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Research Paper

Studies on Phenolic and Polyphenolic Compounds in Winged Bean Mutants

: Science

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ABSTRACT

Winged bean (Psophocarpus tetragonolobus (L) DC.) is one of the unknown plant systems. It has large potential to fulfill the need of staple food that is rich in protein and oil for man and as a fodder for animals. Besides the trypsin and chymotrypsin inhibitors, there are other unfavourable compounds like tannins (proanthocynanidins), phenol and polyphenol which have been reported to be present in seeds of winged bean (de Lumen and Salamat, 1980; Tan et al., 1983; Kantha et al., 1986; Klu et al., 1997). Phenolic and polyphenolic compounds which interact with proteins, form precipitates, and therefore reduce food protein quality (Tan et al., 1983; Cabrera and Martin, 1986). To obtain the quality protein and oil from plants sources, mutagenesis has been considered an important tool which can alter the biochemical composition of plants.

In present investigation efforts have been made to analyse the phenolic and polyphenolic contents of winged bean mutants. The low phenolic mutant lines have been established which are nutritionally improved.

INTRODUCTION

In day to day life, human beings and different animals are suffering from different types of health problems. This is happening due to the quality of food they are consuming especially the low quality proteins in the food and the high antinutritional factors carried by that food.

Many of the scientists and plant breeders have contributed and developed high yielding varieties in different crop plants. This study mainly revolved around the quantitative as well as qualitative characters of the proteins, oils, carbohydrates and vitamins in plant products. The initial approach was to look for quantitative aspect. This was followed by qualitative consideration of food materials. Hence now a days the quality rich protein and oil food items are in demand. To obtain the quality protein and oil from plants sources, mutagenesis has been considered an important tool which can alter the biochemical composition of plants.

Now days nutrition has become a critical issue in medical sciences and the knowledge of nutrition helps modulate the physiopathology and intervention of several diseases. Near about 34% of the world's malnourished children live in India. About 50% of all childhood deaths are attributed to malnutrition. Nearly 30% of all new bornes have a low birth weight making them vulnerable to be further malnourished and diseased (Tekale, 2004).

Despite possessing high nutritional potential the winged bean has remained unfamiliar among society because of the presence of high amount of anti-nutritional factors such as trypsin and chymotrypsin inhibitors, phenols, polyphenols and tannins some undesirable characters of the plant (NAS, 1981).

For achieving the better quality food out of winged bean plant parts the development of low polyphenol and low phenol lines of winged bean has become crucial. It is understood from literature that such lines have been achieved in few other food legume plants like *Vigna mungo* (Sagade, 2008) and *Glycin max* (George, 2006) through induced mutational approach.

Mutants of winged bean

Induced mutational studies in winged bean were initiated by Kulthe (2000) as a part of his doctoral work. He successfully developed different economically important winged bean mutant lines like, large leaf, high yielding, long pod, flat pod, early maturing, and dwarf.

Though these mutant lines were morphologically well studied, their biochemical nature, however, was not well characterized. In winged bean the presence of high amount of anti-nutritional factors is posing the major problem. To overcome this situation it was visualized that induced mutation could be the desirable approach.

It is well established that the induced mutational approach not only creates morphological variation but also alters the biochemical features of plants. It was envisaged that by resorting to mutagenesis the minimization of different undesirable biochemical factors present in all edible plant parts of winged bean mutant plant types would become possible. By keeping this end in view the present studies were organized to assess the status of phenolic and polyphenolic compounds from the mutants of winged bean developed through earlier mutation breeding programme. It was believed that such efforts would lead to an understanding of the exact quantum of improvement in the biochemical and nutritional attributes of different morphologically desirable mutant lines of winged bean.

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:

1. Long pod
2. Early maturing
3. Flat pod/wingless
4. Large leaf/high yield
5. Flat pod/linear leaf
6. Flat pod/large leaf
7. Anthostem
8. Long pod/large leaf
9 Long pod/black seed
10. Flat pod/long pod
11. Dwarf
12. Wingless/small pod
13. Dark green/flat pod
14. Large Leaf/stiff stem

Estimation of phenols

The total phenol was estimated from the seed and tubers using Folin-ciocalteu reagent (Bray and Thorpe, 1954).

