Variation in chlorophyll content of Alfalfa (*Medicago sativa* l.) as induced by gamma irradiation

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Abstract

Alfalfa is one of the most flowering herb plants in the family Fabaceae and botanically known as the Medicago sativa L. Alfalfa leaf contains essential vitamins, proteins and also source of chlorophyll. In the present investigation, seeds of alfalfa exposed to various doses (5, 20, 35, 50, 65 KR) of Gamma irradiation. Chemically, each chlorophyll molecule consists of porphyrin and magnesium at the center and a long chain hydrocarbon which is attached through a carboxylic acid group. Chlorophyll a and b occur in the higher plant. Chlorophyll-a, b and total chlorophyll contents analyzed by Spectrophotometric method at various level of gamma irradiation doses. Result showed that 5KR concentration showed highest chlorophyll a (4.21mg/g) 20 KR showed highest chlorophyll b (1.25 mg/g) and showed highest total chlorophyll (1.40 mg/g) as compared to control and other doses.

Key words Alfalfa, chlorophyll, Gamma radiation.

Introduction

Chlorophyll is a vital component of photosynthesis, which is how plants get their energy. But, photosynthesis isn't just important to plants; it's also essential to most other living things on Earth. Through photosynthesis, plants take in carbon dioxide and release oxygen back into the air, which is the oxygen that we need to breathe. Photosynthesis is also important because many animals eat plants and use the energy that has been converted from the sun to fuel their own bodies. Chlorophyll mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens have been reported in several crops [Kolar et.al 2011] Chlorophyll are structurally methyl phytol esters of dicarboxylic acid consisting of porphyrin head with four pyrol rings centrally linked to magnesium atoms and a phytol tail ($C_{20}H_{39}$ OH) with a long aliphatic chain of alcohol. Chlorophyll 'a' ($C_{55}H_{72}O_5N_4Mg$) and Chlorophyll 'b' ($C_{55}H_{70}O_6N_4Mg$) play major role in the absorption of solar energy for the sake of photochemical reactions of photosynthesis [Murray *et al*., 1986]. Any genic change will affect the nature of chlorophylls which ultimately influence the synthesis of chlorophylls and bring about some alterations in the amount of chlorophyll content [Vishnu Shankar Sinha, *et al.,* 2015].

Various physical mutagens like gamma radiations and chemical mutagens like EMS, MMS, NMU, SA, Colchicine 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 causes severe reshuffling of genetic material and induce different types of variations in the crops than any other radiation [Patil B. M.2015].

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 herbaceous perennial, 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]. It contains chlorophyll, organic acids, saponins, isoflavins, sterols, coumarins, alkaloid and minerals like Calcium, potassium, phosphorus, Magnesium and zinc [Olimpia et.al, 2015]. The objective of present study is to study the effect of gamma radiation on chlorophyll content of Alfalfa.

Materials and Methods

Plant materials:

Experimental plant material selected for the present investigation was Alfalfa commonly known as Lucern [*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 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.

Determination of chlorophyll content:

The chlorophylls are the essential components for photosynthesis and occurred in chloroplast as green pigments in all green plants. They are made up of proteins and which are extracted in organic solvent like acetone. Chemically, each chlorophyll molecule consists of porphyrin (tetrapyrol) and magnesium at the center and a long chain of hydrocarbon (phytyl) which is attached through a carboxylic acid group. Chlorophyll a and b occur in higher plants.

Chlorophyll was extracted in 80% acetone. The 663nm and 645nm wavelength of spectrophotometer was used for different readings of chlorophyll a and chlorophyll b and by using the absorption coefficients; the amount of chlorophyll a, chlorophyll b and total chlorophyll was estimated. Weight 1g of finely cut leaves and mixed well into a clean mortar. Grind the sample to a fine pulp by adding of 20 ml of 80% acetone. The extract was centrifuge at 5000 rpm for 5 minutes and transferred the supernatant to a 100 ml volumetric flask. Grind the residue with 20 ml of 80% acetone and again centrifuge and transfer the supernatant to the same volumetric flask. This procedure was repeated for changed the residue green colour to colourless. Washed the mortar and pestle thoroughly with 80% acetone and collected in the volumetric flask. To make final volume of extract up to 100 ml with 80% acetone. The absorbance of the samples was recorded at the 645 and 663nm against the solvent 80% acetone as a blank. Calculated the amount of chlorophyll a and b and total chlorophyll present in the extract.

The following formula was used for estimation:

Chlorophyll 'a' (mg/g fresh leaves)

= 12.7 (A 663 nm) – 2.69 (A 645 nm) x V /1000 x W

Chlorophyll 'b' (mg/g fresh leaves)

= 22.9 (A 645 nm) – 4.68 (A 663 nm) x V /1000 x W

Total Chlorophyll (mg/g fresh leaves)

= 20.2 (A 645 nm) + 8.02 (A 663 nm) x V /1000 x W

Where,

A = absorbance at specific wavelengths

V = final volume of chlorophyll extract in 80% acetone

W = fresh weight of tissue extracted.

