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Genetic Enhancement of Protein and Methionine Content in Bambara Groundnut (Vigna Subterranea(L.)Verdc.)Through Mutation Breeding

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ABSTRACT

Bambara groundnut (*Vigna subterranea*(L.) Verdc.), is a diploid annual legume, seed is regarded as a completely balanced food and contain quantitatively superior protein 18.0-24.0 % and qualitatively superior amino acids like lysine and methionine. So experiment was undertaken to enhance the protein and methionine content in bambara groundnut through mutation breeding.The protein was estimated by Lowary's method and Methionine content through High performance liquid chromatography. Highest protein content of 25.50% for family 17(SB-42 variety) and Highest methionine content of 3.80 mg/100gm (Family -17-SB-42).Increased protein & methionine percentage in M_3 generation was observed compared to control.

INTRODUCTION

Bambara groundnut (*Vigna subterranea*(L.) Verdc.), member of the family Fabaceae is a self-pollinating diploid annual legume crop with a chromosome number of 2n=22. It is an important food grain grown widely in semi-arid Africa and is closely related to cowpea (*Vigna unguiculata*) with which it shares its origin of genetic diversity. In Africa, Bambara groundnut is the third most important legume after groundnut and cowpea (Sellschope, 1962).

Bambara groundnut is a major source of vegetable protein in Sub-Saharan Africa and constitutes an important part of the local diet, culture and economy (Goli, *et al*., 1997). The seed is regarded as a completely balanced food as it is rich in carbohydrate 51-70 %, fat 5.0-7.0 %, quantitatively superior protein 18.0-24.0 % and qualitatively superior to other pulses due to high lysine and methionine,iron 4.9-48 mg/100 g, ash 3.0-5.0 %, , fiber 5.0-12.0 %, potassium 1144-1935 mg/100 g, sodium 2.9-12.0 mg/100 g, calcium 95.8-99 mg/100 g, oil 6-12%, and energy 367-414 kcal/100 mg (Rowland, *et al*.,1993). The gross energy value of Bambara groundnut seed is greater than that of several other pulses (Anchirina, *et al*.,2001).

Bambara groundnut has not received much research attention as compared to other pulses thus carrying name of under exploited crop. Many of the landraces still remain unimproved (Doku, 1997). Among the constraints mitigating against the development of Bambara groundnut include lack of genetic improvement, inadequate knowledge on the taxonomy, reproductive biology and the genetics of agronomic and quality traits, disease and pest infestation (Anchirina, *et al*.,2001, Lacroix *et al*.,2003). Several workers have reported that the improvement of Bambara groundnut through conventional breeding method is difficult and several attempts on hybridization has failed miserably(Goli, *et al*., 1997, Marandu, *et al*.,1995, Ntunda, 1997, Kone, *et al*.,2007). *Vigna subterranea* is an extreme inbreeder; an autogamous crop with flower that are cleistogamous in nature (Uguru and Agwatu 2006) which gives rise to high percentage selfing since the floral structure is perfect resulting in extreme inbreeding. For effective selection in any genetic enhancement programme, genetic variation must exist. Radiation and other chemical mutagens have been used to induce variability in crop plants (Ahloowalia, *et al*.,2004). Generally, EMS causes alkylation of

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guanine bases (G) leading to mispairing or mismatch pairing in the DNA of a treated organism. Under these conditions, an alkylated G pairs with T in place of C, causing a G/C to A/T transition in the backbone of the DNA (Henikoff and Comai 2003). It can cause allelic mutations, small deletions and other chromosomal rearrangements. These mutations can be used to activate morphometric and reproductive changes in plants; further selection of mutant plants through a number of generations, resulting in introduction of new traits into a treated population (Narottam Acharya, *et al*., 2007).To improve upon the productivity of Bambara groundnut, strategies like genetic recombination and selection, induced mutation and appropriate biotechnological approaches are some of the techniques that could be used. Mutation breeding thus plays an important role in genetic improvement of Bambara groundnut. Hence with an objective to enhance the protein and methionine content in Bamabara groundnut experiment was undertaken to assess protein content (Lowary's method) and Methionine content through High performance liquid chromatography.

