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Gamma radiation effect on protein content of alfalfa (*Medicago sativa* L.) in M_1, M_2 and M_3 generation

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Abstract
Alfalfa is the world's oldest and most important livestock feeding crop belongs to the family fabaceae and botanically known as the *Medicago sativa* L. Alfalfa leaf contains essential vitamins, carbohydrates and also source of proteins. Gamma radiation is one of the physical mutagen that widely used for mutation breeding. In present investigation the seeds of Alfalfa were irradiated with different doses of gamma radiation (5 KR, 10 KR, 15 KR, 20 KR, 25 KR, 30 KR, 35 KR, 40 KR, 45 KR, and 50 KR) and untreated seeds with gamma radiation were used as control. Treatment combinations were arranged in a randomized block design with eleven replication. Leaf protein estimation was done in M_1, M_2 and M_3 generation. Result revealed that in M_1, M_2 and M_3 generation lower doses of gamma radiation had a stimulating effect on enhancing protein content of Alfalfa.

Keywords: Alfalfa, protein, gamma radiation

Introduction
Proteins are biological macromolecules that perform the majority of biological functions in the cell or sometimes in conjunction with other biomolecules. Proteins can thus be thought of as biomolecular devices with natural structural and functional features that are difficult to duplicate in the lab. Spider silk proteins which combine high strength with good elasticity (Altman *et al*, 2003) [1] and bacterial flagella which are minuscule propellers are two classic examples of proteins with unique functionality (Meister *et al*, 1987) [8]. Enzymes are proteins that catalyze processes at a high rate and with a high degree of selectivity. Plant proteins have a wide range of enzymatic, structural and functional functions as a photosynthesis, biosynthesis, transport, immunity, etc. They also serve as storage mediums for seedlings, allowing them to meet their growing and nutritional needs. Proteins carry out various functions in different ways depending on their composition and structural forms such as folding, which can range from compact and well-ordered to unfolded and intrinsically disordered. Despite the fact that some opponents contend that there is no global protein crisis, there are compelling reasons to try to improve the quality and amount of protein available in crop plants through plant breeding (Sarkar *et al*., 2024) [11]. Various physical mutagens like gamma radiations and chemical mutagens like EMS, MMS, NMU, SA, Colchicines could cause genetic changes in the plants through which promising progenies can be obtained which are useful in the improvement of crop plants. Gamma rays cause severe reshuffling of genetic material and induce different types of variations in the crops than any other radiation. (Patil B. M. 2015) [10]. Alfalfa is one of the oldest cultivated fodder crops in the world. It is often grown in fields by farmers for pasturage and forage. Alfalfa is an herbaceous perennial crop; its botanical name is *Medicago sativa* Linn. and it belongs to the family of Leguminosae. Alfalfa is a good source of protein and is rich in vitamins and minerals (Edward *et al*, 2013) [4]. It contains chlorophyll, organic acids, saponins, isoflavins, sterols, coumarins, alkaloid and minerals like Calcium, potassium, phosphorus, Magnesium and zinc (Olimpia *et al*, 2015) [9]. The objective of present study is to study the effect of gamma radiation on protein content of Alfalfa.

Materials and Methods

Plant materials

Experimental plant material selected for the present investigation was Lucerne commonly known as Alfalfa [*Medicago sativa* (L.), Var.: RL-88]. Germplasm (seeds) of this variety was procured from Fodder improvement Division of Mahatma Phule Agricultural University, Rahuri (Ahmednagar district, Maharashtra state, India). The cultivar is desi type, commercially and widely cultivated in the area of Ahmednagar district of Maharashtra.

Gamma radiation

The experiment employed cobalt 60 as a source of gamma radiation (^{60}CO). The facility available at the BHABA Atomic research center, Trombay, Mumbai. (M.S. India) was availed. 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR doses were used for each treatment. Dry uniform 91 gm seeds were irradiated with above mentioned doses of gamma radiation. Untreated seeds with gamma radiation were used as control.

Field layout and seed sowing

Irradiated and control seeds were sown in the experimental fields by using the Randomized Block Design (RBD Method) with eleven replications (Giri *et al*, 2010) [6]. The seeds treated with gamma radiation of selected doses and control were symbolized as T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, T_9, T_{10}, T_{11}.

Determination of Protein content

Freshly collected green leaves were used to extract leaf protein. The sample cleaned with tap water to remove dust or dirt particles (Lowery *et al*, 1951) [7] Leaf protein estimation were done in M_1, M_2 and M_3 generation.

