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Full length Research Paper

$^{7}Li^{+++}$ Heavy ion radiation exposure to mustard seeds induced changes in seed oil

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The heavy ion radiations (HIR)-induced mutations may lead to either lethality or survival; depending on irradiation dose, type of species, physiological status and DNA breaks and their repairibility. In present study, the effects of $^{7}Li^{3+}$ ion irradiation exposure on seeds were studied to elucidate the change in total oil that contains important fatty acid including glucosinolate in seeds of *Brassica juncea* cv RH-30 grown in field for three years. The total seed oil and fatty acid content varied in oil seeds of crop depending on irradiation dose and crop cycle. The palmitic acid, a saturated and cholesterol raising fatty acid decreased at two lower dosage up to M2 generation, but little increased in M3 generation seeds. The unsaturated fatty acids (Oleic, Linoleic, Linolenic and Eicosenoic acid) increased 10-18% depending on dosages over control. There was considerable increase in glucosinolates due to irradiation only in M1 cycle, but thereafter decreased up to M3 cycle. Erucic acid level also varied with dose and crop cycle. The study suggests that mustard could be good potential model system for desirable change in fatty acid composition might be implicated in neutraceutical and HIR specific dose may be exploited for improvement.

Keywords: Heavy ion radiation, glucosinolates, fatty acids, mustard seed oil.

INTRODUCTION

Studies are devoted to understand the plant responses to sparse radiations like, UV and gamma (Rousseaux et al., 1999). Radiations generally cause oxidation of lipids in the biological systems (Pelle et al., 1990).This may disturb metabolism of a cell(s) leading to growth retardation, mutation or even death in plants.

Heavy ion radiation (HIR), an ionizing cosmic radiation are considered to be lethal due to many severe perturbations in metabolic processes (Mishra et al., 2006; Kimura et al., 2007). Moreover, chromosomal aberrations, either addition or deletion of nucleotides after heavy ion irradiations of seeds or plants led to transformation of the cells negatively or positively (Mishra et al., 2006). This is being precisely attributed to unique nature of HIR, i.e. the tendency of linear energy transfer (LET) decreases exponentially after penetrating into the object/target, called as Bragg peak's, implied that HIR have different relative biological efficiency (RBE) (Rakhwal et al., 2008). In view of these reports the HIR has been simulated to evaluate precise responses in plants and animals, and suggested that the HIR has potential to modulate the cell structure and function in animals (Shimano et al., 2001) and plants (Shikazono et al., 2002; Yokota et al., 2003) may vary. Certain useful modifications like dwarf (Honda et al., 2006), semi-dwarf, early maturity and large grain size mutants in rice plants from the seeds exposed to HIR have been reported (Liu et al., 1991). The $^{7}Li^{+++}$ irradiation to mustard seeds have shown increased grain yield in subsequent crop depending on dose (Mishra et al., 2000; Verma et al., 2004). Few studies suggested dose dependent responses in plant species (Shikazono et al., 2002; Mishra et al., 2006). The differential gene expression and dose dependent growth in rice after carbon ion exposure has been also demonstrated (Rakhwal et al., 2008). The oil crops being important source of energy for both human and live stock including use as raw materials for wide range of industrial products such as detergent, cosmetics, lubricant and bio-fuel (Ohlrogge, 1994) have always been focus for further studies for quality and

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quantity improvement under varying conditions where as mustard economic values and consumability of oil in terms of nutritional qualities primarily depends upon the fatty acid composition of the seed oil.

The mustard (*Brassica juncea* RH-30) is widely grown, because of high yield, relatively more tolerant to heat and drought stress, less susceptibility to few major diseases including shattering (Downey, 1990). Moreover, the oil content is governed by number of genes with additive effect; hence the selected oil content gene(s) could be taken to improve oil quality, especially character(s) sensitive to environmental condition (Sharma and Anand, 1994). Therefore, oil composition response(s) are required to be elucidated under different physical conditions to exploit at molecular level. Zidenga et al. (2003) have suggested that genetically modified biochemical pathways could be utilized to produce fatty acids according to human need. Keeping in view of positive effects of heavy ion irradiation effect and few strong characters of RH-30, it is in order to evaluate the heavy ion irradiation effects on total oil and its composition in RH-30 seed.

