of significance respectively, where df = Degrees of freedom, CTD= canopy temperature depression, Tchlo=total chlorophyll content(µg/g), TSS =total soluble sugar (mg/g FW), proline (µg/g FW) and GM=grand mean of each trait.*

Phenotyping for yield and yield related traits

The performance of black gram genotypes under water-stressed and stress-free conditions revealed significant variation among genotypes for all traits. Trial mean, range, standard error of mean difference (SEd), coefficient of variation (CV%) and heritability for various morpho-physiological and biochemical traits was given in Table 3. Under water stress conditions, most traits showed a declining trend, except for canopy temperature depression (CTD), total soluble sugar, and proline content, which were higher compared to the stress-free conditions. This increase is consistent with the expected stress tolerance mechanisms that crops typically exhibit in response to water stress. A high coefficient of variation was noted for traits such as the number of branches, number of clusters, number of pods, seed yield, and pod weight, both under stress-free and stressed conditions. Most of traits studied showed high broad sense heritability (h^2_b) both under stress-free and stressed conditions indicating that these traits are more influenced genetically than environment.

Among the growth parameters, plant height was significantly affected under water stress conditions compared to stress-free across the years. Under stress-free conditions, plant height ranged from 24.25 cm to 35.90 cm, with a mean of 28.01 cm. In contrast, water stress reduced the overall plant height, with a mean of 25.78 cm and a range of 20.77 cm to 34.60 cm. Under stress-free conditions, genotype IPU96-12 recorded maximum plant height of 35.90 cm, whereas genotype IPU7-3 had the shortest height of 20.77 cm under stress conditions. The number of clusters per plant also decreased under water stress conditions. Under stress-free conditions, the cluster number varied from 7.28 to 12.22, whereas under stress, the range dropped to 5.62 to 10.65. Genotypes such as IPU9-13 (12.22), IPU10-21 (12.02), IPU96-12 (11.60), IPU9-2 (11.60), and G-215 (11.52) performed better in stress-free conditions. Under stress, G-215 (10.65), IPU9-26 (9.95), IPU13-7 (9.72), PLU-710 (9.43), and IPU13-3 (9.35) produced more clusters. Notably, genotypes IPU13-5, IPU10-26, and IPU10-117 consistently exhibited higher cluster counts under both conditions.

Table 3: Mean, range, standard error, coefficient of variation and heritability for various morpho-physiological and biochemical characters tested under stress-free and water stressed conditions

Character	GM ± SEd		CV (%)		Range		Heritability	
	Stress-free	Water-stress	Stress-free	Water-stress	Stress-free	Water-stress	Stress-free	Water-stress
RWC	83.12±0.84	77.46±0.87	3.76	3.97	79.62-85.91	73.47-82.95	0.47	0.72
MSI	59.87±1.55	43.81±1.75	18.12	18.27	42.05-71.22	29.23-60.54	0.91	0.92
SPAD	46.59±0.55	43.2±0.5	8.17	9.87	44.35-50.15	39.63-46.62	0.88	0.94
NDVI	0.76±0.01	0.64±0.01	7.29	9.24	0.68-0.81	0.53-0.72	0.93	0.97
Fv/Fm	0.65±0.02	0.59±0.02	4.41	6.42	0.58-0.68	0.49-0.68	0.59	0.65
CTD	-4.38±0.2	-2.34±0.16	7.81	12.07	-6.5--2.4	-4.47--1.24	0.96	0.95
Tchlo	4.16±0.18	3.05±0.13	7.33	7.17	3.6-5.02	2.32-3.6	0.62	0.83
TSS	12.94±0.72	24.15±0.67	9.67	4.78	7.01-21.95	14.82-35.77	0.93	0.98
Proline	7.14±0.26	17.49±0.49	0.45	4.84	4.35-9.11	8.33-34.26	0.96	0.99
DH	35.45±0.32	35.47±0.35	4.20	3.74	33.93-37	33.63-36.83	0.95	0.86
PH	28.01±0.66	25.78±0.58	12.05	11.43	24.25-35.9	20.77-34.6	0.80	0.79
BN	5.4±0.31	4.35±0.3	16.75	19.54	3.97-6.28	3.6-5.32	0.73	0.73
CN	9.97±0.47	7.87±0.4	35.49	32.46	7.28-12.22	5.62-10.65	0.56	0.79
PL	4.65±0.1	4.25±0.11	6.77	7.69	4.07-5.20	3.82-4.77	0.66	0.71
SPP	5.9±0.19	5.34±0.19	10.58	10.92	5.33-6.60	4.53-6.05	0.51	0.77
PN	28.35±0.96	20.41±0.76	25.01	27.41	18-39.05	13.38-27.62	0.90	0.90
PW	9.62±0.28	7.34±0.25	36.83	25.46	7.29-13.96	5.21-10.07	0.89	0.87
SY	6.64±0.28	4.7±0.25	30.66	24.66	5.02-8.73	3.78-6.06	0.88	0.86
TB	18.28±0.39	15.6±0.37	20.21	15.92	14.66-24.04	12.08-19.76	0.93	0.93
HSW	4.59±0.12	3.96±0.14	7.96	11.96	4.18-5.2	3.47-4.39	0.69	0.79
HI	0.36±0.01	0.31±0.01	18.42	18.65	0.28-0.45	0.23-0.39	0.82	0.83

