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Alterations in the sugar metabolism of black gram (*Vigna mungo*) genotypes grown under high temperature stress

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ABSTRACT

Black gram (Urd bean) is an important pulse crop preferred in South India due to its daily dietary component. Often the productivity is limited by environmental stresses particularly of water, salinity and high temperature. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affects the plant growth and development and may lead to a drastic reduction in economic yield. The adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. A thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermo tolerance is imperative. The sugar metabolism is an important parameter and understanding the thermo tolerance in terms of total carbohydrate and starch content might through light on genotypic variability. Among the nineteen black gram genotypes screened under controlled atmospheric studies have shown that VBG – 06 – 002 has shown promising for heat tolerance as the carbohydrate content in leaves and starch content in the grains was least affected by heat stress and deserve to be evaluated further. There were only 2.8 and 5.07 per cent reduction in the carbohydrate and starch content due to heat stress when compared other genotypes which have recorded more reduction in both parameters indicating genotypic superiority for heat stress. Based on the biochemical analysis, black gram genotype.

Key words: High temperature, Black gram, Total Carbohydrates, Starch

Black Gram [*Vigna mungo* (L.) Hepper, Hindi-*Urd*] is a highly prized pulse crop of South India. It is mostly consumed as pulse and in the preparation of typical Indian dishes like *pappad, idli, dosa* and *vada* which are delicious and often preferred by foreign tourists. It is a good source of phosphorus. Pulses are an important component of human nutrition as sources of proteins, carbohydrates, and minor nutrients such as vitamins and minerals. The major pulse seed storage polysaccharide is starch, which is made up of highly branched amylopectin and sparsely branched amylase (Wei *et al.,* 2012).

Pulse starches generally contain a higher concentration of amylose as compared to cereals and tuber starches. The non-starch complex carbohydrates are major components of dietary fiber including cellulose, hemicellulose, and pectic polysaccharides with considerable structural diversity. Diets rich in pulses are associated with health benefits such as reduced calorific content, reduced or no effect on blood glucose levels (low glycemic index), and improved heart health.

Pulses are major sources of proteins among the vegetarians in India, and complement the staple cereals in the diets with proteins, essential amino acids, vitamins and minerals. They contain 22-24% protein, which is almost twice the protein in wheat and thrice that of rice. Pulses provide significant nutritional and health benefits, and are known to reduce several non-communicable diseases such as colon cancer and cardiovascular diseases (Yude *et al*, 1993; Jukanti *et al.*, 2012). India is the largest producer and consumer of pulses in the world. Major pulses grown in India include chickpea or Bengal gram (*Cicer arietinum*), pigeonpea or red gram (*Cajanus cajan*), lentil (*Lens culinaris*), urdbean or black gram (*Vigna mungo*), mungbean or green gram (*Vigna radiata*), lablab bean (*Lablab purpureus*), moth bean (*Vigna aconitifolia*), horse gram (*Dolichos uniflorus*), pea (*Pisum sativum* var. arvense), grass pea or khesari (*Lathyrus sativus*), cowpea (*Vigna unguiculata*), and broad bean or faba bean (*Vicia faba*). It has been estimated that India's population would reach 1.68 billion by 2030 from the present level of 1.21 billion. Accordingly, the projected pulse requirement for the year 2030 is 32 million tons with an anticipated required growth rate of 4.2% (IIPR Vision 2030). India has to produce not only enough pulses but also remain competitive to protect the indigenous pulse production. India ranks first in the world in terms of pulse production (25.5 % of total worlds production) (FAO STAT 2012). Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka and Rajasthan are the major states growing pulses in India. These six states contribute 80% of total pulse production and area (Directorate of Economics and Statistics, Department of Agriculture and Cooperation, 2013). Among the abiotic stresses that limit productivity of crops, high temperature stress assumes second rank next to drought. The global air temperature is predicted to rise by 0.2˚C per decade which will lead to temperatures 1.8˚C to 4˚C higher than the current level by 2100 (IPCC, 2007). Plant growth and development involve numerous biochemical reactions that are sensitive to temperature. Limitations in carbohydrates supplies have been implicated as a factor responsible for reproductive failure under heat stress (Hasanuzzaman *et al.*, 2013). Therefore an investigation was made to determine the responses of black gram genotypes under heat stress particularly concerning the carbohydrate metabolism. In this study screening of black gram genotypes was taken up to analyse the carbohydrate and starch content and implicate to see any genetic variation with reference to heat tolerance. It was earlier reported that heat stress is often causes an imbalance in starch synthesis (Hasanuzzaman *et al.*, 2013).