MATERIAL & METHODS

The experimental material for the present investigation was consisted of two varieties Bambara groundnut having good agronomic base belonging to different accession *viz*., SB-42 (light brown, round shape and white hilum) and S165A(dark brownish, dark spotted surface and oval shape). The seeds were obtained from National Research Centre on Groundnut Junagadh through National Bureau of Plant Genetic Resources. The seeds of two bamabara groundnut varieties were treated with Ethyl methane sulphonate having concentration of (0.1%,0.2%,0.3%,0.4%,0.5%) after treatment M_1 seeds were sown along with the control . M_1 seeds were harvested with individual plants and planted plant to progeny rows in M_2 generation and in M_2 generation high yielding and other desirable characters like resistant were selected and forwarded to M_3 generation having 110 families of SB-42 and 40 families of S-165A, in M_3 generation families performed well for yield and other characters were subjected for protein and methionine content. Protein content of high yielding mutants was determined according to the method of Lowry *et al.,* (1951) using bovine serum albumin (BSA) as standard and Methionine content was determined through HPLC in accordance with the Agilent method (Henderson, *et al.,* 2000).

RESULTS & DISCUSSION

Seed storage proteins of legumes and cereals are the major protein sources for human and domesticated animals. Because of the high level of expression and subsequent accumulation of these proteins, they determine the nutritional value of the seed. Consistent with their role as a reserve of nitrogen, they are rich in nitrogen-containing amino acids and generally deficient in essential amino acids such as lysine and threonine in cereals and sulfur-containing amino acids, methionine and cysteine in legumes. In attempts to improve the nutritional value of crops grains, conventional breeding programs had been largely unsuccessful. Mutation breeding is an alternative approach that allows the direct modification of storage proteins genes to improve their amino acid composition. For that purpose, we initiated a work on mutation breeding for protein and methionine enhancement. Total soluble protein content of selected high yielding mutants (Table: 1) was estimated using Lowry's method. Highest protein content of 25.50% for family 17(SB-42 variety) and minimum protein content of 11.59 % against control of 21.26% was recorded. In the present investigation Methionine content of selected mutants (Table: 1) was estimated using Agilent method (Henderson, et al., 2000). Highest methionine content of 3.80 mg/100gm (Family -17-SB-42) and minimum methionine content of 0.80 mg/100gm was recorded in families of Bambara groundnut. Increased protein & methionine percentage in M3 generation was observed compared to control.

Data indicated that the introduction of a chimeric gene encoding a methionine-rich seed protein into crop plants, particularly legumes whose seeds are deficient in essential sulfur-containing amino acids, represents a feasible method for improving the nutritional quality of the seed proteins. Genetic alteration of specific protein fractions provides a means for increasing protein content, raising limiting essential amino acid concentration by differentially regulating fractions with different amino acid composition.

In the present investigation, M3 generation protein and methionine content was increased at 0.3% of EMS treatment compared to other concentration of EMS. It indicated that protein and methionine content were positive correlation with each other. The increase in values of protein and methionine could due to the occurrence of polygenic mutations with cumulative effects in Vigna radiata (L.).

Induced greater variability in polygenic traits might due to increased mutations and recombination induced by EMS among the concentrations, EMS (0.3%) provided most significant enhancement in of protein and methionine content. So the study, infers that, protein and their fractions are able to increase through mutagenesis. Hence protein & methionine content has been increased through induced mutagenesis. Results of Imran et al, (2011) in Mungbean and Arulbalachandran and Mullainathan (2011) in Black Gram are in confirmation with the present study and protein malnutrition being one of the worst curses to Indian population, requires an immediate substitution of alternate protein rich foods like Bambara groundnut which can play a significant role in alleviating problem associated with malnutrition and on comparison of other pulses like Vigna unguiculata and Vigna radiate the protein content is on par and qualitatively superior to the others due to the presence of higher amount of methionine (Table:2)

Table1. *Estimation of protein and methionine content of high yielding mutants in M_3 generation*

