Effect of induced mutation on some biochemical content of Winged Bean

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ABSTRACT

In present investigation efforts have been made for evaluation of level of carbohydrates and reducing sugar in induced mutants of winged bean like high yielding, long pod, large leaf, flat pod, early maturing and dwarf mutant lines. The level of carbohydrates and reducing sugar of all mutant lines were compared with control plants (EC-38955-A) and improvement in quantity of carbohydrates and reducing sugar was observed. The higher level of seed carbohydrate content was observable in mutant lines flat pod/ wingless-3 (41.70 %), large leaf/ stiff stem-3 (41.41 %) and anthostem-3 (41.21 %) than the control (37.05 %). In tuber highest carbohydrate content was noted in flat pod/wingless-3(31.62 %) mutant lines. The maximum seed reducing sugar could be noticed in large leaf/ high yielding-3 (108 mg/g), and long pod/ black seed-2 (108 mg/g). In case of tuber highest reducing sugar have been noted in wingless/small pod-2 (55 mg/g) and dark green/ flat pod-4 (55 mg/g). From such mutant lines the nutritionally improved material has been established from the pertinent study. This study also indicated that the amount of nutritional components will be increase through the genetic improvement by using induced mutation is very much possible.

Key words: Carbohydrates, reducing sugar, mutants

INTRODUCTION

The winged bean has been cultivated in the humid tropics of India, Srilanka, Bangladesh, Myanmar, Malaysia, Thailand, Vietnam, Laos, Combodia, Phillippines, Indonesia and Papua New Guinea (National Research Council, 1984). In India, the winged bean also popular by the name, Goa/Asparagus bean, is described by various regional names in different states of India. It is known as "Choudhari Wal" in Maharashtra; as rakki/kattuavarai in Karnataka; as boonch/perandai avarai in Tamil Nadu; as Choughal Sem in Madhya Pradesh and as Charpatti Sem in West Bengal, respectively (Banerjee, 1985).

Carbohydrates

The winged bean seeds contain 23.9 to 42 % carbohydrates as well. Not only is the protein content high, but the tubers of winged bean forming a nice source of carbohydrates (27.2 to 30.5 %) that provide energy. This rare combination makes the winged bean tuber unusual among the tropical root crops (Alan, 1983). Analysis has shown that the level of raffinose and stachyose (sugars that lead to flatulence) is less in winged bean than in soybean (A typical comparison of the seed meal of winged bean and soybean has been given in Table 2. (Gunn, 1980).

MATERIALS AND METHODS

Fourteen true breeding M_6, M_7 and M_8 mutant lines of variety EC 38955-A of winged bean obtained from the earlier mutation breeding programme (Kulthe, 2003) were taken for the analysis of carbohydrates and reducing sugar.

The list of mutants of winged bean used in the present study is as follows: Long pod, Early maturing, Flat pod/wingless, Large leaf/high yield, Flat pod/linear leaf, Flat pod/large leaf, AnthostemLong pod/large leaf, Long pod/black seed, Flat pod/long pod, Dwarf, Wingless/small pod, Dark green/flat pod, Large Leaf/stiff stem.

Extraction of total carbohydrates

The 100 mg of seed and tuber powder were weighed into separate boiling tubes and 5 ml of 2.5 N HCL was added to them. All the boiling tubes were placed in boiling water bath for 3 hrs. and after 3 hrs. Tubes were cooled at room temperature.

The boiled suspension was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 3000 rpm for 10 minutes. The supernatant was used for carbohydrate estimation.

Estimation of total carbohydrates

The estimation of total carbohydrates was carried out by Phenol-sulphuric acid method (Dubois et.al., 1956). Glucose working solution (10 µg/ml) was serially diluted (0.2 -2 ml) and volume was made upto 2 ml. The 100 µl sample was taken in separate test tube and volume was made upto 2 ml. The 1 ml of 5 % phenol and 5 ml of 96 % sulphuric acid were added to each test tube and mixed well. After 10 minutes the contents were mixed well. It was later placed in water bath at 25-30 ^{0}C for 20 minutes. The colour absorbance was read at 490 nm. Standard graph of glucose was plotted and total carbohydrate present in the sample was calculated by using the following formula.

$$\text{Total carbohydrate (\%)} = \frac{\text{Sugar value from graph}}{\text{Aliquot sample used (0.1 ml)}} \times \frac{\text{Total vol. of extract (100 ml)}}{\text{wt. of sample (100 mg)}} \times \frac{1}{1000}$$

Estimation of reducing sugar

The total reducing sugar was determined by the Dinitro salicyclilc acid (DNS) method (Miller, 1972). The DNS reagent was prepared by dissolving simultaneously 1 g of DNS, 200 mg of crystalline phenol and 50 gm of sodium sulphite in 100 ml of 1 % NaOH solution by stirring. The 100 mg seed/ tuber powder was taken in boiling tubes and 5 ml (twice) of hot 80 % alcohol was used for extraction of sugar. The supernatant was evaporated in water bath and sugar was dissolved in 10 ml distilled water.