Further, it is also assumed that the understanding of HIR responses on metabolites could provide insight for strategic genetic modification in general and more specifically in economically valuable crops/plants under breeding programs.

MATERIALS AND METHODS

Uniform size seeds of mustard (*B. juncea* cv RH-30) were irradiated with different fluence (10^7, 5×10^7, 10^8 p/cm^2) of $^7Li^{+++}$ using Pelletron accelerator at IUAC, New Delhi. The linear energy transfer at seed surface was 45 MeV/µm. Irradiated seeds were sown for 2-cycle of crop during winter (rabi) season, in split-2 plot (size 1×1m), designed for each treatment without enriching the soil with extra nutrient at KVK Rohtak, regional centre of HAU, Hisar. Seeds obtained from M1 generation were grown for consecutive two seasons to get M2 and M3 cycle of crop. In another set of experiment, seeds of M1 generation (from respective dosages) were again irradiated with dose of 5×10^7p/cm^2 and allowed to grow in same condition to examine changes in total oil and fatty acid profile. Seeds obtained from M1, M2 and M3 generation crop as well as crop from re-irradiated were used for estimation of total oil content. Seeds were dried to 4-5% moisture level in oven at 108°C for 16-18h and than the total oil content of seed samples were determined by a non-destructive method using a Newport NMR analyzer (Model-4000-oxford analytical instruments Ltd. U.K.) after calibrating with pure Brassica oil. The fatty acids were determined by following the method of Mandal et al. (2002). The seed samples were extracted with solvent mixture consisting of chloroform: hexane: methanol (8: 5: 2 v/v/v). The methyl ester of oil prepared and then dried and redissolved in hexane and one micro-litre of this hexane fraction used for fatty acid estimation by using the GLC (Model -Micro-9100). The glucosinolates content by Pd-quick test method (Fette, 1982) followed by ELISA, O.D recorded at 450nm.

RESULTS AND DISCUSSION

HIR effect on total oil in seed

The total oil content in seeds of *B.juncea* cv. RH-30 corroborates with that reported by Mandal et al. (2002). The total oil contents varied in seeds of consecutive generations of crop after $^7Li^{+++}$ irradiation of seeds (Figure 1). The increase in oil contents in M1 generation seed was dose dependent and the maximum increase was 6% at fluence of 10^8 p/cm^2 over control and significant within treatment. In the M2 generation seeds, oil content declined remarkably over M2 at all dosage. While, the oil contents in M3 generation seeds, increased over M1 cycle but dose dependent decline was observed in comparison to M3 control (Figure 1).

The variation in oil content, prompted to re-irradiate M1 seeds (with fluence of 5×10^7 p/cm^2, selection of the re-irradiation dose based on certain positive response Sarita, (2003). This experiment results showed a significant increase in oil content in the crop from seeds exposed to lower dosage10^7 p/cm^2 and 5×10^7 p/cm^2 only (Figure 1). Whereas, re-irradiation caused decline in total oil in those seeds from higher dose 10^8 p/cm^2. The change in total oil content in seeds is implied in change in quality. The changes in oil quality of seeds under changing physical conditions like salinity (Sinha and Yadav, 1991) and drought (Singh et al., 2003) have been observed. Generally, the fatty acids are tolerant to alterations in chemical structure and is a good target for genetic manipulations, without disturbing the physiology of the whole plant (Voelker and Kinney,2001), therefore, it could be exploited to an extent according to the need, as the morphology of plants in these experiments were not different from non-irradiated one.

HIR Effect on fatty acid composition

The nutritional value and utility of oil primarily depend on the composition of the fatty acids (Mandal et al., 2002), with chemical structure are least affected under change physical factors (Voelker and Kinney, 2001). The fatty acid composition of affected oil depends on radiation dose (Figure 2 A-F). The decrease in palmitic acid level upto 10-30% was observed compared to control, except in the seed's oil from 10^8 p/cm^2 exposed ones (Figure 2 A). However, palmitic (16:0) a saturated and cholesterol raising fatty acid showed variation in its level in oil consistently up to M3 generation. However, this fatty acid

