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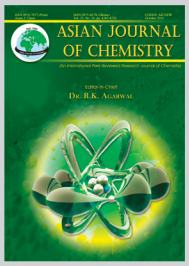

γ-Irradiation Activates Biochemical Systems: Induction of Nitrate Reductase Activity in *Cajanus cajan in vitro* and *in vivo* Conditions

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γ-Irradiation induces various physiological and biochemical alterations in plants. γ-Irradiation lead to activation of biochemical system in *Cajanus cajan* when subjected to absorbed doses 30Gy, 50Gy, 100Gy, 150Gy and 200Gy under *in vivo* and *in vitro* conditions. Nitrate reductase activity enhanced significantly ($p < 0.05$) with increasing doses of γ-radiation under both *in vitro* and *in vivo* conditions. γ-Irradiation induced high levels of nitrate reductase activity in regenerative callus, *in vitro* raised micoshoots and also *in vivo* grown leaves whereas a reverse effect was seen in case of nitrate content. Nitrate content declined significantly with increasing doses of γ-radiation under both *in vitro* and *in vivo* conditions. However, absorbed doses 30Gy and 50Gy of γ-rays had a stimulatory effect on nitrate accumulation.

Key Words: γ-Rays, Irradiation, *In vivo*, *In vitro*, Nitrate, Nitrate reductase.

INTRODUCTION

γ-Irradiation induces various physiological and biochemical alterations in plants. γ-Irradiation can be useful for the alteration of one or a few physiological characters[1]. Photons of γ-irradiation are powerful enough to be completely democratic with regard to molecular species with which they interact. γ-Irradiation leads to changes in the plant cellular structure and metabolism[2,3].

γ-Rays are often used on plants in developing varieties that are agriculturally and economically important and have high productivity potential. γ-Rays are also very important in mutation breeding and in *in vitro* mutagenesis in order to develop required features of plants and increase the genetic variability. The many mutant varieties, which are resistant to diseases, cold, salt and with high quality, have been developed[4].

Pre-sowing seed irradiation is also an effective method of improving production, yield components and chemical composition in plants[5,6]. Prevailing opinion is that physical methods for processing of pre-sowing seed stimulate physiological and biochemical changes in the seeds[7-11]. Studies have also been carried out to elucidate the effects of γ-rays on some aromatic plants and legumes[6,12-16].

The aim of the present investigation is to assess the use of γ-radiation as a physical elicitor to alter the physiological characteristics of *Cajanus cajan* after exposures of pre-sowing (seeds) to variable doses of γ-rays *in vivo* and *in vitro*.

EXPERIMENTAL

Cajanus cajan L. was selected for the present study. The seeds were irradiated with γ-radiation of absorbed doses 30Gy, 50Gy, 100Gy, 150Gy and 200Gy for *in vivo* and *in vitro* studies. The device used was gamma cell GC-5000 BRIT-BOMBAY. The source of gamma radiation was Cobalt-60; with a dose rate 2.08 Kilo Gray per hour (2.08 KGh^{-1}) at Indian Institute of Nuclear Medicine and Applied Sciences (INMAS) New Delhi. Nitrate was estimated by Grover[17] method. The concentration of nitrate was determined against the standard curve prepared by using KNO_3 (potassium nitrate) solution. The nitrate content was expressed in m mol g^{-1} fr.wt. Nitrate reductase activity was estimated using the method given by Klepper[18]. The corresponding concentration of nitrate was determined against the standard curve of nitrite prepared by $NaNO_2$ (sodium nitrate) solution. The nitrate reductase activity was expressed in μ mol g^{-1} fr. wt.

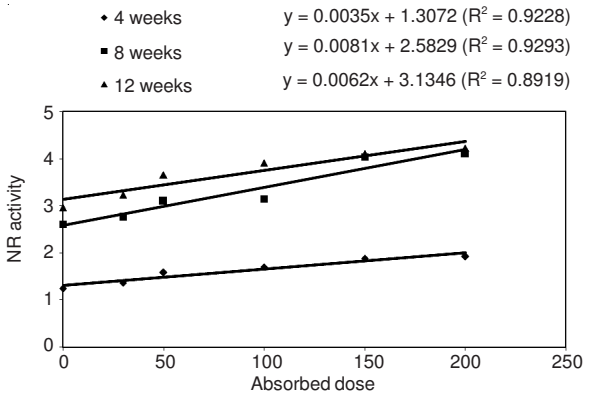
RESULTS AND DISCUSSION

Nitrogen metabolism impairment is one of the primary determinants for growth and development of the plants. Nitrate assimilation is closely linked to carbon assimilation. While the carbon fixation forms carbon skeleton and stores energy, the nitrate assimilation provides reduced nitrogen, which is used in the synthesis of amino acids and proteins. The later is involved in mediating various steps of carbon cycle and