Sl.No	Name	Protein (%)	Methionine (mg Meth/100gm)	Number of pods per plant	Pod yield per plant (g)
Parent	SB-42	21.26	2.40	28.00	22.00
Parent	S-165A	18.63	1.90	30.00	40.00
1	SB-42-F-1(0.3% of EMS)	21.45	2.74	28.80	35.20
2	SB-42-F-2(0.3% of EMS)	20.13	2.47	31.60	32.80
3	SB-42-F-4(0.3% of EMS)	22.59	2.97	40.00	44.24
4	SB-42-F-5(0.3% of EMS)	23.63	3.18	30.00	41.04
5	SB-42-F-8(0.3% of EMS)	23.08	3.07	29.00	33.76
6	SB-42-F-10(0.3% of EMS)	23.08	3.07	33.20	43.60
7	SB-42-F-11(0.3% of EMS)	17.61	1.96	31.20	43.20
8	SB-42-F-12(0.3% of EMS)	20.10	2.46	38.00	52.32
9	SB-42-F-14(0.3% of EMS)	19.53	2.35	37.60	50.96
10	SB-42-F-15(0.3% of EMS)	16.36	1.70	41.60	58.64
11	SB-42-F-17(0.3% of EMS)	25.50	3.80	35.60	44.80
12	SB-42-F-80(0.3% of EMS)	22.96	3.05	47.60	61.12
13	SB-42-F-20(0.3% of EMS)	18.48	2.13	40.00	54.72
14	SB-42-F-21(0.3% of EMS)	21.14	2.67	42.40	44.16
15	SB-42-F-24(0.1% of EMS)	15.50	1.53	37.60	48.88
16	SB-42-F-25(0.1% of EMS)	18.15	2.07	40.40	40.00
17	SB-42-F-26(0.1% of EMS)	18.79	2.20	41.60	63.20
18	SB-42-F-27(0.1% of EMS)	11.59	0.73	44.44	63.20
19	SB-42-F-28(0.1% of EMS)	21.13	2.67	38.40	41.92
20	SB-42-F-30(0.1% of EMS)	11.94	0.80	46.00	67.36
21	SB-42-F-31(0.1% of EMS)	22.69	2.99	48.80	38.32
22	SB-42-F-32(0.1% of EMS)	23.26	3.11	39.20	43.20
23	SB-42-F-34(0.1% of EMS)	22.11	2.87	56.00	62.08
24	SB-42-F-35(0.1% of EMS)	21.55	2.76	36.00	76.72
25	SB-42-F-64(0.1% of EMS)	13.28	1.07	50.80	75.92
26	SB-42-F-62(0.4% of EMS)	20.25	2.49	36.00	83.36
27	SB-42-F-39(0.2% of EMS)	12.13	0.84	42.00	58.56
28	SB-42-F-105(0.5% of EMS)	21.56	2.76	45.60	79.92
29	SB-42-F-41(0.2% of EMS)	23.04	3.06	40.00	60.88
30	SB-42-F-43(0.2% of EMS)	20.53	2.55	36.00	47.12
31	SB-42-F-44(0.2% of EMS)	24.24	3.31	42.40	48.72
32	SB-42-F-46(0.2% of EMS)	21.20	2.69	43.60	48.88
33	SB-42-F-47(0.3% of EMS)	24.29	3.32	44.40	39.20
34	SB-42-F-49(0.3% of EMS)	20.76	2.60	32.80	48.08
35	SB-42-F-50(0.3% of EMS)	20.51	2.55	38.00	69.28
36	SB-42-F-51(0.3% of EMS)	23.55	3.17	57.60	43.60
37	SB-42-F-52(0.3% of EMS)	24.63	3.38	39.60	40.48
38	SB-42-F-53(0.3% of EMS)	15.31	1.49	49.20	56.32
39	SB-42-F-54(0.3% of EMS)	24.31	3.32	50.00	68.24
40	S165A-F-1(0.1% of EMS)	19.75	2.39	56.00	60.00
41	S165A- F-23(0.1% of EMS)	16.96	1.82	40.00	44.00
42	S165A-F-37(0.3% of EMS)	20.76	2.60	38.00	46.00
43	S165A-F-7(0.2% of EMS)	20.10	2.46	104.00	85.00
44	S165A-F-14(0.2% of EMS)	22.60	2.97	80.00	81.00
45	S165A-F-9(0.2% of EMS)	18.53	2.14	74.00	89.00
46	S165A-F-19(0.2% of EMS)	16.90	1.81	70.00	86.00
47	S165A-F-18(0.3% of EMS)	25.50	3.56	48.00	56.00