The standard graph of glucose was prepared from the working glucose solution (10 µg/ml) by taking serially 0.2-2 ml in test tubes. The required amount of extract (0.5 – 3 ml) was used and volume was made upto 3 ml in each test tube. The 3 ml DNS reagent was added and heated for 5 minutes in boiling water bath. The colour was developed, and then 1 ml of 40 % Rochelle salt solution was mixed (when the contents were still warm). All test tubes were cooled and absorbance was read at 510 nm using reagent as blank. The amount of reducing sugar was calculated using standard graph of glucose and following formula,

$$\text{Reducing sugars (\%)} = \frac{\text{Sugar value from graph}}{\text{Aliquot used (ml)}} \times \frac{\text{Total volume of extract (10 ml)}}{\text{wt. of sample (100 mg)}} \times \frac{1}{1000}$$

RESULTS AND DISCUSSION

Carbohydrate content (Table 1)

Induced mutation playing important role in plant breeding for variety development. Induced mutation alters both the morphological as well as biochemical characters (Mukund Kulthe et.al., 2013). The data regarding seed and tuber carbohydrate content revealed substantial variability. The higher level of seed carbohydrate content was observable in mutant lines flat pod/ wingless-3 (41.70 %), large leaf/ stiff stem-3 (741.41 %) and anthostem-3 (41.21 %) than the control (37.05 %). In wingless/ small pod-1 (25.16 %) lowest seed carbohydrate percentage was detected. The values of tuber carbohydrate content ranged from 23.87 % (dark green/flat pod-4) to 31.62 % (flat pod/wingless-3). The tuber carbohydrate content in control (EC-38955-A) was 27.35 %.

Reducing Sugars (Table 1)

The values for seed reducing sugar ranged from 35 to 108 mg/g while tuber reducing sugar varied from 28 to 55 mg/g in different mutant lines. The maximum seed reducing sugar could be noticed in large leaf/ high yielding-3 (108 mg/g), long pod/ black seed-2 (108 mg/g) and large leaf/high yielding-5 (105 mg/g), as compared to control (76 mg/g) while the lowest seed reducing sugar content was revealed by the wingless/small pod-5 (35 mg/g), long pod –2(38 mg/g) flat pod/large leaf-4 (38 mg/g) and wingless/small pod-4 (38 mg/g).

