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ORIGINAL ARTICLE

Morpho-physiological and biochemical changes in black gram (*Vigna mungo* L. Hepper) genotypes under drought stress at flowering stage

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Abstract

The response of drought stress on morpho-physiological and biochemical characters was assessed in black gram genotypes in a pot culture experiment. Water stress was applied at flowering stage of the crop and various morpho-physiological and biochemical characters were analyzed under control and water stress conditions. The genotypes, water levels and their interaction varied significantly for majority of the traits quantified revealing the presence of substantial genetic diversity. Based on these studies, genotypes PGRU95016, COBG05, IPU99209, IPU941 and IPU243 were identified as tolerant to drought stress conditions. Photosynthesis, stomatal conductance, transpiration rate, total chlorophyll, proline content and peroxidase activity could be useful to screen for drought tolerance in black gram.

Keywords Black gram · Biochemical · Morphological and physiological traits · Drought stress · Genetic diversity

Introduction

Abiotic stresses specially drought causes major crop yield losses worldwide (Vinocur and Atman 2005). It is the major impediment for crops to complete its life cycle especially in the context of climate change. Dry spells at different growth stages of crop are more frequent during last few decades. Under drought stress, various morpho-physiological and biochemical responses are triggered in plants to cope with stress. The impact of stress varies with its intensity, duration and phenophases of crop. In majority of germplasm evaluation studies for drought tolerance, seed yield is the major criteria. However, for an effective breeding program identifying characters contributing drought tolerance is equally important. Drought stress affects morphological, physiological and biochemical characters which culminate into poor grain yield (Baroowa and Gogoi 2012, 2013; Baroowa et al. 2016; Maheswari et al. 2016). The depletion of chlorophyll content is more with increased intensity and duration of drought stress (Kiani et al. 2008). Drought stress disturbs turgor pressure and affects cell enlargement due to loss of cell turgidity resulting in poor plant growth (Mondal et al. 2012). It also inhibits rate of photosynthesis and damages photosynthetic apparatus (Manivannan et al. 2007). Drought stress affects almost all growth and development processes. Many morphological and physiological traits which are imparting tolerance to water-deficit stress were reported in crop plants. The reduction in leaf number and size due to drought stress reduces source capacity, thereby impacting realization potential of sink. Drought stress negatively impacts the inherent traits such as leaf water potential (ψ), RWC (relative water content) and OP (osmotic potential) as well as SC (stomatal conductance) and TR (transpiration rate) (Anjum et al. 2011; Subramanian and Maheswari 1990a, b; Shanker et al. 2014). Better root growth under water-deficit conditions is well known to enable plants to match the increased demand of water (Zlatev and Lidon 2012). Hence, it is imperative to identify and characterize traits which are contributing to drought tolerance in the available germplasm to use in breeding program.

Black gram or urdbean (*Vigna mungo* L. Hepper $2n = 22$) is a short duration grain legume and with high protein

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content in seeds. It improves soil fertility by capturing atmospheric nitrogen. It ranks fourth in acreage and production after chickpea, pigeon pea and green gram amongst pulse crop in India and is consumed throughout the country (Singh and Ahlawat 2005). Although, urdbean is considerably grown under a large area, the average national productivity is still very low (550 $kgha^{-1}$) as compared to other pulses. This crop is exposed to water stress at different stages of its growing period. However, an in-depth analysis about responses of this crop under water-deficit stress is not available. Water stress drastically affects production and productivity of pulse crops if exposed to stress at flowering and post-flowering as compared to other phenophases (Cortes and Suidaria 1986; Uprety and Bhatia 1989). In this context, a study was conducted under control (well-watered) and water stress to characterize black gram genotypes for morphological, physiological and biochemical traits at flowering stage for identifying tolerant genotypes and traits for screening germplasm against drought stress.

Materials and methods

Plant material and growing conditions

10 black gram genotypes, namely COBG05, IPU99189, IPU99209, PDU1, PGRU95016, STY2868, UH855, UH99144, IPU243 and IPU941 were characterized at ICAR-Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (latitude 22° 37′N, longitude 75° 5′E, altitude 557 m MSL) under control and water stress conditions in a pot experiment. The experiment was conducted in randomized block design having three replications. Three seeds for each genotype were sown on third week of May in earthen pots having soil and compost in 3:1 ratio. Watering was done regularly and finally having one plant in each pot.

Two water regimes, i.e., well-watered (control: 66% of field capacity) and water stress (stress: 40% of field capacity) were maintained by regulation of watering at 45 DAS (days after sowing). Water stress was imposed by withholding of irrigation. Pots in the water stress treatment were protected from any possible rain water by placing under rainout shelter. Observation was recorded on fifth day after withdrawal of water. Stress was relieved after recording observations on fifth day by re-watering and plants were maintained stress free till harvest.

Measurement of morphological traits

Data were recorded for morphological traits such as plant height (PH), branches/plant (BR), clusters/plant (CL), pods/plant (PN), leaf number/plant (LN), shoot dry weight/plant (SDW), root length (RL) and root dry weight (RDW). Plant height was recorded from base to the tip of main branch. Total number of leaves was counted for each treatment. Shoots and roots were separated and oven dried at 80 °C for 72 h. Dry weight of shoots and roots were measured to determine shoot and root dry biomass.

Physiological traits

Net photosynthesis rate, SC, TR, total chlorophyll content and water use efficiency (WUE) were measured at flowering stage both under control and water stress conditions. Net photosynthetic rate (PS: µmol CO_2 m^{-2} s^{-1}), SC (g_s: mmol m^{-2} s^{-1}) and TR (TR: mmol H_2O m^{-2} s^{-1}) were measured using LI-COR 6400 photosynthesis system in water stress and control treatment. The light intensity of 1500 µmol photons m^{-2} s^{-1} and CO_2 partial pressure of 400 ppm was used for taking observation. Instantaneous water use efficiency (WUE) was measured as the ratio of net photosynthetic rate and transpiration rate (Baburai Nagesh 2006). Total chlorophyll (Tchlo) content was measured according to the method of Arnon (1949).

Biochemical traits

Various biochemical characters were also estimated at flowering stage of all genotypes under control and water stress conditions.

Soluble sugars

The soluble sugar was measured by the phenol–sulfuric acid method. Leaf samples of 100 mg were taken in test tubes and added 10 ml of 70% of ethanol. These samples were stored at 4 °C for one week. The sample was centrifuged for 20 min. 1 ml each of clear solution mixed with phenol and 3 ml sulfuric acid was mixed and allowed to stabilize for 1 h and development of color. In addition, one blank contains 1 ml of distilled water. The absorbance was read at 580 nm for measuring soluble sugars. The amount of soluble sugars was calculated and expressed as mg g^{-1} dry weight (Kochert 1978).

