DISCUSSION
Gamma irradiation has been widely used to develop economically improved different plants i.e. varieties with high productivity potential, salt stress and environmental factors (Jain et al. 1998; Hajduch et al. 1999). Moreover, gamma irradiation has been recommended for quarantine treatment of legumes and as chief and authentic method of decontamination of medicinal herbs (Variyar et al. 1998; Maity et al. 2009). Such radiation practices albeit within certain range of low doses can elevate the physiological activities of cells in plants and photosynthetic microbes by ameliorating germination and growth rates (Thiede et al. 1995; Al-Safadi and Simon, 1996; Korystov and Narimovar, 1997; Lee et al. 1998; Charabaji and Nabulsi, 1999; Kim et al. 1999; 2000; Thapa, 2004; Klarrize, 2005; Melki and Sallami, 2008 and Melki and Marouni, 2009) increasing stress resistance (Zaka et al. 2002; Lee et al. 2002 a, b. 2003 and/or improving crop yields (Weindal et al. 1995; Kim et al. 1998; Al-Safadi et al. 2000). However, irradiation of seeds with higher doses of gamma rays disturbs the synthesis of proteins (Xiuzher, 1994), hormonal balance (Dwelle, 1975; Rabie et al. 1996), leaf gas exchange (Steova and Bineva, 2001) water exchange, enzyme activity (Zaka et al. 2002; Al Rumaih et al. 2008; Vandehove et al. 2009; Stajner et al. 2009) and mutagenesis by means of gamma rays which has played an important role in the producing new mutants with improved properties that can produce higher amounts of commercially important metabolites (Sanada, 1986; Abdalhadey et al. 2008). The morphological, structural and functional changes in plants depends on the strength and duration of gamma irradiation stress. The salient findings obtained during present investigations are discussed in order to correlate it with the earlier findings. Following variable doses of gamma rays, the overall effects experienced by the plant has been assessed in terms of growth, development and synergistic interaction between metabolites and physiological morphological, biochemical and enzymatic chemistry of model plant. Attempts have been made to examine, evaluate and demonstrate the findings of the present investigation
recorded in the course of time in terms of dose and response relationship and to
draw the valid conclusions.

**Effect of Gamma radiation on Seed Germination**

The observations recorded during the present investigations provide some useful
information on the effects of gamma radiation of seeds on the process of
germination in *Psoralea corylifolia* L. Following variable doses of gamma rays
germination of *P. corylifolia* was adversely affected especially at higher dosage of
15 and 20kGy (Table. 9). A significant reduction over control in the remaining
doses could also be noticed under field as well as laboratory conditions. Along
with increasing doses of gamma-rays germination ability decreased significantly
and this decrease was more rapid than non-irradiated seeds. These findings were
in accordance with some earlier reports Pandey et al. (1990) in kiwifruit; Cuny
and Roudot (1991) in melon and Sattar et al. (1992) and Kumar and Priyanka
(2006) in soybean; Cuny (1992), Abak et al. (1996) in some cucurbit crops;
Yanmaz et al. (1999) in snake cucumber; Kurtar et al. (2002) in squash; Mokobia
in rice and Nasab et al. (2010) in barley. Reduction in seed germination with
gamma radiation might be due to the increase in production of active radicals
responsible for seed lethality. The total DNA content and bonds intension
decreased with increasing gamma ray dosage which is anticipated main effect of
gamma irradiation on viability of *Psoralea* seed (Jyoti et al. 2009). Higher
exposures of gamma rays may cause injury in seeds (Mehetre et al. 1994) and
usually shows inhibitory effects on seeds of angiosperms and gymnosperms
(Akhaury and Singh, 1993; Thapa, 2004) or damage to chromosomes and mitotic
retardation (Al Safadi and Simon, 1996). Factors affecting seed dormancy include
the presence of certain plant hormones notably abscissic acid which inhibits
germination and gibberillin which ends seed dormancy. Most work in plant
hormones clearly indicate that dormancy and germination are under hormonal
control (Pillay, 1966). Gamma and X-radiation have been shown to affect a plants’ response to growth regulators as well as to affect hormonal activities directly. Low doses apparently inhibit auxin and gibberillic acid synthesis while larger doses can destroy auxin activities directly (Momiyama et al. 1999; Moussa, 2006) and activate the synthesis of ethylene and abscissic acid (D’Innocenzo and Lajolo, 2001). Ethylene and abscissic acid induce dormancy in seeds while indole acetic acid and cytokinins breaks dormancy (Meinke, 1992; Meinke et al. 1994; Subbaiah and Reddy, 2010).

There are some phenolic compounds such as coumarin, chlorogenic acid and their derivatives present in the seed coats of plant species. These may inhibit germination of the seeds that contains them or once leached out into soil may affect neighbouring seeds. The inhibitory substances shown to be present in the seed coats of *Psoralea corylifolia* L. is a crystalline material isolated from the leachate both from the non-glycosidic fraction and from the hydrolyzed glycosidic fraction and identified as the furocoumarin, psoralen (Baskin, 1967). This compound hitherto known to occur in two other species of *Psoralea* and is believed to be responsible for the inhibition of seed germination and root growth observed in this species. The stimulating and inhibitory effects of coumarin may have been due to the action on the storage materials of the seeds (phosphate oxygen ratio) and the coupling action in germinating seeds either directly or indirectly (Bryant, 1985; Mayer and Poljakoff-Mayber, 1989). Gamma irradiation in *Citrus dementia* L. stimulates the biosynthesis of hesperidin corresponding to the maximum of PAL activity and *p*-coumaric acid content was particularly high in irradiated fruits (Oufedjikh et al. 2000).

Low doses of gamma radiation activated biochemical changes in plants while as higher doses inhibited the metabolic changes as recorded during observations. However, increased levels of germination inhibitor psoralen and isopsoralen impair the germination following all doses of gamma rays (Table 20). Despite
increase in protein and sugar levels following lower doses of gamma rays germination is inhibited because of concomitant increase in psoralen and isopsoralen as revealed by HPLC analysis of irradiated seeds. Psoralens and isopsoralens have specific biochemical properties which may contribute to the survival of certain plant species under stress. Specifically these compounds belong to that group of substances which can inhibit certain plant growth without otherwise harming the plant (Bennett and Bonner, 1953; Rodighiero, 1954). The pronounced accumulation in seeds and reproductive organs matches the location of compounds which are principally allocated to the organ that play key role in plant fitness (Katz et al. 1979).

**Effects of Gamma radiation on Growth, development and yield attributes**

Development is whole series of changes such as growth, differentiation and maturation which an organism undergoes throughout its life cycle. Plant growth and development are greatly affected by the environment. If any environmental factor is less than ideal, it limits a plant's growth and development. These environmental factors are perceived sometimes with exquisite precision and response is affected in terms of growth differentiation, development and so on. Many plant varieties with commercial and agricultural importance have been developed using gamma radiation (Donini and Sonnino, 1998; Ahloowalia and Maluszynski, 2001). Gamma rays belong to ionising radiation and interact on atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially on the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the anti oxidative system and accumulation of phenolic compounds (Kim et al. 2004; Wi et al. 2005).
The use of ionising radiation technology may be considered as revolution in agronomic research especially metabolomics and crop improvement (El-Bazza Zainab et al. 2000; Jamil and Khan 2002; Khan et al. 2003a). The first experiments on seeds exposed to relatively low doses of ionising radiation to stimulate growth and development were performed a few years after discovery of X-rays by Roentgen in 1895. Such exposure mainly stimulated seed germination, plantlet growth, flowering, plant size and yield Breslavets (1946). These different results allowed Patskevich (1961) to conclude that irradiation of seeds prior to sowing held great promise from the view point of its practical application in agriculture. It was generally agreed that low doses of gamma rays stimulate cell division, growth and development in various organisms including plants and animals. This phenomenon, named 'hormesis' has been analyzed and discussed by authors in various plant species (Luckey, 1980; Korystov and Narimanov, 1997). However, the way radiation influences plant growth and development is still unknown and the available data remain controversial. Indeed, the magnitude of the reported hermetic effects of radiation is usually small, being approximately 10% of control values and often not providing critical evidence that crop yield are significantly increased by irradiating seeds (Miller, 1987). Moreover, these effects were often reproducible (Miller, 1987) or only transitory (Marchi et al. 1962). More recently, new studies showed that induced anomalies are related to the stage of development where differentiating tissues being most sensitive (Zaka et al. 2004; Singh and Datta, 2010).

