Gamma Radiation and Calcium Content of Medicago Sativa L

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Abstract

In India, Alfalfa (Medicago sativa L.) is an important fodder crop which is rich in calcium, minerals, proteins and other nutrients. It not only increases fat value, but also increases the milk yield. Gammaradiation has been effectively used to induce useful mutation and enrich the biochemical components in alfalfa.Medicago sativa L. seeds were irradiated with 5KR, 20KR, 35KR, 50KR and 65KR cobalt dosesrespectively. Further the seeds were sown in the fields under a Randomized Block design. Entireplant material was collected at maturity stage, dried and reduced to powder. The powder was analysed to determine the calcium content using Flame photometry. Gamma radiation showed stimulating effect on increasing calcium content of Alfalfa in lower doses of cobalt 60. It was also observed that calcium content in aqueous extract was increased as compared to methanolic extract. From the present investigation it can be concluded that gamma radiation can be effectively used in Alfalfa mutation breeding programmes for fodder quality improvement as it is useful in increasing calcium content.

KEYWORDS: Gamma radiation, cobalt 60, Medicago sativa L., calcium, mutation breeding.

INTRODUCTION

Alfalfa Grass is beneficial for increasing the fat values. 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 a good source of protein and is rich in vitamins and minerals (Edward et.al, 2013). It contains chlorophyll, organic acids, saponins, isoflavins, sterols, coumarins, alkaloid and minerals like Calcium, potassium, phosphorus, Magnesium and zinc (Olimpiaet.al, 2015).Cell wall strength and thickness are increased by calcium addition. Calcium is a critical part of the cell wall that produces strong structural rigidity by forming cross-links within the pectin polysaccharide matrix. With rapid plant growth, the structural integrity of stems that hold flowers and fruit, as well as the quality of the fruit produced, is strongly coupled to calcium availability (Easterwood, 2002).

Gamma rays have generally a shorter wavelength and hence possess more energy. In general, Cobalt-60 and Cesium-137 are the main sources of gamma rays used in mutation induction (Kovacs and Keresztes, 2002).Gamma rays are characterised in ionizing radiation because these radiations cause alterations in the anatomy, morphology, Physiology and biochemistry of the plants (Mohajeret.al, 2014). The influence of these radiations is dose dependent, as these rays stimulate growth in plants at low dose. Therefore, these radiations are important in transforming the plant genome for crop improvement. Radiations have been used successfully to induce useful mutation for plant breeding. Gamma radiation can induce useful as well as harmful effects on crops so there is a need to predict the most beneficial dose for improvement of specific traits of crop

plants (Jamil and khan, 2002).The objective of present study is to study the effect of gamma radiation on calcium content of Alfalfa seedlings after germination.

MATERIALS AND METHOD

Plant materials:

Experimental plant material selected for the present investigation was Alfalfa commonly known as Lucerne [Medicagosativa (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 cultivaris a desi type, commercially and widely cultivated in the area of Ahmednagar district of Maharashtra.

Gamma radiation:

The source of gamma radiation used in the investigation was cobalt 60 (^{60}CO). The facility available at the Department of Biophysics, Government Institute of Science Aurangabad (M.S. India) was availed. The doses employed were 5KR,20KR,35KR,50KR and 65KR. Dry, uniform 50 gm. seeds of Alfalfa were irradiated with different doses of gamma radiation (5KR, 20KR,35KR,50KR and 65KR). Untreated seeds with gamma radiation were used as control.

Germination of Seeds:

To study the effect of different doses of Gamma radiation on calcium content of Alfalfa seedling, between paper methodwas used. The experiment was conducted and the results were averaged in order to get better results. Untreated seeds with gamma radiation were used as control.

Experimental Setup:

Determination of Calcium in various extracts of Alfalfa seedling using flame photometry:

A) Instruments, reagents and glassware used:

Flame Photometer CL 22D, volumetric flasks, pipettes: 1, 2, 5 ml, ten 50 ml beaker for extracting solutions, standards of, Ca+2. Flame Photometer CL 22D is a unit designed for mineral analysis application. The instrument provides automation in operation, measurement and end-result presentation. The unit can do the estimation of calcium (Ca+2) of a sample.

B) Preparation of extractsfor seedling analysis:

For preparation of methanolic extract, 100gram of fresh seedling of Alfalfa was crushed thoroughly, using mortar and pestle. The crushed seedling was completely exhausted by addingsmall quantities of methanol and filtering off every time in a successive manner, to yield final volume of 100 ml. (Shah et.al, 2011). (Aminet.al, 2015).

C) Preparation of standard solution of $Cacl_2$ for flame photometer:

Standard solution of Ca+2 were prepared by dissolving 276.9 mg of CaCl2, so it gives 100 ppm (2.495 millimole/liter) of Ca+2, Similarly standard solution of above element were also prepared using distilled water.

RESULTS AND DISCUSSION

Result for analysis of calcium in methanol extract of Alfalfa in terms of mmol/100ml, ppm and mg/100 g fresh Alfalfa seedling is givenin following table.

Table 1.1- Standardization of Calcium solution:

Sr. No.	ml. of standard (ppm) sol^n	Dilution	Conc. Of Standard (ppm)	Meter Reading (Calcium)
1	20	100	20	20
2	40	100	40	38
3	65	100	60	59
4	80	100	80	79
5	100	100	100	100

Repeatability of method is checked by repeating the same procedure by preparing standard solution for three times on three different days. This is mean of three meter reading for Calcium, Potassium and Sodium which are taken on three different days.