Where, RWC= relative water content (%), MSI= membrane stability index (%), SPAD=chlorophyll meter reading (SCMR), NDVI= normalized difference vegetation index, PH=Plant height(cm), DH= days to 50% flowering, BN=branches number, CN=cluster number, PL= pod length(cm), SPP= number of seeds per pod, PN=number of pods, FY=fodder yield(g/plant), PW= pod weight(g), SY=seed yield(g/plant), TB= total biomass (g/plant), HSW=hundred seed weight (g), HI= harvest index, CTD= canopy temperature depression, Tchlo=total chlorophyll content(µg/g), TSS =total soluble sugar (mg/g FW), proline (µg/g FW), GM= grand mean, SEd= standard error, and CV= coefficient of variance.

For yield-related parameters, including pods per plant (PN), seed yield (SY), and harvest index (HI), genotypic performance varied significantly between treatments. Under stress conditions, genotypes IPU7-3 and IPU10-21 demonstrated strong yield potential, producing 25.62 and 27.62 pods per plant, respectively. In terms of seed yield (g/plant), the highest performers under stress-free conditions were IPU10-26 (8.73), IPU06-02 (8.49), IPU11-2 (8.26), LBG-623 (8.08), and STY-2868 (7.89). Under stress, the top genotypes for seed yield were IPU06-02 (6.06), IPU10-26 (5.91), PLU-710 (5.52), PLU-826 (5.48), and G-215 (5.19). Genotypes IPU2-43, IPU13-1, IPU10-117, and IPU96-7 consistently exhibited higher seed yield under both stress-free and stress conditions, making them promising candidates for yield stability across varying environmental conditions. The harvest index (HI), which indicates the efficiency of plants in allocating source to sink production, showed significant differences between genotypes. Under stress-free conditions, the highest HI values were observed in IPU9-9168 (0.45), IPU11-6 (0.43), IPU10-21 (0.41), WBG-108 (0.41), PSRJ-95016 (0.41), and IPU13-6 (0.40). Under stress conditions, genotypes exhibiting greater adaptability included IPU13-1 (0.39), IPU13-6 (0.36), IPU10-16 (0.36), IPU11-6 (0.35), IPU10-1 (0.35), and IPU13-7 (0.35), demonstrating their ability to maintain a higher harvest index even under adverse conditions. Genotypes were ranked based on the pooled means under water stress conditions, encompassing various morpho-physiological, biochemical, and yield traits showed genotypes PU10-21, IPU9-26, IPU2-43 as tolerant for most traits, followed by genotypes IPU10-23, IPU10-33, IPU9-13-2. Water stress had a notably negative impact on morpho-physiological traits compared to stress-free conditions, although genotypes exhibited differential responses (Bordoloi *et al.,* 2023). Water stress primarily affects cell expansion and growth by reducing cell turgor, leading to stunted plant height. Similar observations have been reported earlier in legumes (Gurumurthy *et al.,* 2019).

