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Effects of Chronic Ionizing Radiation and Interactions with Other Environmental and Climatic Factors on Plant Growth and Development

J.E. Olsen and S.B.Dineva
Trakia University - Stara Zagora; Faculty of Techniques and Technology;
http://tk.uni-sz.bg
Yambol 8602, "Gr. Ignatiev" str. №38, Bulgaria

Abstract

Plants are the main supportive human being system. Under insistently exposure to mutagens, such as low ionizing doses radiation, enhance level UV-B radiation, chemicals, heat, drought, and cold, they are enforces either to adapt either to die. Ordinarily is accepted that the living organisms under the influence of environmental stress factors, always acquire adaptive responses, but the available data still stay controversial. The effects of chronic exposure on living organisms and populations still stay insufficiently explored, and denote a much needed field of research. The aim of a review is to summarize published data for consequences of chronic ionizing radiation on plant growth and development. Epigenetic and genetic alterations were registered in plants arising under combined influence of different environmental stress conditions. Nevertheless, there are still not enough information for the combined effects of ionizing radiation, enhance level UV-B radiation, which are already registered as results from climatic changes and so expected to have important role in the future on plants populations. The increased pollution of the environment is out of the doubt, but the knowledge about mechanisms and the range of plants to adapt is still insufficient.

Keywords: Low ionizing doses radiation, UV-B radiation, combined effects, plant populations.

Introduction

Ordinarily, plant species are mostly and frequently used for mitigation of adverse environment, and for improving living conditions of human. They are recognized as the main supportive system of human life, and will ever take such important place. The mechanisms of which chronic ionizing radiation influence on growth and development of plants is still unknown and the available data remain provocative. The information for the effects of high-levels ionizing radiation (IR) on plants is more available than for chronically low-doses, the reason may be is that studies onchronical effects require many years to be completed, while investigationhigh-level radioactive radiation produce more clear results in a quite short time(Mergen and Stairs, 1962).The literature is also still limited regarding effects of smaller short-term doses on plants, on a range of doses below 10 Gy.With the rising problem of environmental radioactive pollution, generating relatively low radiation doses in polluted areas, it is necessary to collect reliable data on those effects of such radiations on biological organisms (Zaka,*et al*, 2004).

Recently many researchers indicates that ionizing radiation causes persistent genetic effects in the distant progeny of exposed cells (O'Reilly *et al.,* 1994; Barker *et al.,* 1997; Brodsky *et al.,* 2000; Barber *et al.,* 2002; Kiuru *et al*., 2003; Zaka *et al*, 2004).Abramov *et al.* (1995) reported that the peak of mutations observed in *Arabidopsis* populations from Chernobyl appeared two years after an accident. Under conditions of chronic ionizing radiation at low rates, plants are increasing the genetic load in the next generations(Abramov *et al.,* 1995). In some cases very low doses, apparently harmless for G1 plants, induce in G2 the same effects as 10 Gy in G1 plants have been stated from some researches (Zaka *et al.,* 2004). These results are in good agreement with those recently obtained on animals (Barber *et al.*, 2002) and humans (Kiuru *et al.,* 2003).Embryos originating from male and female gametes of G1 irradiated plants usually bear modified genetic information that is expressed at a lower level in G2 individuals compared to the treated (G1) generation. Indeed, in G2, the apparent threshold dose is not 10 Gy but 0.4 Gy, which is the irradiation dose from which male fertility and seed production are significantly disturbed. This indicates that in the range of low and moderate doses, irradiation tends to have greater effect on meiotic activity in the second generation (Zaka *et al*, 2004).

Kovalchuk *et al.* (2000) reported much higher frequency of homologous recombination (HR) in plants of *Arabidopsis thaliana* (L.) Heynh exposed to chronic irradiation when compared to acutely irradiated plants. While acute application of 0.1–0.5 Gy did not lead to an

* Corresponding author : Dr. Snejana Boycheva Dineva
e-mail: sbdineva@abv.bg

increase of frequency of HR, the chronic exposure of the plants to several orders of magnitude lower dose of 200 μGy led to a 5–6-fold induction of the frequency of HR as compared to the control. Also the effect was more pronounced when seedlings were irradiated, due to more active metabolism and higher water content (Kovalchuk *et al,* 2000).Many authors are agree that it is necessary to develop studies on inheritance of the effects of low and very low doses radioactive radiation on plants in order to obtain models potentially useful in conservation biology and radio-protection for humans (Zaka *et al,* 2004).

soil characteristics, with terminal temperatures for most living organisms occurring below 100°C. Through soil heating, fire can directly alter the size, activity, and composition of the microbial biomass. The immediate effect of fire on soil microorganisms is a reduction of their biomass. However, very less information exists regarding how fire affects soil microorganisms over the long-term, and whether any of the changes in the composition or activity of these organism's feedbacks to impact the forest plant community.

Theodorou and Bowen (1982) found, after 4 weeks of a bushfire of moderate intensity, an increase in microbial numbers in the burned soil in comparison with the control. Bauhus *et al*., (1993) found that fire could promote autotrophic bacteria over chemotrophic bacteria because of the soil enrichment in mineral salts. These authors also found a higher bacteria/fungi ratio in the burned soil caused by the rise in pH after fire.

Long-term responses of soil microflora to fire may be primarily due to alterations in plant community composition and production because of the strong interrelationships between plants and soil micro organisms. In fact, the peak temperatures often considerably exceed those required for killing most living beings (DeBanoet *al*., 1998). In extreme cases, the topsoil can undergo complete sterilization. Adverse effects on soil biota can be due to some organic pollutants produced by the combustion processes. Heat also indirectly affects survival and recolonization of soil organisms through reduction and modification of organic substrates, removal of sources of organic residues, buffering and every other eventual change to soil properties (Bissett and Parkinson, 1980). On the other hand, as demonstrated by Wardle *et al*., (1997) for boreal forests of *P. sylvestris*, continued fire suppression may lead to late secondary succession under which microbial activity declines.

Dose-response patternan environmental risk assessment of low doses ionizing radiation

Despite the fact that radiation protection standards and dose limits are legitimately established for humans, there is impending legislation for the protection of the environment (Vanhoudt et al, 2014). The effects of low doses ionizing radiation is a matter of important debate over the last few years (Goldstein and Stawkowski, 2014). The main challenge for environmental risk assessment is the extrapolation of data (Calabrese, 2004). Most discussions concern the validity of the linear dose–response extrapolation for low doses, used by international organizations, to establish radio-protection norms (Zaka *et al*., 2002). In the field of plant studies, doses vary from a few Grays (Gy) and centigray (cGy) up to several hundred Gy and kGy, with an acute or chronic type exposure. In order to develop a framework for the assessment of the environmental impact of radiation, it is necessary to establish the relationship between exposure (dose rate, accumulated dose) and the effects that may be induced. Dose range response is strongly dependent on the species studied, stage of development and etc. (Kovalchuk *et al,* 2000), thus it is difficult to predict a standard response to IR in plants. However, some patterns do emerge.

Practically, it seems impossible to compare the experimental data on plant responses to IR as the models and factor conditions varied greatly. Thus, the type of irradiation, acute or chronic, the dose rate,the applied dose, the plant species (variety, cultivar), the developmental stage at the time of irradiation, and also individual response variations (Zaka *et al.,* 2004; Boyer *et al*., 2009; Kim *et al.,* 2009).The degree of the radiation effects is dependent on the species, age, plant morphology and physiology, genome size and composition. Woody plant species, in general, tend to be less resistant to IR as compared to herbaceous species (Holst and Nagel, 1997).However, it can be broadly concluded that, although minor effects may be seen at lower dose rates in the most sensitive species and systems, the threshold for statistically significant effects in most studies is about 10^2 μGy h^{-1}. The responses then increase progressively with increasing dose rate and usually become very clear at dose rates>10^3 μGy h^{-1} sustained for a large fraction of the lifespan (Real *et al.,* 2004).

The occurrence of hormesis is becoming broadly discussed, especially in toxicology and radiation biology (Luckey, 1980). Various studies report hormesis effects such as growth stimulation following irradiation with relatively low doses of ionizing radiation (Sax, 1954; Miller, 1987;Marcu*et al.,* 2013).A typical hormetic curve is either U-shaped or has an inverted U-shaped dose–response, depending on the endpoint measured. If the endpoint is growth or longevity, the dose–response would be that of an inverted U-shape; if the endpoint is disease incidence, then the dose–response would be described as U- or J-

shaped (Calabrese, 2004). Hormesis is an adaptive response with distinguishing dose-response characteristics that is induced by either direct acting or overcompensation-induced stimulatory processes at low doses. In biological terms, hormesis represents an organismal strategy for optimal resource allocation that ensures homeostasis is maintained (Calabrese and Baldwin, 2002).

