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Effect of waterlogging on physiological traits and yield in black gram (*Vigna mungo* L.) in field condition

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Waterlogging is an important abiotic factor affecting crop productivity worldwide. Black gram (*Vigna mungo* L.) is very sensitive to waterlogged conditions. A field experiment was conducted in randomized complete block design to evaluate three black gram genotypes for waterlogging tolerance. Stress was imposed by maintaining the water level above the soil surface for 10 days after 30 days of sowing. Different physiological parameters including chlorophyll (Chl), chlorophyll fluorescence, normalized difference vegetation index (NDVI), sugars, along with the yield per plant and thousand grain weight (TW) were recorded in control and stressed plants. Results showed that NDVI, Chl, chlorophyll fluorescence, sugars, seed yield and TW reduced significantly during stress. Stress susceptibility index (SSI) for grain yield varied from 0.32 to 2.38. Linear correlation study showed that SSI was negatively correlated with NDVI (0.43),Chl (0.68) and TW (0.42) and grain yield (0.96). NDVI and sugars were correlated to TW under stress. IC530491 and IC519933 (SSI < 0.5) were waterlogging tolerant under field conditions. The study concluded that identified black gram lines may be utilized as trait donors in breeding program.

Keywords: Black gram, Chlorophyll, Flooding, Sugars, Yield

Black gram is a short season nutritious legume. The crop is sown during June/July in India. The crop faces the waterlogging stress at different growth stages due to onset of monsoon, which coincides with the crop growth cycle. Climatic variability may decline black gram production due to unpredictable waterlogging occurrence in future. Though, waterlogging stress impedes crop growth at all the stages, early vegetative and reproductive stages are highly detrimental to crop yield[1]. Waterlogging may occur due to erratic rainfall, poor drainage or undulated lands. The heavy soil texture may be the possible reason for water logging[2]. Diffusion of oxygen is nearly ten thousand times restricted in waterlogged conditions compared to normal. In addition, respiration by plant roots and microorganisms, scavenge the left-out oxygen causing its deficiencyintherhizosphere[3]. Water loggingfor short duration may resultinhypoxic conditionandin long term, it may lead to anoxia. Therefore, low oxygen level in soil forces plants to perform anaerobic metabolism. As a result, it lowers the soil pH and makes it acidic because of increase in the concentration of inhibitory soil products (ethylene, organic acids *etc*.). These changes in the physico-chemical properties of the soil due to water logging makes it difficult for plants to avail essential micronutrients, which hampers plant's growth and development[4-5].

Study of physiological and biochemical changes under waterlogging conditions contributes to an understanding of the adaptive mechanism of the crop. Waterlogging reduced photosynthetic potential, osmoregulation, antioxidative potential, biomass and yield in different crops[6-7]. Different physiological and biochemical traits (chlorophyll content, stomatal conductance, photosynthetic rate, membrane stability index and antioxidant enzyme activity) have been recorded during waterlogging stress in mung bean and black gram[8]. Chlorophyll content and lipid peroxidation were found to be correlated with waterlogging tolerance in black gram[9]. Normalized difference vegetation index was also used to quantify green cover in soybean under waterlogged environment[10]. Depletion of sugar reserve in

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cucumber was observed in response to waterlogged condition[11].

Yield is the most important economic trait and selection based on yield under target environment is the most widely used strategy by breeders. Stress susceptibility index and yield traits were used to assess peanut genotypes under waterlogging[6]. Different studies on correlation between physiological and yield parameters were carried out to identify the criteria for selection of waterlogging tolerant genotypes[6,12]. Comprehensive understanding of morphological, physiological, biochemical and yield responses under waterlogging stress may further assist the breeders in identification of representative traits related to crop yield. Therefore, present study was conducted to evaluate key physiological and yield responses of black gram genotypes to waterlogging stress in field.

Material and Methods

Plant Materials and waterlogging treatments

A field experiment was conducted with 3 black gram genotypes; Uttara, IC530491 and IC519933 obtained from Genebank, National Bureau of Plant Genetic Resources, New Delhi, India. Experiment was conducted in field condition at ICAR-Indian Agricultural Research Institute, New Delhi in randomized complete block design with three replications during 2020 and 2021. Crop was subjected to stress after 30 days of sowing. Standard agronomic practices were used to raise the crop. To implement waterlogging stress, bunds were prepared, and water was filled in the field for 10 days. After 10 days of stress, water was removed with the help of pump. Water level was monitored daily and was maintained constant by supplying the water if required. All the observations were recorded after one week of stress on appearance of yellowing in susceptible genotype. All the physiological and biochemical traits were measured on first fully mature leaf from top. For yield measurement, plants were tagged separately to avoid destructive sampling and grain yield was recorded at maturity.