The 0.5 g seed and tuber powder was ground in 5 ml 80 % acetone, centrifuged at 1000 rpm for 20 minutes and supernatant was collected. The residue was re-extracted with 5 ml 80 % acetone. This process was repitted five times. The collected

supernatant was evaporated to dryness and dissolved in 5 ml distilled water.

The different aliquots (0.2-2 ml) of catechol (100 µg/ml) were pipetted out into test tubes and volume was made up to 2 ml with distilled water. In other test tubes 0.5 ml sample extract was pipetted out. In every test tube 0.5 ml of FCR was added and kept for 3 minutes. After 3 minutes 2 ml of 20 % Na_2CO_3 solution was added to each test tube and mixed thoroughly.

All the test tubes were placed in boiling water bath for exactly one minute, cooled and the absorbance was read at 650 nm against blank. The standard graph of catechol was prepared and total phenol was calculated using standard graph. The total phenol was expressed as mg/g.

Extraction of polyphenols

The defatted seed and tuber powder was extracted in 10 volumes of 2 N HCL and kept in boiling water bath for one hour. Then it was allowed to cool at room temperature for few minutes and centrifuged at 3000 rpm for 10 minutes and supernatant was collected. The above extract was used for further processing. 2 ml of ethyl acetate was added to 2 ml extract, vortexed and allowed to stand for 10 minutes. Ethyl acetate layer was separated in a test tube. Ethyl acetate extraction was repeated thrice and the obtained extracts were kept at 70 ^{0}C in water bath for evaporation of ethyl acetate. Last traces of ethyl acetate were evaporated to dryness by keeping the test tubes in boiling water bath. Dried sample in the test tube was dissolved in minimum amount of ethyl alcohol and centrifuged at 500 rpm for 10 minutes and the clear supernatant obtained was used for the estimation of polyphenols.

Estimation of polyphenols

The amount of total polyphenolic compounds in the extracts was estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959).

RESULTS AND CONCLUSION

Phenol content (Table 1)

In control the seed phenol content (0.490 mg/g) and tuber phenol content (0.195 mg/g) were compared in different mutant lines. Such lines showed maximum variability in seed phenol content and tuber phenol content which ranged from 0.255 to 0.572 mg/g and 0.075 to 0.221 mg/g, respectively.