A perusal of the response exhibited by variable gamma doses in the present study revealed that exposure to low doses of gamma rays 2.5, 5, 10kGy significantly increased plant growth, dry weight of individual plant parts, total plant biomass, number of leaves and branches, yield attributes of the entire *Psoralea corylifolia* L. (Fig. 4-18). Hence it may be used extensively as a convenient criterion of radiation tolerance in medicinal herbs. All vegetative, reproductive
Discussion

and yield attributes exhibited maximum enhancement at 10kGy at flowering stage. An increment in plant height, root and shoot length could positively be correlated with high nitrogen and carbon content associated with increased number of leaves and branches, increased NR activity and/or high pigment content with low doses of gamma rays. Similar cause could be associated with flower number which reached maximum at 10kGy (Fig. 15). Results of this experiment revealed that with increasing gamma ray dosage, the percentage of germination and emergence, plant height, number of leaves and branches, root length, and shoot length decreased. The highest implement gamma ray dosage 15 and 20kGy had negative and hazardous effects on Psoralea development and growth compared to control.

Several investigators are of the view that stimulation provoked with low doses of ionising radiations in winged bean (Haq et al. 1992; Bai and Sunil, 1993; Khan and Wani, 2005), Bambusa arundinacea (Lokesha et al. 1994), Vitis vinifera (Charbaji and Nabulsi, 1999), in Pinus kesiya and P. wallichiana (Thapa, 2004), in Anacardium occidentale (Klarizze, 2005), in Eruca vesicaria (Moussa, 2006) and in Pisum sativum (Zaka et al. 2004). Cepero et al. (2001) observed maximum stimulating effect of low doses (150 and 180 Gy) on seedling height of Leucaena leucocephala cv. Cunningham. The lower doses of gamma radiation produce a stimulus on the growth of the aerial as well as the underground parts. The hormesis phenomenon, quite often mentioned in the literature, is described as the stimulating effect of any factor on the growth of organisms (Szarek, 2005). In the present study, the hormesis phenomenon is provoked by the gamma radiation impact on the Psoralea seeds as a result of their exposure to $^{60}$Co rays. The study reveals that 10 kGy is a stimulative dose. The radio stimulation established in this study is specific to Psoralea corylifolia L. The hypothetic origins of these stimulations are acceleration in cell division rates (Zaka et al. 2004). The increase observed in growth attributes in response to radiation might be
correlated to stimulatory influence of such doses on the photosynthetic machinery. Moreover, Abbas (1994), found that low doses of radiation 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 irradiation, it is likely that these growth factors mediate at least part of NR induction due to irradiation. Pandey and Sabbharwal (1982) provided evidence that irradiation can activate a biochemical system, induction of NR, indirectly, through the activation of growth regulators in *Psoralea* plants. Hageman and Flesher (1960) established a positive correlation between the NR activity and the growth and protein content of corn. Reinink et al. (1987) indicated that when the plant accumulated nitrate as an osmoticum, more carbohydrates could be used to increase the dry matter production. Low doses of gamma irradiation induces growth stimulation by changing hormonal signalling network in plant cells or by increasing the anti oxidative capacity of the cells to easily overcome daily stress factors such as fluctuation of light intensity and temperature in growth conditions (Kim et al. 2006). Further, increased plant vigor caused by irradiation could be explained by the stimulation of biosynthesis of some amino acids like lysine and phenylalanine (Antonov et al. 1989).

In the present study, using higher dosage of 15 and 20kGy, there were significant decrease or depletion of various quantitative and qualitative traits of *P. corylifolia*, decrease was more prevalent in plants at 15 and 20kGy (Fig. 4-18). Reduction in plant growth with increasing doses of gamma irradiation have been reported in various plants such as lentil (Shaikh and Begum, 1991), chick pea (Haq et al. 1992); Winged bean (Veeresh et al. 1995), *Arabidopsis thaliana* (Davies et al. 1994; Rabie et al. 1996), barley (Khan, 2003b), Pinus (Thapa, 2004) and barley (Nasab et al. 2010). Gamma irradiation caused damage to tissues by producing H$_2$O$_2$ and organic peroxy radicals (Viosine et al. 1991; Wi et al. 2007; Alikamanoglu, 2010).
At higher doses the entire cellular metabolism is grossly impaired resulting in enzyme activity. Gamma and X-radiation have been shown to affect a plant's response to growth regulators, as well as to affect hormonal activities directly. Low doses apparently inhibit auxin synthesis while larger doses can destroy auxin activities directly and irradiated tissues often display production of endogenous ethylene (Antoaszewaki et al. 1978; Khan, 2005). Very high doses of ionising radiation can induce the depolymerisation of pectins and cellulososes in vitro and in vivo (Zhao et al. 1996; Zegota et al. 1999) alteration of Chl structure and function, alteration of enzyme synthesis and activity (Jones et al. 2004; Kiong et al. 2008).

Root, stem, leaf dry weight and total plant biomass exhibited maximum enhancement following 10kGy at flowering stage (Figs. 8, 9, 10 and 14). Low dose had stimulatory effect on plant biomass accumulation while as higher doses resulted in depression of said parameters. The increase in leaf biomass was due to increase in number of leaves and branches at flowering stage (Figs. 13 and 14). The leaf density and leaf area seemed to be most sensitive response to radiation of all parameters measured (number of leaves and branches, leaf area and plant biomass) as it was significantly different from control at lower as well as higher gamma doses applied (Figs. 12-15). Increment in plant biomass with subsequent increase in leaf area has already being attributed to enhanced plant metabolism at flowering stage till 10kGy as conjectured from results of biochemical studies in preceding chapter. At higher gamma doses (15 and 20kGy) significant reduction in root, shoot, leaf dry weight, number of branches, leaves and plant biomass, especially at post flowering stage was observed (Figs. 8, 9, 10, 12, 13 and 14). It is probably due to changes in assimilation capacity in plants where the number of leaves and branches is maximum at 10kGy for plant at all developmental stages. However, the decrease in number of branches and leaves following higher gamma dosage from flowering to post flowering stages is distinct. This might be
due to premature abscission induced as result of increased production of ethylene or inhibition of auxin. Similar results were observed with bean and Impatiens, where irradiation of 175 to 323 kilo roentgens (kR) and 175 to 280kR significantly accelerated the onset of abscission (Dwelle 1975; Strydom and Whitehead, 1990; Buanong and Kanlayanarat, 2010). A decreasing trend in plant biomass with increase in exposure to gamma dose suggests that there was radiation effect on carbon gain. Reduced carbon gain following higher gamma dosage has also been supported by various workers (Ursino et al. 1977, Theide et al. 1995, Kim et al. 2005, Jia and Li, 2008).

Effects of gamma irradiation treatments on the production of reproductive traits as mean number of flowers and seeds per raceme and number of seeds per raceme showed a significant (p<0.05) variation in control as well as in gamma irradiated plants (Fig.16). The maximum enhancement was observed with 10kGy gamma dose. However, a dose dependent reduction was observed with higher doses of 15 and 20kGy. Increment in flower number following low doses of gamma rays has been reported in Zinnia elegans (Veankatchalam and Jayabalan, 1997) and in black gram (Mahalik and Routary, 2010). The increase in seed and flower density per raceme may be attributed as potential of gamma radiation in inducing positive effect on the net yield of plant concerned in association with the specific adaptive response and enhanced radio-resistance.

Number of seeds per plant and weight of 100 seeds showed significant (p< 0.05) increase with low doses of gamma rays (2.5, 5 and 10kGy). The maximum enhancement in number of seeds per plant was noticed at 10kGy, while maximum decline was observed at 20kGy (Figs. 17 and 18). Similar variability in seeds/pod have also been recorded by Sharma et al. (1991), Charumathi et al. (1992) and Gupta and Sharma (1996). The increase in average leaf area per plant (Fig. 14) was expected to enhance the rate and efficiency of photosynthesis, leading to a marked rise in plant biomass and consequently, may be associated
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with improved productivity. Various workers have also reported increment in seed yield following gamma irradiation. At higher doses, number of seeds per plant decreased significantly (Dubey et al. 2007; Mishra et al. 2007; Sharma and Mishra, 2007). Similar findings have also been reported by Sharma et al. (1991) in green gram, Charumathi et al. by (1992) in black gram and by Gupta and Sharma (1996) in horse gram.

**Effects of Gamma radiation on pigment and carotenoid concentration of *Psoralea corylifolia* L.:**

Photosynthesis is of fundamental importance in utilizing the carbon locked up in plant material as a source of bio fuel energy. Because of the fundamental biological significance of photosynthesis and its importance in agricultural productivity, a considerable amount of research has been directed toward understanding the effects of environmental factors on the process. Different species, and in some cases even different cultivars of the same species, vary widely in their ability to tolerate the multitude of environmental stresses that a photosynthesizing plant may encounter (Paul and Foyer, 2001). Both traditional plant breeding methods and the techniques of modern molecular biology are being used to produce plants that can maintain their vigour and sustain high rates of photosynthesis when subjected to environmental stress. Gamma irradiation of seeds induces various physiological and biochemical alterations in plants. The irradiation of plants with high dosages of gamma rays ameliorates the hormone balance, leaf gas-exchange, water exchange and enzyme activity (Stoeva, 2002). These effects include changes in the plant cellular structure and metabolism such as dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds (Wi et al. 2007). Photosynthesis is one of most studied process under the effects of gamma irradiation accompanied mainly by growth experiments. Despite the diversity of gamma ray targets in plants, it seems that
the photosynthetic apparatus is among the main action sites of gamma rays (Kulandaivelu and Noorudeen, 1983). Gamma irradiation of seeds to improve photosynthesis by modulating pigment system has been reported (Kim et al. 2004; Hegazi and Hamedeldin, 2010). A stimulative effect of low doses of gamma radiation on chlorophyll synthesis was noticed. The radio-sensitivity of chlorophyll $a$ and chlorophyll $b$ synthesis varied with the experimental conditions, suggesting that chlorophyll $b$ synthesis might occur independent of chlorophyll $a$ synthesis.