Physiological and biochemical parameters

Several physiological parameters showed significant variation between stress-free and stress conditions. SPAD values, which indicate chlorophyll content, ranged from 44.35 to 50.15 µg/g under stress-free conditions but dropped to 39.63 to 46.62 µg/g under stress, suggesting that water stress led to a decline in chlorophyll content across genotypes. Normalized Difference Vegetation Index (NDVI) values were also higher under stress-free conditions, ranging from 0.68 to 0.81, while under stress they declined to 0.53 to 0.72. The highest NDVI value under stress-free condition was recorded in IPU13-4, indicating strong vigour, whereas the lowest values under stress were observed in IPU9-13 and PLU-710, indicating susceptibility to stress.

Relative Water Content (RWC) generally decreased under stress in all genotypes. Under stress-free conditions, RWC ranged from 79.62% to 85.91%, while under stress, it ranged from 73.47% to 82.95%. Genotype IPU10-16 exhibited the highest RWC under stress-free conditions (85.91%), highlighting its superior water retention ability, while IPU9-13 had the lowest RWC (79.62%). Genotypes like IPU10-16, UH-855, PLU96-06, IPU99-168, IPU2-43, and IPU10-23 maintained higher RWC levels under stress free. Under stress conditions, UH-855, COBG-05, IPU2-43, IPU10-16, PLU96-06, and IPU13-3 showed better water retention.

Membrane Stability Index (MSI), a key indicator of cell membrane stability under stress, was notably higher in PSRJ95016 (57.60%) and IPU13-6 (60.54%), indicating stronger membrane integrity compared to more stress-sensitive genotypes like COBG-05 (29.23%) and PLU96-06 (30.26%). Under stress-free conditions, genotypes such as IPU94-4, PLU96-06, IPU9-26, IPU9-13, PLU-826, IPU94-1, and IPU11-6 displayed higher MSI values. Under stress, genotypes like IPU13-7, PLU-826, STY-2868, IPU13-1, IPU10-117, and G-215 consistently maintained higher MSI levels, reflecting their stronger stress tolerance.

Other physiological and biochemical traits, such as Canopy Temperature Depression (CTD), total chlorophyll content (Tchlo), total soluble sugars (TSS), proline content, and chlorophyll fluorescence (Fv/Fm), were recorded in the second year of the experiment. These traits serve as key indicators for understanding the genotypic response to water stress. The CTD values were generally higher under stress conditions, with stress-free values ranging from -6.50°C to -2.40°C and stress values from -4.47°C to -1.24°C, indicating reduced canopy cooling under stressful environments. Chlorophyll content (µg/g) also declined under stress, with stress-free values ranging from 3.60 to 5.02 µg/g and stress values ranging from 2.32 to 3.60 µg/g, reflecting a reduction in photosynthetic efficiency under water deficit.

Among physiological traits, water stress significantly reduced total chlorophyll content and relative water content compared to stress-free conditions, although the degree of impact varied among genotypes, likely due to inherent tolerance mechanisms (Bordoloi *et al.,* 2023). Canopy temperature depression, chlorophyll fluorescence, total soluble sugars, and proline content were also adversely affected by water stress. Reduced chlorophyll content under drought conditions impairs light harvesting, and increasing energy absorption by the photosynthetic apparatus may trigger the production of reactive oxygen species (ROS) (Moosavi *et al.,* 2020). To counteract this, plants degrade light-absorbing pigments to mitigate ROS damage (Herbinger *et al.,* 2002).