Sr. No.	Mutant Name and Number	Tuber carbohydrate (%)	Seed carbohydrate (%)	Seed reducing sugar mg/gm	Tuber reducing sugar mg/gm
1	Control **EC-38955-A**	27.35 ± 0.46	37.05 ± 0.53	76 ± 3.41	48 ± 4.72
2	Long Pod-1	28.55 ± 0.56	41.00 ± 0.87	66 ± 8.12	53 ± 2.51
3	Long Pod-2	26.15 ± 0.78	41.12 ± 0.42	38 ± 2.41	35 ± 3.95
4	Long Pod-3	27.25 ± 0.85	29.16 ± 0.37	70 ± 3.18	30 ± 1.17
5	Long Pod-4	29.91 ± 0.35	34.25 ± 0.52	102 ± 5.61	42 ± 3.14
6	Long Pod-5	26.25 ± 0.33	28.91 ± 0.94	92 ± 4.15	39 ± 2.72
7	Early Maturing-1	24.15 ± 0.49	25.63 ± 0.34	62 ± 2.92	51 ± 3.00
8	Early Maturing-2	28.95 ± 0.38	39.95 ± 1.05	98 ± 3.44	34 ± 4.16
9	Early Maturing-3	30.00 ± 0.94	40.15 ± 0.25	104 ± 5.11	38 ± 2.74
10	Early Maturing-4	26.79 ± 0.42	32.55 ± 0.34	80 ± 4.96	36 ± 3.25
11	Early Maturing-5	28.53 ± 0.53	37.59 ± 0.48	83 ± 3.16	52 ± 2.51
12	FP/Wingless-1	25.91 ± 0.95	25.17 ± 0.85	93 ± 4.14	53 ± 3.72
13	FP/Wingless-2	27.65 ± 0.73	35.25 ± 0.75	84 ± 3.48	51 ± 4.91
14	FP/Wingless-3	31.62 ± 0.92	41.70 ± 1.13	65 ± 2.13	43 ± 2.16
15	FP/Wingless-4	27.28 ± 1.02	34.91 ± 0.58	74 ± 3.34	49 ± 3.58
16	FP/Wingless-5	29.79 ± 0.32	39.62 ± 0.74	46 ± 2.19	37 ± 2.91
17	La.L./high yield-1	29.28 ± 0.74	38.45 ± 0.84	62 ± 3.41	45 ± 1.86
18	La.L./high yield-2	28.34 ± 0.91	40.48 ± 0.53	78 ± 4.52	48 ± 1.77
19	La.L./high yield-3	25.68 ± 0.42	39.20 ± 0.94	108 ± 3.99	51 ± 2.52
20	La.L./high yield-4	29.36 ± 0.75	41.05 ± 0.66	92 ± 2.29	53 ± 3.48
21	La.L./high yield-5	26.37 ± 0.47	40.35 ± 0.98	105 ± 4.18	44 ± 5.32
22	FP/Li.L-1	28.75 ± 0.78	39.90 ± 0.28	78 ± 5.10	54 ± 4.15
23	FP/Li.L-2	26.58 ± 0.98	36.25 ± 0.72	69 ± 2.84	48 ± 2.21
24	FP/Li.L-3	27.45 ± 0.45	38.50 ± 0.77	96 ± 3.58	41 ± 1.65
25	FP/Li.L-4	26.95 ± 0.27	36.78 ± 0.35	82 ± 4.00	32 ± 2.41
26	FP/Li.L-5	28.55 ± 0.48	35.20 ± 0.24	76 ± 3.44	28 ± 3.71
27	FP/La.L.-1	27.77 ± 0.45	35.75 ± 0.52	58 ± 1.92	39 ± 4.38
28	FP/La.L.-2	25.39 ± 0.82	33.80 ± 0.74	69 ± 2.71	46 ± 3.16
29	FP/La.L.-3	28.76 ± 0.44	35.00 ± 0.68	77 ± 5.25	35 ± 2.78
30	FP/La.L.-4	29.14 ± 0.95	36.66 ± 0.25	38 ± 4.85	34 ± 3.92
31	FP/La.L.-5	24.76 ± 0.35	31.12 ± 0.93	63 ± 2.21	29 ± 2.14
32	Anthostem-1	30.10 ± 0.72	39.75 ± 0.75	69 ± 3.15	42 ± 3.33
33	Anthostem-2	29.66 ± 0.58	40.00 ± 0.92	57 ± 2.68	38 ± 4.00
34	Anthostem-3	28.72 ± 0.24	41.21 ± 0.72	64 ± 3.33	43 ± 3.16
35	Anthostem-4	25.25 ± 0.92	38.91 ± 0.25	58 ± 2.58	34 ± 2.95
36	Anthostem-5	27.95 ± 0.74	39.16 ± 0.73	96 ± 3.92	41 ± 2.00
37	LP/La.L.-1	28.20 ± 0.58	36.15 ± 0.54	102 ± 4.48	39 ± 3.21
38	LP/La.L.-2	26.47 ± 0.92	34.74 ± 0.74	68 ± 1.95	40 ± 1.98
39	LP/La.L.-3	29.54 ± 0.24	36.35 ± 0.28	92 ± 3.42	42 ± 2.71
40	LP/La.L.-4	24.98 ± 0.72	30.00 ± 0.72	78 ± 4.41	38 ± 3.91
41	LP/La.L.-5	28.73 ± 0.81	33.19 ± 0.45	65 ± 2.18	45 ± 4.21
42	LP/Black seed-1	29.90 ± 0.75	35.90 ± 0.38	104 ± 4.72	34 ± 4.00
43	LP/Black seed-2	28.21 ± 0.42	29.25 ± 0.94	108 ± 4.05	35 ± 2.56
44	LP/Black seed-3	25.12 ± 0.37	38.49 ± 0.72	92 ± 3.14	44 ± 1.95
45	LP/Black seed-4	31.18 ± 0.72	40.16 ± 0.28	48 ± 2.38	43 ± 2.85
46	LP/Black seed-5	27.65 ± 0.24	34.28 ± 0.15	62 ± 2.92	35 ± 2.16
47	FP/LP-1	26.40 ± 0.54	39.50 ± 0.97	98 ± 4.64	40 ± 3.75
48	FP/LP-2	28.72 ± 0.92	36.18 ± 0.62	44 ± 2.12	37 ± 2.40
49	FP/LP-3	26.23 ± 0.84	29.83 ± 0.38	55 ± 4.11	44 ± 3.51
50	FP/LP-4	27.16 ± 0.75	35.18 ± 0.75	90 ± 2.59	52 ± 2.13
51	FP/LP-5	29.62 ± 1.14	39.75 ± 1.24	47 ± 4.72	50 ± 3.15