Peroxidase assay

The activity of peroxidase (POD) in leaves from control and water stress treatments were measured following the change of absorption at 420 nm due to guaiacol oxidation (Gueta Dahan et al. 1997). Enzyme activity was estimated using extinction coefficient of tetra-guaiacol, an oxidation product of guaiacol ($\epsilon = 26.6$ mM^{-1} cm^{-1}).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was estimated as per Dhindsa et al. (1981). The fresh leaves were grounded in potassium phosphate buffer containing EDTA and 2% polyvinylpyrrolidone (PVP). The sample was centrifuged at 10,000 g for 20 min and the supernatant was analyzed for enzyme activities. Superoxide dismutase was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) following method described by Beauchamp and Fridovich (1971).

Membrane lipid peroxidation (MDA)

Lipid peroxidation was measured by measuring malondialdehyde (MDA) using standard protocol (Heath and Packer 1968). 1 gram of leaf tissue was grounded in 2.0 ml of TBA and centrifuged at 10,000 g for10 min at 4 °C. 2 ml of supernatant and 0.5% TBA were added to 20% trichloroacetic acid solution. The reaction mixture was incubated in hot water bath for 30 min. The mixture was allowed to cool for 5 min. The absorbance was read at 532 and 600 nm ($\epsilon = 155\ \mathrm{mM}^{-1}\ \mathrm{cm}^{-1}$).

Proline content

One gram of fresh leaf tissue was homogenized in 3% aqueous sulphosalicylic acid and centrifuged at 10,000 g for 10 min. Acid-ninhydrin solution (1.25 g ninhydrin in 30 ml glacial acetic acid) was added and was heated at 90 °C for 1 h. It was allowed to cool and then extracted with 4 ml of toluene by vortexing for 1 min. The absorbance was read at 520 nm, using toluene as a blank and expressed as µM proline/g of fresh tissue (Bates et al. 1973).

Cell membrane stability (CMS) and cell membrane injury

Membrane stability index was estimated by measuring electrical conductivity of leaf leachates in distilled water at room temperature after soaking overnight at 100 °C (Deshmukh et al. 1991). 100 mg leaves were cut into discs of equal size and put in tubes with 10 ml of distilled water in two sets. The first set was kept overnight at room temperature and second set at boiling water bath (100 °C) for 15 min and their electric conductivities C_1 and C_2 were measured. The cell membrane stability and cell membrane injury were calculated using the following formula:

$$\text{Cell membrane stability} = [1 - (C_1/C_2)] \times 100;\ \text{cell membrane injury}\ \% = 100 - \text{CMS}\%. \quad (1)$$

Statistical analysis

Data were analyzed using SAS software (Version 9.3; SAS Institute, Cary, NC). Analysis of variance and least significant difference (LSD) test was applied to compare genotypes for each character in individual trial and combined over different water regimes. Genotypes (σ^2_g) and error (σ^2_e) variances and standard errors were used for estimating broad sense heritability (h^2_b) for all traits.

Broad sense heritability was estimated as:

$$h^2_b = \sigma^2_g/(\sigma^2_g + \sigma^2_e), \quad (2)$$

where σ^2_g is the variance due to genotypes; σ^2_e is the variance due to error component.

Cluster analysis using SAS 9.3 software was done to summarize genetic variation among genotypes under control and water stress conditions using mean data of all characters recorded. Mean data of all characters were also used for calculation of Pearson's correlation coefficient among these characters.

Results

Analysis of variance

The combined analysis of variance (ANOVA) showed significant genotypic differences ($p \leq 0.05$) for all morphological traits except leaf number and root length (Table 1), physiological traits except total chlorophyll content (Table 2) and biochemical traits except malondialdehyde, superoxide dismutase (Table 3), indicating the magnitude of differences in genotypes was sufficient to select superior genotypes against water-deficit stress. The genotype into management interaction was also significant for shoot and root dry weight, chlorophyll content, rate of photosynthesis, SC, TR and WUE among morpho-physiological traits. Among biochemical traits, proline content, soluble sugar, POD, MDA and cell membrane injury percentage showed significant genotype into management interaction. The significant genotype, management and genotype × management interaction variances revealed that most traits were influenced by both genetic and management conditions.

Table 1 Combined ANOVA for morphological traits in black gram evaluated under well-watered and stressed condition

Source of variation	d.f.	PH	BR	PN	CL	LN	SL	SDW	RL	RDW
Management	1	43.35**	0.15	442.82	0.60	345.6**	28.02**	63050.4*	14.70	280166.66**
Rep (management)	4	5.83	0.28	298.26*	48.58	1.83	5.17	7839.47	16.87*	11283.97
Genotypes	9	35.13**	2.79**	370.85**	90.04**	12.11	8.49*	43987.57**	5.80	26786.51**
Management*genotypes	9	5.57	1.04	138.78	25.15	10.19	4.68	63731.60**	12.01	25500**
Error	36	5.94	0.69	95.54	27.52	9.00	3.87	11697.39	5.99	5411.80

PH Plant height, *BR* branches, *PN* pod number, *CL* cluster number, *LN* leaf number, *SL* shoot length, *SDW* shoot dry weight, *RL* root length, *RDW* root dry weight

*, ** significant at the 5% and 1% level of significance, respectively

Table 2 Combined ANOVA for physiological traits in black gram evaluated under well-watered and stressed condition

Source of variation	d.f.	PS	g_s	Tchlo	TR	WUE
Management	1	2478.12**	0.91**	0.08	285.58**	0.59**
Rep (management)	4	3.37	0.002	2.87	0.42	0.06
Genotypes	9	222.56**	0.12**	2.78	38.38**	1.22**
Management*genotypes	9	126.25**	0.05**	8.97**	33.29**	1.230**
Error	36	2.71	0.00	2.55	0.32	0.06

PS photosynthetic rate, g_s stomatal conductance, *TR* transpiration rate, *Tchlo* Total chlorophyll, *WUE* water use efficiency

*, ** significant at the 5% and 1% level of significance, respectively

Table 3 Combined ANOVA for biochemical traits in black gram evaluated under well-watered and stressed condition

Source of variation	Proline	Soluble sugar	POD	MDA	SOD	CMS	Injury %
Management	22694.64**	35.94**	33.88**	0.81**	12.85*	127.05	528.00**
Rep (management)	396.79**	5.35**	4.96	0.15	1.03	681.59**	180.52**
Genotypes	219.68**	13.50**	9.82**	0.23	4.31	514.88**	715.92**
Management*genotypes	249.77**	7.80**	10.82**	0.37**	1.56	58.63	251.16**
Error	65.04	1.40	2.62	0.12	2.89	110.40	35.03