MATERIALS AND METHODS

Nineteen black gram genotypes were raised in pots and kept in the temperature controlled chamber on 03.07.2013 at four leaf stage. The temperature regime of 40^0 C + 2^0 C was maintained from 10 am to 4 pm (6 hours daily) from 16.7.13 onwards. The crop was irrigated at 50% ASM. A similar set of pot culture was maintained in an identical chamber as that of heat chamber but without heat so as to serve as control for comparison.

Estimation of Total Carbohydrates

Total carbohydrate was estimated by the method of Hedge and Hofriter (1962) in fresh leaves. The dry organic matter was reacted with 2.5N HCl on water bath for 3 hour to convert into 5 hydroxy methyl furfural. This gives green colour with anthrone reagent. The carbohydrate was estimated by the standard curve prepared by different concentrations of glucose using the formula below and expressed as mg g^{-1} FW.

$$\text{Total carbohydrates} = \frac{\text{mg of glucose}}{\text{Volume of the test sample}} \times 100$$

Estimation of starch content

The starch content was estimated by following the method prescribed by Hedge and Hofreiter (1962) in the fresh leaves. One hundred mg of the sample was homogenized in hot 80 percent ethanol to remove sugars. The residue was retained after centrifugation. The residue was washed with hot 20 percent ethanol till the washings did not give colour with anthrone reagent. The residue was dried well in a water bath. To the residue, 5 ml of water and 6.5 ml of percent perchloric acid were added. Starch is extracted at 0˚C for 20 minutes. The extract was retained after centrifugation. The extraction was repeated with fresh perchloric acid. The extracts were pooled after centrifugation and the volume was made up to 100 ml with 52 percent perchloric acid. To 0.2ml of the extract, 0.8ml of distilled water and 4ml of anthrone reagent were added. The reaction mixture was heated for 8 min in a boiling water bath and cooled rapidly. The colour intensity as read at 630nm using a spectrophotometer. D – glucose was used as a standard and the starch content was expressed as mg g^{-1} DW

$$\text{Starch content} = \frac{\text{mg of glucose}}{\text{Volume of the test sample}} \times 100$$

RESULTS AND DISCUSSION

Estimation of Total Carbohydrates

The total carbohydrate content ranged from 19.33 mg/g in VBG-04-003 to 30.02 mg/g in VBG-04-005 in the control. Under heat stress the same genotypes recorded 16.57 mg/g and 25.66 mg/g respectively. The lowest percent reduction in total carbohydrate content over control was recorded in the genotype VBG-06-002 (2.8 %) and the highest percent reduction was observed in the genotype VBG-06-009(20.4%) (Table 1; Fig.1). It is therefore seen that the black gram genotype VBG-06-002 appear to be heat tolerant as far as the carbohydrate content is concerned. Varietal variation with respect to heat tolerance in black gram was reported by Manasi Dash and Dhara Shree (2012). The present study showed heat tolerance nature of VBG-06-002 and deserves further evaluation.

Estimation of Starch content

The starch (mg g^{-1} DW) content in control and heat treated genotypes ranged from 9.96 -15.64 and 7.52 - 14.77 mg/g respectively. The highest starch content in control and het treated was recorded by the genotypes VBG-04-003(15.64) and VBG-04-005, Co-6(7.52, 7.52) respectively. The lowest percent reduction was recorded VBG-06-002(5.07) (Table.2; Fig.2) and the highest percent reduction was recorded by the genotypes Co Bg-10-05(33.84). The temperature during germination, seedling and reproductive stage in black gram is very critical (Manasi Dash and Dhara Shree, 2012). The black gram variety VBG-06-002 performed best in terms of least decline in starch content due to heat stress similar to that of carbohydrate content. It has been reported that VBG-06-002 is a good performer under heat stress as far as the yield was concerned and designated as heat tolerant genotype (Vijayaraghavan *et al.*, 2013).