Study the percentage of cells with chromosome aberrations or micronuclei induced by low doses of acute (dose rate of 47 cGy/min) or chronic (dose rate of 0.01 cGy/min) gamma-irradiation in vitro in Chinese hamster fibroblasts, human lymphocytes, and *Vicia faba* seeds and seedlings, revealed that the sensitivity of the indicated biological entities to low doses was greater than expected based on linear extrapolation from higher doses. Authors supposed that the induction of DNA repair occurs only after a threshold level of cytogenetic damage and that the higher yield of cytogenetic damage per unit dose at low radiation doses is attributable to an insignificant contribution or the absence of DNA repair processes. The dose-response curves for cytogenetic damage that were obtained were nonlinear when evaluated over the full range of the doses used. At very low doses, the dose-response curves appeared linear, followed by a plateau region at intermediate doses. At high doses the dose response curves again appeared linear with a slope different from that for the low-dose region. There was no statistically significant difference between the yields of cells with micronuclei induced by low doses of acute versus chronic irradiation (Zaichkina *et al.,* 2004).Dose-effect curves on chromosome aberrations in root meristem cells of *Pisum sativum* plantlets in the dose rangeof 0-10 Gy also showed non-linear responses with a plateau for doses up to 1 Gy (Zaka *et al.,* 2002). In *A. thaliana*, Kovalchuk *et al.* (2007) showed that the genome is regulated differently depending on whether the irradiation was chronic or acute. Growth responses of *Arabidopsis thaliana* (L.) Heynh, to a gradient of chronic gamma-radiation demonstrated a significant, but non-linear, response for three variables, number of seedlings emerging, plants flowering, and plant volume. Flowering and plant volume were the most sensitive indicators of radiation exposure. The response of number leaves per plant was not related to daily exposure. LD50 values ranged from 66 R/20 hour day for plant volume to 1231 R/20 hour day for seedling emergence (Daly and Thompson, 1975).Joiner *et al.* (2001) showed that most cell lines have hyperradiosensitivityto very low radiation doses, which is not predicted by back extrapolation of the cell survival curve from higher doses. Such nonlinear data have led to the recent view that biological effects of ionizing radiation should not be extrapolated from high to low doses based on the LNTmodel.Manyyears ago it was shown by Russel (1965) that the mutation yield per unit dose was higher at low doses of radiation than at high doses. Similar results were obtained by studying radiation-induced cytogenetic damage (Luchnikand Sevankaev, 1976; Pohl-Rulling *et al.,* 1983; Lloyd *et al.,* 1988; Zaichkina*et al.,* 1997), transformation (Oftedal, 1990), and cell survival (Joiner, 1994;Joiner *et al.,* 1996).

For carcinogens, regulatory agencies accepted that risk is directly proportional to exposure in the low-dose zone and consequently, there is no safe level of exposure, no level is completely harmless. This so-called linear non-threshold (LNT) dose–response model has become the standard model for assessing the health risks of chemical carcinogens and radiation by regulatory agencies in many countries (Calabrese, 2004). The LNT model is in conflict with three other models, the threshold model, which proposes that low doses are harmless; the radiation hormesis model proposes that small doses can be beneficial; the supralinear model suggests that ionizing radiation at very low doses is more harmful per unit dose than radiation at higher doses (Moore, 2002; Tredici, 1987).

Currently, radiation protection of the environment and conservation of ecosystem sustainability is of a special concern. Nevertheless, the information on dose-effect relationships at low doses for non-human species is limited despite its importance. The development of a harmonized approach to human and biota protection has been recognized as a challenge for modern radiobiology and radioecology. In this framework, much more information on non-human species response to low level radioactive radiation exposuresis needed.

Plant-test models using for carry-onphysiology, epigenetics and genetics research

Radiation safety standards limiting radiation exposure of man and doses at which radiobiological effects in non-human species were not observed after the Chernobyl accident (Fesenko *et al.,* 2005).

A methodological approach for a comparative assessment of ionizing radiation based on the use of Radiation Impact Factor (RIF). However, no internationally agreed criteria or policies for protection of the environment from ionizing radiation till now exist. It is difficult to determine or demonstrate whether or not the environment is adequately protected from potential impacts of radiation under different circumstances (ICRP, 2003). In the framework of ICRP a task group has been established aimed at substantiating a representative set of critical species and indicators for estimating radiation effects (Williams, 2003).

Table 1. Commonly used plants as biomonitoring system

Plant-test model	Used for monitoring	Endpoint	References
Arabidopsis thaliana(L.) Heynh.	gamma radiation; chemical mutagenesis;	germination rate, survival rate and growth; embryonic test; gene expression; comet assay; enzyme capacity responsible for antioxidative defence mechanisms (SOD, APOD, GLUR, GPOD, SPOD, CAT, ME)	McKelvie A.D., 1965; Daly andThompson, 1975; Abramov *et al.,* 1995; Kim *et al.* 2014; Kovalchuk*et al.* 2000; 2007;Vandenhove *et al.,* 2010a, b; 2014;
Pinus sylvestris L.	gamma radiation	cytogenetic alterations in seedling root meristem; enzymatic locipolymorphism; abortive seeds; cytogenetic alterations in coleoptiles of germinated seeds; length of sprouts;	GeraskinandVolkova , 2014; Geraskin *et al.,* 2010, 2011, 2012; Arkhipov *et al.* 1994; Kalchenkoand Fedotov 2001; Kalchenko *et al.* 1993a, b; Kovalchuk *et al.* 2003; RubanovichandKalchenko 1994; Shevchenko *et al.* 1996
Vicia faba	chemical mutagenesis; chronic and acute gamma radiation	chromosomal aberations; micronuclei;	Amer *et al.,* 1969; Rank *et al.,* 1994; Ma *et al.,* 2005; Zaichkina *et al.,* 2004;
Allium cepa L.	gamma radiation chemical mutagenesis	growth parameters - germination rate, survival rate and growth; mitotic index and micronuclei %; chromosomal aberrations; chromosome fragmentation; chromosome stickiness and clumping;	Vaijapurkar *et al,* 2001; Mohandas and Grant, 1974; KumariandVaidyanath,1989; Grant, 1978; Fiskesjo, 1995; Ma *et al.* 2005;
Phaseolus vulgaris	gamma radiation	stem elongation; number of internodes and leaf dry weight; photosynthetic pigment composition;ribulose 1,5-bisphosphate carboxylase(Rubisco) activity;glutathione S	Arena *et al*., 2014

Pisum sativum	low doses of short-term gamma irradiation	germination rate, survival rate;growth (plant size andweight); reproduction (podnumber per plant,seed number per pod); meiotic anomalies(micronuclei); qualitative biochemicaltraits(seed storage proteins);	Zaka *et al.,* 2004

Many studies have shown that air, water, soil and food are often contaminated with mutagens and carcinogens, which increase environmental carcinogenic hazards. For that reason monitoring of genotoxic compounds in the environment has become an important objective of public health. Plants are used for monitoring the presence of chemical and physical mutagens in polluted habitats. Higher plants provide valuable genetic assay systems for screening and monitoring environmental pollutants (Ecobichon, 1997). The assessments with higher plants confirmed that plant genotoxicity assays are highly sensitive, only with few false negatives in predicting carcinogenicity of test agents (Ennever et al. 1988). There are about 233 plants that have been used in various aspects of mutagenic research (Sherby 1976). Some of them as onion (Allium cepa, 2n=16), Mouse ear cress (*Arabidopsis* thaliana, 2n=10), Hawks beard (Crepis capillaris, 2n=6), Soybean (Glycine max, 2n=40), Barley (Hordeum vulgare, 2n=14), Spiderwort (Tradescantia clones, 2n=12), Broad bean (Vicia faba, 2n=12) and maize (Zea mays, 2n=20) are the best worked out assays for gene mutation, mitotic and meiotic chromosome aberrations, micronucleus (MNC), sister chromosal exchange (SCE) and the comet assay that evaluates DNA damage (PandaandPanda, 2002). Several numbers of assays have been validated and standardized to stimulate routine use in the detection of environmental mutagens (Grant, 1994).

The International Program on Chemical Safety (IPCS) collaborative study on higher plant genetic systems for screening and monitoring environmental pollutants was initiated in 1984. It is a cooperative venture of the United Nations Environment Program, the International Labour Organization and the World Health Organization. Its goal was to develop methodologies for improving the assessment of risks from chemical exposure (Grant and Salamone, 1994; Gopalan, 1999). Under the sponsorship of the IPCS, 17 laboratories from diverse regions of the world participated in evaluating the utility of four plant bioassays for detecting genetic hazards of environmental chemicals (Sandhu *et al.,* 1994). For screening and monitoring environmental pollutants, are choosing the *Arabidopsis thaliana* white embryo and the *Tradescantia* stamen hair test for gene mutation assays, while the *Vicia faba* root tip and *Tradescantia* micronucleus test were chosen for chromosal aberration (Ecobichon,1997).

Plant bioassays for detection and screening hazardous environment, chemical-induced cytogenetic aberrations and gene mutations existed from many years (Grant, 1994). Many tests have been recommended to regulatory authorities, the advantages of these assays to make them ideal for screening potential mutagens and carcinogens are shown on table 2 (Grant, 1994).

Table2. Selection criteria for IPCS Collaborative Study on Higher Plant Genetic Systems.

1. Ease of use.
2. Well-developed methodology
3. Used by a number of investigators
4. A large data base on chemical mutagens
5. Adaptability of protocols to different climatic conditions
6. Ease of distribution of source material

From Grant (1994)

The most commonly assaysused for studying mutagenicity of various pollutants in plants are based on the detection of chromosomalaberrations in *Allium cepa* (Fiskesjo, 1995, Ma *et al.* 2005),*Tradescantia* (Ichikawa, 1992), *Vicia faba* plants (Kanaya *et al.,* 1994) or *Zea mays* (Grant and Owens, 2006).*Allium cepa*roots chromosomal aberration (AL-RAA) and micronuclei (AL-MCN) tests are widely employed toevaluate the genotoxicity of many chemical compounds and environmental pollutants. These assaysare are good and sensitive methods for

monitoring clastogenic effects (Grant, 1982; Ma *et al.,* 1995). An *Allium cepa* chromosome aberration test thatcan serve as a rapid screen for toxic effects of chemicals is among them (Grant, 1994м Bolle *et al.* 2004).The advantages of the *Allium cepa* test are that it is a fast and inexpensive method, easy to handle,gives reliable results. Dueto its sensitivity, the *Allium cepa* test was the first of nine plant assay systems evaluated by the Gene-Tox. Not only known chemicals but also water-soluble compounds (e.g. saltsolutions), heavy metals and complex environmental mixtures are studied by the *Allium cepa* test:river and lake waters, waters of well, chlorinated drinking water, domestic and industrialwastewaters, leachate of landfill, industrial waste, soil samples and soil extracts have been studiedusing this test (Fiskesjö, 1985; Cabrera *et al.,* 1999; Monarca *et al.,* 2003).Furthermore, the test can be used to measurealso toxicity, studying macroscopic parameters as length of roots, variations in form, colour andconsistency of roots, presence of broken root tips, tumors and hooks (Fiskesjö, 1985).*Allium cepa* was exanimated as test plant model for study ionizing effects on morphological features such as the number of root and length of root formation, and shoot formation but the evidence were not enough confidence to accept them as a biological indicator for lower gamma dose measurement (Vaijapurkar *et al.,* 2001).