Photosynthetic pigments can be destroyed by higher doses gamma irradiation, with concomitant loss of photosynthetic capacity (Strid et al. 1990). Chlorophylls and carotenoid may be adversely affected by relatively high dosages of gamma irradiation, with carotenoid generally being less affected than the chlorophylls (Pfundel et al. 1992). However, Kim et al. (2004) contradicted this statement by proposing that, chlorophylls are virtually insensitive to low dosage gamma irradiation whereas the levels of carotenoid increase in a dosage dependent manner at the same degree of irradiation. In present study, the chlorophyll contents of gamma irradiated $P. corylifolia$ displayed a gradual decrement as the gamma dosage increased (Tables 10-12). However, low dose had modulatory effect on the concentration of chlorophyll $a$, $b$ and total chlorophyll especially at flowering stage following 10kGy. In addition to that, it can be observed that the concentration of chlorophyll $b$ was relatively higher than chlorophyll $a$ in both irradiated and non-irradiated plants. Earlier it has been reported that gamma irradiation resulted in greater reduction in the amount of chlorophyll $b$ as opposed to chlorophyll $a$ (Strid et al. 1990). This statement contradicts the results of our study. The reduction in chlorophyll $b$ is due to a more selective destruction of chlorophyll $b$ biosynthesis or degradation of chlorophyll $b$ precursors (Marwood and Greenberg, 1996).
In a study on the sensitivity of *Nicotiana tabacum* seedlings to gamma irradiation by Wada et al. (1998), it was found that chlorophyll content was significantly reduced by gamma irradiation when seedlings were exposed to 20 and 50 Gy. In this study, plants raised from seeds irradiated at 15kGy and 20kGy exhibited 32.50% and 41.12% decrement in total chlorophyll content at post-flowering stage as compared to the non-irradiated plants. The negative effect of the gamma rays on the growth limiting factors was mainly related to changes in the chlorophyll content and hence photosynthesis. Loss of Chl contents indicated that Chl accumulation was inhibited by gamma radiation, which improved the activities of chlorophyllase, promoted degradation of Chl and ultimately reduced photosynthetic activity of plants (Chowdhury and Johri, 2003). Similar results recorded in pea seeds when irradiated at (80 and 100 Gy) 23 and 52% decrease in the chlorophyll content was observed, as compared to the non-irradiated seeds. This could be one of the reasons for the reduced photosynthesis rate. However, El-Sayed et al. 1994, found that 10 Krad gamma rays increased chlorophyll a and b and carotenoids in tomato hybrids. In another study by Alikamanoglu et al. (2007), an increase in chlorophyll a, b and total chlorophyll levels was observed in *Paulownia tomentosa* plants that were exposed to gamma irradiation. When compared to the non-irradiated plants, the total chlorophyll increased by 20.6% in *P. tomentosa* that were exposed to 80Gy gamma dosage. Furthermore, in a study on the effects of gamma irradiation on red pepper plants by Kim et al. (2004), the plants irradiated at 16Gy had some significant increase in their chlorophyll content that can be correlated with stimulated growth. Modulation in photosynthesis in irradiated plants might partly contribute to increase in growth (Kim et al. 2004). It was previously shown that gamma irradiation significantly influences the cell metabolism and mitosis in plant meristem cells (Alikamanoglu et al. 2007). Marked varietal differences were not present in the expression of induction of chlorophyll mutations at different doses of gamma rays. All photosynthetic pigment contents were significantly increased as a result
Discussion

of water soaking and gamma irradiation treatments in *Abelmoschus esculentus* (Hegazi and Hamideldin, 2010).

Following higher doses of gamma rays, 15 and 20kGy, there was increase in total carotenoid content (Table 13). Carotenoid functions both as photosynthetic pigments and endogenous antioxidants, through absorbing surplus energy and quenching active oxygen in addition to protecting Chl by absorption of photon energy (Sakaki et al. 1983, Fukuzawa et al. 1998). Similar to this, increase in the level of carotenoid content at low dose under dimethoate (Mishra et al. 2008) and UV-B (Strid and Porra, 1992; Lingakumar and Kulandaivelu, 1993) exposure were observed. Furthermore, plants adapt to environment more widely through high photon energy use efficiency by increasing the proportion of Chl b in reaction centres involved in photosynthesis. These findings are consistent with our results showing positive correlation with growth attributes of *Psoralea corylifolia*. The increase in pigment concentration may also cause increment in phytochemical value of plant, considering the fact that plant biomass and nitrogen content was also increased at flowering following low doses gamma rays.

**Effects of Gamma radiation on soluble protein content of *Psoralea corylifolia* L.**

Besides being an integral factor for plant growth, proteins are involved in signal transduction, anti-oxidative defence system, anti-freezing and heat shock, anti-pathogenesis or osmolyte synthesis which is essential for plant development and physiology (Gygi et al. 1991). Gamma irradiation has been reported to have predilection for proteins, enzymes, DNA, RNA, amino acids, and nucleoproteins in its effects on biological materials (Uwaifo, 1983).

An examination of the average mean values over treatments revealed that the content of leaf soluble protein attained peak values in flowering plants raised from seeds irradiated with 10kGy (Table 14). Values varied from 32.12 mg g⁻¹fw
at the flowering stage to 27.50 mgg$^{-1}$fw at post-flowering stages. Higher gamma doses had depressing effect on protein content at all developmental stages. Again, data for leaf soluble protein and photosynthesis demonstrated that low doses of gamma rays resulted in significant enhancement over the control values for both parameters. The interrelationship between these two has also been reported with other species (Evans, 1983; Makino et al. 1984; Lawlor et al. 1987, 1989; Sinclair and Horie, 1989). Findings have been similar in oilseed crops (Ahmad et al. 1999; Fazli et al. 2005a, b; Jamal et al. 2005). The better response by plants of *P. corylifolia* to low doses of gamma rays may have been due to activation of enzymes and release of energy (in form of packets 'photon') throughout the growth period, especially because they are all involved in the biosynthesis of amino acids, the building blocks of proteins.

It was previously shown that gamma irradiation significantly influences the cell metabolism and protein synthesis in plant meristem cells (Casarett, 1968). According to the results obtained in the present study, it was observed that increased gamma dosage caused a reduction of total soluble protein content especially at post-flowering stage. However, plants irradiated at relatively low dosage (2.5, 5 and 10kGy) displayed a higher total soluble protein content compared to their non-irradiated counterparts. This result demonstrated that there was a direct correlation between gamma dosage and protein content. According to Bajaj et al. (1970) gamma irradiation caused inhibition of tissue culture growth along with failure of RNA and subsequently the failure of protein synthesis. In accordance with the results obtained by Stajner et al. (2007) in soybean seeds, 10Gy dosage caused a slight increase in total soluble protein content, an increase of 11.0% as compared to the non-irradiated seeds. His study further demonstrated that quantity of carbonyl groups in oxidatively modified proteins significantly increased. Introduction of carbonyl groups into amino acid residues of proteins was a hallmark for oxidative modification due to gamma
rays exposure. Gamma irradiation creates oxidative stress and affects biomolecules by causing conformational changes, oxidation, rupture of covalent bonds and formation of free radicals such as the hydroxyl and superoxide anion that were generated by radiation (Variyar et al. 2004). Data obtained by Cho and Song (2000) showed that gamma irradiation, did not induce significant loss in water soluble components such as total soluble proteins, minerals, nitrogenous constituents, and sugars. These free radicals could modify the molecular properties of the total soluble proteins causing oxidative modifications of the proteins (Wilkinson and Gould, 1996). The chemical changes caused by gamma irradiation in proteins were fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals generated in the radiolysis of water (Davies and Delsignore, 1987). These changes depend on the chemical nature of the protein, its physical state, and the irradiation condition (Woods and Pickaev, 1994).