Table 1. Effect of temperature on Total carbohydrates (mg g^{-1} FW) content of black gram tolerant genotypes

S. No	Genotype	Control	Heat stress	% Reduction
1	VBG-04-003	19.33	16.57	14.3
2	VBG-04-005	30.02	25.66	14.5
3	VBG-06-002	21.56	20.96	2.8
4	VBG-06-003	22.61	19.40	14.2
5	VBG-06-005	21.79	20.40	6.4
6	VBG-06-009	27.34	21.76	20.4
7	VBG-06-010	26.10	24.69	5.4
8	VBG-07-001	26.14	24.70	5.5
9	VBG-08-003	25.91	23.11	10.8
10	VBG-10-008	19.87	18.18	8.5
11	VBG-10-024	26.53	23.52	11.3
12	VBN-6	25.32	23.47	7.3
13	VBN-7	22.77	20.78	8.7
14	CO-6	28.80	23.43	18.6
15	COBG-11-02	20.41	18.24	10.6
16	COBG-11-03	21.28	19.57	8.0
17	COBG-10-05	26.54	23.21	12.5
18	COBG-10-06	23.76	21.68	8.8
19	COBG-759	23.62	21.26	10.0
	Mean with standard error	**24.19 ± 0.706**	**21.61 ± 0.506**	

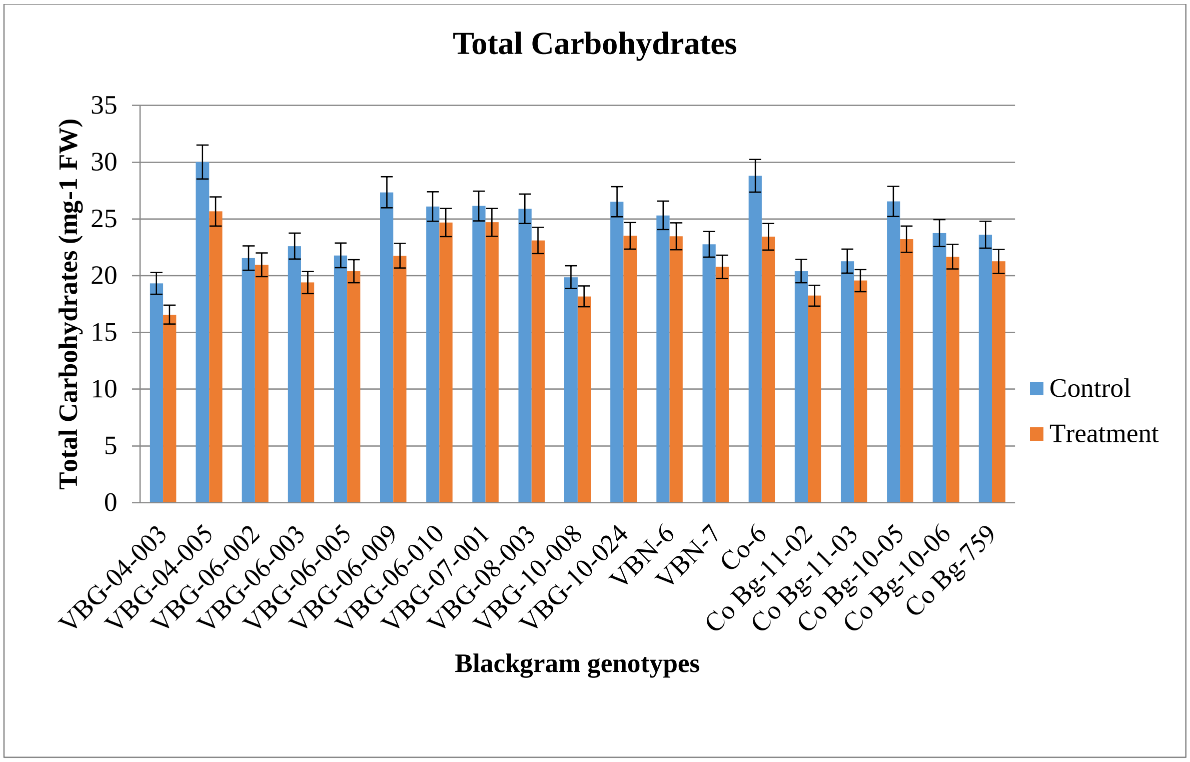

Fig 1. Effect of temperature on total carbohydrates content of black gram genotypes

Table 2. Effect of temperature on Starch content (mg g^{-1} FW) content of black gram genotypes.