Another suitable plantfor detecting especially different types of hazardouscondition in the environment is *Tradescantia* (Ma *et al.,* 1996). There are two maintests: the stamen hair mutation (Trad-SH) test and themicronucleus assay (Trad-MCN). The first is based onthe heterozygosity for flower colour in Tradescantiaclones. Clone 4430 is a hybrid of *T. hirsutiflora*and*T. subacaulis* reproduced only asexually, throughcloning. The visual marker for mutation induction is aphenotypic change in the pigmentation of the stamencells from the dominant blue colour to recessive pink(Ma *et al.,* 1994a). The Trad-MCN test is based on the frequencyof micronuclei in tetrad cells induced in male meioticcells by the tested mutagen (Ma et al., 1994b). These tests may beused under laboratory, or in situ exposure conditions,for monitoring air or water, or for testing radioactiveor chemical agents (GichnerandVeleminský, 1999; Knasmuller, 2003; Cebulska–WasilewskaandPlewa, 2003).

Among plant based bioassays, the *Vicia faba* is considered as favorable for evaluating the environmental quality, by DNA damages and abnormalities in cell division. Various chemicals have scored positive in the *Vicia faba*-based sister chromatide exchange assay (Rank *et al.* 1994, Ma *et al.* 2005).The use of *V. faba* chromosome aberration has been ongoing for decade.*Vicia faba* seeds (cv. Giza 1) were planted in gamma radiation field and chronically irradiated with gamma-rays (392—2075r)during the whole life of the plant. Chronic irradiation of *Vicia faba* plants did not reduce pollen fertility. The percentages of the induced abnormal pollen mother cells (P.M.Cs) as well as the frequency of abnormal P.M.Cs in the different meiotic stages were proportional with the given doses. The main types of chromosome aberrationswere anaphase and telophase bridges, fragmentation and lagging chromosomes. Thenearest plants to the source showed an inhibition of shoot growth, flower and seed sterility andirregular branching. The most dominant type ofanomaly was the presence of micronuclei in the different stages of mitosis and in the restingcells (Amer, 1969).*Vicea faba* offers many advantages and is ideal for use by scientists in the field of environmental mutagenesis for screening and monitoring of genotoxicity, cytotoxicity and mutagens according to the standard protocols and genetic makeup is similar to other living organisms (LemeandMarin-Morales, 2009; Kristen, 1997).

In some systems, e.g. in testswith maize, morphological changes of the pollen are used, or in the case of *Arabidopsis thaliana*, changes inthe color of the embryos. Soybean (*Glycine max*) and tobacco (*Nicotiana tabacum*), formation of mosaicismwhich leads to leaf spots varying in their color and morphology; detection of somatic crossing over,chromosome deletions, nondisjunction and point mutations are used (Vig, 1982).

A new approach to biomonitoring, which involve stransgenic plants is based on the integration intothe plant genome of a marker gene of knownsequences that will serve as target for mutagenic influences.Essential progress in generation and development of transgenic plants asbiomonitors has been made (Lebel *et al*. 1993; Kovalchuk *et al.* 1998 and 1999; Ries *et al*. 2000; Kovalchuk*et al*. 2001; Li *et al.* 2006; Boyko *et al*. 2007; Van der Auwera *et al.* 2008). Two different transgenic systems were designedto study mutagenic influence via point mutationsand homologous recombination events (HR).One of theimportant advantages of transgenic biosensors is the ability to customize the assay in accordance withmonitoring needs. Transgenic plant biomonitors used for the evaluation of genotoxicity are *Arabidopsis thaliana*and *Nicotiana tabacum* plants (Kovalchuk and Kovalchuk, 2008).

Arabidopsisthaliana (L.) Heynh,(Mouse-ear Cress, or Thale Cress) is currently the most popular plant-test model, with first sequenced genome.*Arabidopsis thaliana* (L.) Heynh is self-compatible weedy species with a worldwide distribution, often used as a model, because of its small genome, easy growth in lab

conditions, and also it is self-fertile. It has proved to be a useful organism for mutationresearch because of its short life cycle and morphologically distinctivemutants that can be induced. Approximately 1000 mutants were produced in an attempt tolook for mutagenic agents giving high mutation rates and offering prospects of mutationspecificity (McKelvie A.D., 1965).Mutant of*Arabidopsisuvh1*, is hypersensitive to bothUV-B and UV-C light wavelengths and to ionizing radiation.*Uvh1* plants showed chlorosis, wilting, and extensive cell deathfollowing exposure of leaves to small, acute fluencies of UV-Bor UV-C light that did not affect wild-type plants. In addition, irradiation of uvh1 seeds with y-rays inhibited the productionof the first true leaves at much lower doses than those neededto similarly affect wild-type plants. These hypersensitive mutantphenotypes are due to a single, recessive mutationprobably located on chromosome 3.Additional *uvh* mutants, and five of these mutantsare currently being characterized in detail (Greg *et al.,* 1994). Otherradiation-sensitive mutants of *Arabidopsis* have recently beendescribed. A UV-B-hypersensitive mutant was isolated usinga root bending assay and was shown to have a defect in repairof 6-4 pyo (Britt *et al.,* 1993).Its small stature and short generation time facilitates rapid genetic studies. It grows from far north to equatorial location within a wide climatic and latitudinal range that makes it an excellent model for studying natural variation in adaptive traits. Most examples of heritable epigenetic variation for plants have come from experimental models such as maize (*Zea mays* L.), *Pinus sylvestris* L., and*Arabidopsisthaliana* (L.) Heynh (Richards, 2006;Mousseau *et al.,* 2013).Surveys on genomic consequences of gamma radiation and chemical induced mutagenesis have been widely applied with plant test model *Arabidopsisthaliana*(L.) Heynh(McKelvie, 1965; Abramov et al, 1995; Kovalchuk et al, 2007).

*Pisum sativum*is determined as radiosensitive plant mentioned in the NATO document AC/25-WP/79 about the effects of radioactive fallout on food and agriculture (Zaka *et al,* 2004).*Pisum sativum* has been used for studying all the cytological endpoints that followtreatment of chromosomes by chemical and physical agents. (Grant&Owens, 2002). Detailed descriptions of these assays can be found in Plewa (1982), Sandhu *et al.* (1989), Grant (1994) and Kanaya *et al.* (1994), Ma *et al.* (1994 and 1995).The relevance of higher plant genotoxic bioassays has been discussed in detail (Fiskesjo,1995; Grant, 1994; Grant&Owens, 2002).The advantages in utilizing plant systems have been reviewed by many authors(MannandStory, 1966; Nilan, 1978; Conte *et al.* 1998).The most serious disadvantage of a plant system forthe detection of genetic risks to man is the lack of similarity between vegetative and mammalian metabolism.

Pinus sylvestris(Scots pine) have been widely used as a study organism for estimation the consequences after ionizing radiation, because it is common and widespread in the regionnear Chernobyl, also these pines are more susceptible to the negative impact of radiation than many other species of trees (Arkhipov *et al.,* 1994; Kalchenko and Fedotov, 2001; Kalchenko *et al.,* 1993a, b; Kovalchuk *et al.,* 2003; Rubanovichand Kalchenko, 1994; Shevchenko *et al.,* 1996).*Pinus sylvestris*, L. has become one of the primary test objects for ecological and genetic monitoring due to its widespread distribution, similarity of its radio sensitivityto that of humans, reproducibility and sensitivity of the available experimental endpoints (Geraskin *et al.,* 2003).Coniferousplants generally show a high retention capacity and low turnoverrate for contaminants taken up by the aerial biomass from the atmosphere, an assessment of cytogenetic anomalies in the intercalar meristem of young needles alsoappears to be a promising test system. In either case, the damage to the DNA mainlyappears as chromosome aberrations at the first mitosis (Geraskin *et al.,* 2003).

1. Biological indicators measuring consequencesof gamma radiation

Ionizing radiations induce morphological, genetical, physiological and biochemical changes, that vary with plant species, irradiation dose and type.