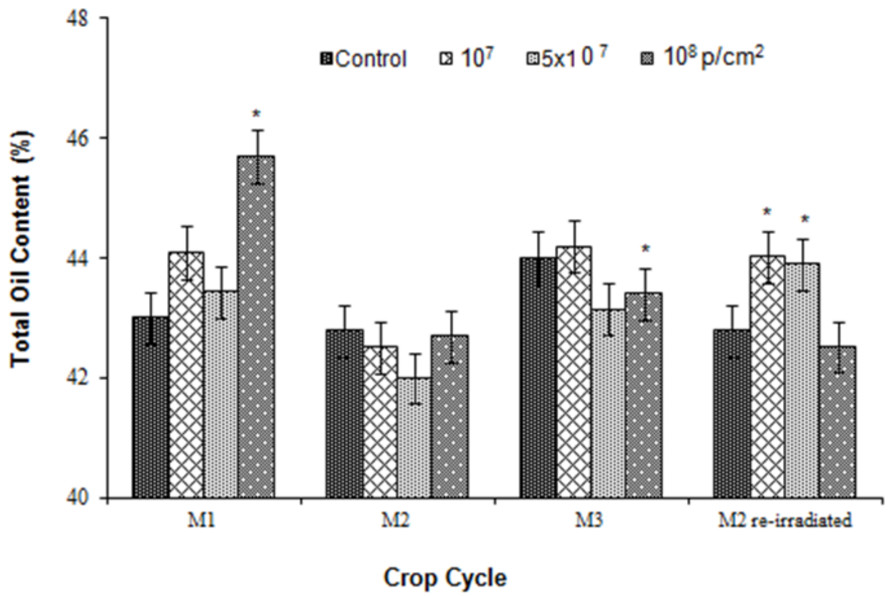

Figure 1. Total oil content in seeds of *Brassica juncea* obtained from different cycles of crop emerging from irradiated/re-irradiated treatment. Data are the mean value of (n=3)± SD. (*) indicate the significance of difference at $p<0.05$. Re-irradiated; the seeds obtained from different exposure were irradiated with $5x10^7 p/cm^2$ of $^7Li^{+++}$.

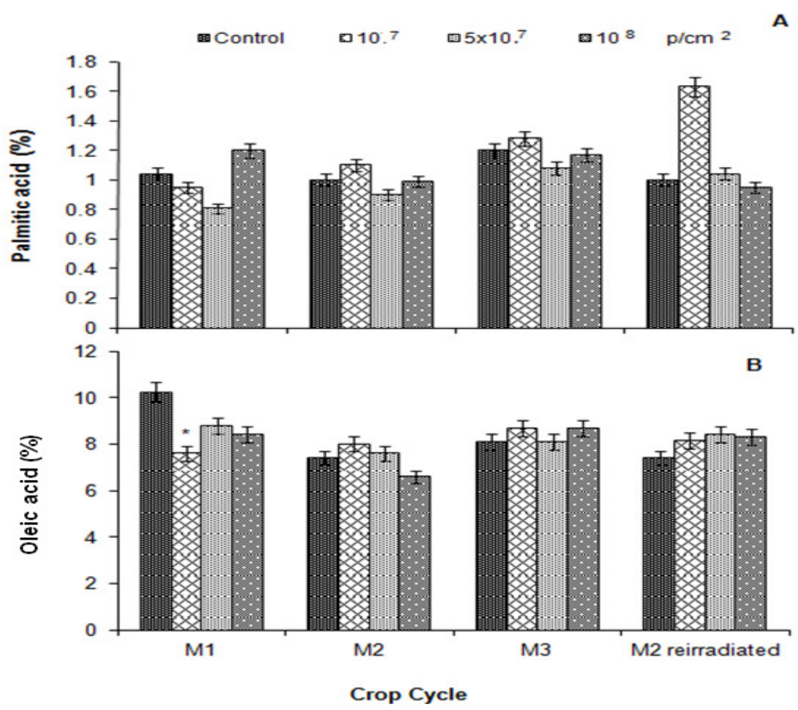

Figure 2 A-B. Palmitic acid (A) and Oleic acid (B) composition in seeds of *Brassica juncea* obtained from different cycles of crop emerging from $^7Li^{3+}$ exposed seeds. Data are the mean value of (n=3)±SD. (*) indicate the significance of difference at $p<0.05$ between treatment and (a) indicate the significance with in the treatment. Reirradiated: The seeds obtained from different exposure in M1 generation were irradiated $5x10^7 p/cm^2$ of $^7Li^{3+}$

