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Research Article

EFFECT OF CHEMICAL MUTAGENS ON LEAF AND SEED PROTEIN CONTENTS IN M_3 MUTANTS OF WINGED BEAN

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ABSTRACT

In the present investigation, efforts have been made for evaluation of level of proteins in leaf and seed of M_3 mutants of winged bean.For the present research work, the seeds of winged bean (*Psophocarpus tetragonolobus* (L.) DC.) of variety (cultivar) namely II-EC-178313 and 2I-EC-38825 were treated with the chemical mutagens such as Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA) and successively different nine type of mutants were obtained in M_3 generation of both the varieties (cultivars). Further these nine mutants were selected to find out the content of protein in leaf and seed. The mutants like early flowering and long pod displayed an increase in the leaf protein percentage as compared with control (Variety II-EC-178313). While in case of variety 2I-EC-38825, only long pod mutant shows increased level of protein content in leaf. Regarding seed protein (crude) content, in variety II-EC-178313 out of nine mutants, only three mutants demonstrated the enhancement in crude protein content as compared with control. While in case of variety 2I-EC-38825, the anthostem mutant showed increased protein content than the control. The present investigation therefore has provided significant insights for further improvement of winged bean germplasm for its level of protein content in leaves and seeds.

INTRODUCTION

Legumes have always enjoyed a special place in the human diet because of their high protein content as compared with cereals. They are the principal source of dietary proteins for vegetarians. According to (Siag *et al*., 2005) pulses being rich in quality protein, minerals and vitamins are inseparable ingredient of the diet of majority of Indian population. Pulses also occupy an important position in world agriculture because of their high protein content, several essential amino acids and their capacity for fixing atmospheric nitrogen.

Of the legumes, the winged bean (*Psophocarpus tetragonolobus* (L.)DC.) is considered to be a potentially important crop because of its high protein and oil contents. It is popularly known as winged bean, Goa bean, Asparagus bean and Manila bean is a tropical legume found growing abundantly in hot humid equatorial countries like Sri Lanka, Bangladesh, India, Thailand, Philippines, Burma, Indonesia, and Papua New Guinea. It is also called a wonder legume as it has the high amount of protein content in the seeds and therefore considered as a versatile legume, (Anon, 1981). It has demonstrated potentialities of a new food crop in different regions of the world. Every part of the winged bean plant is quite edible and immensely nutritious. The nutritional value of the winged bean is mainly due to the various positive attributes carried by its mature seeds. The seeds contain high amount of proteins (20%- 42%) and edible oil (15%- 20%),(NAS, 1981).

Though it possesses several positive attributes as mentioned above, but it is neglected throughout the world due to few shortcomings. The shortcomings such as labour intensive nature of crop, the relatively long duration of its life cycle and the presence of some pharmacologically active –anti-nutrients such as trypsin, chymotrypsin and amylase inhibitors along with tannin in its seeds and tubers. Therefore the objective of present study is to develop improved genotype with the highest nutritional content such as grain yield, protein, oil and lowest antinutritional factors.

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MATERIALS AND METHODS

The authentic seeds of winged bean (*Psophocarpus tetragonolobus* (L.)DC.) cultivars namely II –EC- 178313 and 2I-EC-38825 were procured from National Bureau of Plant Genetic Resources, Regional Station PKV, Akola (M.S.).

Mutagens Used

The chemical mutagen such as Ethyl Methane Sulfonate (EMS) and Sodium Azide (SA) was used in the present study. Details of Mutagenic Treatments

The pilot experiments were conducted for determining the suitable concentrations for further studies. Prior to mutagenic treatments seeds were immersed in distilled water for 6 hours. The presoaking enhances the rate of uptake of the mutagen through an increase in cell permeability and also initiates metabolism in the seeds for treatment. Such presoaked seeds were later on immersed in the mutagenic solutions for 6 hours with an intermittent shaking. Seeds soaked in distilled water for 12 hours served as control. All the chemical mutagenic treatments were given at room temperature of 25 ± 2^{o} C.

The different concentrations used for the chemical treatments were 0.05%, 0.10% and 0.15% for EMS and 0.01%, 0.02% and 0.03% for SA respectively. Immediately after the completion of treatment the seeds were washed thoroughly under tap water. Later on they were kept for post soaking in distilled water for 2 hours. After the completion of treatment process the seeds were sown in field following randomized block design (RBD) with three replications along with control as the M_1 generation. Seeds were harvested separately from each plant of M_1 progenies, stored in polythene bags and used for M_2 generation. Collected seeds were sown to raise M_2 generation. Later on M_2 population was screened carefully for selection of different types of viable mutants. Further the seeds of viable mutants from M_2 population were harvested from each mutant to raise M_3 generation to obtain promising nine viable macromutants. Thus these nine mutants such as early flowering, late flowering, early maturing, *chlorina*, linear leaflet, flat pod, long pod, anthostem and high yielding were selected for estimation of crude leaf/seed proteins.

Estimation of Crude Leaf/Seed Proteins

The crude protein content of leaves/seeds of M_3 mutants of winged bean was estimated by Microkjeldahl method. The dry defatted leaf/seed powder weighing 300 mg , was digested with 7.5 ml of concentrated sulphuric acid (H_2SO_4) in presence of a catalyst. The sample was heated for about 8 hours until the mixture became clear. The digested material was diluted up to 50 ml in volumetric flask with distilled water.