POD peroxidase activity, *MDA* malondialdehyde, *SOD* superoxide dismutase, *CMS* cell membrane stability

*, ** significant at the 5% and 1% level of significance, respectively

Morpho-physiological and biochemical characterization and heritability under control and water stress conditions

Trial mean, range, standard error of mean difference (SE_d), coefficient of variation (CV) and heritability for various morpho-physiological and biochemical characters are given in Table 4. Overall mean of most of these traits showed a decreasing trend with water stress except total chlorophyll, proline, POD, MDA, SOD and injury percentage. The shoot and root dry weight, rate of photosynthesis and transpiration, stomatal conductance, WUE, proline, soluble sugar, POD and cell membrane injury percentage showed high broad sense heritability (h_b^2) both under control and water stress, indicating that these traits are more influenced genetically than environment. The coefficient of variation percentage was high for pod number, cluster number and leaf number under control conditions. Under water-stressed condition, CV was high for number of branches, cluster number, root dry weight, stomatal conductance, proline, MDA and CMS.

The pooled means of genotypes under control and stress conditions for all characters studied are given in Tables 5, 6 and 7. Based on overall ranking of genotypes across all traits, genotype PGRU95016 was found to be tolerant to water stress for most of these traits followed by COBG05 and IPU99209.

Morphological traits

Plant height of all genotypes was affected under water stress except COB05, PGRU95016 and UH99144 (Table 8). The number of leaves, pods, shoot and root dry weight were

Table 4 Trial mean, range, standard error, coefficient of variation and heritability for various morpho-physiological and biochemical characters tested under well-watered and water stressed conditions

Sl	Characters	GM		Range		SE_d		CV (%)		Heritability	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
1	PH	13.67	11.97	11.33–17.00	9.00–17.67	1.98	2.00	17.77	20.45	0.55	0.78
2	BR	4.53	4.63	3.33–6.33	4.00–5.67	0.54	0.81	14.52	21.02	0.84	0.11
3	PN	42.50	37.07	22.00–56.33	21.67–45.33	10.83	3.17	31.21	10.48	0.51	0.90
4	CL	18.67	18.47	12.00–24.67	14.33–24.33	5.16	3.17	33.85	21.06	0.48	0.61
5	LN	17.67	12.87	14.33–23.00	11.67–13.67	2.92	1.88	20.21	17.80	0.39	0.00
6	SL	13.27	11.90	11.67–15.33	9.00–14.00	1.74	1.46	16.03	15.08	0.06	0.62
7	SDW	788.60	723.77	591.67–1009.67	515.00–1040.33	64.31	107.05	9.99	18.12	0.84	0.75
8	RL	16.53	15.54	14.70–21.13	14.13–17.77	2.25	1.71	16.67	13.51	0.39	0.16
9	RDW	403.30	266.63	273.33–534.33	185.33–527.67	25.49	81.03	7.74	37.22	0.95	0.71
10	PS	30.54	17.69	25.77–36.57	5.83–29.70	0.79	1.73	3.16	11.97	0.98	0.99
11	Tchlo	14.68	14.76	11.98–16.95	13.37–17.90	1.01	1.54	8.46	12.79	0.77	0.30
12	g_s	0.57	0.32	0.36–0.88	0.07–0.52	0.02	0.08	5.34	28.81	0.99	0.91
13	TR	11.72	7.36	9.07–14.53	2.27–15.10	0.31	0.58	3.22	9.61	0.98	0.99
14	WUE	2.62	2.82	2.44–2.84	1.25–4.66	0.09	0.27	4.46	11.76	0.80	0.95
15	Proline	10.58	49.48	7.91–13.96	32.20–65.10	0.78	9.28	9.09	22.97	0.92	0.72
16	Starch	10.46	8.91	8.67–12.57	5.13–12.50	1.15	0.74	13.49	10.19	0.71	0.94
17	POD	10.12	11.63	8.16–12.89	8.73–15.03	0.81	1.69	9.77	17.79	0.88	0.65
18	MDA	0.80	1.03	0.60–1.41	0.70–2.00	0.12	0.38	18.38	45.09	0.87	0.52
19	SOD	10.71	11.63	9.14–12.24	10.10–12.40	0.87	1.72	10.71	18.17	0.68	0.00
20	CMS	62.06	59.15	51.53–73.97	46.87–82.87	5.23	10.95	10.31	22.67	0.77	0.54
21	Injury %	32.89	38.82	23.16–49.33	7.30–59.67	3.22	6.03	12.01	19.01	0.95	0.92

PH Plant height, *BR* branches, *PN* pod number, *CL* cluster number, *LN* leaf number, *SL* shoot length, *SDW* shoot dry weight, *RL* root length, *RDW* root dry weight, *PS* photosynthetic rate, g_s stomatal conductance, *TR* transpiration rate, *Tchlo* Total chlorophyll, *WUE* water use efficiency, *POD* peroxidase activity, *MDA* Malondialdehyde, *SOD* superoxide dismutase, *CMS* cell membrane stability, *WW* well watered, *WS* water stress

Table 5 Combined means of genotypes for various morphological traits evaluated under well-watered and water stressed condition

Sl.	Genotypes	PH	BR	PN	CL	LN	SL	SDW	RL	RDW
1	COBG05	17.00	3.67	32.33	17.17	15.00	13.50	763.17	14.87	303.50
2	IPU243	11.17	4.83	50.83	20.83	15.83	13.17	796.00	15.40	435.50
3	IPU941	11.00	4.00	46.67	23.17	13.83	14.00	824.17	17.73	413.17
4	IPU99189	10.50	4.83	27.33	14.17	15.33	11.00	653.17	16.57	293.00
5	IPU99209	12.67	4.33	32.67	15.50	15.33	11.83	881.50	15.87	229.33
6	PDU1	10.33	4.17	34.83	15.00	15.17	11.83	665.33	16.82	336.67
7	PGRU95016	15.83	3.83	40.17	15.17	14.00	13.33	855.67	14.47	251.67
8	STY2868	11.17	5.67	47.67	23.33	13.33	11.00	638.67	16.77	341.67
9	UH855	13.33	5.33	39.33	17.33	16.67	12.00	719.17	16.17	383.33
10	UH99144	15.17	5.17	46.00	24.00	18.17	14.17	765.00	15.70	361.83
	GM	12.82	4.58	39.78	18.57	15.27	12.58	756.19	16.04	334.97
	SE_d	1.41	0.48	5.64	3.03	1.73	1.14	62.44	1.41	42.47
	CV	19.02	18.13	24.56	28.25	19.65	15.63	14.30	15.27	21.96
	LSD	2.85	0.97	11.44	6.14	3.51	2.30	126.64	2.86	86.13