Proline, an important osmo-protectant, increased substantially under stress, aiding in mitigating its effects. Proline levels under stress-free conditions ranged from 4.35 to 9.11 µg/g FW, with genotypes like IPU9-13-2 (9.11 µg/g FW), IPU13-7 (9.10 µg/g FW), IPU11-6 (9.03 µg/g FW), IPU13-5 (8.81 µg/g FW), and IPU10-23 (8.80 µg/g FW) showing higher proline content. Under stress, proline levels rose significantly, ranging from 8.33 to 34.26 µg/g FW, with genotypes like IPU13-3 (34.26 µg/g FW), IPU13-1 (24.73 µg/g FW), G-215 (24.73 µg/g FW), PLU-710 (24.58 µg/g FW), and PLU96-06 (21.44 µg/g FW) showing the highest proline accumulation. The Fv/Fm ratio, which indicates the efficiency of photosystem II and photoinhibition resistance, was significantly higher in genotypes like PLU-826 (0.68) and IPU13-1 (0.66) under stress, suggesting less photoinhibition compared to more susceptible genotypes such as IPU99-168 (0.49). Proline content increased sharply by more than 50% under stressed conditions, with most genotypes exhibiting more than a two-fold increase. However, there was considerable variation in proline levels among genotypes under stress (Baroowa *et al.,* 2012). Proline helps protect cells by accumulating and balancing osmotic potential in response to external water stress, as reported in wheat (Pireivatloum *et al.,* 2010). Its accumulation plays an adaptive role, imparting stress tolerance (Verbruggen and Hermans, 2008). Proline not only acts as an osmo protectant during water stress but also regulates water loss from plant cells (Yokota *et al.,* 2006). Additionally, it supplies energy for plant survival and growth under stress. Therefore, proline accumulation is an important selection criterion in drought-tolerance screening of germplasm. These findings support the role of proline as a key factor in drought adaptation and stress resilience in crop plants.

Total soluble sugars (TSS) were lower under stress-free conditions, with genotypes such as PLU-826 (21.95 mg/g FW), IPU06-02 (19.24 mg/g FW), PLU-710 (16.13 mg/g FW), PDU-1 (15.98 mg/g FW), and IPU11-2 (15.71 mg/g FW) showing comparatively higher TSS levels. Under stress conditions, however, TSS values increased significantly. Genotypes like IPU9-16 (35.77 mg/g FW), PSRJ-95016 (32.23 mg/g FW), IPU10-33 (31.25 mg/g FW), WBG-108 (30.43 mg/g FW), and LBG-787 (29.95 mg/g FW) exhibited the highest TSS under stress. The markedly higher TSS values under stress (14.82–35.77 mg/g FW) compared to stress-free (7.01–21.95 mg/g FW) suggest a metabolic adjustment to cope with water stress. Overall, the data suggest that black gram genotypes responded to water stress through physiological and biochemical adaptations, such as reduced chlorophyll content, increased soluble sugars, and enhanced proline accumulation, enabling them to better tolerate stressful conditions.

Cluster analysis

The cluster analysis performed using morpho-physiological, biochemical, and yield traits under both stress-free and stress conditions, reveals distinct hierarchical groupings of genotypes. Using the Ward's method, the forty genotypes were categorized into five major clusters in both stress-free and stress (Fig. 1) conditions. Under stress, some genotypes shifted into different clusters, indicating altered associations caused by stress, while certain genotypes remained consistent across both the conditions. This divergence highlights the impact of stress on clustering patterns. The distance between clusters also varied, reflecting degrees of dissimilarity in response to stress-free and stressed conditions.

In our study, genotypes exhibited significant variability in morphological, physiological, biochemical, and yield traits under both stress-free and water stress conditions. Genotypes were ranked based on their performance for each trait under water stress, and an overall average rank was calculated for each genotype. Based on these rankings, genotypes such as IPU10-21, IPU9-26, and IPU2-43 demonstrated superior performance across most traits, followed closely by IPU10-23, IPU10-33, and IPU9-13-2. Clustering analysis further categorized these genotypes into distinct groups, revealing their diversity. Genotypes with outstanding performance from different clusters can be utilized in hybridization programs to create genetic variability, facilitating the selection of superior plant types, and improving stress tolerance in black gram.