The distillation was carried out by using the Markhams steam distillation apparatus. The amount of nitrogen content was calculated. The crude protein content was worked out by multiplying the value of nitrogen content by 6.25.

RESULTS AND DISCUSSION

In variety II-EC-178313 the value for leaf protein in control was 26.87%, out of nine mutants only two mutants displayed an increase in the leaf protein percentage. These mutants were early flowering and long pod. The remaining seven mutants like late flowering, early maturing, *chlorina*, linear leaflet, flat pod, anthostem and high yielding revealed reduced level of leaf protein content. The highest leaf protein percentage (27.62%) was found in early flowering mutant, while the lowest value (21.54%) was noticeable in linear leaflet mutant.

In case of variety 2I-EC-38825, a considerable amount of variation could be detected in leaf protein values of mutants. The control showed 27.39% leaf protein. The maximum leaf protein percentage (28.24%) could be noticed in the long pod mutant while the lowest value (21.57%) could be observed in the late flowering mutant. The remaining mutants like early flowering, early maturing, *chlorina*, linear leaflet, flat pod, anthostem and high yielding revealed the decreased values of leaf protein content as compared with control. (Table -1).

Table 1 Leaf protein percent in M_3 mutants of variety II-EC-178313 and variety 2I-EC-38825 of *Psophocarpus tetragonolobus* (L.) DC

Sr. No.	Name of mutant	Leaf protein % *	
		II-EC-178313	2I-EC-38825
1	Control	26.87	27.39
2	Early flowering	27.62	26.81
3	Late flowering	22.86	21.57
4	Early maturing	23.72	26.16
5	*Chlorina*	23.97	22.67
6	Linear leaflet	21.54	23.46
7	Long pod	26.91	28.24
8	Flat pod	24.76	22.69
9	Anthostem	25.16	24.52
10	High yielding	22.62	23.19

*Mean of three replication values.

The mutants of variety II-EC-178313 of winged bean, exhibited variability for seed crude protein content values. The value for the crude protein content in control was 29.89%, majority of the mutants showed reduction in crude protein content over the control. Out of nine mutants, only three mutants such as early maturing, flat pod and anthostem demonstrated the enhancement in crude protein content as compared with control. The highest crude protein content could be noticed in the anthostem mutant (31.87%). The lowest value for crude protein (24.23%) could be observed in the linear leaflet mutant.

In variety 2I-EC-38825 the seed crude protein content was 29.12% in its control. The anthostem mutant showed increased seed crude proteins (32.59%) than the control. The values for the seed crude protein content in other mutants ranged from 24.46% to 31.81%. The maximally reduced seed crude protein content (24.46%) could be seen in the late flowering mutant. (Table -2).

Table- 2 Seed crude protein content in M_3 mutants of variety II-EC-178313 and variety 2I-EC-38825 of *Psophocarpus tetragonolobus* (L.) DC

Sr. No.	Name of mutant	Seed protein % *	
		II-EC-178313	2I-EC-38825
1	Control	29.89	29.12
2	Early flowering	26.34	28.16
3	Late flowering	29.32	24.46
4	Early maturing	30.19	31.72
5	*Chlorina*	27.64	26.42
6	Linear leaflet	24.23	27.56
7	Long pod	28.37	29.74
8	Flat pod	30.89	27.29
9	Anthostem	31.87	32.59
10	High yielding	28.09	31.81

*Mean of three replication values.

The leaf/seed protein content in the present investigation revealed an enhancement in few mutants of both II-EC-178313 and 2I-EC-38825 varieties (cultivars) of winged bean As against this, some of the mutants of both the varieties demonstrated a declining nature as well pertaining to the same parameter.

The data regarding the effect of physical and chemical mutagens on the protein content have been recorded by (Swaminathan *et al*., 1968), (Tanaka, 1969), (Rabson and Bhatia, 1975), (Denic *et al*., 1976) and (Reddy, 1977).

Induced mutants with altered protein content have been reported earlier by (Haq *et al*., 1970), (Gaul *et al*., 1973), (Narhari *et al*., 1976), (More, 1992), (Satpute, 1994) and (Harsulkar, 1994) in different crop plants.

(Swaminathan, 1983) stated that the quality of storage proteins can be changed by site directed mutagenesis such that all the essential nutritional requirements of human can be met by the grain protein only. This has become important as an appropriate scientific integration of conventional and emerging techniques of plant breeding can help in successfully facing the challenge of malnutrition problems.

According to (Gottschalk and Muller, 1970) an improvement in protein content and composition through genetic manipulation is a permanent improvement.

(Bliss, 1973) reported a negative correlation between yield and percent protein of seed instead of increase in protein content. (Bhagwat *et al*., 1979) have recorded existence of negative correlation on protein content and some other traits like days to maturity, number of grains and size and the grain yield. (Palamarcuk, 1961) has recorded an increase in protein content after gamma ray treatments in red clover, similar findings have been noted by (Sirsov and Sain, 1973). The pertinent results have conclusively indicated that the genetic improvement of protein quality and quantity is indeed possible through mutation breeding.

CONCLUSION

The biochemical analysis of different promising M_3 mutants in regard to leaf and seed protein contents have demonstrated differences in their levels as compared to control.

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