Sr. No	Mutant Name and Number	Seed phenol (mg/gm)	Tuber phenol (mg/ gm)	Seed phenol (mg/gm)	Tuber polyphenol (mg/gm)
1	Control EC-38955-A	0.490	0.195	0.152	0.115
2	Long Pod-1	0.365	0.182	0.122	0.085
3	Long Pod-2	0.425	0.105	0.110	0.080
4	Long Pod-3	0.570	0.171	0.115	0.110
5	Long Pod-4	0.425	0.146	0.120	0.119
6	Long Pod-5	0.385	0.191	0.122	0.130
7	Early Maturing-1	0.290	0.175	0.180	0.157
8	Early Maturing-2	0.560	0.130	0.120	0.075
9	Early Maturing-3	0.370	0.145	0.127	0.090
10	Early Maturing-4	0.492	0.132	0.150	0.125
11	Early Maturing-5	0.428	0.192	0.125	0.110
12	FP/Wingless-1	0.456	0.120	0.115	0.080
13	FP/Wingless-2	0.375	0.140	0.125	0.110
14	FP/Wingless-3	0.298	0.128	0.110	0.077
15	FP/Wingless-4	0.378	0.139	0.115	0801
16	FP/Wingless-5	0.481	0.171	0.120	0.115
17	La.L./high yield-1	0.405	0.103	0.150	0.120
18	La.L./high yield-2	0.431	0.160	0.125	0.115
19	La.L./high yield-3	0.392	0.125	0.135	0.875
20	La.L./high yield-4	0.455	0.095	0.125	0.090
21	La.L./high yield-5	0.362	0.082	0.160	0.131
22	FP/Li.L-1	0.415	0.110	0.185	0.160
23	FP/Li.L-2	0.398	0.132	0.122	0.120
24	FP/Li.L-3	0.348	0.128	0.172	0.135
25	FP/Li.L-4	0.465	0.195	0.160	0.085
26	FP/Li.L-5	393	0.205	0.115	0.80
27	FP/La.L.-1	0.411	0.182	0.115	0.095
28	FP/La.L.-2	0.572	0.175	0.165	0.127
29	FP/La.L.-3	0.425	0.105	0.170	0.135
30	FP/La.L.-4	0.382	0.082	0.165	0.117
31	FP/La.L.-5	0.398	0.075	0.162	0.140
32	Anthostem-1	0.456	0.127	0.150	0.105
33	Anthostem-2	0.442	0.121	0.185	0.142
34	Anthostem-3	0.391	0.114	0.190	0.137
35	Anthostem-4	0.255	0.085	0.150	0.122
36	Anthostem-5	0.385	0.108	0.192	0.135
37	LP/La.L.-1	0.429	0.172	0.160	0.120
38	LP/La.L.-2	0.552	0.221	0.194	0.140
39	LP/La.L.-3	0.503	0.135	0.160	0.105
40	LP/La.L.-4	0.280	0.079	0.150	0.100
41	LP/La.L.-5	0.425	0.092	0.210	0.150
42	LP/Black seed-1	0.372	0.185	0.180	0.135
43	LP/Black seed-2	0.433	0.212	0.185	0.117
44	LP/Black seed-3	0.396	0.198	0.165	0.120
45	LP/Black seed-4	0.416	0.203	0.170	0.140
46	LP/Black seed-5	0.381	0.081	0.155	0.125
47	FP/LP-1	0.522	0.132	0.150	0.110
48	FP/LP-2	0.502	0.145	0.100	0.045
49	FP/LP-3	0.428	0.209	0.175	0.130
50	FP/LP-4	0.390	0.201	0.167	0.115
51	FP/LP-5	0.421	0.172	0.120	0.100
52	Dwarf-1	0.475	0.185	0.145	0.115
53	Dwarf-2	0.482	0.216	0.122	0.092
54	Dwarf-3	0.325	0.191	0.135	0.115
55	Dwarf-4	0.448	0.112	0.165	0.120
56	Dwarf-5	0.366	0.143	0.110	0..080
57	Wingless/Small pod-1	0.495	0.185	0.117	0.110
58	Wingless/Small pod-2	0.382	0.131	0.140	0.117
59	Wingless/Small pod-3	0.418	0.124	0.110	0.072
60	Wingless/Small pod-4	0.365	0.089	0.175	0.070
61	Wingless/Small pod-5	0.569	0.102	0.125	0.082
62	Dark green/flat pod-1	0.360	0.142	0.155	0.130
63	Dark green/flat pod-2	0.422	0.117	0.145	0.125
64	Dark green/flat pod-3	0.473	0.138	0.107	0.087
65	Dark green/flat pod-4	0.384	0.215	0.160	0.140
66	Dark green/flat pod-5	0.435	0.182	0.185	0.117
67	La.L./Stiff stem-1	0.515	0.125	0.110	0.080
68	La.L./Stiff stem-2	0.371	0.075	0.135	0.085
69	La.L./Stiff stem-3	0.457	0.083	0.120	0.075
70	La.L./Stiff stem-4	0.385	0.109	0.160	0.082
71	La.L./Stiff stem-5	0.292	0.162	0.115	0.060
	Mean	0.420	0.144	0.145	0.109
	S. D.	0.068	0.042	0.027	0.025
	S. E.	0.008	0.005	0.003	0.003
	C. D.	0.016	0.010	0.006	0.006

In flat pod/large leaf-2 (0.572 mg/g) and long pod-3 (0.570 mg/g) highest seed phenol level was obtained while in anthostem-4 (0.255 mg/g) and long pod/large leaf-4 (0.280 mg/g) lowest seed phenol level could be noted as compared with control.

