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BIOCHEMICAL ANALYSIS OF SEEDS FROM INDUCED MUTANTS OF *VIGNA MUNGO* L.

GIRISH RAISINGHANI* AND S.K.MAHNA

Cytogenetics Laboratory, Department of Botany, Maharshi Dayanand Saraswati University, Ajmer-305 001, India.

*Department of Botany, Government College, Tonk-304 001, India.

Biochemical estimations of seed storage substances like protein, sugar and phenol were carried out in 9 mutants of *Vigna mungo* L. var T9 induced by DES, DMS and gamma rays. The isolated mutants were tall, dwarf, fasciated, round leaves, excessively branched, high nodulating, low nodulating, large sized nodules and xanthina virescens. Investigations on seed biochemistry revealed that amount of seed storage substances altered due to the influence of mutated gene otherwise controlling the root/shoot characters. Negligible to significant variations in estimated metabolites as compared to the mother variety were recorded in the induced mutants.

Keywords: DES; DMS; Gamma rays; Mutants;Protein.

Introduction

Induced mutagenesis is an integrating tool in genetics and plant breeding to generate desired variablility in plants. *Vigna mungo* L. (black gram) an important pulse crop, occupies unique position in Indian agriculture. Although many reports are available regarding mutations affecting protein content of seeds in different legumes[1-4] but a very few reports are available on mutated gene influencing carbohydrates and phenols.[5-7]. Therefore, the present study was designed to estimate protein, sugar and phenol from seeds of induced mutants of *V.mungo* L. after mutagenic treatments with diethyl sulphate (DES) and dimethyl sulphate (DMS), the two well known alkylating agents and gamma rays.

Materials and Methods

Presoaked seeds of *V.mungo* L.were treated with freshly prepared solutions of DES (0.05%, 0.07%, 0.09% and 0.10%) and DMS (0.02%, 0.04%.0.06% and 0.08%). For gamma rays treatments, dry seeds were exposed to cobalt 60 source at the doses of 10kR, 20kR, 40kR and 60kR. Chemically treated seeds after thorough washing under running tap water and gamma irradiated seeds as such were immediately sown in experimental fields to obtain M1 generation. The seeds obtained from M1 generation were used to raise M2 population. Mutants were isolated from M2 segregating families and were later confirmed in M3 generation. The seeds obtained from the induced mutants in both the generations were biochemically analysed for protein, sugar and phenol using standard techniques.

Results and Discussion

Nine mutants namely tall, dwarf, fasciated, round leaves, excessively branched, high nodulating, low nodulating, large sized nodules and xanthina virescens were recovered in *V.mungo* after mutagenic treatments. The salient features of these induced mutants have been presented in Table1. The data was recorded on biochemical estimations of protein, sugar and phenol from seeds of these induced mutants in M2 and M3 generations. The observations from both the generations did not show much variation (Table2). Total protein stored in the seeds of control plants was 26.0%. Enhanced values in the protein percentage was recorded in majority of the mutants except for low nodulating and xanthina virescens which showed lower percentage. Noticible vairations were observed in estimated total soluble sugar percentage.Maximum percentage of sugar (0.08%) was recorded from tall and round leaves mutants when compared to control (0.05%). The amount of total phenol recorded from seeds of normal plants was 2.2%. Variations in amount of phenol in the seeds of mutants were quite insignificant. All the mutants investigated except for dwarf showed low phenol percentage in the seeds.

As per protein and sugar percentage (the two important seed metabolites), the enhanced values were observed in mutants like tall, excessively branched and high nodulating. Thus these mutants could be grouped as beneficial mutants.

An interesting correlation between mutant phenotype and amount of seed storage substances was observed. The mutants with inferior phenotypes showed lower values of protein and sugar while mutants with improved phenotypes had high percentage of two metabolites.With regard to variations recorded in phenolic content,mutants showing improved vegetative characters possessed low amount of phenol in general, while dwarf mutant with inferior vegetative characters synthesised greater amount of phenol. Similar findings were reported by Shaikh[2], Singh and Yadav[8], Ignacimuthu and Babu[9], Mahna and Gupta[10] and Garg[11] and have correlated the inferior/superior vegetative growth due to high/low amount of seed storage substances.

Table 1. The salient features of the isloated induced mutants of *Vigna mungo* L.

Mutant types	Salient Features
Tall	Increased plant height (110.0% over control)
Dwarf	Reduced plant height (45.0% decrease over control)
Fasciated	Flattened and bended stem and with changed orientation of branches.
Excessively branched	50-70% increase in the number of axillary branches than control.
Round leaves	Leaflets round in shape instead of ovate shape in control.
High nodulating	Increased nodulation frequency (90% increase over control)
Low nodulating	Low nodulation frequency (70% reduction over mother variety)
Large sized nodules	The size of the nodules was doubled compared to control.
Xanthina virescens*	Initially leaves were complete yellow but gradually turned to normal green.

* The terminology used to describe chlorophyll deficient mutant is as per Muszynski[13] scheme.

Table 2. Biochemical analysis of seeds from induced mutants of *Vigna mungo* L. in M3 generation (Average of 10 plants in 2 replicates).

Values in paranthesis indicate M2 data

Mutant types	Protein (%)	Sugar (%)	Phenol (%)
Control	26.0±0.18	0.05±0.001	2.2±0.02
	(26.0±0.22)	(0.05±0.002)	(2.2±0.04)
Tall	28.5±0.22	0.08±0.004	2.0±0.01
	(27.8±0.34)	(0.08±0.006)	(2.1±0.03)
Dwarf	26.0±0.30	0.05±0.002	2.3±0.04
	(26.6±0.88)	(0.04±0.001)	(2.0±0.08)
Fasciated	26.8±0.62	0.04±0.003	2.0±0.04
	(25.2±0.58)	(0.03±0.004)	(1.6±0.08)
Round leaves	32.0±0.41	0.08±0.006	2.1±0.04
	(31.7±0.38)	(0.07±0.004)	(2.0±0.02)
Excessively branched	28.0±0.26	0.06±0.002	1.8±0.02
	(27.5±0.33)	(0.06±0.001)	(1.4±0.04)
High nodulating	34.5±0.54	0.07±0.003	2.1±0.02
	(33.8±0.94)	(0.07±0.006)	(2.0±0.02)
Low nodulating	24.0±0.18	0.03±0.002	1.8±0.01
	(25.0±0.15)	(0.03±0.001)	(1.8±0.06)
Large sized nodules	26.2±0.22	0.06±0.003	2.0±0.02
	(25.6±0.63)	(0.05±0.004)	(1.9±0.03)
Xanthina virescens	25.8±0.52	0.04±0.001	1.6±0.01
	(24.2±0.38)	(0.05±0.008)	(1.6±0.04)

The altered values of seed storage substances of the mutants observed in the present work could be a part of pleiotropic pattern of mutant gene otherwise controlling either root or shoot trait. Similarly Gottschalk and Wolff[12] have also demonstrated that the amount of nutritionally valuable seed substances in the mutants of legumes can be positively altered under the influence of mutated gene.

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