In general, radiation causes the irreversible changes of protein conformation at the molecular level by breakage of covalent bonds of polypeptide chains (Kume and Matsuda, 1995). The general inhibition of protein-synthesis prevents plastid transformation from one type into the other and that is why both greening and degreening could be inhibited by irradiation (Kovacs and Keresztes, 2002). This provides further evidence for decreased photosynthetic pigments following higher doses of gamma rays. During gamma irradiation of tomatoes, protein synthesis was not stopped, but restored to form different set of proteins called as gamma induced proteins (GIP). The function of these proteins is not yet known; but they may be involved in physiological disorders triggered by irradiation or in repair process (Triantaphylides et al. 1994).

**Effects of Gamma radiation on soluble sugar content of Psoralea corylifolia L.**

In addition to essential roles as substrates in carbon and energy metabolism and in polymer biosynthesis, sugars have important hormone-like functions as primary messengers in signal transduction. Biochemical, molecular, and genetic
experiments have supported a central role of sugars in the control of plant metabolism, growth, and development and have revealed interactions that integrate light, stress, and hormone signalling (Roitsch, 1999; Sheen, 1999; Smeekens, 2000; Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002) and coordinate carbon and nitrogen metabolism (Stitt and Krapp, 1999; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001). Variations in the environment can decrease photosynthetic efficiency and result in sugar-limited conditions in parts of the plant, which down-regulate biosynthetic activity to conserve energy and protect cells against nutrient stress while upregulating starch degradation and protein and lipid catabolism to sustain respiration and metabolic activity (Yu, 1999; Fujiki et al. 2001). Soluble sugar has been postulated to act as regulatory molecules that help to control seed and embryo development (Borisjuk et al. 2004).

In present investigation, the sugar content increased at low doses with maximum enhancement at flowering stage, declined thereafter and there was a marked drop in sugar levels of plants raised from seeds irradiated with 15 and 20kGy (Table 15). Such a decline in sugar content at post flowering stage following higher gamma dosage is correlated with observed suppression in pigments. Increased soluble sugar content at flowering stage may be associated with increased photosynthetic activity. The maximum increase in sucrose content of both potato tubers and sweet potato roots was achieved by an irradiation dose of 3 to 4 kGy for potatoes and 0.8 to 2 kGy for sweet potatoes (Ezekiel et al. 2007). Further, free sugar, glucose, sucrose and maltose were significantly increased by 7.5 and 10 kGy gamma irradiation in fruits (Byun et al. 1997). A statistically significant increase in reducing sugars following low doses of gamma rays may be due to some changes in starch distribution. The increase in sugar content supports some changes in the grain starch granules which have been reported earlier (Blaszczak et al. 2002; Gralik and Warchalewski, 2006), where ionising
radiation caused visible changes in the microstructure of kernel starchy-endosperm at 10kGy. MacArthur and D'Appolonia (1983), supported increase of endogenous amylolitic enzymes which enhance sugar content. Machaiah and Pednekar (2002) also noted distinct increase in reducing sugar over control in whole wheat flour irradiated with 1kGy. In our study, 15 and 20kGy, there was gradual drop in soluble sugar content especially at post-flowering stage (Table 15). Water-soluble components such as total solids, nitrogen, sugars, and minerals were appreciably eluted in the highly-irradiated samples at above 10 kGy (Byun et al. 1997). The ratio is low in dry seeds and increase during germination. Increment and decrease in soluble sugar content following low and high gamma doses respectively has been reported in *Eruca vesicaria* subsp. *sativa* (Moussa, 2006).

The decrease in sugar contents of plants under maturation stage can be due to two factors, the leaf senescence induced by the gamma radiation and the cycle end of the crop, because the reserves as nitrogen compounds and sugars are transported to the sink. Other explanation is the photosynthetic reduction through stomatal closure and consequent lower entrance of CO$_2$ in leaf tissue, as well as degradation of photosynthetic pigments promoted by oxidative stress during irradiation. Drought, salinity, low temperature and flooding, in general, increased soluble sugar concentrations, whereas high light irradiance (PAR, UVB), heavy metals, nutrient shortage and ozone decreased sugar concentrations (Dubey and Singh, 1999; Gill et al. 2001). Increased simple organic molecules such as sugar, free amino acids, and total soluble phenols might act as an osmotica for the regulation of plant osmosis, and decrease in nitrate accumulation in the cell vacuoles and consequently better plant growth and yield (Muller and Touraine, 1992; Imsande and Touraine, 1994). Sugar sensing and signalling are also implicated in the regulation of leaf senescence that coincides with a decline in chlorophyll content and photosynthetic activity (Jiang et al. 1993; Bleecker and Patterson, 1997; Quirino et al. 2000).
Effects of Gamma radiation on free amino acid in *Psoralea corylifolia* L.: 

Free amino acids (AA's) represent a significant source of available N for plants and soil microorganisms. Amino acids contribute to carbohydrate synthesis via gluconeogenesis, to fat synthesis or energy production via acetyl-CoA and special nitrogen compounds such as alkaloids, hormones and contribute to nucleic acid and phospholipids synthesis as nitrogen group donor. A considerable volume of literature is available concerning the influence of radiation on protein and protein constituents in animals and animal products. However, very little is available concerning the radio sensitivity of such materials in living plant tissue. Plants synthesize all of their essential amino acids in highly complex and normally coordinated array of enzymes and processes. After gamma and neutron irradiation of pea seeds, an inverse correlation between growth and degrading enzyme activity has also been reported (Bagi et al. 1988). Gamma radiation has varied effects on free amino acids of oil bearing seeds. The present study describes the variation of free amino acid in growing plants raised from seeds irradiated at different gamma dosage. Irradiation of grains and legumes resulted in some loss of essential amino acids, particularly those containing sulphur (Khattak and Klopfenstein, 1989).

Radiation resulted in significant changes in the free amino acid concentrations in growing plants. In our study, free amino acid pool increased in concentration with increasing doses of gamma rays, with significant increment following 10kGy dose at flowering stage. At 15 and 20kGy, there was varied response of free amino acid pool (Table 16). Thus the free amino acid content of growing plants showed a varied response to ionising radiation characteristic of each of the amino-acids and the dose received. Although the concentration of all aminoacid decreased ultimately with increasing radiation, the different thresholds and the rates of disappearance indicated different radiation effects for each amino acid. A decrease in the amino acid concentration would be expected following...
irradiation if only radiochemical events were occurring. Such has been reported after the irradiation of numerous solutions of pure amino acids (Iqbal et al. 1974; Moussa, 2006) and proteins (Ciesla et al. 2000). The deviation of the results obtained in this study from those obtained in pure solution represents the influence of both the complex chemical mixture and the activity of the biological systems within the cell. Because of increase in the process of photosynthesis at low gamma doses, carbon precursors necessary for the increase of amides will result in increase of free amino acid. Increased simple organic molecules such as sugar, free amino acids, and total soluble phenols might act as an osmotica for the regulation of plant osmosis (Moussa, 2006).

**Effects of Gamma radiation on nitrate content and nitrate reductase activity in *Psoralea corylifolia* L.**

The nitrogen metabolism impairment must be one of the primary determinant for growth and development of the plants. Nitrate assimilation pathway 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 latter is involved in mediating various steps of carbon cycle and nitrogen is involved in the development and maintenance of photosynthetic machinery and leaf organs (Srivastava, 2001). 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 (Cheng et al. 1992, Vincentz et al. 1993, Huber et al. 1994, Kaiser and Huber, 1994). NR activity is positively correlated with protein content, nitrogen accumulation, free amino acid content and better yield in several crops (Ahmad et al. 1998, 1999). Furthermore, leaf 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 and NR activity in leaves were higher at flowering stage following low doses of gamma rays raised to peak values at 10kGy. NR activity in *P. corylifolia* L. decreases significantly with increase in gamma dosage applied, maximum reduction being observed at 20kGy (Table 17 and 18). This observation is in agreement with results noticed in *Haworthia* callus, *Ustilago*, *Agrostemma* gethago by exposure to varied doses of gamma rays (Moussa, 2006). The induction of nitrate reductase could be due to hormone production in these tissues. The above hypothesis is supported by our observations that exogenous hormones induce nitrate reductase in un irradiated tissues. Moreover, Abbas (1994) found that low doses of -radiation 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 irradiation, it is likely that these growth factors mediate at least part of NR induction due to irradiation (Pandey and Sabhrawal, 1982). This study provides evidence that irradiation can activate a biochemical system, induction of NR, indirectly which may be through the activation of growth regulators in *Psoralea* plants. The activity of NR is directly correlated to nitrate concentration and plays an important role in protein synthesis.

Nitrate content in *Psoralea corylifolia* leaves decreased significantly at high doses at all the developmental stages. However, low doses of gamma rays had a stimulatory effect on nitrate accumulation. Similar results were found in Sakha rice cells exposed to variable doses of gamma-rays before culturing on PEG. It was slightly stimulated, the accumulation of total nitrogen, increase in protein synthesis or to increase in nitrate absorption and assimilation in these cells relative to the control (El Shafey et al. 2009).