[Source: Sadasivam and Manikam, 2008]

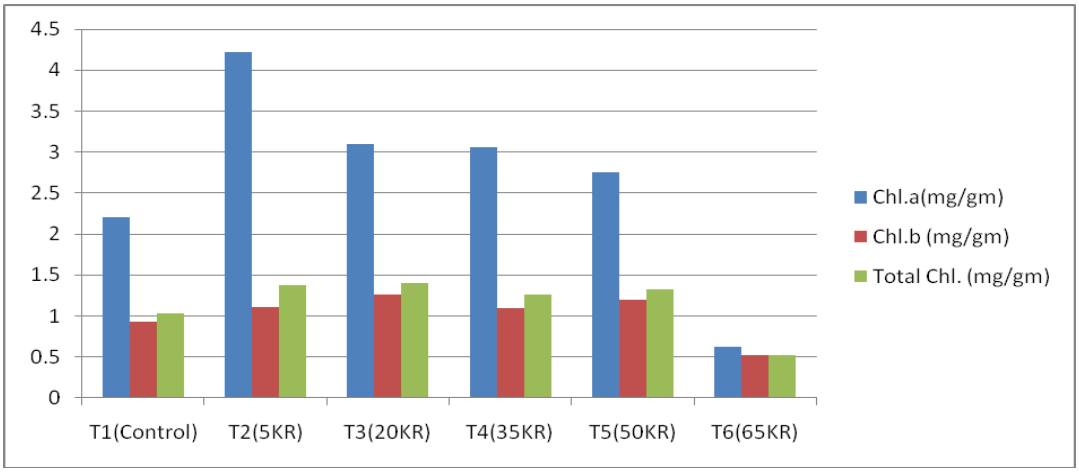
Result and Discussion

The effect of gamma rays on chlorophyll content was measured in terms of chlorophyll content mg/gm fresh weight of leaf. The data presented in Table 1 showed that 5KR dose caused an increase in the content of chlorophyll a, and 20KR caused an increase in the content of chlorophyll b and total chlorophyll in Alfalfa as compared to that in control leaf. It caused a decrease in chlorophyll content at 65KR dose. The maximum increase and decrease were noted at 5KR dose and 20KR, 35KR, 50KR and 65KR dose respectively (Table 1).It means that chlorophyll content was decreases from lower to higher doses of gamma radiation as compared to control. Chlorophyll content is found to be sensitive to higher doses of radiation.

The chlorophyll a and chlorophyll b content was significantly increased as a result of mutagenic treatments and in some mutants the content of chlorophyll was linearly decreased [Mejri*et al*., 2011] reported that chlorophyll a and chlorophyll b content was significantly decreased after Gamma radiations. Gamma radiation induces various physiological and biochemical alteration in plants. The irradiation of plants with high dose of gamma rays disturbs the hormone balance, leaf gas-exchange, water exchange and enzyme activity [Kiong*et al*., 2008]. These effects include changes in the plant cellular structure and metabolism such as dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidant system, and accumulation of phenolic compounds. Based on transmission electron microscope observations, chloroplasts were extremely sensitive to gamma radiation compared to other cell organelles, particularly thylakoids being heavily swollen [Wi*et al*., 2007]). In this study, the chlorophyll content of gamma irradiated Alfalfa displayed a gradual decrease at 65KR dose. In addition, it can be observed that the concentration of chlorophyll *a* was relatively higher than chlorophyll *b* in irradiated and non-irradiated plants. Kiong*et al*., 2008 reported that the reduction in chlorophyll *b* is due to a more selective destruction of chlorophyll *b* biosynthesis or degradation of chlorophyll *b* precursors.

Table 1: Effect of gamma radiation on chlorophyll content of Alfalfa.

Sr. No.	Dose (KR)	Chl.a (mg/gm)	Chl.b (mg/gm)	Total Chl. (mg/gm)
1	T1(Control)	2.210	0.925	1.029
2	T2(5KR)	4.214	1.103	1.378
3	T3(20KR)	3.095	1.259	1.408
4	T4(35KR)	3.059	1.100	1.264
5	T5(50KR)	2.753	1.203	1.326
6	T6(65KR)	0.628	0.526	0.524



Conclusion

In conclusion, from the above estimation, there is a lot of scope for improvement through induced mutation breeding and biochemical studies like chlorophyll content in Alfalfa. The estimation of biochemical components indicated the application of mutation breeding in the development of superior genotypes carrying improved nutritional values in Alfalfa.

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