PH Plant height, *BR* branches, *PN* pod number, *CL* cluster number, *LN* leaf number, *SL* shoot length, *SDW* shoot dry weight, *RL* root length, *RDW* root dry weight

Table 6 Combined means of genotypes for various physiological traits evaluated under well-watered and water stressed condition

Sl.	Genotypes	PS	g_s	Tchlo	TR	WUE
1	COBG05	16.62	0.25	15.99	6.43	2.49
2	IPU243	28.43	0.62	14.23	11.80	2.42
3	IPU941	30.30	0.56	15.23	11.82	2.57
4	IPU99189	29.47	0.46	14.42	10.32	2.94
5	IPU99209	32.78	0.67	13.59	12.02	2.79
6	PDU1	27.05	0.49	15.24	10.58	2.56
7	PGRU95016	18.27	0.44	14.26	12.08	2.04
8	STY2868	17.17	0.29	14.38	6.42	2.99
9	UH855	20.15	0.35	14.99	7.32	2.67
10	UH99144	20.93	0.31	14.86	6.60	3.75
	GM	24.12	0.44	14.72	9.54	2.72
	SE_d	0.95	0.04	0.92	0.33	0.13
	CV	6.82	15.45	10.85	5.94	9.14
	LSD	1.92	0.08	1.87	0.66	0.29

PS photosynthetic rate, g_s stomatal conductance, *Tchlo* total Chlorophyll, *TR* transpiration rate, *WUE* water use efficiency

Table 7 Combined means of genotypes for various biochemical traits evaluated under well-watered and water stressed condition

Sl.	Genotypes	Proline	Starch	POD	MDA	SOD	CMS	Injury %
1	COBG05	25.94	9.47	9.51	1.30	10.85	50.53	45.01
2	IPU243	33.42	7.59	12.87	0.85	11.88	63.08	32.94
3	IPU941	29.32	9.28	10.58	0.80	11.31	49.73	54.50
4	IPU99189	34.26	10.94	11.36	0.75	12.20	53.15	42.57
5	IPU99209	34.42	9.24	12.53	0.77	9.80	72.05	30.53
6	PDU1	36.24	10.25	11.77	0.74	11.95	63.92	35.77
7	PGRU95016	38.11	6.90	10.72	0.97	10.25	57.44	24.88
8	STY2868	21.93	11.06	9.83	1.21	11.33	56.08	38.70
9	UH855	24.80	10.91	8.90	0.80	11.90	78.42	15.23
10	UH99144	21.85	11.25	10.68	0.94	10.23	61.63	38.44
	GM	30.03	9.69	10.88	0.91	11.17	60.60	35.86
	SE_d	4.66	0.68	0.93	0.20	0.98	6.07	3.42
	CV	26.85	12.25	14.90	37.68	15.22	17.33	16.50
	LSD	9.44	1.38	1.89	0.40	1.99	12.30	6.93

POD peroxidase activity, *MDA* Malondialdehyde, *SOD* Superoxide dismutase, *CMS* cell membrane stability

drastically affected under water stress than control, while reduction was more prominent in case of shoot and root dry weight. High pod and cluster number was observed in genotypes STY2868, UH99144, IPU243 and IPU941 in control and water stress conditions. This indicates their ability to maintain better performance under water stress conditions. Shoot dry weight was relatively high in IPU243, IPU941, UH855 IPU99209, PGR95016 and UH99144. High root dry weight was recorded in IPU243, IPU941 and UH99144.

Physiological traits

Means of physiological characters in control and water stress conditions along with grand mean, SE_d, CV and LSD are given in Table 9. Among these characters, a decreasing trend was observed in rate of photosynthesis, TR and SC in water stress as compared to control (Table 9). Adverse impact of water stress was prominent for rate of photosynthesis, SC and TR although there was significant variability between genotypes. The decrease in chlorophyll accumulation under water stress may lead to reduced photochemical activities of chloroplast. The reduced CO_2 assimilation rate under water stress could be due to decreased stomatal conductance.

Table 8 Means of various morphological traits of black gram genotypes evaluated under well-watered and water stress condition

Sl.	Genotypes	PH		BR		PN		CL		LN		SL		SDW		RL		RDW	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
1	COBG05	16.33	17.67	3.33	4.00	43.00	21.67	19.33	15.00	17.00	13.00	13.00	14.00	830.33	696.00	14.70	15.03	390.33	216.67
2	IPU243	13.33	9.00	4.67	5.00	56.33	45.33	24.33	17.33	18.33	13.33	15.33	11.00	859.00	733.00	15.63	15.17	343.33	527.67
3	IPU941	12.67	9.33	4.00	4.00	55.00	38.33	24.67	21.67	16.00	11.67	14.33	13.67	856.67	791.70	21.13	14.33	459.00	367.33
4	IPU99189	11.33	9.67	4.33	5.33	22.00	32.67	14.00	14.33	17.33	13.33	13.00	9.00	786.67	519.70	16.43	16.70	378.67	207.33
5	IPU99209	12.00	13.33	4.00	4.67	29.33	36.00	12.67	18.33	19.00	11.67	12.67	11.00	722.67	1040.30	15.50	16.23	273.33	185.33
6	PDU1	11.33	9.33	4.00	4.33	36.00	33.67	12.00	18.00	16.67	13.67	12.67	11.00	815.67	515.00	15.87	17.77	456.00	217.33
7	PGRU95016	17.00	14.67	3.67	4.00	42.33	38.00	15.00	15.33	14.67	13.33	13.33	13.33	1009.67	701.70	14.80	14.13	315.67	187.67
8	STY2868	12.33	10.00	5.67	5.67	50.67	44.67	22.33	24.33	14.33	12.33	11.67	10.33	591.67	685.70	18.87	14.67	445.33	238.00
9	UH855	15.00	11.67	6.33	4.33	42.67	36.00	18.00	16.67	20.33	13.00	11.67	12.33	719.33	719.00	15.17	17.17	534.33	232.33
10	UH99144	15.33	15.00	5.33	5.00	47.67	44.33	24.33	23.67	23.00	13.33	15.00	13.33	694.33	835.70	17.20	14.20	437.00	286.67
	GM	13.67	11.97	4.53	4.63	42.50	37.07	18.67	18.47	17.67	12.87	13.27	11.90	788.60	723.78	16.53	15.54	403.30	266.63
	SE_d	1.98	2.00	0.54	0.81	10.83	3.17	5.16	3.17	2.92	1.88	1.74	1.46	64.31	107.05	2.25	1.71	25.49	81.03
	CV	17.77	20.45	14.52	21.01	31.21	10.48	33.85	21.06	20.21	17.80	16.02	15.07	9.98	18.11	16.66	13.50	7.74	37.21
	LSD	4.16	4.20	1.12	1.67	22.75	6.66	10.84	6.67	6.12	3.92	3.64	3.07	135.12	224.91	4.72	3.60	53.56	170.24