Similarly, NR activity in control conditions, increased with plant age upto flowering but after that in post flowering it again declines. Decline in NR activity following higher gamma radiation might be a result of reduced uptake of
nitrogen or adverse effects of NR enzyme structure itself (Tukendorf and Rauser, 1990). Hageman and Flesher (1960) established a positive correlation between the NR activity and the growth and protein content of corn. Reinink et al. (1987) indicated that, when the plant accumulated nitrate as an osmoticum, more carbohydrates could be used to increase the dry matter production. Seginer et al. (1998) concluded that there was a negative correlation between the concentration of soluble carbohydrates and nitrate in the cell sap and so, as irradiation increased the carbohydrate content (Nouri and Toofanain, 2001) and nitrate accumulation decreased. Thus, based on our and previous works, we believe that pre-sowing irradiation at the dose of 10kGy can be used for preventing excessive nitrate accumulation, yield increase and yield quality improvement of *Psoralea corylifolia* L.

**Effects of Gamma radiation on proline content in *Psoralea corylifolia* L.**

It is evident from results that free proline, known to accumulate in plant tissues following all doses of gamma rays would contribute to scavenging of surplus of free radicals produced under a high gamma dosage applied (Fig. 19). Carletti et al. (2003) found similar results in UV-B-irradiated maize seedlings. They claim that it is worth nothing that proline may play a regulatory part in plant metabolism and even small increase in proline biosynthesis might have a large impact on the extent to which the cellular NADP pool was reduced. In addition, Fedina et al. (2006) suggested that the response of barley seedlings to UV-B depended both on the content of UV-B-induced compounds and on the level of free proline. Baek et al. (2006) observed that contents of proline and abscissic acid (ABA) were higher in the gamma irradiated rice plants.

Proline accumulation may reduce stress-induced cellular acidification or prime oxidative respiration to provide energy needed for recovery. High levels of proline synthesis during stress may maintain NAD(P)+/NAD(P)H ratios at values compatible with metabolism under normal conditions. The increased
NADP+/NADPH ratio mediated by proline biosynthesis is likely to enhance activity of the oxidative pentose phosphate pathway. This would provide precursors to support the demand for increased secondary metabolite production during stress as well as nucleotide synthesis accompanying the accelerated rate of cell division upon relief from stress, when oxidation of proline is likely to provide an important energy source for ADP phosphorylation (Hare and Cress, 1996).

Interaction of nitrogen, sulphur and carbon metabolism

The nutrients available for plant uptake may be present in ratios much different from those needed by plants. Sulphur is primarily a constituent of amino acids and proteins. The stoichiometry between nitrogen and sulphur is fairly constant, indicating that on average there are 36 atoms of nitrogen for each atom of sulphur in proteins. Net carbon storage in any ecosystems is determined by the balance among three distinct processes: (1) the net amount of carbon fixed by the plant (i.e. carbon fixed in excess of plant respiratory demand); (2) the relative amounts of this fixed carbon allocated to vegetation growth increment versus plant litter; and (3) the decomposition of carbon compounds entering the soil system. All three of these processes are regulated by temperature, moisture and the availability of key nutrients such as nitrogen, phosphorus, and sulphur (Dijkshoorn and Van Wijk, 1967).

The absorption of sulphate from excess supply may be faster than its reduction and assimilation of the sulphur atoms into organic compounds. Thus, an appreciable fraction of total sulphur in plants may be in the form of sulphate (Epstein, 1972; Turner et al. 1980). Several studies have established regulatory interactions between sulfate and nitrate assimilation in plants (Brunold, 1990; Takahashi and Saito, 1996; Kim et al. 1999; Koprivova et al. 2000). The two assimilatory pathways are well coordinated so that deficiency for one element represses the other pathway. The activities of ATP sulfurylase, APR, and OAS
(thiol) lyase decreased under nitrogen-deficient conditions in *Lemma minor* and cultured tobacco (*Nicotiana tabacum*) cells (Reuveny et al. 1980; Smith, 1980; Brunold and Suter, 1984). On the other hand, in plants, sulfur deficiency results in a reduction of nitrate reductase (NR) activity and an accumulation of amino acids (Reuveny et al. 1980; Migge et al. 2000; Prosser et al. 2001), whereas in cyanobacteria, NR is decreased and nitrite accumulates. However, the reduction of NR activity and mRNA levels seems to be a relatively late process in plant adaptation to sulfur-limiting conditions (Prosser et al. 2001).

Very little is known about the interactions of sulphur and carbon assimilation. It is clear that sulfate assimilation is dependent on photosynthesis as a direct or indirect source of reduction equivalents, as demonstrated by the light dependency of sulfate reduction by broken chloroplasts (Schmidt and Trebst, 1969). The flux through sulfate assimilation is lower in the dark than in the light (Kopriva et al. 1999). On the other hand, sulfur limitation reduced growth and photosynthesis of the green alga *Dunaliella salina* (Giordano et al. 2000). The addition of Sulphur to Arabidopsis plants in the dark induced the accumulation of APR mRNA, protein, and enzyme activity, revealing that sulfate assimilation is directly regulated by carbohydrates (Kopriva et al. 1999).

**Effect of gamma irradiation on nitrogen content in various plant parts at different developmental stages**

Leaf and stem nitrogen content in *Psoralea corylifolia* L. plants were highest during flowering stage following low doses of gamma rays but declined at higher doses (Fig. 27) i.e. plants irradiated with 15 and 20kGy. The content of nitrogen in stem was less than that of the leaves. However, roots show lowest nitrogen at all developmental stages. Our results are in agreement with findings of Maman et al. (1999) that content of nitrogen in most tissues of crop plants were high during early stage of growth and decreased with advancement of plant age. The demand for nitrogen is closely related to plant growth, and
nitrogen deficiency, after water stress is the most frequently reported limitation to growth (Kozlowski, 1971; Kramer and Kozlowski, 1979). The ratio of total nitrogen to total sulphur in plants is close to this value under conditions where there is no luxury consumption of either element (Epstein, 1972). Many studies have shown that nitrogen can be taken up in excess and stored as amino acids, for example, arginine (Barnes and Bengtson, 1968; Kramer and Kozlowski, 1979). Nitrogen is known to affect the plant growth primarily through leaf expansion and secondarily, through rate of photosynthesis (Angus and Moncur, 1985).

Low doses of gamma rays played modulatory role by increasing total nitrogen content in all plant parts at different phenological stages with maximum enhancement following 10kGy at flowering stage (Figure 27). However, higher doses 15 and 20kGy resulted in depression of plant nitrogen especially at post-flowering stages. Maximum nitrogen was observed in leaf at flowering stage indicating increment in nitrogen available for incorporation into reproductive parts. Gamma irradiation was reported to affect the mineral content of several plants. An increase in nitrogen content was found by Maltseva and Kuzin (1975) when *Vicia faba* seeds were irradiated with 0.1 and 10 k-rad of gamma rays. Nitrogen and phosphorus contents of cabbage, onion and carrot were also increased by 0.1 to 1.25 k-rad gamma irradiation (Rennie and Nelson, 1975). Habba (1989) exposed seeds of *Hyoscyamus* and *Atropa* spp. to 1-2.5 k-rad gamma irradiation and also reported an increase in nitrogen percentage. Deaf (2000) reported an increase in nitrogen, phosphorus and potassium contents of lemongrass when seeds exposed to 1-4 k-rad gamma irradiation. Mahmoud (2002) indicated that gamma irradiation increased phosphorus and potassium content of *Delphinium* plants. Contradictory results were reported; for example a decrease in nitrogen content was found in *Capsicum annuum* (Iqbal, 1974), Zham and Voloozh (1976), however, reported that irradiating tomato seeds with 2.5 k-rad gamma rays did not affect the concentration of nitrogen or phosphorus which is contradictory to our findings.
Effect of gamma irradiation on sulphur content in various plant parts at different developmental stages

Sulphur content in leaf and stem of *Psoralea corylifolia* L. plants were highest during flowering stage following low doses of gamma rays but declined thereafter. This decline coincided with plants irradiated with 15 and 20kGy (Figure 28). The content of sulphur in stem was less than that of the leaves. However, roots show lowest nitrogen at all developmental stages. Significant increase in sulphur content of leaves was recorded with low doses of gamma rays. Maximum sulphur content was recorded in plants raised from seeds irradiated at 10kGy. Sulphur content was found to be highest in leaves, stem and roots respectively. Roots showed minimum sulphur content as compared to other plant parts. Ionising irradiation has a pronounced effect on protein sulphydryl group as investigated in animal, there is a paucity of research done on sulphur metabolism in plants (Jo and Ahn, 2000). Cysteine, lysine and glutathione are known to increase after low dose gamma irradiation (Khare et al. 1982). Ionising radiation inhibited the utilization of methionine (Horvatic and Grüner, 1993). Ionising radiation caused an increase in the levels of glutathione (GSH) and the activity of a GSH synthesizing enzyme and some superoxides. Similarly an increased level of the glutathione peroxidase activity (Marchenko et al. 1996) after low doses of gamma irradiation has also been reported in corn (*Zea mays* L.).