52	Dwarf-1	30.68 ± 0.93	38.20 ± 0.93	68 ± 3.25	48 ± 2.33
53	Dwarf-2	29.95 ± 0.78	35.52 ± 0.42	85 ± 2.49	51 ± 3.30
54	Dwarf-3	31.50 ± 0.54	40.05 ± 0.74	74 ± 4.22	39 ± 2.71
55	Dwarf-4	25.29 ± 0.94	34.19 ± 0.35	92 ± 2.99	33 ± 2.00
56	Dwarf-5	27.91 ± 0.48	37.15 ± 0.38	75 ± 3.44	51 ± 3.11
57	Wingless/Small pod-1	26.29 ± 0.92	25.16 ± 0.97	72 ± 4.17	41 ± 1.76
58	Wingless/Small pod-2	25.96 ± 1.05	36.40 ± 0.25	84 ± 2.91	55 ± 2.38
59	Wingless/Small pod-3	28.24 ± 0.38	38.48 ± 0.28	52 ± 2.38	31 ± 2.82
60	Wingless/Small pod-4	29.49 ± 0.78	35.70 ± 0.84	38 ± 4.14	43 ± 4.12
61	Wingless/Small pod-5	24.98 ± 0.64	27.35 ± 0.29	35 ± 2.14	35 ± 2.71
62	Dark green/flat pod-1	30.41 ± 0.35	39.35 ± 0.74	92 ± 3.66	47 ± 3.15
63	Dark green/flat pod-2	26.75 ± 1.15	30.26 ± 0.99	81 ± 4.16	38 ± 2.71
64	Dark green/flat pod-3	28.71 ± 0.98	31.92 ± 0.28	44 ± 2.95	42 ± 3.91
65	Dark green/flat pod-4	23.87 ± 0.38	26.56 ± 0.75	72 ± 3.38	55 ± 3.61
66	Dark green/flat pod-5	28.53 ± 0.54	29.23 ± 0.68	98 ± 4.84	39 ± 2.18
67	La.L./Stiff stem-1	30.05 ± 0.96	36.28 ± 0.82	52 ± 2.24	41 ± 3.46
68	La.L./Stiff stem-2	29.43 ± 1.32	40.29 ± 0.94	78 ± 4.47	50 ± 2.67
69	La.L./Stiff stem-3	28.79 ± 0.48	41.41 ± 0.38	84 ± 2.99	49 ± 3.15
70	La.L./Stiff stem-4	29.77 ± 0.75	38.34 ± 0.75	93 ± 4.14	29 ± 2.72
71	La.L./Stiff stem-5	29.96 ± 0.58	40.75 ± 0.54	89 ± 2.26	51 ± 3.17

In case of tuber reducing sugar the increased reducing sugar was demonstrated by mutant lines of wingless/small pod-2 (55 mg/g), dark green/ flat pod-4 (55 mg/g) and flat pod/linear leaf-1 (54 mg/g) while decreased level of reducing sugar was noticed in flat pod/linear leaf-5 (28 mg/g), flat pod/large leaf-5 (29 mg/g) and large leaf/ stiff stem-4 (29 mg/g).

Among the macro mutant lines, the flat pod/wingless-3 could be found as a high seed carbohydrate mutant of winged bean. The mutant flat pod/wingless -3 could be inferred as a high tuber carbohydrate content carrying mutant line.

The reducing sugar studied in different mutant lines and mutants showed increase in seed and tuber reducing sugar content in some mutant lines. The maximum increase in seed reducing sugar was observed in mutant lines large leaf/high yielding-3, long pod/black seed-2 and large leaf/high yield-5. In wingless/small pod-2, dark green flat pod-4 and flat pod/linear leaf-1, the highest tuber reducing sugar was noticeable.

From above study it is concluded that the carbohydrate and reducing content in winged bean has been increased through the induced mutagenesis. From the foregoing account it can be concluded that the varied mutant lines developed in winged bean can be considered as morphologically and nutritionally desirable and such lines would have scope at commercial level as direct varieties.

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How to Cite this Article:
Bhalerao AL and VS Kothekar, 2013. Effect of induced mutation on some biochemical content of Winged Bean. *Biosci. Disc.,* **4**(2):182-186.