1.1. Morphological criterion

Typically as morphological parameters for estimation radiosensitivity are used several characters describing plant growth: germination, root test analysis, percentage ofplant survival,seedling lengthand weight, growth reduction or stimulation. The frequently observed symptoms at low dosages are early germination and inhibition at high dosages (Sax, 1963; Luckey, 1980; Sagan, 1987; Planel *et al.,* 1987; KorystovandNarimanov, 1997; CharbajiandNabulsi, 1999; Kim *et al.,* 2000; Toker *et al.,* 2005; Wi *et. al,* 2007; Ling *et al.,* 2008; Melki and Marouani, 2009; Borzouei *et al*., 2010; Wi *et al.,* 2005;Minisi *et al*, 2013; ChaudharyandAgrawal, 2014), and reduced growth characteristics parallel with increasing the radiation dosages (Dwelle, 1975; ChandorkarandClark, 1986; Kim *et al.,* 2000; Zaka *et al*., 2004; Toker *et al.,* 2005; Wi *et al.,* 2007; Kon *et al.,* 2007; Vanhoudt *et al.,* 2014; ChaudharyandAgrawal, 2014). In most cases fluctuations in growth criteria are observed, but with not clear pattern and dose-dependent curve. Treatmentof *A. thaliana*(L.) Heynh seedlings with different gamma radiation doses resulted on variations of root and leaf

fresh weights but no dose-dependent growth inhibition have been detected (Vanhoudt *et al.,* 2014). Therefore, authors supposed that those fluctuations are mostly due to biological dissimilarities rather than a distinct radiation effect. Hence, when aiming to construct a dose-response curve, higher total absorbed gamma radiation doses need to be applied on a more sensitive developmental stage of the seedlings. The results from the investigation show low doses irradiated dry and wet seeds of *Molucellalaevis* (L.), at 2.5, 5, 7.5 and 10 Kr, all doses of seeds except 20 Kr had the same plantsurvival percentage 100% in both seasons. On otherhand, the higher doses (12.5, 15, 17.5 and 20 Kr) of wetseeds decreased the plant survival percentage withthe increase of gamma radiation doses in both seasons (Minisi *et al*, 2013).The results of the experiments with higher dosage of gamma radiation indicated a pronounce decrease of germination percentage, number of survival plants and plant height (Vaijapurkar *et al.,* 2001; Minisi *et al,* 2013). Also, wet treatments of radiation caused a simulative effect in most characters. The high doses 12.5 to 17.5 Kr of wet seeds caused some morphological variations. The genetic relationship of the morphological variations can be determined by using RAPD analysis (Minisi *et al,* 2013).

According to theVaijapurkar *et al*. (2001), when study ionizing effects on irradiated *Allium cepa*(onion -red globe-Mathania Desi) concluded that themorphological features such as the number of root and length ofroot formation, and shoot formation cannot give a confidenceto accept themas a biological indicator forlower gamma dose measurement. It can be only used forqualitative measurement for gamma dose evaluation.Analysisshowed a relationwith delivered gamma-radiation ononions at lowerdoses, i.e., 50–2000 cGy. The differences in the root numbersand root length of irradiated *Allium cepa* (onions-red globe-Mathania Desi) atdifferent intervals was extremely significant (P<0, 0001) at doses above 500 cGy. Comparatively, root numbers and rootlength of controlled onions were much enhanced. But significant reduction in growthrate of root length and root numbers cannot be observedbelow 500 cGy. Thus that parameter can provide qualitative data only (Vaijapurkar et al., 2001).Number of roots formedand lengthening of roots with dose delivered to onion bulbswas unable to establish any definite correlation at doses500, 1000, 1500 cGy.Asmall shoot tip appeared after 72 h incontrolled onions, irradiated onions (with doses500, 1000, 1500 cGy) showed induction of shooting after144 h. No shoot appeared in onions irradiated with 2000 and2500 cGy dose even after 300 h (Vaijapurkar *et al.,* 2001).Although lowdose rates and low total absorbed doses wereapplied by Vandenhove *et al.* (2010) and the effects on growth were already visible on 24-day-old plants.

Chronic exposure to low dose rates can be more effective on the growth than a short exposure to higher dose rates. Indeed, Vandenhove *et al*. (2010) reported for gamma radiation effects on growth of A. thaliana chronically exposed during a full life cycle to dose rates ranging from 81 μGy h-1 to 2336 μGy h^{-1}. The dose rate estimated to result in a 10% reduction in growth (EDR-10) ranged between 60 and 80 μGy h^{-1} for *Arabidopsis* (Vandenhove *et al.,* 2010a), germination of seeds was not hampered. It had been reported by Kovalchuk *et al*. (2007) that the genome of *A. thaliana* is regulated differently depending on whether the irradiation, was chronic or acute. Nevertheless, controversial data still exist. The demonstration of a variable threshold at low levels of IR exposure indicates that in nature A. thaliana may be exposed to environmental radiation throughout its life-cycle without significant modification of growth or development (DalyandThompson, 1975).

1.1. Cell alterations

Gamma rays have high penetration and energy, so easy interact withatoms or molecules producing free radicals in cells that changed plant cellular structure and metabolism. The ultra-structural observations of the irradiated plant cells of *Arabidopsis*shown thatlow-dose irradiation of 1 or 5 Gy did not affect significantly the ultrastructure of cell organelles, and that chloroplasts were more sensitive compare with other cellorganellesto a high dose of gamma rays 50 Gy (Wi et al., 2007). Analogous results induced by other environmental stressfactors such as UV, heavy metals, acidic rain and high light have been reported (Molas, 2002; Barbara *et al.,*2003; Quaggiotti *et al.,* 2004). Based on transmission electron microscope observations, chloroplasts were extremely sensitive to gamma radiation compared to other cell organelles, particularly thylakoids being heavily swollen (Wi*et al.,* 2007).However, the low-dose irradiation did not cause thesechanges in the ultra-structure of chloroplasts.

Under radiation stressplants have registered modifications of growth and development, decreasing of reproduction capacity, metabolic amendments and DNA damages (DalyandThompson, 1975; Esnault *et al*., 2010; Kovalchuk *et al*., 2007; Vandenhove *et al.,* 2010; Wi *et al.,* 2000).Usually, Ionizingradiation produced chromosome aberrations. Vaijapurkar *et al*.(2001) reported for a number of cellular injuries that can be seen under microscope after a small dose of ionizing radiation. Commonly that are chromosomal stickiness, permanent bridges formation,not clean chromosomal separation, suppression of anaphase movements, displacementof constriction relative to

chromosomes, unequal division of chromosomes between daughters, chromatids and chromosome breaks, production of micronuclei in the next cell cycle. According to Vaijapurkar *et al.(*2001) micronuclei formation can be taken as a biological indicator to measure gamma doses.The two cytological parameters, mitotic index and percentage micronucleican be considered as good biological indicator for low-level γ-dose measurements. The rate of micronuclei formation with dose is faster compared to mitotic index reduction with dose. The formation of micronuclei is significant at a gamma dose of 400 cGy even though micronuclei formation initiated at a dose of 200 cGy itself. The observed micronuclei percentage at a dose of 200 cGy is 0.67% and at a dose of 400 cGy is 1.04%. In unirradiated sprouted onions the % of micronuclei in has not shown any significant change, i.e., <0. 02%. It is observed that up to 100 cGy the mitotic index lies between 4.4 and 5.0 which somehow matches with the mitotic index of unirradiated onions (control) (Vaijapurkar *et al.,* 2001).

A chromosome condensation in shape and size can only be used as a biological indicator to confirm that the plant (*Allium cepa*) has received the gamma dose, but cannot be taken as biological indicator because their variation with dose is not well pronounced (Vaijapurkar *et al.*, 2001). A significant chromosome condensation has registered above 500 cGy. There were observed a gradual increase in chromosomal condensation in shape and size towards higher dose, but measurement for gamma dose below 1000 cGy has not significant statistically trends and any well-defined relation. Chromosomal abnormalities like polyploidy was significant only after 1000 cGy (Vaijapurkar *et al.,* 2001).

Coniferous plants generally show a high retention capacity and low turnover rate for contaminants taken up by the aerial biomass from the atmosphere, an assessment of cytogenetic anomalies in the intercalar meristem of young needles also appears to be a promising test system. In either case, the damage to the DNA mainly appears as chromosome aberrations at the first mitosis (Geraskin *et al*., 2003).Cytogenetic effects inScots pine (*Pinus sylvestris* L.) populations growing in the BryanskRegion have been investigated since 2003 (Geraskin *et al.,* 2011). Chronic radiationexposure at dose rates 0.8 μGy/h has been demonstrated tocause a significant increase in cytogenetic effects (Geraskin *et al.,* 2011). Nevertheless, sometimes decrease in reproductive ability was observed under dose rate that did not increase cytogenetic alterations significantly and vice versa (Geraskin et al., 2014).

1.2. DNA damage

Recently introduced molecular cytogenetic methods allow analysis of genotoxicity, both at the chromosomal and DNA level. Changes in chromosomal morphology are often detected with classical cytogenetic techniques. However, the traditional methods of chromosome staining can fail in the analysis of small changes in chromosome structure. Fluorescent *in situ* hybridization (FISH) gives new possibilities to study chromosomal aberrations in plant mutagenesis, detection and analysis of chromosomal rearrangements in a great detail. The sister chromatid exchange (SCE) test is a well-known, highly sensitive cytogenetic tool for detecting DNA damage. The test is based on DNA segregation, which occurs in chromosomes according to a semiconservative model of DNA replication. SCE involves symmetrical exchange at one locus between sister chromatids that does not alter chromosome length and genetic information. Sister chromatids are visualized through the methods of incorporating bromodeoxyuridine (BrdU) into chromosomal DNA and different staining of chromatids containing DNA with BrdU and chromatids without BrdU (Painter, 1980). The frequency of SCEs per chromosome set increases after treatment with genotoxic agents DNA fragmentation can be estimated using the TUNEL test and the single cell gel electrophoresis (Comet assay). The advantages of the TUNEL test include detection of DNA breaks at a single nucleus, short time of assay and easy screening of labelled nuclei. This test is recommended for the preliminary evaluation of genotoxicity of any new tested agent (Juchimiukand Maluszynska, 2003).

Single Cell Gel Electrophoresis (SCGE) orComet assay, was firstreported by OstlingandJohansons (1984). The Comet assay was established for investigatingthe process of apoptosis in animal cells and subsequently adapted and validated to plant cells (McKelvey-Martin, 1993; Collins, 2004). It allows thequantitative and qualitative study of DNA damage in nuclei isolated from single cells that are embedded inagarose and transferred on microscope slides.The SCGE approach iscurrently usedto investigate the cell response togenotoxic agents that lead tooxidativeDNA damage.Advantages and limitations ofSCGE in ecogenotoxicological and biomonitoring studies have been largely discussed in animal systems(Kumaravel *et al*., 2009).The Cometassay was used to detect DNA damage in nuclei ofseveral plant species isolated from leaves or root tissueafter mutagenic treatment (Navarrete, 1997).The use of SCGE asbiomarker ofexposure toionizing radiation(IR) for environmental

and occupational purposes is well established in human cells (Collins, 2008). There is attention on emerginghighly reliable and low-cost environmental plant-based methods able to complement and support the conventional techniquesso far routinely used for pollution assessment.Inthis context,the multiple SCGE versions need to beextensively tested inlarge-scale representative screenings (Ventura *et al.,* 2013).