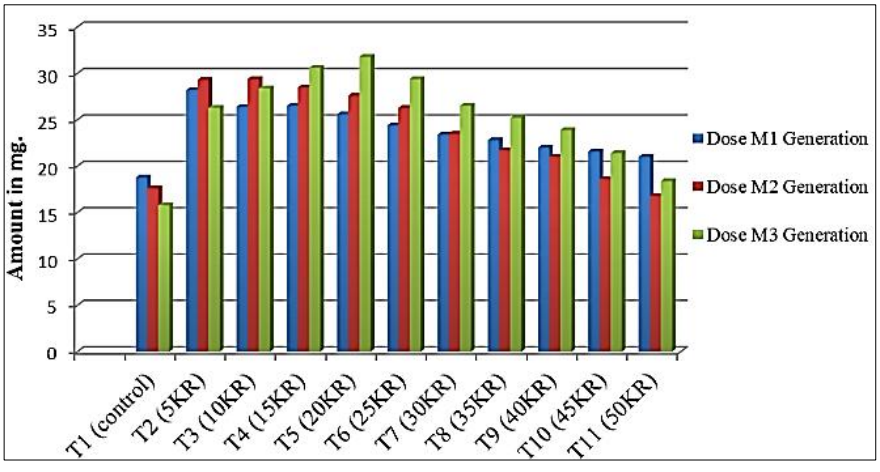
BSA solution of 0.2 to 1.0 ml was pipetted into 1 to 8 test tubes to make a series of standard protein. By adding a suitable volume of distilled water to each tube, the volume in each tube was changed. In test tubes 7 and 8, 0.1 ml and 0.2 ml of protein extract was pipette out. Each tube with 5 ml of freshly prepared regent C was filled. The contents were thoroughly mixed and incubated the tubes at room temperature for 10 minutes. Further 0.5 ml of reagent D to each tube was added and incubated for another 30 minutes at room temperature. A spectrophotometer was used to measure the absorbance of the samples at 660 nm. Test tube No.1 was used as a blank to standardize the instrument. A graph was plotted for the number of proteins versus absorbance at 660 nm to compute the amount of proteins present in the sample (Sadasivam and Manikam, 2008) [13].

Results and Discussion

Plant protein contents of Alfalfa were quantitatively estimated in M_1 generation. Data presented in table showed a maximum increase in plant protein content is also observed in T_2 which are 28.2 mg/100ml with SD 0.73 as compared to T_1 showed 18.8 mg/100ml and T_{11} reported 21.0 mg/100ml protein. In M_2 generation the average protein content was ranging from 16.8 mg/100ml to 29.4 mg/. The average maximum protein content was 29.4 mg/100ml with SD 1.10 reported in T_3 treatment followed by T_2 treatment (29.3 mg/100ml with SD 0.87) as compared to T_1 (Control) 17.6 mg/100ml with SD 1.04 while the average minimum content of gamma radiation observed in higher dose of gamma radiation T_{11} was 16.8 mg/100ml with SD 1.05 in M_2 generation. Treatment T_2 and T_3 showed slightly variation in protein content of Alfalfa. The range of protein content in M_3 generation was 15.8 to 31.8 mg/100ml. The average maximum protein content for all the treatments in this experiment was 31.8 mg/100ml with SD 1.16 at T_5 treatment of gamma radiation and average minimum protein content was 15.8 mg/100ml with SD 0.71 at T_1 (Control) and higher dose of gamma radiation T_{11} was 18.4 mg/100ml with SD 0.99 respectively.

Table 1: Effect of gamma radiation on protein content of Alfalfa plant protein in M_1, M_2 and M_3 generation.

Dose (KR)	Plant Protein (mg/100ml)					
	M1 Generation		M2 Generation		M3 Generation	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
T_1 (control)	18.8	1.11	17.6	1.04	15.8	0.71
T_2 (5KR)	28.2	0.73	29.3	0.87	26.3	0.88
T_3 (10KR)	26.4	0.92	29.4	1.10	28.4	0.77
T_4 (15KR)	26.5	1.48	28.5	1.02	30.6	1.10
T_5 (20KR)	25.6	1.21	27.6	1.00	31.8	1.16
T_6 (25KR)	24.4	0.97	26.3	0.96	29.4	0.92
T_7 (30KR)	23.4	0.96	23.5	0.81	26.5	1.02
T_8 (35KR)	22.8	0.56	21.7	0.92	25.2	0.77
T_9 (40KR)	22.0	0.55	21.0	0.92	23.9	0.96
T_{10} (45KR)	21.6	0.81	18.6	1.05	21.4	0.94
T_{11} (50KR)	21.0	0.86	16.8	1.05	18.4	0.99



Graph: Effect of gamma radiation on protein content of Alfalfa in M1, M2 and M3 generation

It is concluded that the protein content of Alfalfa in various doses of gamma radiation was increased from the treatment T_2 to T_5 while it was decreased when increase the doses of radiation. It means that lower doses showed the stimulatory effect and higher doses showed the inhibitory effect on protein content of Alfalfa. The similar result was obtained by researchers like Desai and Rao, 2014 [3]. Pavadai *et al*, 2010 [12] reported that increased seed protein content in Soybean. Auti and Apparao, (2008) [2] reported that seed protein content increased in viable mutants after mutagenic treatments in Mungbean. Sri Devi and Mullainathan, (2012) [14] recorded that increased seed protein content of mutant in Black gram. Similar result was reported by many researchers in viable mutants of different crop plants like in Pea (Satpute and Dhulgande, 2010) [14] and in Cowpea (Gaikwad, 2013) [5]. These proteins play an important role in anti-pathogenesis and signal transduction, which were necessary to plants growth and function (Zolla, *et al*, 2003) [16].

Conclusion

In M_1, M_2 and M_3 generation lower doses of gamma radiation had a stimulating effect on enhancing protein content of Alfalfa. The acquired results indisputably validate the use and efficacy of the induced mutational strategy in biochemical improvement of Alfalfa (*Medicago sativa* L.) for obtaining superior plant types with increased protein content. Alfalfa a type of green fodder is the principal diet for dairy cows. Alfalfa has high calcium, protein content and other nutrients. It not only increases fat value but it also enhances the milk nutrition. As a result, high yielding forage crop types and imaginative forage production abilities are needed to increase fodder crop productivity and nutritional value.

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