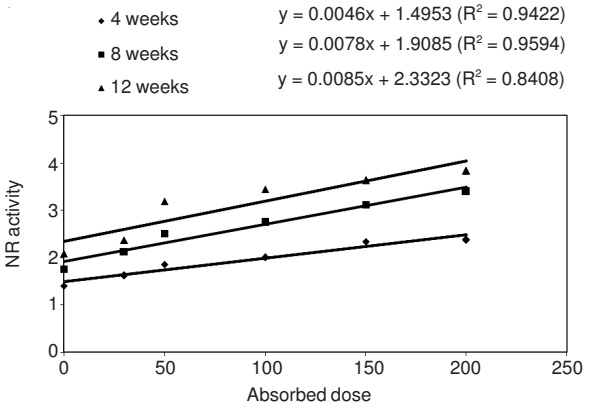
nitrogen is involved in the development and maintenance of photosynthetic machinery and leaf organs[19]. Nitrate reductase is a key enzyme of the nitrate assimilation pathway and its activity is controlled at both the transcriptional and post-transcriptional level by a number of metabolites and stimuli, such as light, nitrate availability and sucrose content[20-22]. Nitrate reductase activity is positively correlated with protein content, nitrogen accumulation, free amino acid content and better yield in several crops[23]. Nitrate reductase activity in the present study, enhanced significantly with increasing doses of γ-radiation, *in vitro* and *in vivo* (Fig. 1). In both *in vivo* and *in vitro*, the maximum enhancement was observed with 200Gy. Present results are in agreement with Lemnott[24] and Moussa[25]. The induction of nitrate reductase could be due to hormone production in these tissues. Abbas[26] found that optimum doses of γ-radiations markedly increased the endogenous growth hormone (IAA, GA and cytokinins) in lupine. Because cytokinins (kinetin) and auxins (IAA) induce nitrate reductase activity and because endogenous cytokinins (and presumably other growth factors) are induced by γ-irradiation, it is likely that these growth factors mediate at least part of nitrate reductase induction due to γ-irradiation[27]. This study provides evidence that irradiation can activate a biochemical system, induction of nitrate reductase, indirectly which may be through the activation of growth regulators in *Cajanus cajan* plants. The activity of nitrate reductase activity is directly co-related to nitrate concentration and plays an important role in protein synthesis. Nitrate reductase is a key enzyme of the nitrate assimilation pathway and its activity is controlled at both the transcriptional and post-transcriptional level by a number of metabolites and stimuli, such as light, nitrate availability and sucrose content[20-22,28]. Nitrate reductase activity is positively co-related with protein content, nitrogen accumulation, free amino acid content and better yield in several crops[23]. Furthermore, nitrate assimilation depends on the photosynthetic process for the provision of reducing equivalents and ATP, whereas in roots the same process depends on the carbohydrate availability deriving from the shoots. Nitrate content under both *in vivo* and *in vitro* conditions decreased significantly at high doses in all developmental stages (Fig. 2). However, low doses of γ-rays had a stimulatory effect on nitrate accumulation. Similar results were found in sakha rice cells exposed to variable doses of γ-radiation, the accumulation of total nitrogen, increase in protein synthesis, increase in nitrate absorption and assimilation in these cells relative to control[29]. Tukendorf and Rauser[30], Hageman and Fischer[31] established a positive correlation between the nitrate reductase activity and growth and protein content of corn. Reinik *et al.*[32] indicated that, when the plant nitrate acts as an osmoticum, more carbohydrates could be used to increase the dry matter production. Seginer *et al.*[33] concluded that there is a negative co-relation between the concentration of soluble carbohydrates and nitrate in the cell sap and so irradiation increases the carbohydrate content[34] while the nitrate accumulation decreases.

Conclusion

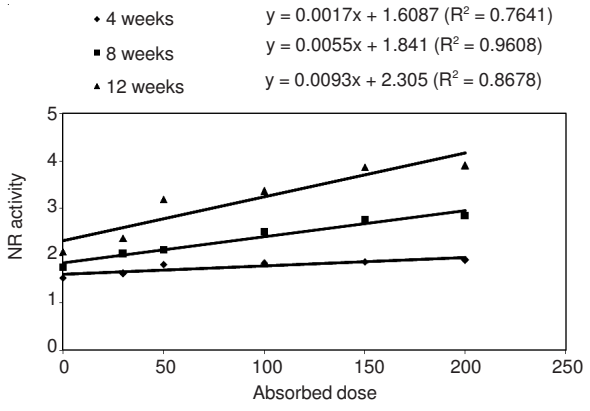
Biochemical analysis confirmed that nitrate content, nitrate reductase activity are very sensitive to γ-radiation and are good indicators of tolerance. The differences between various cultures