Arabidopsis thaliana exposed to low-dose chronic gamma irradiation during a full life cycle (seed to seed) with applied dose rates 2336, 367 and 81 μGy h^{-1} were analyzed with comet assay, but did not reveal any effect of gamma dose rate on DNA integrity(Vandenhove *et al.,* 2010a).

KoppenandAngelis (1998) demonstrated that *Vicea faba* roots exposed to X-ray (30 Gy) could repair DNA strand breaks, estimating that approximately 50% of DNA damage was repaired in less than 20min. Similarly, Gichner *et al.* (2000b) showed that DNA damage induced by γ-rays in the range 20–40 Gy was completely repaired in non-replicating tobacco leaf nuclei after 24 h. According to these results, SCGE (Single Cell Gel Electrophoresis) analysis of nuclei from plant leaves is not suitable for biomonitoring the late effects of IR, since DNA damage is readily repaired.

1.3. Physiological and biochemical parameters

1.3.1. Photosynthetic pigment content

Gamma radiation is an electromagnetic radiation of high frequency and consistsof high-energy photons, that have a high penetration and can interact directly and indirectly with biological matter causing ionizations andinduces various physiological and biochemical alterations. Photosynthetic pigments were found to be highly sensitive toradiation as γ-radiation and may modify the plastid structures likethylakoids and altered photosynthetic pigment content (KovacsandKeresztes, 2002). Dosesof 50 and 100 Gystrongly inhibited both chlorophyll and carotenoid synthesis (Arena *et al*. 2013; Al-EneziandAl-Khayri 2012; Alzahrani, 2012).Ling *et al.* (2008) also obtained lowerchlorophyll content from γ-irradiated plantlets as compared tonon–irradiated plantlets of sweet orange (*Citrus sinensis*).Lower chlorophyll content with irregular distribution was obtained from irradiated plantlets as compared to non-irradiated plantlets (Kim *et al.,* 2004; Ling *et al.,* 2008). The highest amount of total chlorophyll content was obtained in seedlingsirradiated at 100 Gy (Borzouei *et al.,* 2010).Conversely, in studies of *Citrus sinensis*on-irradiated plantlets demonstrated the highest amount of chlorophyll content as compared to plantlet irradiated at 10, 20, 30, 40 and 50Gy (Ling *et al.,* 2008).According to Arena *et al.* (2014) in their study with *Phaseolus vulgaris* up to 10 Gy, chlorophyll andcarotenoid content did not change. In *Arabidopsis thaliana,*after exposing 2-weeks-old seedlings for 7 days to total doses of 3.9 Gy, 6.7 Gy, 14.8 Gy and 58.8 Gy, the capacity of photosystem II (PSII measured as Fv/Fm) remainedintact, plants started optimizing their photosynthetic process at the lower radiation doses by increasingthe PSII efficiency (4PSII) and the maximal electron transport rate (ETRmax) and by decreasing the non-photochemicalquenching (NPQ). At the highest radiation dose, 58.8 Gy, photosynthetic parameters resembledthose of control conditions (Vanhoudt *et al*, 2014). Although, the effects of ionizing radiationon photosynthetic pigments vary among plant species and cultivars(Kim *et al*., 2004).

Undernatural conditions plants are exposed to changing light intensities and the photosynthetic antenna complexes have important rolescapturing light energy to drive photosynthesis, andalso to protect the photosynthetic apparatus from photo-oxidative damage due to ROS formation.It dissipated the excess light energy as heat, non-photochemical quenching (NPQ) (Niyogi, 1999). The NPQ kinetics can also be related to the formation of zeaxanthin and antheraxanthin as shown by D'Haese *et al*. (2004). The xanthophyll zeaxanthinis known to be an important player in NPQ through the deactivation of excited singlet chlorophyll(Jahns and Holzwarth, 2012). Recent studies already indicated that NPQ is generally inhibited under gamma radiation in a plant species-dependent way (Kim *et al.,* 2005, Kim *et al*., 2010 and Moon *et al.,* 2008). The decreased NPQ under gamma radiation was partly associated with altered xanthophyll cycle activities (Moon *et al.,* 2008).Carotenoids play an important role in the photoprotection of photosystem II (PSII) through the deactivation of triplet chlorophyll and singlet oxygen. The pigments such carotenoids and flavonoids save plant cell against UV-B and gamma irradiation (KovachandKeresztes, 2002). Kim *et al.* (2005) demonstrated for the first time that carotenoid pigments are the most radiosensitive and fastest recovering compounds in plants. In study of combined effect of gamma-radiation and elevate levels of ozone, dry and healthy seeds of clover cv Wardan was irradiated with 0, 5, 10, 20 and 25 krad dose of gamma rays (60Co), and plants germinated from γ-irradiated seeds were exposed with two levels of O3 - non filtered ambient air (AO) and non–filtered ambient air +10 ppb elevated O3 (EO). Total chlorophyll and carotenoids in plants showed varying degree of reductions with all the treatments. The extent of reduction in total chlorophyll was maximum in EOγ20 (32.7%) followed by EOγ10 (26.8%), EOγ0 (23.0%) and minimum in EOγ5 (11.0%) at 40 DAG. Elevated O3 exposure exhibited reduction

in carotenoid content and magnitude varied with γ-irradiation doses with maximum reduction in EOγ20 (17.9%) and minimum in EOγ5 (6.8%) at 100 DAG. Three–way ANOVA revealed significant variations in total chlorophyll due to A, γ, T, A×γ and γ×T and carotenoids due to all the individual factors and their interactions except due to A×γ×T (Chaudhary and Agrawal, 2014).

1.3.2. Antioxidative defense

The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Stoeva, 2002; Al-RumaihandAl-Rumaih, 2008; Hammed *et al.,* 2008; Ling *et al.,* 2008; Kiong *et al.,* 2008). These effects include alteration in photosynthesis, modulation of the antioxidant system, and accumulation of phenolic compounds. Many biochemical differences have been registered in gamma irradiated seedling, very often which is accumulation of phenolic compounds (KovachandKeresztes, 2002; Kim *et al.,* 2004; Wi *et al.,* 2007; Ashraf, 2009; ChaudharyandAgrawal, 2014).Total phenolscorrelated with plant resistance against many stresses and itsincrement of total phenols in γ irradiated plants has also beenreported by Lee *et al*. (2009).

Plantcells contain a significant quantity of water, exposed to gamma radiation the production of reactive oxygen species (ROS) initiate that will cause cellular damage. Gammaradiation oxidative stress appears with overproduction of ROS, such as superoxide radicals, hydroxyl radicals and hydrogen peroxide that react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids initiating disturbance of cellular metabolism (Al-Rumaih and Al-Rumaih, 2008; Ashraf, 2009; Noreen and Ashraf, 2009).Superoxide dismutase (SOD) represents the first line of ROS-defense as it catalyzes the disproportionation of superoxide into hydrogen peroxide (McCordandFridovich, 1969).In the roots, SOD generally increased its capacity after irradiation, but only significantly for the lowest and highest radiation doses, and leaves appeal to be less radiosensitive as most enzymatic capacities remain unaffecteddenoted Vanhoudt *et al*. (2014). Al-Rumaih and Al-Rumaih (2008) reported that when dryseeds are subjected to gamma rays (0, 40, 60, 80, 100 Krad) from a cobalt source ^{60}Co at a dose rate of 233.5 rad/min,they displayed a dose dependent increase in the activities of ascorbate peroxidase (APOX), superoxide dismutase (SOD) and glutathione reductase (GR) observed in both shoots and roots of the studied species. On the contrary, catalase activity was repressed, particularly at the higher applied dosesγ-radiation. Shoots were more significantly affected by irradiation than roots (Al-RumaihandAl-Rumaih, 2008).In studied on 2-weeks-old seedlings of *Arabidopsis thaliana* threatened for 7 days with total doses of 3.9 Gy, 6.7 Gy, 14.8 Gy and 58.8 Gy, on subcellular level, roots showed increased superoxide dismutase (SOD) and ascorbate peroxidase (APX) capacities under gamma irradiation but catalase (CAT), syringaldazine peroxidase (SPX) and guaiacol peroxidase (GPX) activities, on the other hand, decreased. In the leaves no alterations were observed in SOD, CAT and SPX capacities, but GPX was highly affected. Based on these results it seems that roots are more sensitive for oxidative stress under gamma radiation exposure than leaves (Vanhoudt *et al,* 2014).

Several of the antioxidative defence enzymes have been studied in *Arabidopsis thaliana* exposed to low-dose chronic gamma irradiation during a full life cycle (seed to seed) with applied dose rates 2336, 367 and 81 μGy h^{-1}. The enzyme capacity of enzymes involved in the antioxidative defence mechanisms (SOD, APOD, GLUR, GPOD, SPOD, CAT, ME), was generally stimulated towards flowering but generally no significant effect of dose rate on enzyme capacity was observed. Gene analysis revealed a significant transient and dose dependent change in expression of RBOHC indicating active reactive oxygen production induced by gamma irradiation. Low dose γ-radiation led to an efficientinduction of antioxidative enzymes involved in ROS scavenging (Zaka *et al.,* 2002) as these radicals do not kill the cells, but ratherproduce genetic abnormalities and immediately triggers anti-oxidativedefense systems by modulating the activities. Therefore, positive correlation was observed between antioxidative enzymesand γ irradiation treatment (Chaudhary and Agrawal, 2014).