WW well-watered, *WS* water stressed, *PH* plant height, *BR* branches, *PN* pod number, *CL* cluster number, *LN* leaf number, *SL* shoot length, *SDW* shoot dry weight, *RL* root length, *RDW* root dry weight

Table 9 Means of various physiological traits of black gram genotypes evaluated under well-watered and stress condition

Sl.	Genotypes	PS		Tchlo		g_s		TR		WUE	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
1	COBG05	26.90	6.33	14.08	17.90	0.42	0.07	10.07	2.80	2.67	2.31
2	IPU243	34.17	22.70	12.73	15.73	0.78	0.45	14.03	9.57	2.44	2.39
3	IPU941	30.90	29.70	16.02	14.43	0.63	0.50	12.47	11.17	2.48	2.66
4	IPU99189	31.07	27.87	14.53	14.30	0.61	0.31	12.23	8.40	2.54	3.33
5	IPU99209	36.57	29.00	11.98	15.20	0.88	0.45	14.53	9.50	2.52	3.06
6	PDU1	28.33	25.77	16.95	13.53	0.49	0.48	11.17	10.00	2.55	2.58
7	PGRU95016	25.77	10.77	15.15	13.37	0.36	0.52	9.07	15.10	2.84	1.25
8	STY2868	26.80	7.53	14.42	14.33	0.45	0.13	10.57	2.27	2.54	3.43
9	UH855	34.47	5.83	15.65	14.33	0.56	0.14	12.33	2.30	2.80	2.54
10	UH99144	30.47	11.40	15.29	14.43	0.49	0.14	10.73	2.47	2.84	4.66
	GM	30.54	17.69	14.68	14.76	0.57	0.32	11.72	7.36	2.62	2.82
	SE_d	0.79	1.73	1.01	1.54	0.02	0.08	0.31	0.58	0.09	0.27
	CV	3.15	11.97	8.46	12.79	5.33	28.81	3.22	9.61	4.46	11.76
	LSD	1.65	3.63	2.13	3.23	0.05	0.15	0.65	1.21	0.20	0.56

WW well-watered, *WS* water stressed, *PS* photosynthetic rate, g_s stomatal conductance, *TR* transpiration rate, *WUE* water use efficiency, *Tchlo* total chlorophyll

Transpiration rate and stomatal conductance decrease in most of the genotypes under water stress. Plants restrict gas exchange between atmosphere and leaves through stomatal closure. As compared to control conditions, the highest reduction of photosynthetic rate was recorded for genotype UH855, while minimum reduction was observed in IPU941 and IPU99209 under water stress. Similarly, IPU941, IPU99209, PDU1 and PGRU95016 maintained higher stomatal conductance, transpiration rate and WUE. It shows that these genotypes have better tolerance mechanism under water stress conditions. The water stress did not have significant impact on chlorophyll a and chlorophyll b (data not shown). However, genotypes COBG05, IPU243, IPU941 and IPU99209 recorded higher chlorophyll content under control and water stress conditions.

Biochemical traits

Biochemical characters (Table 10) mostly over expressed in water stress as compared to control plants. There was over

Table 10 Means of various biochemical traits of black gram genotypes evaluated under well-watered and stress condition

Sl	Genotypes	Proline		Soluble sugar		POD		MDA		SOD		CMS		Injury (%)	
		WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS	WW	WS
1	COBG05	9.08	42.80	9.21	9.73	9.09	9.93	0.60	2.00	9.74	11.97	54.19	46.87	44.91	45.10
2	IPU243	12.91	53.93	8.84	6.33	11.68	14.07	0.66	1.03	11.95	11.80	63.59	62.57	36.74	29.13
3	IPU941	7.91	50.73	9.65	8.90	8.16	13.00	0.87	0.73	10.55	12.07	51.53	47.93	49.33	59.67
4	IPU99189	8.85	59.67	12.57	9.30	11.61	11.10	0.79	0.70	12.24	12.17	54.63	51.67	40.74	44.40
5	IPU99209	11.30	57.53	11.02	7.47	12.89	12.17	0.70	0.83	9.14	10.47	73.07	71.03	24.20	36.87
6	PDU1	9.68	62.80	12.03	8.47	8.51	15.03	0.77	0.70	11.77	12.13	68.03	59.80	25.20	46.33
7	PGRU95016	11.11	65.10	8.67	5.13	10.88	10.57	0.77	1.17	10.40	10.10	59.00	55.87	23.77	26.00
8	STY2868	11.67	32.20	10.94	11.17	10.93	8.73	1.41	1.00	10.26	12.40	62.87	49.30	24.57	52.83
9	UH855	13.96	35.63	9.31	12.50	8.17	9.63	0.66	0.93	11.80	12.00	73.97	82.87	23.16	7.30
10	UH99144	9.33	34.37	12.37	10.13	9.33	12.03	0.70	1.17	9.22	11.23	59.68	63.57	36.28	40.60
	GM	10.58	49.48	10.46	8.91	10.12	11.63	0.79	1.03	10.71	11.63	62.06	59.15	32.89	38.82
	SE_d	0.78	9.28	1.15	0.74	0.81	1.69	0.12	0.38	0.87	1.72	5.23	10.95	3.22	6.03
	CV	9.08	22.97	13.49	10.18	9.76	17.79	18.37	45.08	10.70	18.16	10.31	22.67	12.00	19.01
	LSD	1.64	19.50	2.42	1.55	1.69	3.54	0.25	0.79	1.96	3.62	10.98	23.04	6.77	12.66

WW Well-watered, *WS* Water stressed, *POD* peroxidase activity, *MDA* Malondialdehyde, *SOD* Superoxide dismutase, *CMS* cell membrane stability

expression of proline content, MDA and SOD in stressed plants. The increasing level of proline, POD, MDA and SOD in stressed plant may be attributed to the triggering of tolerance mechanism in plants, so that it can reduce adverse effect of water stress and works as an osmo-protectant. Proline content increased for all genotypes, while highest increase was registered with PGRU95016 followed by PDU1, IPU99189 and IPU99209. Peroxidase activity was highest in PDU1 followed by IPU243, IPU941 and IPU99209 under water stress conditions. Malondialdehyde content was high in case of COBG05, PGRU95016 and UH99144, while the activity of SOD was highest in STY2868 followed by IPU99189 and PDU1 under water stress condition. Based on biochemical characters, the genotypes PGRU95016, COBG05, IPU99189, PDU1, IPU243 and IPU99209 had better tolerance to water stress conditions.