Effect of Gamma Radiation on Calcium content in Fresh Alfalfa seedling (Methanolic Extract):

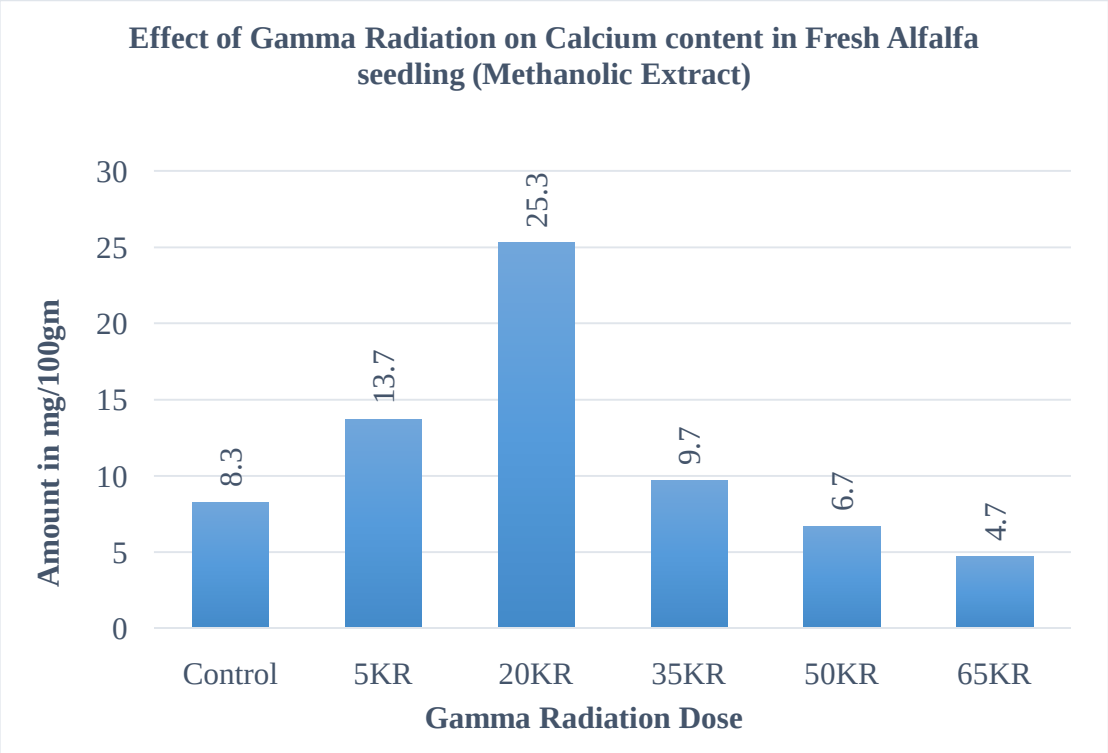
The result reveals that average maximum content of calcium in the Alfalfa seedling was 25.3 mg at 20KR dose of gamma radiation. However, minimum average content of calcium was reported in 65KR and control with 4.7 mg and 8.3 mg respectively. It is quite noticeable that lower doses of gamma radiation shows maximum content of calcium while higher doses of gamma radiation shows minimum content of calcium. 5KR and 20KR doses of gamma radiation show maximum content of calcium. When increasing the dose of gamma radiation from 35KR to 65KR the amount of calcium goes on decreasing.The amount of calcium in Alfalfa shows variations in all the doses of gamma radiation and control.

Table 1.2-Effect of Gamma Radiation on Calcium in Fresh Alfalfa seedling (Methanolic Extract):

Sr. no.	Sample Methanolic Extract	Amount of Calcium in mg/100gm			Mean	S.D.
		1st Day	2nd Day	3rd Day		
1	Control	08	09	08	8.3	0.5
2	5KR	14	14	13	13.7	0.5
3	20KR	26	24	26	25.3	1.2
4	35KR	10	09	10	9.7	0.5
5	50KR	07	06	07	6.7	0.5
6	65KR	05	04	05	4.7	0.5

(Three times with the same extract on three different days by repeating the calibration with freshly prepared standards.)

Graph 1.2- Effect of Gamma Radiation on Calcium content in Fresh Alfalfa seedling (Methanolic Extract):



However, the method of sample preparation in methanolic shows relatively variation. Similar result also obtained by Sanni T.A., (2015) who studied the effect of gamma Irradiation on mineral, vitamin and cooking properties of Sorrel (Hibiscus Sabdariffa L.) and recorded that calcium was significantly increased with increasing radiation dose to 2.0KGy but sodium was significantly reduced at 2.5KGy than at any other dose level. Along with the stimulation of cell division and enzymatic activity there is an increase of mineral and water uptake, which can explain the increase if assimilatory pigments in plants derive from seeds exposed at lower doses (Majeedet.al, 2010). Borzoueiet.al, 2013 also observed that lower doses will be significant as compared to higher doses. Mohajeret.al, 2014 also reported that lower doses of gamma radiation have stimulatory effects on nutritional composition of Sainfoin. Low doses of Ionizing radiation have modulatory role in the metabolic and biochemical processes of seedling (Sumira Jan et.al, 2013).

CONCLUSION

It is evident from this study that lower doses of gamma radiation i.e. 5KR and 20KR is mostly effective as compared to higher doses with 35KR, 50KR and 65KR and control. The stimulatory effect at a lower dose is due to the fact that mutagens at lower concentration stimulate the role of enzyme and growth hormone responsible for growth and inhibitory effect is due to the fact that biological damage increased at a faster rate in higher concentration of mutagen.It is concluded from this study that appropriate mutation

or variation in Calcium content of Alfalfa can be created through gamma rays and it can be improved in various genotypes through various gamma ray doses.

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