Peroxidase (POD) plays an important role of hydrogen peroxide (H_2O_2) detoxification in cells, protecting cellular components such as proteins and lipids against oxidation (Rumaih, 2007). Thehighest amount of specific activity of peroxidase was obtained in plantletsof *Citrus sinensis* irradiated at 50Gy (Ling *et al.,* 2008).No effect of irradiation was observed on concentration or reduction state of the non-enzymatic antioxidants, ascorbate and glutathione. The level of lipid peroxidation products remained constant throughout the observation period and was not affected by dose rate (Vandenhove *et al.,* 2010a).

Plants have developed various protective mechanisms to avoid oxidative damage (Kiong *et al.,* 2008). One of the protective mechanisms in the synthesis of osmolytes which is essential to plant growth was proline synthesis (Esfandiari *et al.,* 2008). The increase in proline content was reported to cope with the problem of oxidative reagents (Falahati *et al.,*

2007).The increase in proline content was observed in irradiated plants. There was a convincing evidence which showed that the osmolyte synthesis such as proline involved in protective mechanisms were altered with several environmental stresses, including gamma irradiation (Al-Rumaih and Al-Rumaih, 2008).Proline is a compatible osmolyte and it may interact with enzymes to preserve enzyme structure and activities. Indeed, proline has been shown In vitro to reduce enzyme denaturations caused due to heat, NaCl stress, gamma stress, etc.(Kavi Kishor *et al.,* 2005; AshrafandFoolad, 2007). Seedling irradiated at 100 Gy contained highestamount of proline (1.71 mg/g FW), whereas only 0.92 mg/g FW of proline was detected in non-irradiatedseedlings.

1.3.3. Hormone and protein content changes

The most crucial function of plant cell is to respond to gamma stress by developing defenses mechanisms. This defense is fetched by alteration in the pattern of gene expression (Corthals *et al,* 2000).This led to modulation of certain metabolic and defensive pathways (Zolla *et al,* 2003). Due to gene expression altered under gamma stress, qualitative and quantitative changes in total soluble protein content were obvious (Corthals *et al,* 2000). Biochemical differentiation based on total soluble protein content revealed that plantlet irradiated at 50Gy contain the highest amount of total soluble protein, 21.03±1.82mg/gFW, whereas only 14.49±4.04mg/gFW of total soluble protein was detected in 10Gy. Comparing total protein content of control plantlets and 10Gy irradiated plantlets, the control plantlets exhibited significantly greater than those of 10Gy irradiated plantlets. Plantlets irradiated at 10Gy exhibited a total soluble protein content of 14.49±4.04mg/gFW which was 20.90% lower than that of the non-irradiated plantlets, 18.32±1.39mg/gFW (Ling *et al.,* 2008).These proteins might play a role in signal transduction, anti-oxidative defense, anti-freezing, heat shock, metal binding, and anti-pathogenesis or osmolyte synthesis which were essential to a plant's function and growth (Gygi *et al,* 1999).Humera (2006) stated that the stress reaction of plants often results in the alteration of protein metabolism. Several proteins are synthesized and accumulated in plant tissues under a range of stress conditions. Such proteins, referred to as stress proteins, have been noted to be induced in response to several stress factors.

Generally, radiation causes the irreparable changes of protein conformation at the molecular level by breakage of covalent bonds of polypeptide chains (Kumeand Matsuda, 1995). Fragmentation involves reaction of alpha-carbon radicals with oxygen to form peroxyl radicals which decompose to fragment the polypeptide chain at the alpha carbon (DaviesandDelsignore, 1987). Hydroxyl radical and superoxide anion radical generated by radiation could modify primary structure of proteins, which resulted in changes of molecular weight distribution (Garrison 1987). Besides fragmentation, aggregation of protein fragmented is also observed, as well as cross-linking of proteins by irradiation (Filali, 1997). Covalent cross-linkages are registered between soluble proteins and between peptides and proteins (Garrison 1987).

The growth inhibition after gamma irradiation with high or low doses correlated to a more or lesss evere decrease of production of the growth hormone, indolacetic acid (AIA) (Dwelle, 1975; Chandorkar and Clark, 1986). But the mechanisms involved in plant response are still unclear (Kuzin*et al.,* 1984, 1991).

2. Effects of ionizing radiation on plant growth, development and production

An environmentally important type of ionizing radiation is gamma radiation. Gamma irradiation is a physical mutagens widely used for mutation breeding, food sterilization and medicinal healing. Traits induced by mutagenesis include plants size, blooming time, fruit ripening, fruit color, self-compatibility, self-thinning and resistance to pathogens (Predieri, 2001). The effects of ionizing radiation on plants growth and development are well studied, and they vary from stimulatory effects at very low doses (Sax, 1963;Luckey, 1980; Sagan, 1987;Planel *et al.,* 1987; KorystovandNarimanov,1997; Charbajiand Nabulsi, 1999; Toker *et al.,* 2005; Wi *et. al,* 2007; Ling *et al.,* 2008; MelkiandMarouani, 2009; Borzouei *et al.,* 2010; Minisi *et al*, 2013) toward inhibition of growth (Dwelle, 1975; Chandorkar and Clark, 1986; Kim *et al.,* 2000; Zaka *et al.,* 2004; Toker *et al*., 2005; Wi *et al.,* 2007; Kon *et al*.,2007) up to obviousreductions in reproductive effectiveness athigh gamma radiation levels (Chaudhuri, 2002;Minisi *et al,* 2013; Zaka *et al.,* 2004). Nevertheless, ionizing radiation may have different effects on plant metabolism, growth and reproduction, depending on radiation dose, plant species, developmental stage and etc. Somestages of seedlings are most radio sensitive with comparison to dry seeds, wet seeds or mature plants.Studyon development of *P. vulgaris* leaves exposure to X-rays (0.3, 10, 50, 100 Gy), registered effects that were not only dose-dependent butalso have been under strong influence by leaf age. Mature leaves have been more radio-resistantthan young leaves (Arena *et al.,* 2014).Kurimoto *et al.* (2010) reported that older plants are less radiosensitive as they already developed a basic internal structure and large amount of biomass by the time they are irradiated while immature

plants were less capable of tolerating radiation stress. When seeds of red pepper (*Capsicum annuum* L.) were gamma-irradiated, their growth was stimulated at doses from 2 to 8Gy but was hardly affected at 16Gy (Kim *et al.,* 2004). Relatively low-doses ionizing radiation on plants are manifested as acceleration of cell proliferation, germination rate, cell growth, enzyme activity, stress resistance, and crop yields (ChakravartyandSen, 2001).Studies on *Arabidopsis thaliana* (L) Heynh seedlings exposed to low-dose gamma rays (1 or 2 Gy) showed slightly increased a growth compared to the control, while under high-dose irradiation of 50 Gythe seedling growth noticeably decreased (Wi *et. al,*2007).For *Citrus sinensis* stimulation of plant growth was detected at 10Gy and inhibition occurred above 10Gy (Ling *et al.,* 2008). Using in vitro mutagenesis techniques to investigate the effects of gamma irradiation on physiological changes of*Citrus sinensis* at 0, 10, 20, 30, 40 and 50Gy, studies revealed that the LD50gamma doses that killed 50% of the plantlets were achieved at 27Gy (Ling *et al.,* 2008).Actually, in all reported cases the observed effectshighly depend on the species,age, plant morphology, physiology, and genome organization.

Study on survival rate, plant development and seed production of *Pisum sativum* var. Belinda (Fabaceae) showed decreasing of survival rate for seedlings and reducing the fertility. Regardless of the dose, irradiation led to a significant decrease in pod number per plant, as well in G1 as in G2 plants. Plants irradiated with the dose ranging from 0.4 to 10 Gy had only 60 to 80% of the pod set in the control plants. Above 10 Gy, the irradiated plants were unable to reach the flowering stage. None of plants reached the flowering stage of those irradiated with 40 Gy and 60 Gy, they become yellowish and finally dying prematurely. All G1 seeds were germinated two days after imbibition (DAI). About 81–93% of the G1 seedlings irradiated with doses of 0–10 Gy grew and developed normally. The survival rate for seedlings irradiated with 40 and 60 Gy was significantly lower after 28 DAI. Only 15% of the plants irradiated with 40 Gy and 9% of those irradiated with 60 Gy survived after 48 DAI. At 96 DAI the number of surviving plants was 28, 29, 31, 30 and 27 out of 33 for 0, 0.4, 3, 6 and 10 Gy, respectively (Zaka *et al.,* 2014). In the next generation, all G2 plants originating from irradiated G1 individuals displayed a reduced pod set compared to the control, which was roughly of the same magnitude as for G1 (Zaka *et al.,* 2014).

There is a critical evidence that ionizing radiation stimulate plant growth at certain stages of development, induce earlier flowering, stimulate lateral bud development, probably by auxin inactivation (Sax, 1963).The stimulating causes of gamma ray on germination may be certified to theactivation of RNA or protein synthesis, which occurred during the early stage ofgermination after seeds irradiated (Abdel-Hady *et al.,* 2008). Though, no certain explanations for thestimulatory effects of low-dose gamma radiation are available until now. Thereis a hypothesis that low doseirradiation induce the growth stimulation by changing the hormonal signalingnetwork in plant cells or by increasing the anti-oxidative capacity of the cells to easilyovercome daily stress factors such as fluctuations of light intensity and temperature in thegrowth condition (Wi *et al.,* 2007).