Effect of gamma irradiation on carbon content in various plant parts at different developmental stages

Carbon content in leaf and stem of *Psoralea corylifolia* L. plants were highest during flowering stage following low doses of gamma rays but declined thereafter. This decline coincided with plants irradiated with 15 and 20kGy (Figure 29). The content of carbon in stem was less than that of the leaves. However, roots show lowest carbon at all developmental stages. Minimum
carbon content was recorded in roots at post flowering stage. Significant carbon gain in leaves was recorded with low doses of gamma rays. Results of our experiment indicate that the photosynthesis and sugar content was increased in plants raised from seeds irradiated with low doses of gamma rays. These results were almost in agreement with those of Saccardo et al. (1991); Asmahan (1993); Cailian et al. (1993); El-Sayed et al. (1994); Al-Oudat and Razzouk (1994); Zeerak et al. (1994); Al-Kobaissi et al. (1997); Gautam et al. (1998); Asmahan (2000); Rasico et al. (2001) and Osama (2002), who reported that the improvement of yield components and carbon containing compounds (chlorophyll parameters and carotenoid) in various plants such as tomato, maize, rice and wheat was induced after gamma rays. The decreasing trend in average biomass with higher doses suggests that there was a radiation effect on carbon gain. Reduced carbon gain was also supported by Ursino et al. (1974) who found that photosynthesis was reduced by half in Pinus strobes exposed to 10Gy. Higher doses of gamma rays were not lethal and they caused very little visual damage to the plants. Presence of significant differences between total biomass would suggest change in the rate of fixed carbon. Our study exhibited that radiation resulted in decrease in leaf area at higher doses of gamma rays, while showing negative relationship to photosynthesis and a strong positive relationship to leaf density. Similar results have been observed in sunflower plants exposed to variable doses of gamma rays (Thiede et al. 1995).

**Effect of gamma irradiation enzymatic activities in leaf tissues of Psoralea corylifolia L. at different developmental stages**

Exposure to ionising radiation results in generation of reactive oxygen species leading to cytotoxic, mutagenic and carcinogenic effects (Kuroda et al. 1999). The hydroxyl (HO·) and superoxide (O₂·) radicals that are generated by irradiation could modify the molecular properties of proteins and lipids, causing oxidative modifications of proteins and lipid peroxidation (Halliwell and Gutteridge, 1989;
Stajner et al. 2007). Plants respond defensively to oxidation by removing the ROS and maintaining antioxidant defence compounds at levels that reflect ambient environmental conditions (Scandalios, 1993). ROS themselves play a role in intracellular redox sensing, activating antioxidant resistance mechanisms, among other adaptive processes (Toledeno and Leonard, 1991; Karpinski et al. 1997; May et al. 1998). Enzyme capacities of leaves significantly increased with increase in gamma dosage and decrease with plant age. In present study, gamma irradiated plants showed significant enhancement in superoxide dismutase (SOD) activity (Fig. 21), especially during flowering stage. The relatively low activity during post-flowering stage could be because of old age leaves generally contain low concentration of antioxidants and this is why the old leaves are more prone to enhanced oxidative injury than the young leaves (Polle, 1997). In the present study all anti oxidative enzymes like SOD, APX and GR (Figs. 21-23) respectively showed an increasing trend up to flowering stage followed by decreasing trend during senescence of plants, which is in accordance with study of Morimura et al. (1999) on roots of Japanese radish and Donahue et al. (1997) in pea plant.

Several reports with other plants provided evidence of enhanced activities of APX (Singh et al. 1993; Zbikowska et al. 2006, SOD (Pramanik, 1997; Wada et al. 1998; Zaka et al. 2002; Stanjer et al. 2009) and GR (Foyer et al. 1991) by gamma radiation treatment. Involvement of ascorbate peroxidase, (Sharabash et al. 1988) SOD, Blokhina (2003), attributed induction of peroxidases, SOD and GR activities to enhanced production of toxic ROS levels in living organisms under stress. It is well established that oxygen-derived free radicals are produced on radiation exposure to living cells. Peroxidases and SOD are known to have a profound role in production and breakdown of hydrogen peroxide and other superoxide molecules (Klotz and Lagrimini, 1996). According to Karpinsiki et al. (1997), the APX activity induction in Arabidopsis submitted to oxidative stress conditions
such as high light intensity, takes place by induction of apx1 and apx2 gene transcription.

The stimulation of SOD activity is possibly due to positive encoding allele, in response to low radiation doses as shown in different biological models (Schiavone and Hassan, 1988; Scandalios, 1993; Inze and Van Montagu, 1995). The increase in SOD activity could be consequence of denovo synthesis of enzymatic protein (Slooten et al. 1995; Allen et al. 1997). It is important to emphasize that significant SOD activation following low doses of gamma rays depends on the dosage and plant developmental stage or just plant age in the original environment of plants, indicating acquisition of SOD gene regulation as well as that observed for GR and APX in the same conditions, is function of the severity of the ionising radiations. Cho et al. 2000 confirmed this finding indicating increased transcript levels of the genes controlling the biosynthesis of SOD, GST and POX enzymes upon irradiation of *Nicotiana tabacum* seeds. Zaka et al. (2002), have also suggested the possibility of irradiation induced SOD and APX activities at low doses. This over expression probably occur by an efficient regulatory mechanism, adjusting when necessary enzyme expression by positive regulation of corresponding genes to provide cells with resistance.

GR may be a rate limiting enzyme for defence against active oxygen toxicity (Tanaka, 1994). Increase in GR activity in higher plants is result of enhancement of the transcription rate of encoding gene (Foyer et al. 1991). Increased activity of this enzyme was reported earlier in cotton when subjected to elevated atmospheric O₂ (Foster and Hess, 1980) in Mg⁺⁺ deficient bean leaves (Cakmak and Horst, 1991) and in peas fumigated with ozone (Hurkman and Tanaka, 1987). The reduced glutathione pool has to be maintained at high levels so that it does not become limiting for the synthesis of phytochelatins. GR radio-induction in *Stipa* was reported by Zaka et al. 2002) due to its key role in the ascorbate-
Discussion

glutathione pathway. Over expression of APX, SOD and GR activity could be compared to that observed in various plant or animal system submitted to a pre-radiation leading to an increase in free radical scavenging ability at higher doses. The results presented in preceding chapter, revealed induction of APX activities concomitant with suppression of CAT activity in leaves at all developmental stages following variable doses of gamma rays. Peroxidases was considered to be key enzyme for decomposition of endogenous H$_2$O$_2$ content especially under CAT inactivation (Fig. 24). Decline of catalase activity and increase of peroxidase activity was also reported by Rathore et al. (2003) in Triticum aestivum, in sugar cane (Singh et al. 1993), tobacco under ionising radiation stress (Willekens et al. 1994; Liang et al. 2000). CAT is irradiance-sensitive and easy to be inactivated by irradiating (Feierabend and Engel, 1986). There exists a rapid turnover of CAT-like D1 protein of PS2, which is generally sensitive to stress conditions combined with irradiance (Streb et al. 1993). Decreased levels of CAT and increased levels of peroxidase induced by oxidative stress, reflected a general strategy required to overcome the stress and protect the cells against damage.

Lipid peroxidation products in leaves were expressed as TBA (thiobarbituric acid) reactive compounds (mainly malondialdehyde, MDA). The lipid peroxidation process, causing membrane disintegration, is believed to be initiated by free radicals and has extensively used as a marker of oxidative stress (Huggett et al. 1992). It has been reported that prolonged irradiation of wheat seeds with UV led to an increase in level of lipid peroxidation in sprouts (Rogozhin et al. 2000). A dose dependent decrease in triacyl glycerol content and concomitant increase in free fatty acids was observed in chickpea (Hameed et al. 2008). Though lipid peroxidation found to be maximum at post flowering stage, however, there was not a significant injury to plant (Fig. 20). This suggests radioresistivity of Psoralea corylifolia L. which may be because of better adaptability or acclimatization to the doses of gamma rays applied.
Effect of gamma irradiation on anti-oxidant system in leaf tissues of *Psoralea corylifolia* L. at different developmental stages

Plants have developed a complex antioxidant system that includes reduced glutathione (GSH), ascorbic acid, α-tocopherol, carotenoids and enzymes that protect the plant against oxidative damage (Salin, 1991; Dalton, 1995). Ascorbate is classically described as an excellent reducing agent. It readily undergoes two consecutive, yet reversible, one electron oxidation processes to form ascorbyl radical (Asc•−) as an intermediate. Because Asc•− has its unpaired electron in highly delocalized π-system, it is relatively unreactive free radical. These properties make ascorbate a superior donor and small-molecular antioxidant (Demming and Adams, 1992; Zbikowska et al. 2006). Ascorbate can readily repair all oxidizing free radicals with greater reduction potential, which includes hydroxyl radicals as major target. In addition to its role in the scavenging cycle, ascorbate acts as reductant in the regeneration of anti-oxidants and in the zeaxanthin cycle (Foyer, 1993). In the present study, ascorbate content was increased with increase in exposure to gamma dosage and plant age. The maximum increase in total ascorbate content was observed at post-flowering stage with 20kGy (Fig. 25).