It is well established that adequate water availability is critical for optimal crop growth and productivity. However, crops are often subjected to drought stress at various phenological stages, which can severely affect yield. Crop productivity under water stress is often reduced due to alterations in physiological and biochemical processes at the cellular and molecular levels, as plants employ survival mechanisms under stress conditions. This study demonstrated the differential responses of black gram genotypes to water stress, particularly in terms of morpho-physiological, biochemical, and yield-related attributes.

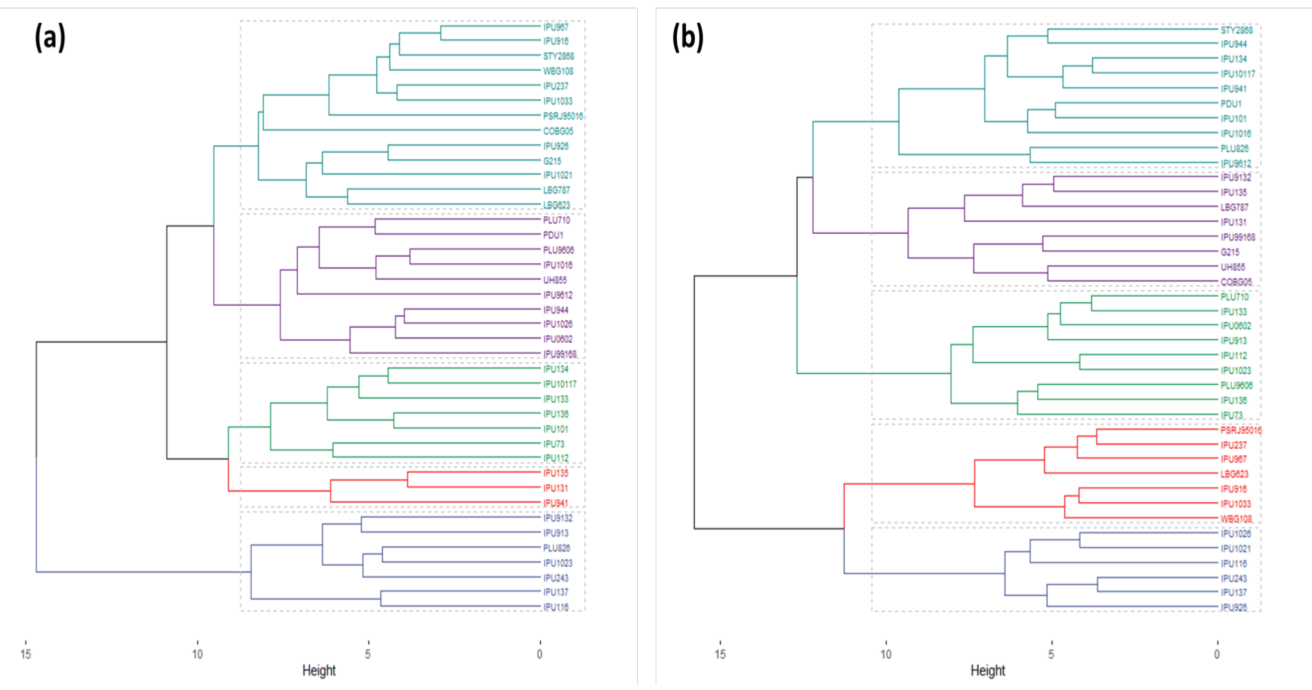

Fig. 1: Clustering of genotypes under well-watered (a) and water stressed condition (b) based on ward's method

Conclusion

The present study revealed that water stress had a clear negative impact on plant growth, reducing traits such as branch number, pod production, and overall yield. However, traits such as proline content, total soluble sugars, relative water content, and canopy temperature depression exhibited considerable variation among genotypes under both stress-free and stress conditions. Genotypes that maintained higher levels of these physiological and biochemical activities under stress can serve as valuable donors for improving productivity in drought-prone environments. This study provides valuable insights for developing strategies to genetically enhance black gram, making it more resilient to drought stress.

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