Seeds of *Vigna sesquipedalis* (long bean) were treated with 300, 400, 500, 600 and 800 Gy gamma rays. All the doses from consistently reduced long bean height compared to the control. Plant height significantly decreased with increasing dosage with 800 Gy having the most pronounced effect. The study revealed that germination percentage, plant height, survival percentage, root length, root dry weight and shoot dry weight decreased with increasing dose of gamma ray. The 800 Gy gamma ray dose in particular had a pronounced effect on these morphological characteristics probably because of injury it might have caused to the seeds. As a result, poor growth and development was noticed. The LD50 for survival and height ranged between 600-800 Gy and 400-500 Gy, respectively. Generally, higher gamma ray doses particularly 800 Gy significantly affected the morphological characteristics of long bean seedlings obtained from irradiated seeds (Kon *et al.,* 2007).Thegrowth inhibition after gamma irradiation with high or low doses is correlated to a decrease of production of the growth hormone, indolacetic acid (AIA)(Dwelle, 1975; ChandorkarandClark, 1986). The high-dose irradiation that causedgrowth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somaticcell division and/or various damages in the entire genome (Preussa and Britta, 2003).Nevertheless, themechanisms involved in plant response growth inhibition after gamma irradiation is still unclear (Kuzin *et al.,* 1984; Kuzin *et al.,* 1991).

The effect of ionizing radiation (IR) is divergenton plant genome stability and total genome expression, depending of exposure pattern, acute or chronic. It had been reported by Kovalchuk *et al.(*2007) that the genome of *A. thaliana* is regulated differently depending on whether the irradiation, was chronic or acute.Analysis of homologous recombination (HR) in plants from chronic and acute exposure, receiving equal dose of radiation 1Gy, revealed a significantly higher increase in frequency of homologous recombination in the group under chronic exposure, as compared toacute one (Kovalchuk *et al.,* 2007).Geraskin *et al.* (2014)

reported in plant populations inhabiting heavily contaminated territories cytogenetic damage is accompanied bydecrease in reproductive ability.

The plant reaction is generally dose-dependent, rangingfrom pronounced damage at high doses, harmful consequencesat intermediate levels and stimulatory effects at very low doses.According to KozubovandTaskaev (2002), after Chernobyl accident,fourzones were identified varying in the extent of radiation damage. First zone of lethal affects with absorbed dose 60–100 Gy, with mass mortality of pine trees, radiation crown damage of birch (*Betula pendula* Roth.) and black alder (*Alnus glutinosa* L.). Zone of sublethal effects, around 3800 ha, where 40–75% of trees driedand absorbed dose was 30–40 Gy. Necrosis of meristems and young shoots were 90–95% in observedpine trees, together with the death of tree tops and suppression of growth. Zoneof medium damage with absorbed doses approximately 5–6 Gy, for over 11,900 ha. In thiszone have been exhibit suppression of growth, partial abscission of needles on the shoot tops and damaged reproductive buds. The so called zone of slight damage, which covered the rest of the forest in the 30-km zone, had absorbed dose approximately 0.5–1.0 Gy. Suppression of pine trees growth in some sites was observed, along with increasing by 10–12%of the number of hollow seeds in cones.Therefore, Chernobyl accident has become a source ofactually unique informationfor the effects of acute and chronicexposure of plants to ionizing radiation.The consequences of the Chernobyl accident demonstrateddeleterious effects of radiation, resulting inalterations at single organ level, such as modification of DNAand protein function, an enhanced rate of mutagenesis andheritable genetic mutations, to damage at the ecosystem level, changes to the whole population dynamics and structure (Kalchenko *et al.,* 1991; Shevchenko *et al*, 1992; Syomov *et al,* 1992; RubanovichandKalchenko, 1994; Real*et al.,* 2004; Fedotov *et al.,* 2006; Geraskin *et al*., 2008; KalchenkoandFedotov, 2001).

Mousseau *et al.* (2013) found it that the degree of suppression ofgrowth of *Pinus sylvestris* during 1986–1990 (post-Chernobyl) compared to1981–1985 (pre-Chernobyl) of individual trees was causedby radiation interacting with tree height, with radiationeffects being disproportionately greater in small comparedto large trees, and they made several possible interpretations.First, more than 90 % of all radioactive material is locatedin the topmost 20 cm of the soil. Short trees having shallowroot systems mayextract more radionuclides than tall trees with deep rootsystems. Second, growth rate effects may be more readilydiscerned in small trees given the larger absolute growthrates in short trees (*Koch et al.,* 2004). Third, this may becaused by an interaction related to differential effects ofradiation on mycorrhizae, which may significantly influenceradionuclide uptake (Dighton *et al.* 2008).The detected variance ingrowth can be a consequence of increasing age and stemdiameter (Fritts, 1976; CarrerandUrbinati, 2004). Assumed that radiationeffects were not observed in all years after 1986, authors concluded that other stressors interacted with radiation tosuppress growth (Mousseau *et al.,* 2013).

3. Effects of chronic ionizing radiation with other stress environmental factors on plants

In the field, plants aresimultaneously challenged by many different factors leading toalmost unpredictable complexity in their response.Although ionizing radiation, enhance level of UV-B rays, and as background the dramatic climate changes, nowadays with different technogenic pollution, causes primary damage at the molecularlevel, but there are emergent effects at the level of plant populations.The Chernobyl Nuclear Power accident provided a unique opportunity to study the effects of ionizing radiation under field conditions.Tree growth has been accepted as areliable indicator of the state of the external environment. Monitoring of tree growth of 105 Scots pine (*Pinus sylvestris*L.), located near Chernobyl, Ukrainenegative effects of radioactivecontaminants particularly pronounced insmaller treeswere registered (Mousseau *et al.,* 2013). Mean growthrate was severely depressed and more variable in1987-1989 and several other subsequent years, followingthe nuclear accident in April 1986 compared to the situationbefore 1986.The higher frequency of years with poorgrowth after 1986 was not caused by elevated temperature,drought or their interactions with background radiation.These findings suggest that radiation hassuppressed growth rates of pines in Chernobyl, and thatradiation interacts with other environmental factors andphenotypic traits of plants to influence their growth trajectoriesin complex ways (Mousseau *et al.,* 2013).

4. UV-B and radioactive radiation

Plants are subjected to a variety of stress condition sassociated with their natural environment. Depletion of stratospheric ozone in the last decays, as a result of anthropogenic influences on the environment, caused constantly increasing of solar UV-B radiation (KerrandMcElroy, 1993; Madronich *et al.* 1998; McKenzie *et al.,* 1999; Andrady*et al.,* 2006; Rowland, 2006).Plants are likely to be exposed in thefuture to enlarge UV radiation from sunlight dueto reductionof stratospheric ozone levels by chemical pollution. The effects that such increased UV-B exposure might have

on plant life are largelyunknown. Therefore, UV-B radiation is one of the main factors that can interfere with low levels ionizing radiation,in naturally conditions. The UV spectrum is commonly divided into three ranges: UV-C(<280 nm), UV-B (280 to 320 nm), and UV-A (320 to 400 nm).No significant UV radiation of wavelength less than 295 nmreaches the earth's surface. Sunlight, which is required for photosynthesis, exposes plants to damaging levels of UV-B radiation and toheat stress (Green, 1983).

Plant growth can be inhibited or stimulated by different levels of UV radiation, depending on the same factors as radioactive radiation, such as species sensitivity and different growth conditions (StaxenandBornmann, 1994). UV damageand heat induce a common stress response that leads totissue death in plants (Jenkins *et al.,* 1997). UV radiation has been shown to cause alterations in physiological and biochemical processes and to alter plant growth and morphology (Hollosy, 2002). UV-irradiated seedlings exhibit increase growth due to stimulation of gibberellins synthesis (Ballare *et al.,* 1991) and also ethylene (RosandTevini, 1995).It has been reported by Lin *et al.* (2009) that ethylene regulates many developmental processes of the plant life cycle including seed germination, flower development, senescence and responses to biotic and abiotic stresses such as drought, chilling and wounding. Ethylene and auxin signaling pathway contribute to local adaptation of high-latitude accessions of *A. thaliana*.It has been shownrecently that the metabolic and signaling regulation of auxin andGA by photoreceptors appear to determine the hypocotyl growthpattern in *A. thaliana* (Tsuchida-Mayama *et al.,* 2010). This indicates the importance of interactionsbetween plant hormones and photoreceptors in determiningadaptation to various environments. Ethylene and auxinsignaling pathway genes are important for plant adaptation todiverse photoperiodic conditions, and maybe a link between environmentalcues such as chilling, drought, length of the winter, and floweringtime responses (Lewandowska-Sabat *et al*., 2012).

UV-B light has a strong effect on surface or near-to-surface area in plant cells. UV-B radiation influences plastid structure and photosynthesis (Kovach and Keresztes, 2002). Adsorption of UV-B radiation byDNA generates two major photoproducts, cyclobutyl pyrimidinedimers and pyrimidine (6-4) pyrimidinonedimers, which block DNA replication and transcription(Britt, 1995). DNA-repair and damage-tolerance mechanisms, whichprovide resistance to UV damage in other organisms, alsooccur in plants (Britt, 1995). These mechanisms includephotoreactivation (PangandHays, 1991; Chen *et al.,* 1994;Ahmad*et al.,* 1997; Landry *et al.,* 1997), post replicationrepair (Cerrutti *et al.,* 1992), and nucleotide excision repair(McClennanandEastwood, 1986). These mechanisms removecyclobutyl pyrimidine dimers and pyrimidine (6-4) pyrimidinone dimers from UV-damaged plants or provideways to avoid their lethal effects (Britt, 1995).

Plants alsorespond to UV radiation exposure by increased flavonoidbiosynthesis (BeggsandWellman, 1985).Some kinds of pigments, such as carotenoids, flavonoids save plant cells against UV-B and gamma irradiation (KovachandKeresztes, 2002).