Chlorophyll

Chlorophyll (Chl) extraction was done using the non-maceration protocol[13]. Leaf tissue (0.1 g) was kept in 5 mL DMSO and incubated at 65°C for 2 h. Then the tubes were cooled at room temperature and absorbance was recorded at 645 nm and 663 nm using a spectrophotometer. Chl content was calculated as follows[14].

$$\text{Chl (mg g}^{-1}\text{)} = [20.2\ (A645) + 8.02\ (A663)] \times V/1000 \times W$$

V= volume of solvent W= fresh weight of tissue extracted

Chlorophyll fluorescence

Chlorophyll fluorescence was measured from the fully expanded leaves using a handheld chlorophyll fluorometer. Data was recorded on the flag leaves after full dark adaptation for 30 min. Fluorescence data represents the maximum quantum yield of PSII and is the ratio of variable fluorescence (difference between maximum and minimum fluorescence, Fv) and maximum fluorescence (Fm).

Normalized Difference Vegetation Index

Normalized difference vegetation index (NDVI) represents the crop vigor and greenness. The values were recorded using Greenseeker handheld crop sensor. During the measurements, the sensor was held approximately 60 cm above the canopy to ensure the accuracy of data.

Soluble sugars

Water soluble sugars were measured using anthrone menthod[15]. 0.5 g sample was extracted with 80% ethanol. Extract was centrifuged at for 30 min. Supernatant was collected and mixed with freshly prepared anthrone reagent. Samples were incubated at 80°C for 20 min. After cooling, absorbance was recorded at 620 nm using spectrophotometer.

Seed yield, thousand grain weight and Stress susceptibility index

Seed yield per plant was measured by harvesting all the pods from tagged plants at maturity. Thousand grain weight (TW) was recorded after drying and threshing of pods. Stress susceptibility index (SSI) for yield for each genotype was calculated as follows[16].

$$SSI = (1-Ys/Yns)/SII,$$

where, Ys = Mean single plant yield of a given genotype under stress
Yns = Mean single plant yield of a given genotype under control condition

$$SII = 1- Xs/Xns$$

Xs = Mean of all the genotypes under stress
Xns = Mean of all the genotypes under normal condition

Statistical analysis

All the experimental data were analyzed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). Physiological and biochemical parameters were recorded with three replications with two independent observations. Differences at P <0.05 were considered statistically significant. Linear correlation analysis was performed to study the relationship between studied parameters.

Results

Effect of waterlogging on physiological parameters

Our results showed significant genotypic changes in the studied parameters under waterlogged conditions. We observed significant treatment, genotype and treatment × genotype interactions for all the traits at P <0.05.

Chl content decreased in response to waterlogging in all the genotypes (Fig. 1A). We reported significant variation for Chl content among the studied genotypes. Percent reduction ranged from 8.6-32.1 in different genotypes compared to control condition. Chl content was the highest in waterlogging tolerant genotypes i.e. IC519933 (14.38 mg g^{-1} fresh weight) and IC530491 (14.25 mg g^{-1} fresh weight). Chlorophyll fluorescence reduced under waterlogging in all the genotypes (Fig. 1B). Fluorescence ranged from 0.75-0.84 among different genotypes. Percent reduction ranged 6.0-10.7 in waterlogging compared to well-watered condition. Reduction in chlorophyll fluorescence was statistically non-significant. Results showed the functioning of PSII was not affected adversely by stress. NDVI decreased significantly in response to waterlogging in all the genotypes (Fig. 1C). NDVI ranged from 0.55-0.72 under control and 0.36-0.63 under waterlogging among different genotypes. We observed significant variation in sugar content under control as well as stress conditions (Fig. 1D). Soluble sugars reduced from 19.9-52.5% under waterlogged