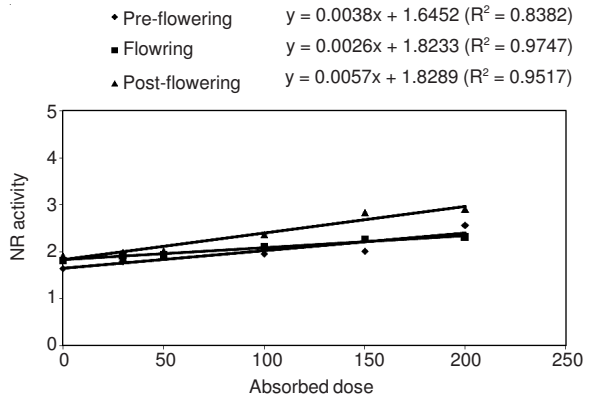
(a) Non-regenerative calli (*in vitro* grown)



(b) Regenerative calli (*in vitro* grown)

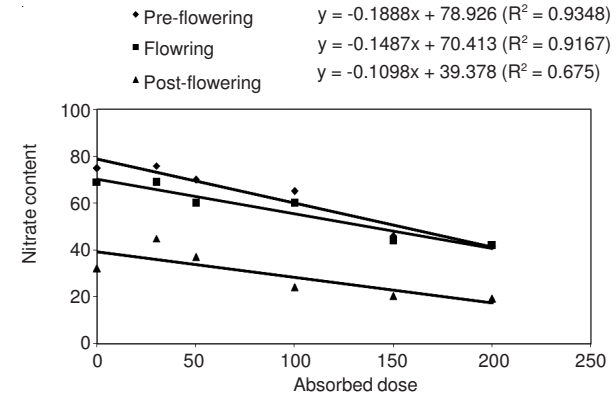


(c) Leaves (*in vitro* grown)

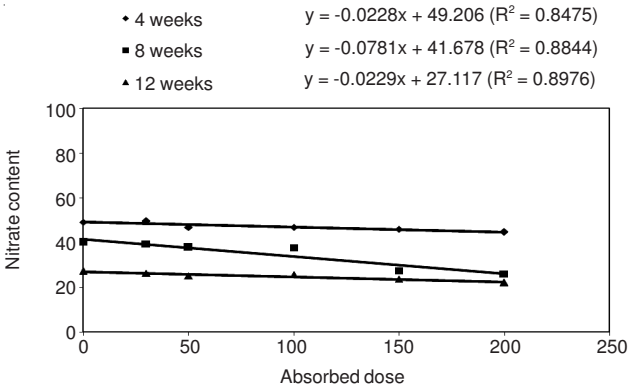


(d) Leaves (*in vivo* grown)

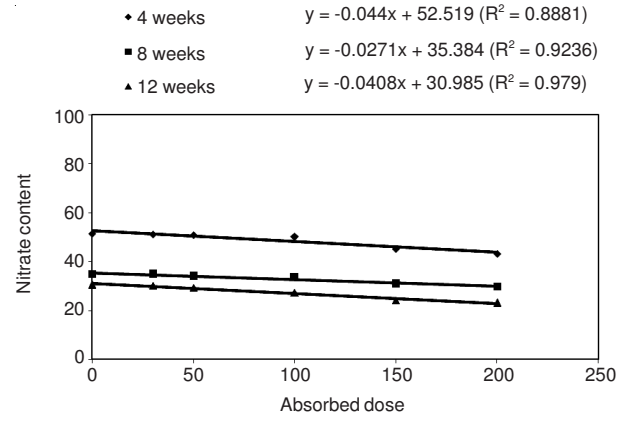
Fig. 1. Variation in nitrate reductase activity in *Cajanus cajan* L. at various growth stages *in vivo* and *in vitro* treated with different doses of γ-radiation



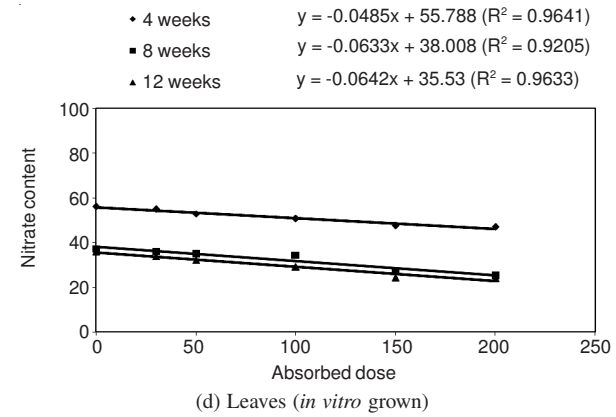
(a) Leaves (*in vivo* grown)



(b) Non- regenerative calli (*in vitro* grown)



(c) Regenerative calli (*in vitro* grown)



(d) Leaves (*in vitro* grown)

Fig. 2. Variation in nitrate content in *Cajanus cajan* L. at various growth stages *in vivo* and *in vitro* treated with different doses of γ-radiation

in their ability to accumulate such compounds were evident with γ-irradiation but not under controlled conditions. γ-Irradiation is a powerful method for *Cajanus cajan* improvement as suitable doses of γ-rays besides inducing various physiological alterations also activates a biochemical system.

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