In case of tuber, maximum phenol content was found in mutant lines like long pod/large leaf-2 (0.221 mg/g) and dwarf-2 (0.216 mg/g) while low phenol content was noticeable in flat pod/large leaf-5 (0.075 mg/g) and large leaf/stiff stem-2 (0.075 mg/g).

Polyphenol content (Table 1)
The results pertaining to polyphenol content have shown a good amount of variation in seeds and tubers of different mutant lines than the control.

The seed polyphenol content ranged from 0.100 to 0.210 mg/g while the tuber polyphenol content ranged from 0.045 to 0.160 mg/g.

The highest seed polyphenol content was noted in long pod/ large leaf-5 (0.210mg/g) and long pod/ large leaf-2 (0.194 mg/g) whereas the lowest values regarding that could be recorded in flat pod/long pod-2 (0.100 mg/g) and dark green/ flat pod-3 (0.107 mg/g), when compared with control (0.152 mg/g).

The tuber polyphenol content increased in mutant lines of flat pod/linear leaf-1 (0.160 mg/g) and early maturing –1 (0.157 mg/g) while it decreased in flat pod/long pod-2 (0.045 mg/g) and large leaf/stiff stem-5 (0.060 mg/g) than control (0.115 mg/g).

In nutritional biology tannins and phenolics are considered as antinutritional compounds, hence low tannin and low phenolic lines are considered as nutritionally improved lines. 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.

Though the winged bean is a highly nutritious plant material it is still a neglected crop in many of the countries. It is hoped that the foregoing efforts will definitely help boost the cultivation of winged bean and its systematic consumption as a high protein, nutritionally versatile food legume crop.

REFERENCE

George Manju (2006). Induced mutations in soybean [Glycine max (L) Meer.] parent MACS- 450, screening and biochemical characterization of selected mutant lines affecting lectin levels. Thesis, University of Pune, Pune, (M.S.) India. | Kulthe M. P. (2003). Induced mutational and biochemical studies in winged bean. Ph. D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, India. | NAS (1981). "The winged bean- A High Protein crop of the Tropics", U.S. govt. Printing Off. Washington DC. Edn-II. | Sagade (2008). Genetic improvement of urdbean (Vigna mungo L. Hepper) through mutation breeding. Ph.D. thesis, University of Pune, India. | Tekale N.S. (2004). Human health and nutrition: A biotechnological approach: is something missing? In: Proc. Nati. Conf. on Human health and nutrition: A biotechnological approach. 12-13 Dec. 2004. | Bray H.G. and Thrope W.V. (1954). Estimation of total carbohydrates by phenol – sulphuric acid method. Methods Biochem. Anal.1: 27-52. | de Lumen B.O. and Salamat L.A. (1980). Trypsin inhibitors activity in winged bean (Psophocarpus tetragonolobus) and the possible role of tannin. J. Agric. Food Chem. 28:533-537. | Kantha S.S., Hettiarachchy and Erdman J.W. (1984). Nutrient, antinutrient contents and solubility profiles of nitrogen, phytic acid and selected minerals in winged bean flour. Cereal chemistry 63:9-13. | Klu G.Y.P., Jacobsen E. and Van Harten A.M. (1997). Induced mutations in winged bean (Psophocarpus tetragonolobus) L.DC) with low tannin content. Eyphytica 98:99-107. | Cabrera A. and Martin A. (1986).Variation in tannin content in Vicia faba L. J. Agric. Sc. Cambridge 106:377-382. | Swain T. and Hills W.E. (1959). Phenolic constituent of Purnus domesticaI Quantitative Analysis of phenolic constituents. J. Sci. Food Agric: 10-63. | Tan N.H., Rahim H.T., Khor and Wong K.C. (1983). Winged bean (Psophocarpus tetragonolobus) tannin level, phytate content and hemagglutinating activity. J. Agric. Food Chem. 31:916-917. |