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Estimation of Soluble Protein by Bradfords Method in Gamma Radiated (wild) Pea

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Abstract: ***In recent year grain legume play important role and primary role in the search of protein owing to the high protein content of seed ranging from 20% to 40% in pea. They can therefore be considered good substitution to animal protein in human diet, especially in the third world. Howe ever the seed storage protein of these legume containing amino acid and plant breeding have to consider this problem in any improvement programs. Mutagenesis started utilized experiment mutagens in altering seed protein in many cereals both quantitatively and qualitatively with view to bridge protein gap cause of malnutrition.***

Keywords: Mutagen, gamma radiation, seed protein

1. Introduction

Field pea is significant pulse crop in both India and Australia. There are a number of similarities that include nationally co-ordinate breeding programs, moisture stress in rained crops, low yield significant of powdery mildew and an interest in developing lodging resistant varieties. Peas are of great nutritional importance due to their high content of protein complex carbohydrates, dietary fibre mineral, vitamins and antioxidant compounds.

The principal of applied mutagenesis through initiated nearly 16 year ago in legume has contributed insignificantly in induced high protein mutant beans among legume shows considerable range of variability (Amrishi and Tavakolib 1970, Rutgure, 1971; Woolfe and Hamblin, 1983) their adapatedable nature , mutation breeding programme for higher protein content and quality under the assistance from university grant commission (Grant No F-23-118179 (sr II) was intimated in 1979 in *Phaseolouse vulgaries*.

Increased interests in plant proteins for feed and food led to the evolution of field pea as high protein crop such as soybean are processed into flour, protein concentrated and protein isolated suitable for various foods brewages sales of soya protein isolated containing 90% protein are expanding because of its higher protein content , functionally , nutritional properties and improved flavour.

Germplasm is vital source in generating new plant types having desirable traits that help in increasing crop quality and production as well thus improved level of human nutrition.

The use of mutagenic agent to induced variability has been a practical tool especially where natural variability is not available. Many investigators used make used of gamma radiation used to induce mutation to generate variability in morphological traits and electrophortic profile of seed storage protein.

2. Material & Methods

Coommassive brilliant blue G-250 is one of the numbers of dyes that combine with protein to give an absorption maximum in the region of 595 nm wave length. The practical advantages are that the reagent is simple to prepare that colour develop rapidly and in stable. Although it is sensitivity down to 20ug protein per cm it is only relative method as the amount of dyes binding appear to vary with the content of basic amino acids residues in the proteins .This makes the choice of standard difficult (Bradford, 1976).

3. Reagents

a) Standard protein solution
b) Protein reagents (0.01%)

a) Standard protein solution:
Dissolve 25mg of bovin serum albumin in 0.15M Nacl and make up the volume to 25ml (1mg/ml).

b) Protein reagents (0.01%):
Dissolve 100mg of comassive brilliant blue G-250 in 50ml of 95% alcohol and add 100ml of 85% (wt/vol) phosphoric acid and dilute to 1 liter in water.

4. Method

1) Pipette out 0.01, 0.02, 0.04---------- 0.1ml of standard protein solution . Make up volume in each tube to 0.1 ml with and phosphate buffer 0.1 ml of buffer alone serves as the blank.
2) Add 5ml of protein reagent and mix thoroughly by inversion or vortexing.
3) Measure the absorbance at 595 nm after 2min and before 1 hour against the reagent blank.
4) Plot a standard graph and calculate the amount of protein in unknown, sample treated in same manner.

Preparation of seed sample
For extraction of protein individual seeds irradiated with particular gamma doses were ground to fine powder with mortal and pestle. To extract protein in 0.01 gm of seed flour, 400ml of the protein extraction buffer (0.05 M tris-HCl, 0.2%, SDS, 5Murea and 1%b- Mercaptoethanol) was added to tube and mixed well by vortex . Then centrifuged at 15,000 rpm for 5 min at room temperature. The extracted

crude protein was recorded as clear supernatant and was stored at 20C for further use.

For complete analysis the explants were used. The extract protein in 30 mg of seed flour 1000 u of 0.1 M NaOH was added to tubes and mix by vortex. Then centrifuge at 10, 000 rpm for 20 minutes at room temperature. The extracted crude proteins are recovered as a clear supernatant. The extracted protein sample was estimated for seed protein content using BSA (0.25 mg/ml) as a standard. To estimation total seed soluble protein in all three wild verities of *Pisum*, dye-binding method (Bradford's method) is used. The result was presented in **table**.

Gamma doses	**Wild *Pisum* (protein nc.ug/ml)**		
	Seed	Shoot	Root
Control	310	260	120
5Kr	400	520	160
10Kr	470	670	240
15kr	420	420	250
20Kr	420	420	220
25Kr	300	530	200
30Kr	250	300	220

5. Result and Conclusion

The phylogenetic differentiation and geographical distribution of genetic variation in term of total seed protein in seed treated with different doses of gamma as compare to control the lower gamma doses were found to increase the considerable protein concentration in 5Kr, 10 Kr and 15Kr doses in *Pisum* verities as compare to other doses were increased in protein concentration.

Although the higher doses shows higher concentration but are associated with decreased or fatal morphological characters.

6. Discussion

The plenty of information on genetic fortification through applied mutagenesis of future variation for higher protein content and quality has been publish. Variation in range of total protein, protein sub-fraction and band pattern in the parental line of wild pea mutant verities indicate that the improvement in production with pea is possible and this is supported by our finding in the mutant deriving from mutationally treated by gamma irradiation, where appreciable increased in their total seed protein has been demonstrated. Nevertheless protein mutant in exhibit negative correlation between total protein and yield has been reported in cereals as well as in legumes (Johnson et.al, 1970, Kaul,1980) as well as (wood et.al, 1979, sjodin et.al 1981) such observation reflect difficultly in selecting high protein mutant associated with no change in yield (Gridley and Evans, 1971). The induction of high protein mutant may be attributed to micro mutation with positive effect and low seed yield to micro mutation with negative effect.

7. Conclusion

The observation of no correlation in pea (Gottschalk and wolff, 1983) and our finding of non significant correlation in lower doses irradiated pea mutant indicate that such correlation is not universal and is dependable on genetic architecture of the experiment on material. Existence of non significant negative correlation suggested that breeding for improvement protein may not have much negative influence on the yielding ability of genotype.

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