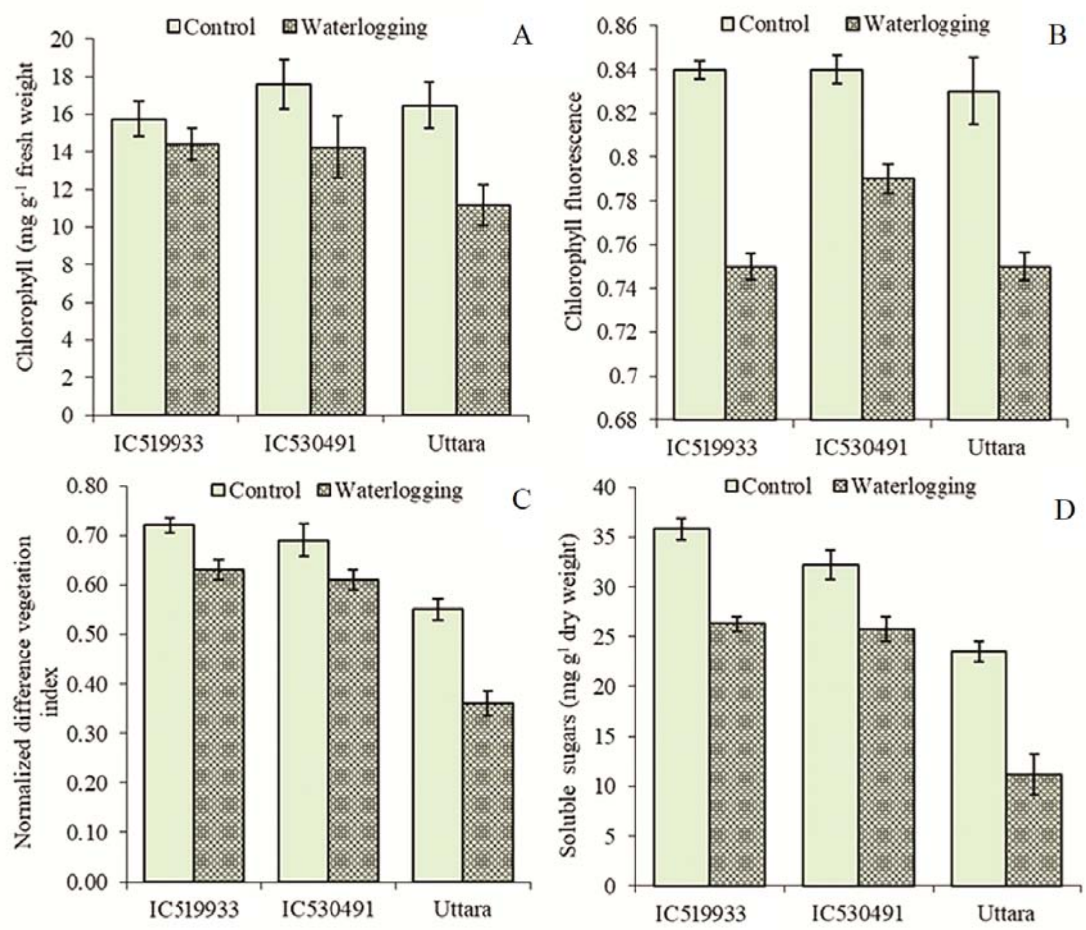

Fig. 1 — Changes in physiological traits (A) chlorophyll content; (B) chlorophyll fluorescence; (C) normalized differential vegetation index; and (D) soluble sugars in black gram genotypes in response to waterlogging

environment depending on the genotype. IC519933 showed the highest sugar content (26.3 mg g^{-1} dry weight) followed by IC530491 (25.8 mg g^{-1} dry weight). Uttara had the lowest sugar content under stress and non-stress condition.

Effect of waterlogging on yield, grain yield and stress susceptibility index

Significant variation among the genotypes with respect to grain yield was very high under control and stress conditions (Fig. 2A). Percent reduction in grain yield reduced from 12.1 to 79.5 depending upon the genotype. Under stress condition, IC519933 reported the highest yield (5.8 g $plant^{-1}$) followed by IC530491 (5.4 g $plant^{-1}$). TW reduced from 18.9 to 72.4% in response to waterlogging (Fig. 2B). IC530491 registered the highest TW (33.50 g) and Uttara (11.33 g) showed the lowest TW under stress.