Table2.*Nutritional composition of Bambara groundnut in comparison to other pulses*

Crop	Protein (%)	Methionine (mg/100 gm)
Bambara groundnut (*Vigna Subterranea* L.)	11.59-25.50	0.73-2.70
SB-42-F-17	25.50	3.80
S165A-F-18	25.50	3.56
Cowpea (*Vigna unguiculata* L.)	12-24.1	0.7-1.84
Green gram (*Vigna radiate* L.)	18-24	0.6-1.80

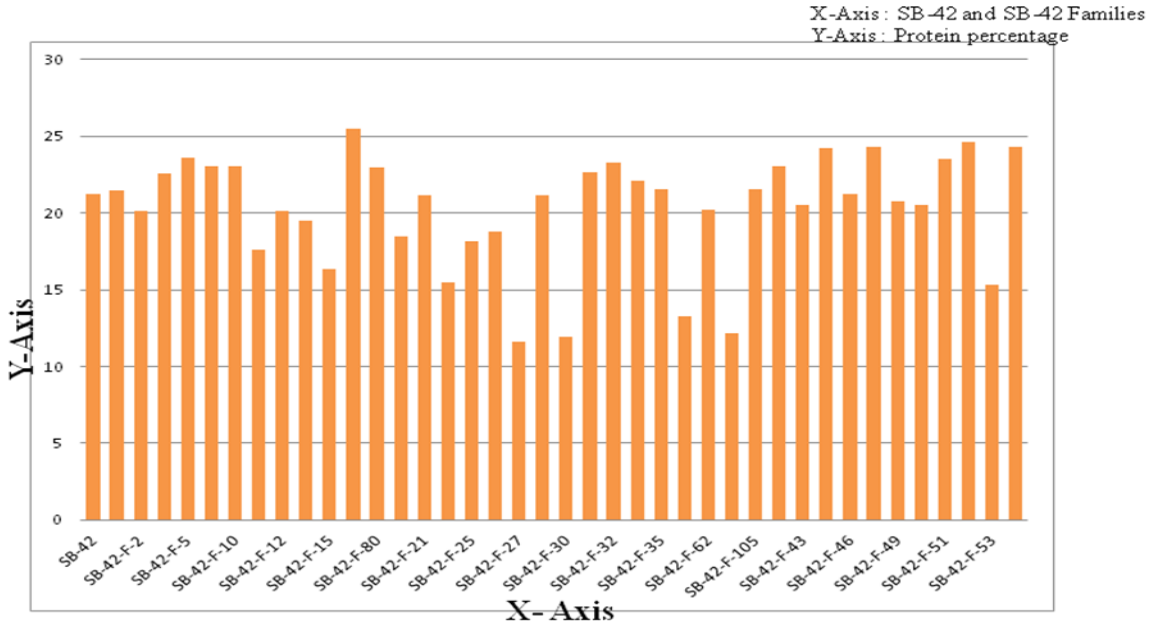

Fig .1: Protein content in selected high yielding varieties of SB-42

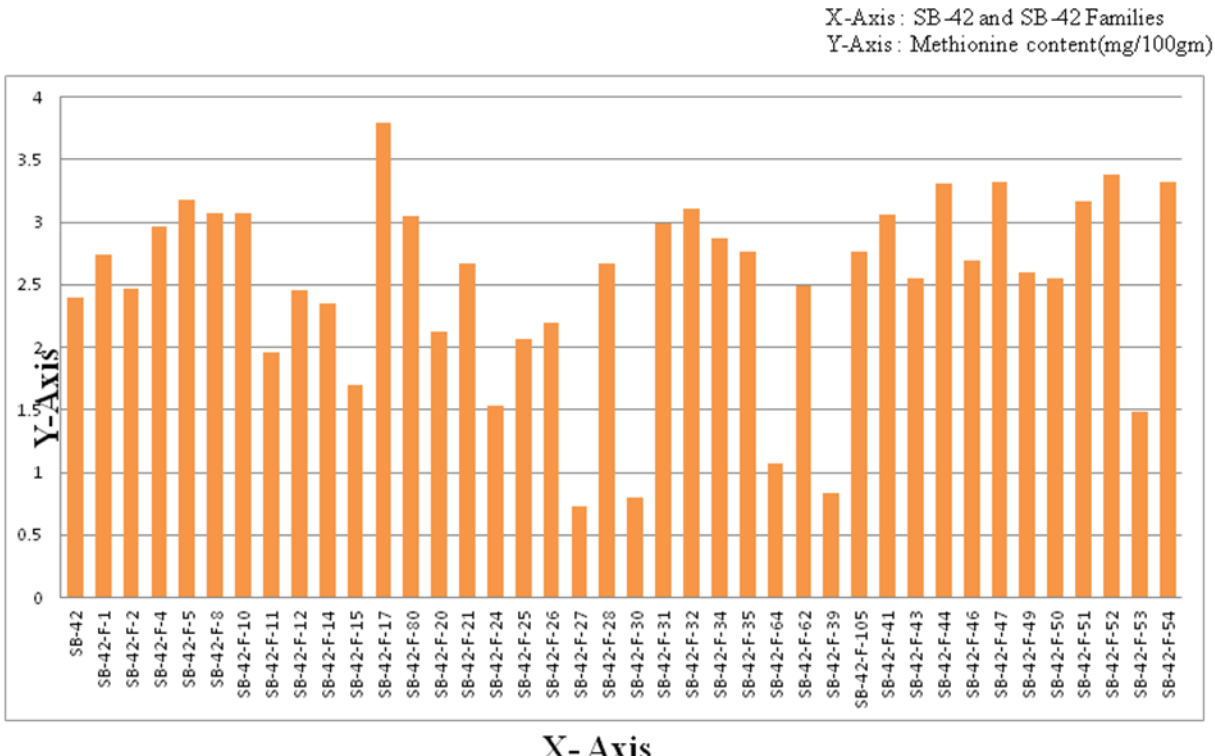

Fig. 2: Methionine content in selected high yielding varieties of SB-42