S. No	Genotype	Control	Heat stress	% Reduction
1	VBG-04-003	15.64	14.77	5.56
2	VBG-04-005	10.72	7.52	29.85
3	VBG-06-002	10.05	9.54	5.07
4	VBG-06-003	11.29	10.6	6.11
5	VBG-06-005	11.25	10.59	5.87
6	VBG-06-009	10.7	8.54	20.19
7	VBG-06-010	11.33	10.63	6.18
8	VBG-07-001	11.45	10.63	7.16
9	VBG-08-003	11.18	10.21	8.68
10	VBG-10-008	10.68	9.63	9.83
11	VBG-10-024	14.27	11.65	18.36
12	VBN-6	10.65	9.59	9.95
13	VBN-7	12.82	11.63	9.28
14	CO-6	9.96	7.52	24.50
15	COBG-11-02	12.01	10.52	2.41
16	COBG-11-03	13.7	11.6	15.33
17	COBG-10-05	14.54	9.62	33.84
18	COBG-10-06	11.34	9.64	14.99
19	COBG-759	12.37	10.51	15.04
	Mean with standard error	**11.89 ± 0.40**	**10.26 ± 0.40**	

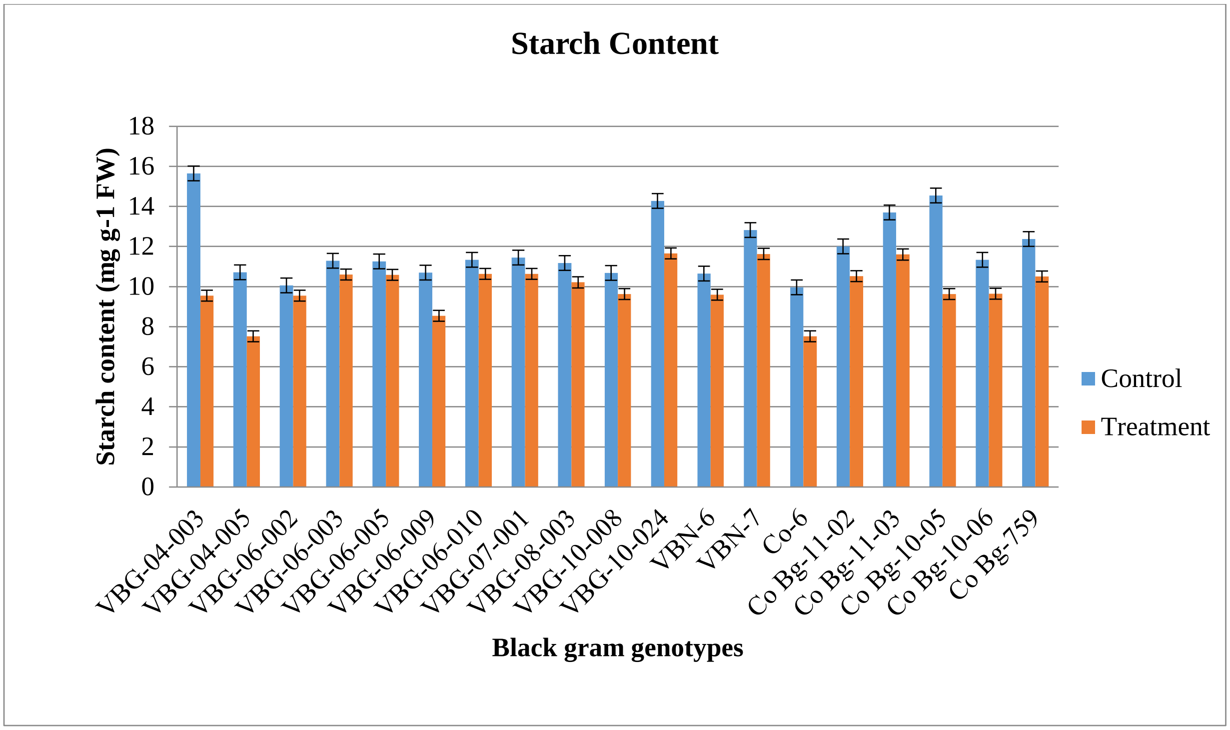

Fig 2. Effect of temperature on starch content of black gram genotypes

CONCLUSION

Considering the total carbohydrate and starch content, VBG – 06 – 002 has shown promising for heat tolerance as these biochemical character was least affected by heat stress and deserve to be evaluated further.

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