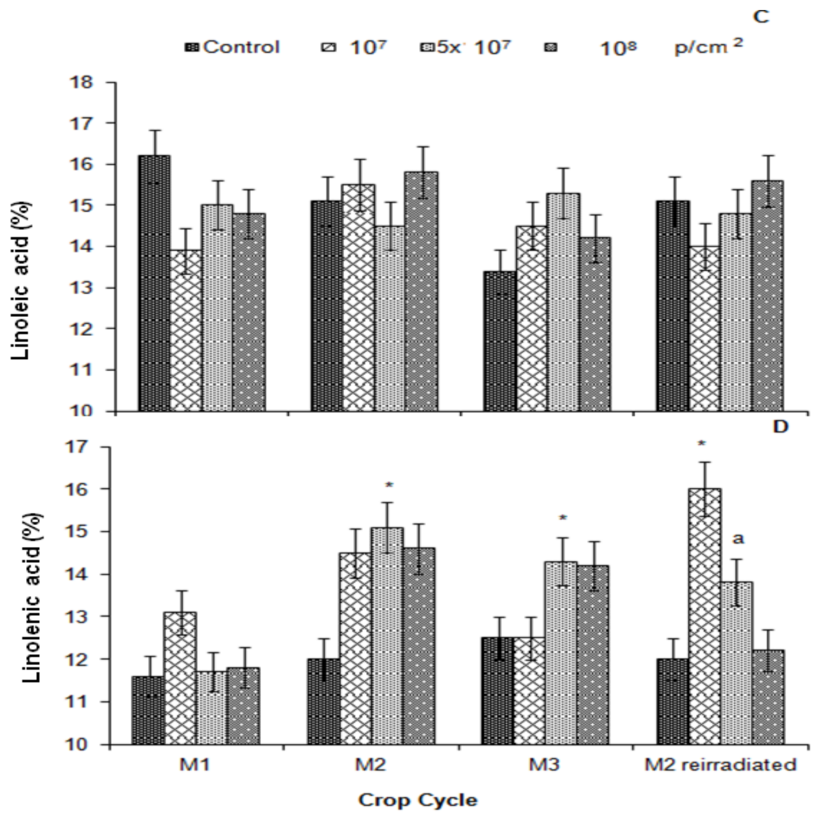

Figure 2 C-D. Linoleic acid (C) and linolenic acid (D) composition in seeds of *Brassica juncea* obtained from different cycles of crop emerging from $^{7}Li^{3+}$ exposed seeds. Rest legend same as figure 2 A-B.

showed gradual increase up to M3 at dose $5x10^7$ p/cm^2, but always remained lower than any treatment as well as control. Re-irradiation caused dramatic increased at 10^7 p/cm^2. The monosaturated fatty acid category underlines the cardiovascular diseases and insulin resistance could be exploited for nutritional quality of aspecific oil (Lovejoy, 2002). The oleic acid (18:1) content in oil also declined significantly in M1 and M2 except M3 cycle seeds as well re-irradiation (Figure 2 B). However, there was little increase (8%) in oleic acid in M2 seeds of crops raised from seeds exposed to 10^7 p/cm^2 over control. A dose dependent change in linoleic acid content in oil was observed from M1 to M3 cycle seed (Figure 2 C). Surprisingly, the linoleic increase over control was observed at M3 cycle. Although, there was total decline in each stage and dose over M1 control. Linolenic acid, an essential fatty acid play role in reducing plasma cholesterol was found to be increased (20%) in M1 cycle and more than 25% in M2 cycle seeds irradiated at 10^7 p/cm^2 of $^{7}Li^{+++}$ irradiation (Figure 2 D). The irradiation induced increase in linoleic in M2 and M3 cycle seeds, specially at higher doage in corroboration of observation made by Bhat et al. (2008) in *Mucuna pruriens* seeds exposed to 30kGy gamma radiation, otherwise lacking in unexposed seeds. Interestingly, the linolenic acid increase was 14% in oil of those seeds, generally exposed to higher fluences. The linolenic acid presence more than 10% of oil is considered good quality (Trautwein and Erbersdobler, 1997). Except M1 cycle and its two higher dosage, there was almost linear increase. The eicosenoic acid decreased 5-10% in M1 generation but increased 10-15% in successive generation seeds (Figure 2 E). The re-irradiated seeds also maintained higher level of eicosenoic acid at different dosage over control, but less than M1 cycle. This monounsaturated fatty acid (20:1) have been implicated in diabetic and lipid metabolism regulation (Iwamura et al., 1984). However, erucic acid level increased prominently due to radiation over control in M1, whereas in M2 cycle seeds either decreased at lower dosage or changed little over control. In M3 cycle seeds (Figure 2 F) the decrease was prominent with higher dose over control. The extremely contrast change due to radiation (linear decrease) in M3 over control, but level apparently same to M1 control suggests remarkable nutritional control. The re-irradiation also caused sign-