SSI based on seed yield was calculated to categorize the stress response performance of different genotypes under stress environment (Fig. 3). Genotypes with SSI < 0.5 were identified as stress tolerant and were IC519933 (0.32) and IC530491 (0.43). Uttara was found highly susceptible (1.08) to waterlogged conditions.

Correlations among different traits

Linear correlation study revealed the significant correlations between different physiological and yield traits (Table 1). Under control conditions, there was a moderate positive correlation (P <0.05) between sugars and TW (r = 0.48), sugars and grain yield (r = 0.47) and grain yield and TW (0.41). When the correlations were studied under stress conditions, more relationships among the traits became evident. NDVI was positively correlated with TW (r = 0.45) and negatively correlated with SSI (0.43). TW exhibited a strong positive correlation to sugars (0.61 at P <0.01) and moderate positive relationship with grain yield (0.41) and negative relationship with SSI (0.42) at P <0.05. Grain yield was strongly correlated to Chl (r = 0.63 at P <0.01). SSI showed strong negative relation to Chl (r = 0.68) and g rain yield (r = 0.96) at P <0.01 and NDVI (r = 0.43) and TW (r = 0.42) at P <0.05.

Discussion

All the studied parameters reduced significantly under stress. Decrease in Chl and NDVI in response to waterlogging indicated decline in greenness in all black gram genotypes. Reduction in Chl content has been earlier reported by different workers in soybean, mung bean and black gram and was suggested as a potential selection criterion for waterlogging tolerance[7-8]. In the

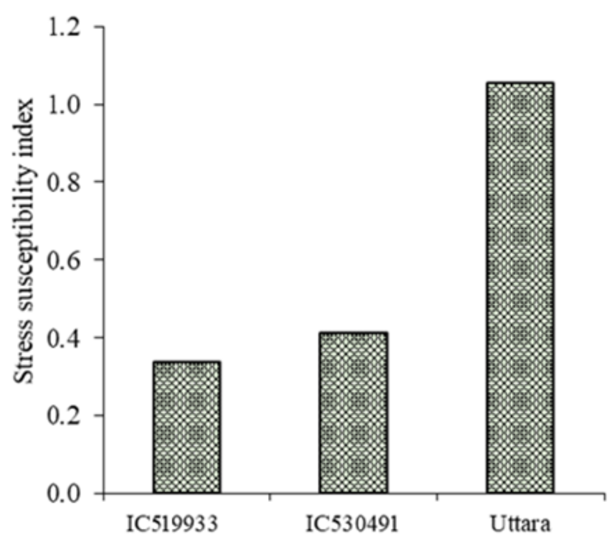

Fig. 3 — Stress susceptibility index of black gram genotypes in response to waterlogging stress

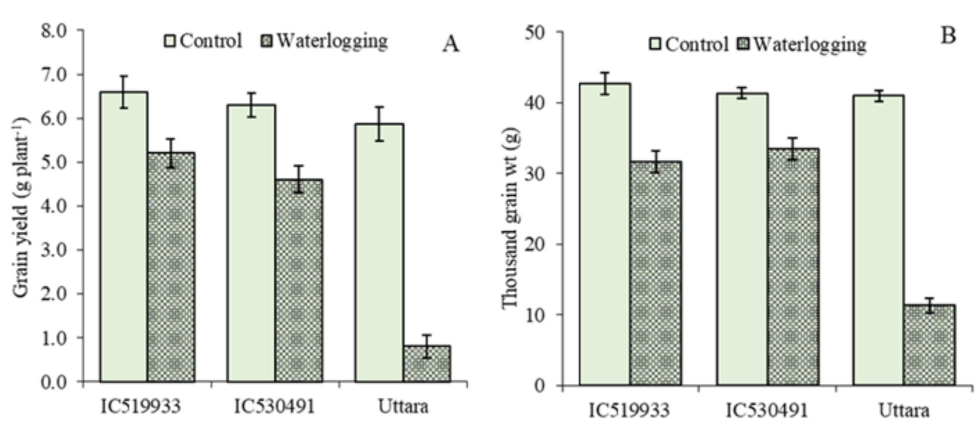

Fig. 2 — Effect of waterlogging stress on (A) grain yield; and (B) thousand grain weight in black gram genotypes