Correlation

The morpho-physiological and biochemical characters of control and water stress treatments were used to calculate Pearson correlation (Table 11). There was significant positive correlation between pod number with cluster number under both control and water stress conditions and negative correlation between shoot dry weight and branches under control conditions. The correlation between branches and shoot length and shoot dry weight with leaf number was negatively significant in water stress conditions. The correlation clearly indicated physiological (PS, SC, TR and Tchlo) and biochemical (proline content, soluble sugar, POD and MDA) characters had a significant association in water stress conditions. Significant positive correlation was found between PS and SC; TR with SC and negative correlation between CMS with cell membrane injury in control and water stress conditions.

Cluster analysis

Cluster analysis using average linkage method of clustering was done for the morpho-physiological and biochemical characters of control and water stress treatments. Genotypes were classified into three clusters following average linkage method of clustering (Figs. 1, 2) in control and water stress conditions, respectively. Genotypes grouped into distinct classes indicate the presence of greater genetic diversity among materials and may be utilized in hybridization program in creating variability for genetic enhancement for drought tolerance in this important legume crop.

Discussion

The study revealed significant genotypic variances for morpho-physiological and biochemical characters except for leaf number; total chlorophyll content; malondialdehyde and superoxide dismutase. Genotype and management interaction was also found to be significant for most of the characters. The study indicated influence of both genotype and environment in expression of these characters. In our study, water stress reduced the linear growth of agro-morphological traits as compared to control conditions. The water stress drastically affects cell expansion and cell growth that is associated with loss of cell turgor resulting reduced plant height. Similar findings were also reported earlier in legumes (Bhatt and Srinivasa Rao 2005; Baroowa and Gogoi 2012). Drought stress leads to defoliation and termination of new leaf production resulting in lower number of leaves (Mwale et al. 2007). The maximum reduction was evident for shoot and root dry weight in water stress conditions.

Among physiological traits, water stress significantly affected rate of photosynthesis, total chlorophyll content, stomatal conductance, transpiration rate and WUE as compared to control conditions, although there was differential behavior among genotypes possibly due to the inbuilt tolerance mechanisms. Water stress adversely affected the photosynthetic apparatus and chlorophyll pigments resulting in reduced photosynthetic activities. In addition, under drought stress, stomata start closing resulting in reduced stomatal conductance which may lead to reduced photosynthetic rate (Cornic and Massacci 1996; Marcinska et al. 2013; Salwa and Ali 2014). Earlier reports also suggest that decrease in stomatal conductance in drought-stressed plants decreases photosynthesis (Tenhunen et al. 1987; Nilsen and Orcutt 1996). Stomatal closure under water stress and reduced CO_2 availability in chloroplast is major reason for decreased photosynthetic activity (Flexas and Medrano 2002). The effect of drought stress on transpiration and photosynthetic rate was similar (Basu et al. 2004). Plants tend to close stomata when experience drought stress condition which also leads to reduced transpiration and restrict exchange of gases inside leaves and environment. Total chlorophyll content was not adversely affected under water stress, although there was significant genotype × environment interaction for chlorophyll content. The duration and extent of water stress determine the chlorophyll level in plants (Kpyoarissis et al. 1995). The reduced chlorophyll content under drought conditions results in poor light harvesting by plants. Increasing energy absorption by photosynthetic apparatus may trigger production of reactive oxygen species. To avoid this situation, plants may degrade absorbing pigments (Herbinger et al. 2002).

Among biochemical characters, proline content increased sharply by about four-to-sevenfold under water stressed

Table 11 Simple correlation coefficient between various morpho-physiological and biochemical traits evaluated under well-watered (upper diagonal) and water stressed (lower diagonal) conditions

Characters	PH	BR	PN	CL	LN	SL	SDW	RL	RDW	PS	g_s	TR	WUE	Tchlo	Proline	Starch	POD	MDA	SOD	CMS	Injury %
PH	1	− 0.04	0.32	0.23	0.15	0.15	0.38	− 0.40	− 0.02	− 0.34	− 0.54	− 0.57	0.797**	0.05	0.15	− 0.58	− 0.26	− 0.36	−0.35	−0.17	0.01
BR	− 0.39	1	0.24	0.35	0.42	− 0.24	− 0.680*	0.16	0.61	0.30	0.01	0.13	0.24	0.12	0.59	0.13	− 0.16	0.32	0.19	0.46	− 0.37
PN	− 0.46	0.53	1	0.866**	− 0.06	0.44	0.04	0.43	0.30	− 0.15	− 0.13	− 0.10	− 0.02	0.10	0.17	− 0.54	− 0.35	0.22	−0.12	−0.19	0.22
CL	− 0.18	0.39	0.654*	1	0.17	0.55	− 0.18	0.56	0.31	− 0.02	− 0.02	0.01	− 0.05	− 0.02	− 0.02	− 0.27	− 0.25	0.21	−0.17	−0.39	0.50
LN	0.07	0.05	− 0.08	− 0.40	1	0.33	− 0.33	− 0.21	0.17	0.59	0.30	0.34	0.35	−0.10	0.14	0.29	−0.12	−0.55	− 0.17	0.33	0.03
SL	0.635*	−0.746*	−0.20	0.09	−0.09	1	0.39	0.18	−0.26	0.14	0.24	0.18	−0.11	−0.13	−0.29	− 0.08	0.04	−0.38	− 0.08	− 0.44	0.57
SDW	0.35	−0.09	0.25	0.34	−0.672*	0.32	1	−0.28	−0.38	−0.24	−0.15	−0.21	0.10	0.13	−0.18	− 0.52	−0.04	−0.48	0.21	−0.36	0.22
RL	−0.35	0.02	−0.35	−0.43	0.24	−0.54	−0.36	1	0.37	−0.08	0.04	0.07	−0.38	0.29	−0.43	0.19	−0.25	0.60	− 0.11	−0.42	0.41
RDW	−0.43	0.13	0.51	0.21	0.01	0.05	0.09	−0.30	1	−0.11	−0.38	−0.21	0.20	0.734*	0.02	0.11	− 0.833**	0.21	0.31	0.05	0.05
PS	−0.56	0.03	0.05	−0.07	−0.28	−0.41	0.06	0.29	0.23	1	0.894**	0.926**	−0.29	−0.47	0.40	0.03	0.28	−0.39	0.12	0.54	−0.06
g_s	−0.42	−0.30	0.18	−0.19	−0.12	−0.11	0.06	0.06	0.22	0.735*	1	0.981**	−0.63	−0.636*	0.22	0.05	0.50	−0.24	0.07	0.36	0.06
TR	−0.25	−0.37	0.05	−0.34	−0.02	−0.03	−0.03	−0.05	0.10	0.61	0.954**	1	−0.63	−0.53	0.27	0.05	0.38	−0.23	0.20	0.41	0.06
WUE	−0.05	0.645*	0.34	0.636*	−0.10	−0.23	0.20	−0.04	0.02	0.05	−0.46	−0.59	1	0.33	0.14	−0.10	−0.33	−0.25	− 0.29	0.07	−0.29
Tchlo	0.48	−0.13	−0.50	−0.23	−0.13	0.27	0.23	−0.16	0.20	−0.20	−0.43	−0.39	−0.03	1	−0.33	0.18	−0.825**	0.11	0.29	−0.18	0.02
Proline	−0.20	−0.31	−0.21	−0.58	0.16	−0.23	−0.20	0.26	−0.07	0.658*	0.853**	0.906**	−0.54	−0.26	1	− 0.42	0.24	0.01	0.24	0.749*	−0.678*
Starch	−0.02	0.25	−0.10	0.34	−0.10	−0.03	−0.13	0.25	−0.22	−0.43	−0.799**	−0.865**	0.52	0.08	−0.805**	1	0.10	0.20	− 0.02	0.03	−0.04
POX	−0.42	−0.17	0.16	0.00	0.16	−0.09	−0.03	0.24	0.48	0.729*	0.680*	0.51	−0.01	−0.16	0.55	− 0.49	1	0.12	− 0.12	0.15	−0.24
MDA	0.812**	−0.29	−0.47	−0.20	0.17	0.58	0.08	−0.41	−0.08	−0.659*	−0.55	−0.39	−0.17	0.762*	−0.33	0.04	−0.42	1	− 0.06	− 0.09	−0.22
SOD	−0.51	0.30	−0.10	0.17	0.08	−0.29	−0.57	0.30	0.25	−0.01	−0.40	−0.49	0.26	0.16	−0.40	0.640*	−0.02	−0.09	1	0.07	−0.01
CMS	−0.01	−0.04	0.21	−0.10	0.07	−0.06	0.36	0.48	−0.03	−0.10	−0.04	−0.16	0.07	−0.20	−0.13	0.18	0.06	−0.27	−0.27	1	−0.813**
Injury	−0.18	0.17	−0.06	0.45	−0.35	−0.06	−0.10	−0.28	0.03	0.39	0.08	0.05	0.32	0.09	0.03	0.00	0.19	−0.06	0.35	−0.805**	1