Increment in ascorbic acid and riboflavin was also observed in case of soya bean seeds, where soyabean seeds were irradiated at 0.20kGy gamma dosage (Sattar et al. 1992; Kim et al. 2005). Ascorbate produced in response to gamma rays at higher doses may be because of its role as reductant in the APX-mediated scavenging of H₂O₂. Contrary to our findings, irradiation reduced the ascorbic acid concentration, primarily in frozen corn. Irradiation converts ascorbic acid to dehydroascorbic acid, which has biological activity similar to that of ascorbic acid. Therefore, the loss in total ascorbic acid (ascorbic acid and dehydroascorbic acid) is likely to be much smaller than the loss in ascorbic acid alone (Fan and Sokorai, 2002). Further, Singh (1996) also reported significant reductions in the...
ascorbic acid content of three leguminous plants (Vigna radiata L., Phaseolus mungo L., and Glycine max L.) after exposure to enhanced UV-B radiation. Increased radioresistivity of P. corylifolia, can be partly explained by higher levels of SOD and APX and ascorbate content. Because limits on ascorbate, a substrate for APX and an antioxidant itself, can inhibit APX activity thereby contributing to improved stress resistance. Increment in ascorbic acid is parallel to decrease in number of leaves and branches at post-flowering stage. An increase in ascorbic acid, decrease reduced form of glutathione (GSH), total glutathione, total non-protein thiol (NPSH) and non-glutathione thiol (RSH) occurred as a result of induction of dormancy, whereas dehydroascorbic acid and oxidized glutathione (GSSG) decreased during the same period.

GSH, a disulphide reductant that protects thiols of enzymes, regenerates ascorbate (As) and reacts with singlet oxygen, hydrogen peroxide and hydroxyl radicals. In some plants, such as legumes, homoglutathione (glutamyl cysteinyl alanine) may partly or wholly replace glutathione. It acts as a protein disulphide reductant, which detoxifies herbicides by conjugation, either spontaneously or by the activity of one of a number of glutathione-S-transferase and regulates gene expression in response to environmental stress and pathogen attack. GSH is depleted by ionising radiation whether X-rays or UV rays from sunlight (Biaglow et al. 1989). All forms of corn system, investigated responded to gamma radiation by increasing the level of the glutathione peroxidase activity and fall of GSH pool. The GSH quantity increased as irradiation dose increased to 140 Gy (27.0% greater than control) and then decreased at highest irradiation dose in soyabean seeds (Stajner et al. 2009). However, No effect of irradiation was observed on concentration or reduction state of the non-enzymatic antioxidants, ascorbate and glutathione in Arabidopsis thaliana (Vandenhove et al. 2010).

The decreased levels of GSH in Psoralea corylifolia L. following exposure to higher doses especially at post-flowering (Fig. 26) suggests depression in sulphur
metabolites as well as detrimental effects on thiol or sulphydryl groups. It is assumed that GSH (Wingate et al. 1988) or GSSG (Winglsee and Karpinsiki, 1996) or change between GSH and GSSG (Foyer et al. 1997) may function as signals for activating stress-responsive gene expression in stress situation.

**Effect of Gamma radiation on protein profile of seeds exposed to variable doses of gamma rays**

Regardless of the irradiation dosage, electrophoretic patterns are qualitatively and quantitatively quite similar. It was evident in this study that the major protein bands were at 17, 26, 34, 43, 55, 76, 95, 130 and 170 kDa (Fig. 30). Protein related functional properties in the cell were influenced by various factors, such as protein denaturation, size, structure and conformation, charge, amino acid composition and amino acid sequence of the protein molecules (Zayas, 1997). He also demonstrated that the denaturation of globular proteins due to gamma irradiation tends to promote the exposure of previously buried non-polar protein sites, leading to increased hydrophobicity and, subsequently, decreased solubility. In accordance to the results obtained by Abu et al. (2005) in the study of *Vigna unguiculata*, gamma irradiation caused an alteration in the protein banding profile which was probably due to the fact that irradiation caused an increase in the ratio of exposed hydrophilic to hydrophobic amino residues. Regarding the radiation damage to proteins, there are two types of damages observed, fragmentation and aggregation (Filali-Mouhim et al. 1997). Gamma irradiation caused the breakdown of polypeptide chain and formation of small molecular weight molecules (Cho and Song, 2000). Similar results were observed in another study of the effect of gamma irradiation on plant proteins by Le Maire et al. (1990). Usually, breakage of covalent bonds in irradiated proteins is shown as new bands below the major band. Also, proteins irradiated may be converted to higher molecular weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, and the formation of disulfide bonds (Davies and Delsignore, 1987).
The formation of the high molecular weight protein aggregates was negligible at low dosages, but increases significantly with increasing dosage. As observed in the present study, the 26kDa band was only present in the seeds irradiated at 2.5kGy, 5 and 10kGy. This may indicate that 26kDa protein had association with other proteins leading to the formation of higher molecular weight polymers that probably could not pass through the gradient gel and thus absent in protein extracts of plants irradiated at higher dosages. According to Sharabash et al. (1988) SDS-PAGE profiles of the irradiated wheat plants showed that gamma-irradiation, at low dosages, caused a slight breakdown of the polypeptide chain with a concurrent decrease of major band intensity under loading of the same amount of the protein. At high dosage above 100Gy, there were cross-linked products of the degraded protein molecules that could not penetrate the running gel (Sharabash et al. 1988). The degradation of protein could be attributed to the direct effect of radiation since 10% of the radiation energy is absorbed directly by the proteins. In the cases of radiation dosages of 35 and 45Gy, drastically different protein profile was observed (Vu Kovij et al. (2005). He indicated that protein fragmentation was more pronounced at the lower radiation dosages up to 100Gy, while protein cross-linking prevailed at the radiation dosages above 150Gy. However, present study doesn’t describe any fragmentation of protein even at higher dosage of 15 and 20kGy as evident in (Fig. 30).

Effect of Gamma radiation on Furanocoumarins content in different plant parts at various phenological plant parts

Psoralea corylifolia L. is known to synthesize diverse phenylproponiod such as furanocoumarins, isoflavanoids, cinnimic acid derivatives etc. (Bourgaud et al. 1989). Psoralen is the major and most active furanocoumarins, possessing biological properties as they exert antiproliferative and photosensitising actions used in dermatological practice (Pathak et al. 1981; Matern, 1999). Psoralen has been found to intercalate of long wavelength UV light and thus are used for the
treatment of hypo-pigmented lesions of the skin like leucoderma (Vaidya, 2006). Previous studies on *Psoralea* species dealt with furanocoumarins distribution in different plant organs regarding their chemotaxonomic role, as well as their feeding deterrent activity (Cappelletti et al. 1982; Zobel and Brown, 1991; Innocenti et al. 1997; Calcagno et al. 2002).

The morphology of *P. corylifolia* led us to separate the plants into vegetative and reproductive parts at various phenological stages. Seeds were collected at harvest stage. In order to cultivate *P. corylifolia* L. for psoralen production, biomass and psoralen content of each vegetative and reproductive part need to be evaluated. Psoralen has been reported to accumulate primarily in leaves, roots and seeds (Table 19). The variation in distribution of psoralen was found associated with developmental stages of leaves, roots and seeds in view of changes in the defensive value of in all said parts. Rapid increase in psoralen synthesis was observed in seeds especially in those irradiated at higher dose rates. Such findings are consistent with defence role of psoralen. The co-occurrence of psoralen and isopsoralen in seeds seem to be chemical marker of genus *Psoralea*. Furanocoumarin distribution in vegetative and reproductive parts of *Psoralea corylifolia* L. following variable doses of gamma rays at different phenological stages was significantly increased. Using TLC and HPLC methods, in present study, it was observed that the accumulation of psoralen is closely correlated with developmental stages of leaves and seeds. Furanocoumarin distribution patterns within reproductive structures are of special interest in connection with their suggested role as germination regulators for plant itself, as allelopathic agent, and as chemical defence against biotic and abiotic stress. The importance of recording phenological changes of furanocoumarins content with vegetative and reproductive structures in order to evaluate the physiological and/or ecological significance of these compounds has been stressed (Berenbaum and Zangrel, 1986; Nitao and Zangrel, 1987).
During 80's the influence of gamma-irradiation on different properties of some aromatic and pharmaceutical plants was investigated by various authors (Aladjadjian and Svetleva, 1997; Aladjadjian and Ylieva, 2003, Aladjadjijian, 2007) and the possibilities of improving parameters of *Verbacum pseudonohile* L. (alkaloids content), *Calendula officinalis* L. (carotenoids content), *Hissopus officinalis* L. (flavonoids, saponins, etheric oil), *Salvia sclarea* (flavonoids content) were recorded. Pre-sowing seed irradiation is one of the most effective methods to improve plant production, yield components and chemical composition (Khan, 1970; Selena and Stepanenko, 1979). Studies have been carried out to elucidate the effect of gamma rays on some aromatic plants such as chamomile (Selena and Stepanenko, 1979; peppermint (Zheljazkov et al. 1996), Youssef and Moussa, 1998), lemongrass (Deaf, 2000 and *Mathiola incnna* and *Delphinium ajacis* (Mahmoud, 2002).