Table 1 — Correlation among different physiological and yield parameters in black gram

	Chl	FLU	NDVI	SUG	TW	GY	SSI
Chl	1	0.23	0.13	−0.08	−0.31	0.06	0.06
FLU	0.30	1	−0.14	0.10	0.05	0.08	0.08
NDVI	0.13	0.03	1	0.02	0.01	−0.04	0.08
SUG	0.24	0.07	0.26	1	0.48*	0.47*	−0.26
TW	0.30	−0.06	0.45*	0.61**	1	0.41*	−0.03
GY	0.63**	−0.22	0.36	0.33	0.41*	1	0.22
SSI	−0.68**	0.09	−0.43*	−0.35	−0.42*	−0.96**	1

Above Diagonal-Correlation coefficient among traits in control condition; below diagonal- Correlation coefficient among traits under stress; * and ** denotes significance at 5 % and 1 % level, respectively, Chl: Chlorophyll; FLU: chlorophyll fluorescence; NDVI: Normalized difference vegetation index; SUG: Soluble sugars; TW: Thousand grain weight; GY: grain yield; SSI: Stress susceptibility index

present study, Chl content was higher in both the waterlogging tolerant genotypes (IC519933 and IC530491) compared to susceptible genotype. NDVI, which reflects the proportion of green vegetation decreased in all the studied genotypes. Similar to Chl, NDVI was also high in waterlogging tolerant genotypes under stress, which reflects that these genotypes were able to maintain photosynthetic ability on exposure to stress. There were positive correlations between yield and Chl, yield and NDVI as well as TW and NDVI. Relationship between yield and both the parameters showed that these parameters can be used for selection of waterlogging tolerant genotypes in field conditions as well. As our previous study revealed that Chl can be used as selection trait for waterlogging tolerance in pot studies[9], present study validated the findings observed earlier. Though Chl and NDVI are associated with the green canopy, we could not observe any relationship between both the parameters. It may be attributed to the fact that NDVI may provide a reflection of greenness of whole canopy, while, only leaves are taken in to consideration during Chl measurement.

Chlorophyll fluorescence reduced in all the genotypes, but responses were genotype dependent. Fluorescence varied non-significantly under stress condition in all the genotypes showing no significant effect on the photochemistry of PSII. Our results agreed with the previous report indicating the comparative resistance of maximum fluorescence to waterlogging in sorghum[17] and was suggested the only severe stress can affect the PSII photochemistry[18]. However, there are conflicts in results on sensitivity of PSII to waterlogging stress as reported in cowpea[19].

Sugars are the key source of energy required for plant growth and development. We also observed 19.9-52.5% reduction in soluble sugars under stress. Waterlogging tolerant genotypes had the higher sugar reserves under stress condition compared to susceptible genotype. During waterlogging, hypoxic conditions trigger shortage of energy supply. We also noted that genotypes with high soluble sugar yielded better compared to susceptible. Significant positive correlation between sugars and TW further confirmed the importance of remobilization of sugars in stress tolerance[2].

Selection for yield potential complemented by physiological traits under stress condition is the most reported strategy used by the breeders[20].We observed significant reduction in grain yield as well as TW under waterlogging condition also observed by different workers[21-22]. Since grain yield showed a positive correlation with TW, maintaining the higher TW contributed to higher yield potential under waterlogged conditions. Different indexes have been used for the selection and study of comparative performance of different genotypes under well-watered and stress conditions[6]. Based on SSI, we concluded that IC519933 and IC530491 were stress tolerant genotype. We observed that waterlogging tolerant genotypes maintained higher yield by effective green cover, sugars mobilization to grains leading to higher grain weight and grain yield under stress condition as observed in peanut[6] and soybean[7].

Present study indicated that to assess the sensitivity of a genotype to waterlogging stress, physiological traits along with the yield should be taken into consideration. Though some physiological traits were correlated to yield, TW and SSI under stress conditions, the contribution of individual trait to stress tolerance vary depending on the genotypes.

Conclusion

We concluded that Chl, NDVI and sugars are key physiological traits resulting in waterlogging tolerance in black gram genotypes. To tackle the narrow genetic base of black gram varieties[23], identification and utilization of novel trait donors is urgently required in breeding program.

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Conflict of interest

All authors declare no conflict of interest.

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