WW well-watered, *WS* water stressed, *PH* plant height, *BR* branches, *PN* pod number, *CL* cluster number, *LN* leaf number, *SL* shoot length, *SDW* shoot dry weight, *RL* root length, *RDW* root dry weight, *PS* photosynthetic rate, g_s stomatal conductance, *TR* transpiration rate, *WUE* water use efficiency, *Tchlo* Total chlorophyll, *POD* peroxidase activity, *MDA* Malondialdehyde, *SOD* Superoxide dismutase, *CMS* cell membrane stability

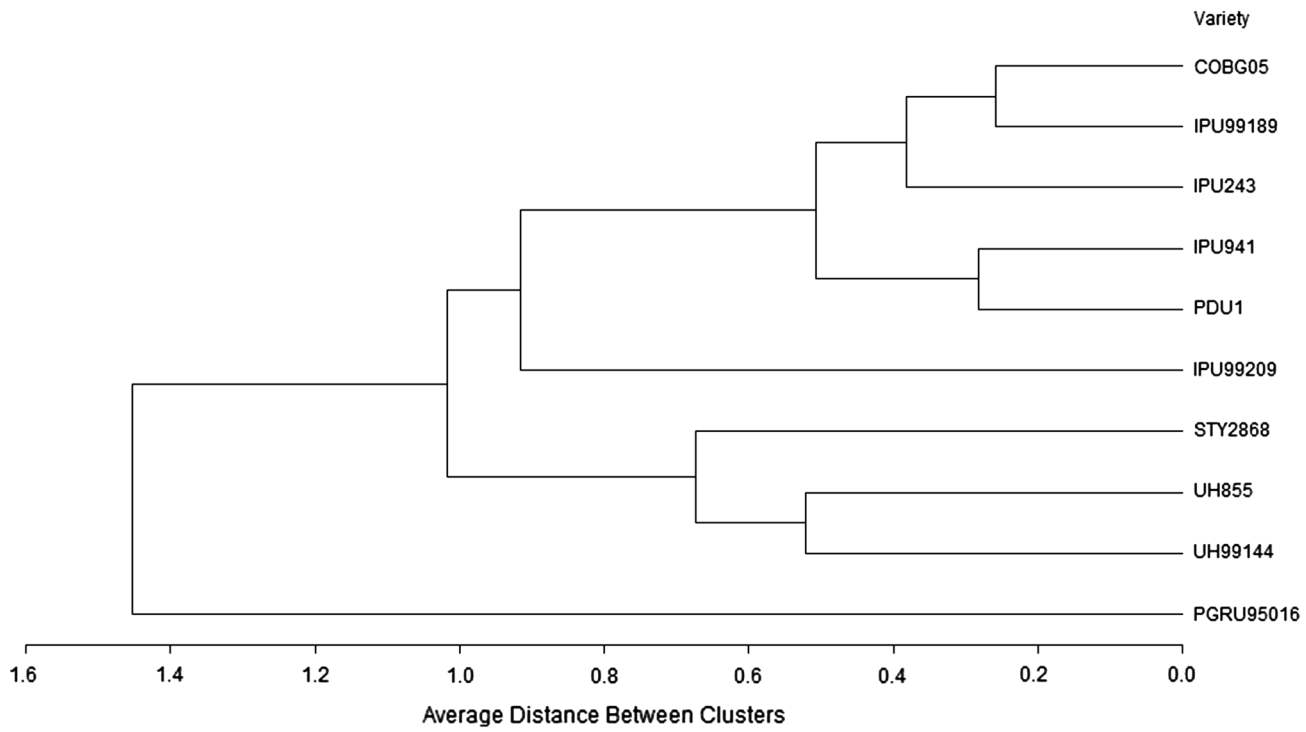

Fig. 1 Clustering of genotypes (well water conditions) based on average linkage method