Increment in psoralen content following doses of gamma rays may be due to photo activation of certain enzymes utilized in the production of psoralen, as many furanocoumarins have photosensitizing activity (Hamerski and Matern 1988; Eckey-Kaltenbach, 1994). Most plants accumulating furanocoumarins possess a highly inducible biosynthetic pathway, which can be triggered by various biotic (Katz et al. 1979) and abiotic stresses (Perez-Rodriguez, 2003). Further, enhancement in psoralen content may be conjectured as plant defence against gamma radiation. The pronounced accumulation in seeds and reproductive organs matches the location of compounds, which are principally allocated to the organ that play key role in plant fitness (Katz et al. 1979). Moreover, gamma irradiation was known to increase the activity of phenylalanine ammonia lyase, which is responsible for the synthesis of polyphenolic acids (Oufedjikh et al. 2000). The ability of gamma irradiation to increase polyphenolic acids in plant material has also been observed in soybeans. Soybean samples treated with gamma irradiation at levels ranging from 50 to
150Gy had increase free polyphenolic acids (Variyar et al. 2004). Siddharaju et al. (2002) attributed such increase in polyphenolic acids to higher extractability by depolymerization and dissolution of cell wall polysaccharides due to gamma irradiation. As coumarins absorb UV radiation and thereby protect plant from excess sunlight (Chong et al. 2002), some coumarins like scopolamine also display radical scavenging properties towards reactive oxygen and may be involved in the reduction of oxidative stress in plant cells (Chong et al. 2002). The evolution towards furanocoumarins accumulation must have occurred under strong selection pressure, since the biocidal and enzyme inactivation properties of furanocoumarins appear to be lethal to plants unless quick adaptation can be accomplished. The increase in the quantity of some constituents as affected by gamma irradiation may be attributed to the fact that some natural chemical constituents are released from their precursors due to the degradation resulting from irradiation. Adamo et al. (2004) and Harrison and Were (2007) reported that phenols are increased by gamma irradiation due to the release of phenolic compounds from glycosidic components and the degradation of polyphenolic compounds into soluble phenols as well as other small metabolites.

**Effect of gamma radiation on essential oil content and oil composition of seeds**

The volatile oils were obtained by hydro distillation of seeds in *P. corylifolia*, collected at harvest stage. Comparing the integration peaks, there were significant differences in total volatile oil content between treatments. Overall, gamma radiation enhanced total volatile oil content of seeds (Table 22 - 26). Differences in the relative content of furanocoumarins and flavonoids between the gamma irradiated and control treatments were significant. Gamma irradiation enhanced the levels of these major non-volatiles oils significantly, in line with the increase in total content of volatile oils. The extracts from the untreated and gamma-irradiated samples of *Psoralea* seeds were pale yellowish liquids with a characteristic, terpenic, powerful odour of babchi trait. The
Discussion

Qualitative compositions of volatile oils obtained from the control and from irradiated samples of seeds at various doses were not identical. These results were also confirmed by GC-MS analysis. The steam-volatile babchi oil consisted primarily of monoterpenes and sesquiterpenes hydrocarbons, and phenylpropanoids compounds (Table 22-26). The GC/MS and SPME analyses revealed more than five hundred compounds from which more than fifty four compounds were identified by the mass spectra and linear retention indices published. Tricyclene, α and β-pinene, sabinene, germacrene D are the important components of the monoterpenes fraction. β-Caryophyllene is the major sesquiterpene and angelicin, psoralen and bakuchiol are the main compounds of volatile oil of Psoralea seeds according to the quantity. Psoraleon composition increase (49.35%) two fold with 15 and 20 kGy as described in Table 25. The gamma irradiation dose of 15 and 20 kGy induced a significant increase in other volatiles in comparison with the control. The control sample had fewer levels of all fifty four compounds Table (22-26) as confirmed by GC-MS and SPME analyses. There were exceptional changes as observed in the volatile oil compounds contents at radiation doses of 5 kGy and 10 kGy (toxicologically and nutritionally confirmed as safe maximal dose). These findings are in agreement with the results of (Calucci et al. 2003 and Seo et al. 2007).

The most important change could be observed at an ionising dose of 20 kGy (2-times excess of the authorised dose) resulting in triple increase of caryophyllene, double increase in psoralen, bakuchiol in comparison with the control. These changes can be explained through the irradiation effects on terpenes. Though, irradiation does not show any thermal effect on the flavour compounds but via one of the following reaction pathways, i.e. via oxidation or hydroxylation of the aromatic ring of terpene, or via an indirect effect, which can generate free radicals from the water contained in seeds (approx. 10%) (Urbain, 1986). These radicals can react with terpenes to produce terpene oxides and terpene alcohols.
On the other hand, terpenes, which are incorporated in most of the essential oils, have the same skeleton structure but differ in their functional groups, such as OH, CHO, or COOH. Therefore, configurational changes can occur by high doses, including changes in the positions of the double bonds and the functional groups to produce different compounds (Sadecka, 2010). There is evidence that phenol contents of medicinal plants are increased when they are irradiated with ultraviolet B (Kumari et al. 2010) while gamma and electron beam ionising radiation did not induce any detectable qualitative or quantitative significant changes in the contents and yields of essential oils immediately after ionising radiation of some medicinal herbs (Haddad et al. 2007). Flavonoids in methanol extract of medicinal plants relatively resist the effect of ionising radiation compared with other extracts but our results describe the radio sensitivity of isoflavones like bakuchiol. This finding is against the early investigations that flavonoids are instable in aqueous solution (Scalia and Mezzena, 2009). Moreover, Kozlowski et al. (2007) reported that radiolysis of flavonoids in ethanol or methanol solution resulted in the formation of new antioxidants depsides.

Gyawali et al. (2006) reported that though the content of majority of volatile compounds of dried Welsh onion was increased after different doses of gamma irradiation (1, 3, 5, 10 and 20 kGy), the content of major sulphur-containing compounds such as 1-propanethiol, 5-methyl thiazole, propenyl propyl disulfide, dipropyl disulfide and 3,5-diethyl-1,2,4-trithiolane was decreased after the process. But γ-irradiation up to 20 kGy did not bring qualitative change in the volatile constituents of dried Welsh onion. Sulphur-containing compounds such as dimethyl disulfide, dimethyl trisulfide, methyl propyl disulfide and methyl propyl trisulfide were highly increased by irradiation at 10 kGy. In a study on nutmeg, Variyar et al. (1998) found that the essential oil constituents showed clear quantitative differences upon gamma irradiation. Thus the content of α-
In contrast to our study, significant quantitative changes were noted by Variyar et al. (1998) in some of the phenolic acids in clove and nutmeg upon irradiation with 10kGy dose. They reported that the content of gallic and syringic acids in irradiated clove was increased, whereas that of p-coumaric, ferulic and synapic acids decreased to approximately half to that of control spice and that of caffeic and gentisic acids remained unchanged. Fan and Gates (2001) found that irradiation of orange juice reduced the concentration of acyclic monoterpenes such as geranial, neral, myrcene and linalool 1 and 7 days after irradiation. The concentrations of monocyclic monoterpenes such as α-pinene, were not influenced by irradiation while as valencene, a bicyclic sesquiterpene was also resistant to irradiation. It appears that the ring structure of terpenes protect these compounds from degradation induced by gamma irradiation. Also, it was reported that the content of several volatile compounds was increased after γ-irradiation of licorice. In comparison to nonirradiated licorice, 10 kGy dose of γ-irradiation induced the maximum level of total yield of volatile compounds such as 2-ethoxy-1-propanol, ethyl acetate, hexanol, hexan ol, [E]-2-tetradecenal, γ-nanolactone, ρ-cymen-8-ol, acetic acid, 2-pentylfuran and α-terpineol (Gyawali et al. 2008).