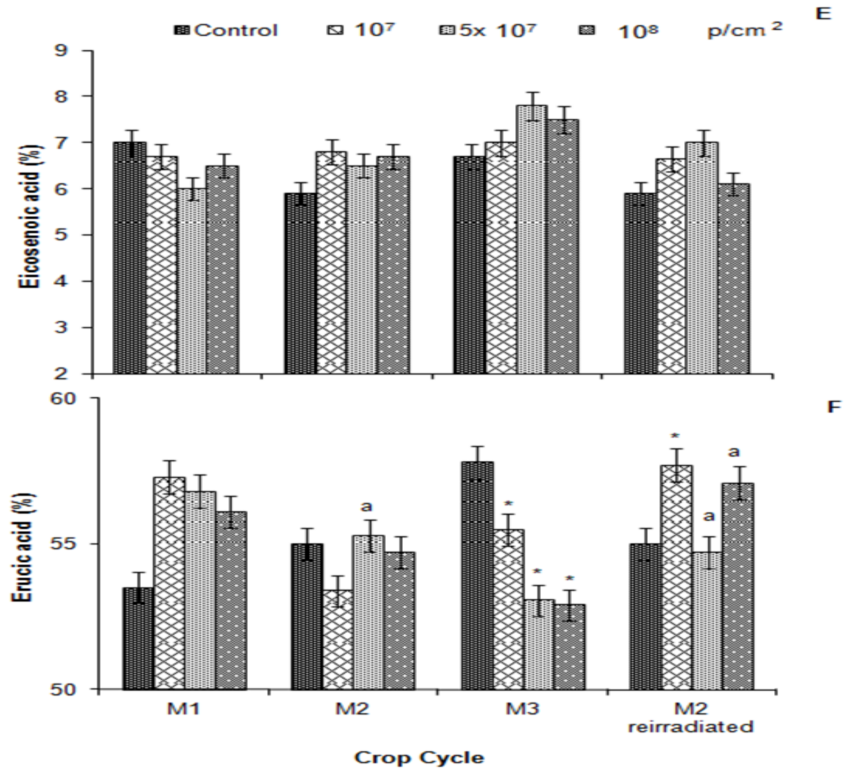

Figure 2 E-F. Eicosenoic acid (E) and erucic acid (F) composition in seeds of *Brassica juncea* obtained from different cycles of crop emerging from $^7Li^{3+}$ exposed seeds. Rest legend same as figure 2A, B

ificant change in erucic acid content in oil. It could be due to induction of enzyme fatty acid elongase (FAE 1,2,3,4) responsible for conversion of oleic to erucic acid (Kanvar et al*.,* 2006) and eicosenoic acid.

It is interesting to note that the unsaturated fatty acids (Oleic 18:1, Linoleic 18:2, Linolenic 18:3 and Eicosenoic acid 20:1) increased 10-18% over control and depending on dosages. While saturated fatty acid (Palmitic acid) increased by 63% in seeds from low (10^7 p/cm^2) exposure of HIR. These results indicate that selected dosage may be useful in developing selective oil trait in plants, depending on its physiological state. The gamma radiations have been implicated in change in hydrocarbon (Kim et al., 2010) and alteration in nutritional quality (Bhat et al., 2008). $^7Li^{+++}$ ion beam could be applied for metabolic alteration and thus selective exposure for improvement in nutritional quality of seed oil (Sinha et al*.,* 2007) which can sustain for many generations.