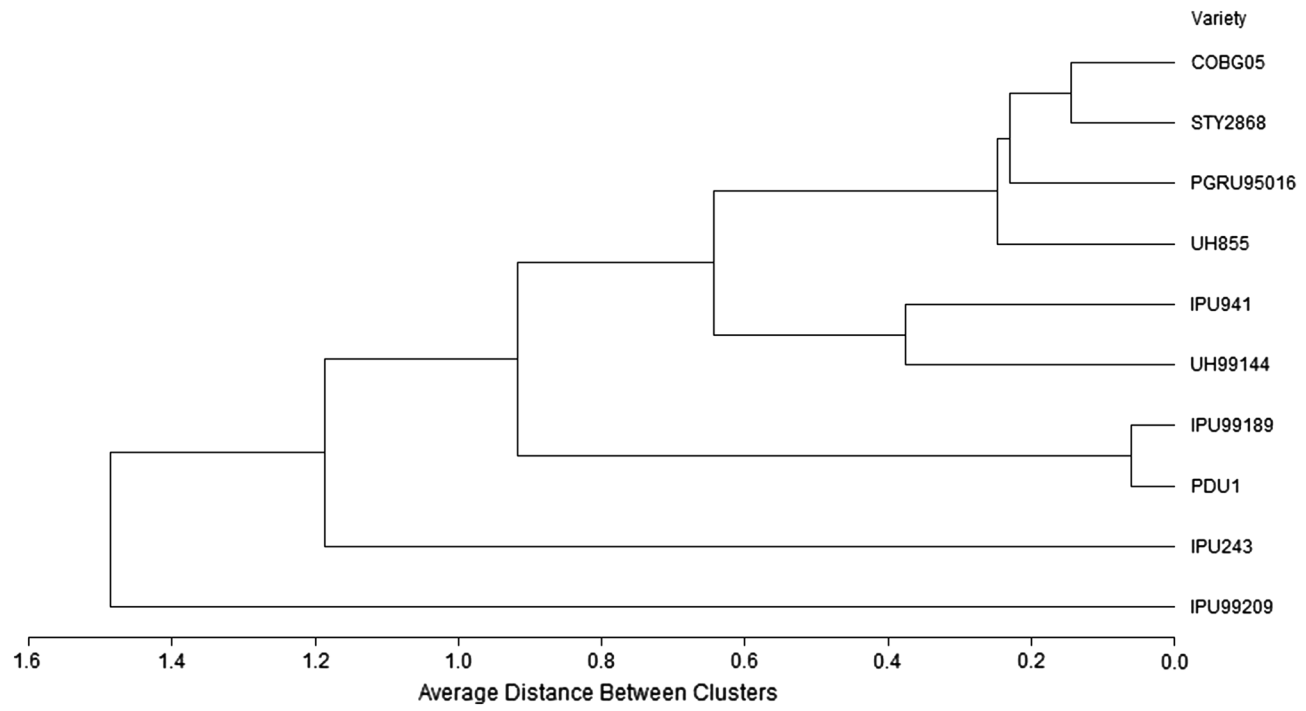

Fig. 2 Clustering of genotypes (well stress conditions) based on average linkage method

conditions. Plants can protect cells through increased proline accumulation and balancing osmotic potential with external environment under water stress as reported in wheat (Pireivatloum et al. 2010). The increased proline accumulation plays adaptive role to impart tolerance in plants (Verbruggen and Hermans 2008). Proline accumulation during water stress also acts as a compatible solute thus regulating water loss from plant cell (Yokota et al. 2006). It also helps in supplying energy for survival and growth of plants (Kumar et al. 2011). Thus, in germplasm screening studies for drought tolerance, the accumulation of proline content is used as an important selection criterion (Bayoumi et al. 2008; Kumar et al. 2011; Rahdari et al. 2012; Yancy et al. 1982 and; Jaleel et al. 2007).

Plants also develop a complex mechanism of antioxidant to deal with oxidative stress. Tolerant genotypes express high ROS-scavenging enzymes activities than susceptible genotypes, indicating its crucial role in abiotic stress tolerance in plants. The higher antioxidant enzyme activities under water stress are helpful in imparting tolerance in genotypes. The SOD, POD and MDA are efficient in metabolizing H_2O_2, which increased significantly in the tolerant genotypes as compared to others. The SOD helps in detoxification of anion-free radicals (O_2^-) by forming H_2O_2, which can be eliminated by POD. The POD also involved in phenolics oxidation (Largrimini 1991), cell elongation regulation (Mohammadkhani and Heidari 2008) and detoxification of H_2O_2 (Chaparzadeh et al. 2004). The drought-tolerant pigeon pea (Kumar et al. 2011), wheat (Hasheminasab et al. 2012) and black gram (Pratap and Sharma 2010) showed higher SOD and POD activities than drought-sensitive genotypes. The lipid peroxidation (MDA), another important biochemical character, used as an indicator for free radical which damages cell membranes in stress conditions. In the present study, MDA increased in majority of genotypes though the magnitude varied between genotypes under drought stress. It is used to assess stress injury in plants (Jain et al. 2001; Katsuhara et al. 2005). The low MDA content in tolerant plants could be due to free radical generation and low membrane damage.

In our study, genotypes showed wide variability for morphological, physiological and biochemical characters both under control and water stress conditions. Based on overall performance for various morpho-physiological and biochemical characters, genotypes PGRU95016, COBG05, IPU99189, PDU1, IPU243 and IPU99209 were found to be tolerant to drought stress as compared to others. The clustering analysis helped in grouping these genotypes into different groups which can be used in genetic enhancement program for further improvement for stress tolerance in black gram.

Conclusion

It is well known that adequate water is essential for optimal growth and productivity of crops. However, crops often get exposed to drought stress at different phenological phases affecting productivity. Crop productivity under water stress might be reduced due to changes in the physiological/biochemical processes at the cellular and molecular levels of plants as it uses it as a survival mechanism under stress. The present study showed differential responses for morpho-physiological and biochemical characters of black gram genotypes. The adverse impacts of drought stress were observed on morpho-physiological and biochemical characters. The study helped in developing strategies for genetic enhancement of this important crop. Drought stress reduced plant growth, branches, pod numbers, shoot and root dry weight, rate of photosynthesis and transpiration, stomatal conductance among morpho-physiological traits. Proline content and activities of POD and SOD among biochemical characters showed enhanced tolerance in plants. Genotypes maintaining higher levels of physiological and biochemical activities can be used as a stress marker. These characters are useful for selecting tolerant genotypes and using them in improving the productivity under drought prone environments.

Authors' contribution statement SG: conduct of experiment, data generation and tabulation, and manuscript editing. BS: data compilation, statistical analysis, manuscript writing, and editing. MV: guidance in conducting experiments and manuscript editing. NJ: manuscript editing and guidance in conducting experiments. SKY: manuscript editing. MM: interpretation of results and manuscript editing.

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