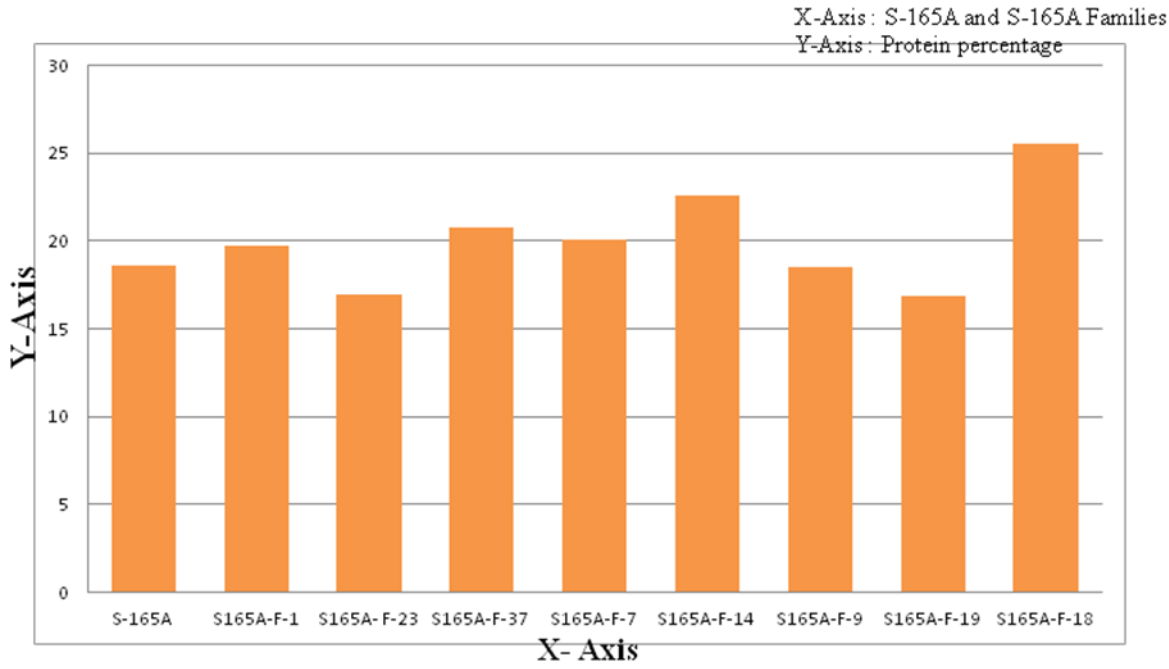

Fig. 3: Protein content in selected high yielding varieties of S-165A

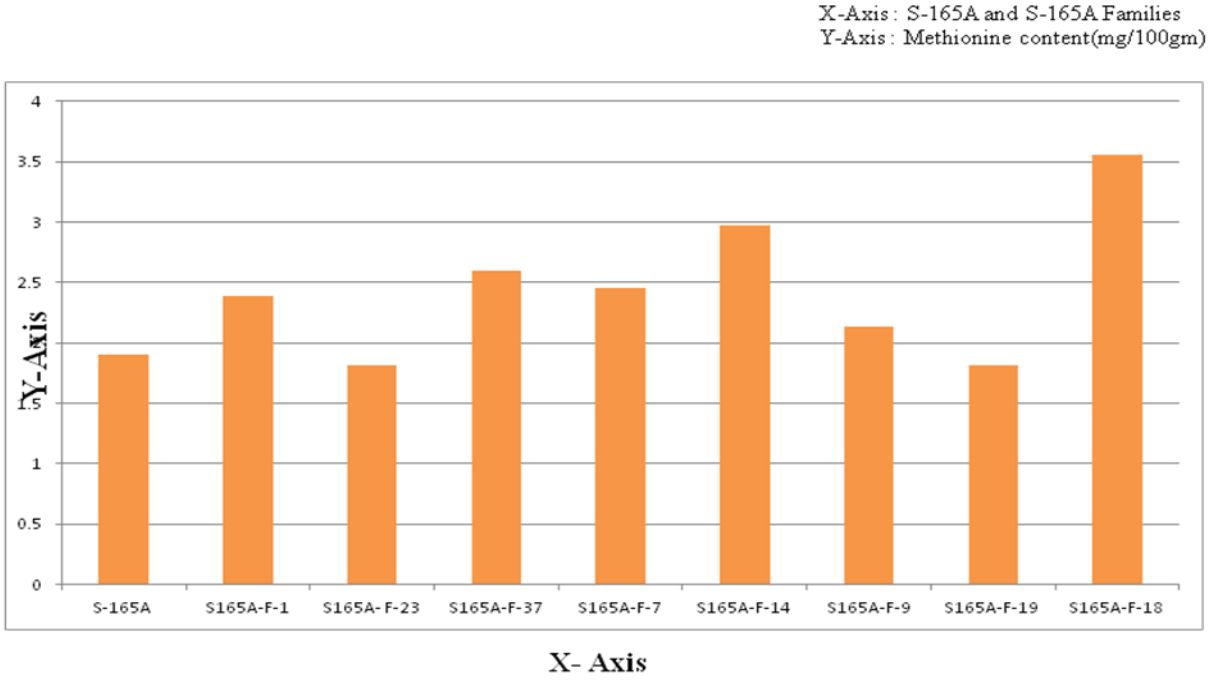

Fig. 4: Methionine content in selected high yielding varieties of S-165A

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