HIR effect on glucosinolates level

Glucosinolates level in seeds decreased 30 to 50% depending on dose of $^7Li^{+++}$ exposure. The dose dependent quantitative decrease was up to M3 cycle (Figure 3). The decrease was more prominent in the seeds exposed to 5 x 10^7 p/cm^2 of $^7Li^{+++}$, whereas 15% increase over control was noted. The re-irradiation with 5 x 10^7 p/cm^2 of $^7Li^{+++}$ of those M1 cycle seeds from having different exposure of HIR showed considerable linear increase in glucosinolates (Figure 3). This was extremely contrast over M1, M2 and M3 cycle seeds. The glucosinolate having properties like flavor, insect protector biofumigation, anticancer **(**Halkier and Gerhenzon, 2006) and mustard being good source of glucosinolate (Verkerk et al*.,* 2009) makes it potential candidate for mor precise desirable changes, especially when an attempt to alter expression of cyp79 gene of enzyme for conversion of amino acids to aldokitone having limited molecular clue for glucosinolates modulation (Mikkelsen et al*.,* 2002). The varied change in level of glucosinolates due to irradiation could be of great economical significance in vegetable oil. Furthermore, because of glucosinolate causing goitrogenicity in livestock and liver hemorrhage (Fenwick, 1984) could be altered by applying HIR exposure to those plants under neutraceuticals (Mithen and Dekker, 2009).

However, the presence of high level of glucosinolates and significant amount of erucic acid in oil makes Indian

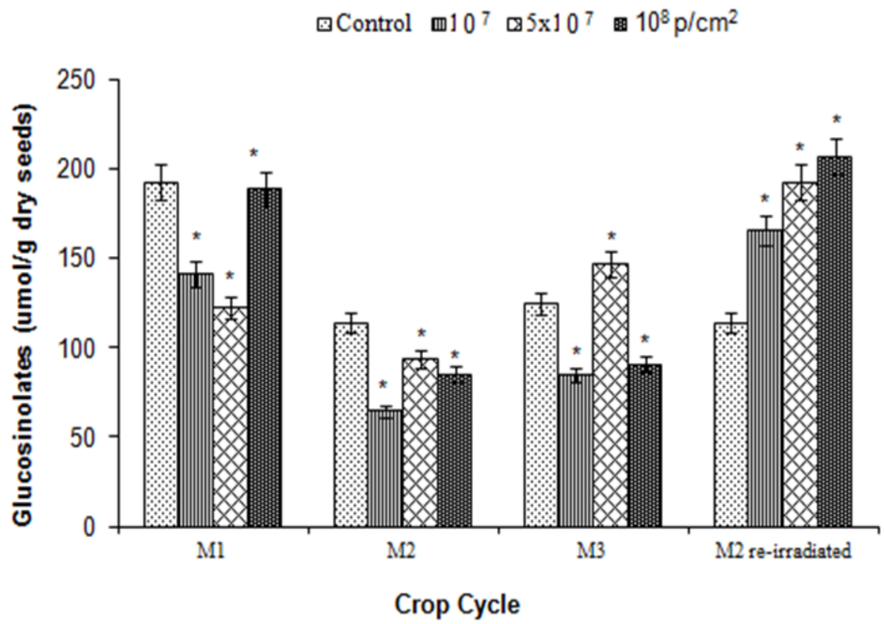

Figure 3. Glucosinolates level in seeds of *Brassica juncea* obtained from various cycles of crop emerging from irradiated treatment. Rest legend same as figure1.

mustard (*Brassica juncea*) unsuited for the market (Tomar et al*.,* 2002). The present experiments showing high erucic acid over low oleic acid may be upregulation of fatty acid elongase (FAE 1,2,3,4). The earlier attempts to reduce glucosinolates by developing mutants through breeding in view of number of physical factors are with minimum success (Mailer and Cornish, 1987). However, the development of low and/or zero erucic acid level varieties is one of the major objectives in quality improvement program (Jambhulkar et al*.,* 1998). In case of heavy ion exposed seeds, tend to increase erucic acid is pressing the need of study of 3 ketoacyl-coA synthase (Fatty acid elongase FAE 1,2,3,4) for its down regulation but at the same time oil quality, nutritionally healthy should have more unsaturated F.A. i.e. in irradiated seeds.

CONCLUSION

The HIR modulation in glucosinolates level indicates possibility to improve the quality of livestock feed vis-à-vis to be employed in value addition in all edible oils seeds, in turn human health management. The differential quantitative change in fatty acids with HIR suggests its evaluation in other economically oil seed crops for their response and also generating curiosity for molecular study.

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