4.1. Chemical pollution and radiation

In the field, plants are simultaneously challenged by many different factors leading toalmost unpredictable complexity in their response.In current years, besides increases in UV-B radiation,a great interest has been engendered on studies relatedto the toxic effects of heavy metals on plants. Nowadays any radionuclide releases or radiation accidentsare occurring at a time when many naturalpopulations are already under pressure from habitat destructionand chemical pollution.Heavy metalpollution is increasing in the environment due to mining, industrialization and other anthropogenic activities. In that case theeffects of lower levels of radioactivecontamination may become more harmful, leading toan appearance of adverse effects in plant populations. At uncertain levels of anthropogenic influences many populationsmay be able to cope through phenotypic plasticity or geneticchanges, but with increasing intensify, plasticity andgenetic adaptation may be pushed to their limits (Geraskin, 2014). However, there are situations (Hoffmanand Hercus, 2000) when resistance to environmental changes hasnot evolved or has not persisted. Moreover, adaptation is often observed in one species but not found in others, despite anequivalent opportunity and exposure conditions (Bradshaw, 1991).

The numerous experimental data from epide-miological studies have revealed that with regard to combine effects between radiation and multiple chemical substances, responses can vary. Genotoxic substances as ionizing radiation that cause initial DNE-damage, generally lead to additive effects following combine exposes, but this particularly true in the low doses. Substances that impair the repair processes of DNA damage following exposure to ionizing radiation can cause super-additive effects. That is true for heavy metals and caffeine. Nevertheless, relatively high concentrations are needed in order suppression of enzyme systems of DNA repair to occur. Other cases are substances that reduce the effect of radioactive exposure. In that case the primary radical reactions are

blocked and diminish. For substances that alter the regulation of cell proliferation subsequent to radiation exposure can cause super-additive effects. These substances reduce DNA repair by shortened radiation-depended delay processes in proliferation cycle of the cell, which normally allow repair processes. Substances that can have hormonal effect, can stimulate cell proliferation and on the basis of these mechanisms to amplify the development of cancer following radioactive exposure. For the interaction between the development of the effects of radiation and substances, the sequence of exposure in time is of considerable significance (Streffer et al., 2013).

When study mature and healthy seeds irradiated with 4 doses (0, 100, 200, 300 Gy) under 3 salinity doses (0, 5, 15 and 25 mmosh/lit NaCl), author detected the lowest percentage of germination for gamma radiation 300 Gy with salinity 15 mmosh/lit. The minimum length of radicle was observed in treatments under 300 Gy gamma irradiation combined with all salinity concentrations. Moreover, the lowest percentage of callus regeneration was recorded in the treatments of various doses of gamma radiation in the salt concentration 25 mmosh/lit. The callus length of 100 Gy seedlings in 5, 15 and 25 mmosh/lit salinity was highest compared to other group. With increasing irradiation and salt concentration proline content was increased. The protein content on the other hand, decreased with increasing irradiation and salinity concentration. These results show that the up - regulation of some physiological characteristics and seedling growth of rice following gamma radiation treatment may be used to control abiotic stresses such as drought and salt (Dehpour *et al,* 2011).

Essential micronutrient forplants as Ni can be strongly phytotoxic at higher concentrations (BoominathanandDoran, 2002). For example, deactivation ofproteins including antioxidant enzymes, lipid peroxidationand membrane function induced from Nihave been reported in plants (MadhavaandSresty, 2000). Also, enhanced zinc concentrations caused an increase in theactivities of SOD, CAT and POD in the roots and trunks of Scots pineseedlings (Ivanov *et al.,* 2012).Shweta & Agrawal (2006) concluded from their study on*Spinacia oleracea* L. that's UV-B and heavy metal treatments caused oxidative stressin plants leading to reductions in photosynthetic pigmentsand consequently the biomass of spinach plants. The combinedeffects of sUV-B and Cd caused the strongest reduction inbiomass at final harvest. Prolineaccumulation appears to be an additional defense againstUV-B and metal-induced oxidative stress. Although an increasing number of chemical pollutants are suspected to give rise to cancersof different types, still there is a lack of data which can be used forrisk analysis. Such carcinogens as inorganic substances like asbestos, arsenic, chromium, nickel and organic substancessuch as benzo(a)pyrene, benzidme, vinyl chloride and coal tar. There are needs of experimental screening of a wide range of chemical agents from our dailyutility list and from our immediate environment that can have mutagenic and carcinogenic properties. Research on biological effects of low-level radiation andradionuclides should continue to re-evaluate the health safety consequences, but more attention now needs to be paid urgently to safeguard humanhealth and environment against the chemical pollutants (Environment, health and quality of life, 2010).

5. Environmental changes and plant adaptation

All living organisms tend to adapt under the influence of environmental stress factors. Amongthem plants rdeserve special attention since they are unableto leave the hazardous habitat.Hence, they cannotavoid harmful environmental influencesmustadapt to life in severe environments. Persistent exposure to low doses mutagenssuch as UV and ionizing radiation, chemicals, and stress environmental conditions as heat, drought, and cold, are expected to push plants to adapt.The ability of plantsto acclimate or adapt after a single generation exposurepreviously has been observed in several studies(Cortes *et al*., 1990; Boyko *et al.,* 2007).As example, afirst exposure to X-rayreducedthe effects seen at the time of a second exposure, accepted as an adaptive response (Cortes *et al.,* 1990). It is assumed that repeated treatments to stress factors allow plants to adapt, but the data about adaptation to chronic radiation, are controversial (Dmitrieva, 1996). In*A. thaliana*, Kovalchuk *et al*. (2007) showed that the genomeis regulated differently depending on whether the irradiation waschronic or acute. Ling *et al*. (2008) reported that irradiation increases plant sensitivity to gamma rays. This may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of break down, or lack of synthesis, due to irradiation (Omar, 1988).

Consequently, in long-term and especially during chronic irradiation,IR affects the genetic structure of populations. Genetic variability is often reduced. This reductionmay be associated with the demonstration of an adaptive process where, in particular, a species is subjectto chronic stress. Thus, the genomic effects of IR demonstrate their likely involvement in speciesevolution.DNA holds clues to climate change adaptation (Holmes, 2012). Researchers believe that which gene to be expressed within individual cells and to what degree dependent on the environment and that is

how epigenetics works.Epigeneticvariation occurs in natural systems (Richards, 2008).Thus, one of the most important reactions of a population to moderate stress is an increase in the genetic and phenotypic variability (Mengoni *et al.,* 2000; Slomka *et al.,* 2011; Geraskin *et al.,* 2013). However, severe stressmay cause a loss of genetic diversity when population size is reduced or due to a bottleneck effect (Deng *et al*., 2007; Kozyrenko *et al.,* 2007). It has been reported for that asignificant latitudinal clinein hypocotyl responses to redand far-red light of *A.thaliana* with northern accessions was found being morede-etiolated than southern accessions (Stenøien *et al,* 2002).Light quality at higher latitudes is rich in blue and far-red light (Nilsen, 1985), whichsuggested an adaptationto differences in light quality that the plant encounters in itsnatural local habitat.

Epigenetic states can be susceptible to environmental influence (Jirtle and Skinner, 2007). Epigenetics cannot change what has already been inherited from the parents, but can modify which gene from the genome pool to be express and to what extent. Environmentally-induced epigenetic variants can be inherited from one generation to the following, able to be passed down through generations (Richards, 2008; Llamas *et al,* 2012). This would allow rapid adaptation to a changed environment, and disseminate a phenotype throughout a population without any genetic change (Llamas *et al.,* 2012). A huge number of evidence indicates that epigenetic states are influenced by the environment. For example, prolonged cold-temperature treatments in plants can lead to both chromatins (Bastow*et al.,* 2004.) and DNA methylation changes at specific genomic loci (Steward, *et al*, 2002). Treatment with DNA damaging agents also change epigenetic states (Axtell and Brink,1967; IvarieandMorris, 1982; Stokes *et al.,* 2002).Epigenetic modifications have the potential to create phenotypic diversity in response to environmental cues, and unlike genetic changes, can be induced in multiple individuals in a population simultaneously (Llamas *et al,* 2012). This increases the possibility that environmentally-induced epigenetic changes in natural populations might produce new heritable phenotypes and by natural selection to provide rapid adaptation to climate and environmental change without the requirement for DNA sequence alterations (JablonkaandLamb, 1989).In all cases a remarkable phenotypic divergence is created by variable epigenetic silencing of individual loci.

Geraskin and Volkova (2014) observed that total mutation frequencysignificantly increased along with the level of radioactive contamination.Therefore, long-term chronic radiation exposure at doserates greater than 0.8 μGy/h led to significant increases in enzymaticloci mutations in Scots pine populations.The pine populations were grownunder chronic radiation exposure for more than 20 years,to some extent, these resultsindicate that chronic radiation exposure at dose rates from 10.4 μGy/hmight play an important role in the genetic differentiation of Scotspine populations. According tothe estimates, the dose accumulated from 1986 to 2008 in the crownsof the test trees amounted to 0.2–1.0 Gy, which is in good accordancewith the results of an independent study (Ramzaev *et al*., 2008) at asite very close to one of study sites.The appearance of new alleles leads to a significant increase inthe relative proportions of rare alleles in Scots pine populations growing under chronic exposure conditions. The frequency of rare alleles increases along with the level of radioactive contamination,whereas their numbers remain the same. Therefore, their proportions at impacted sites do not differ significantly fromeach other (Geraskin and Volkova, 2014).

Certainly the best-studied inherited epialleles in plants were derived from chemical mutagenesis experiments (Jacobsen and Meyerowitz, 1997; Soppe *et al.,* 2000). Investigations of plant adaptations to radioactive contamination provide an excellent opportunity to observe microevolution atwork. Radioactive contamination can change not only mean population characteristics, but also destabilize their temporal dynamics and modify genetic structure of populations (Gerask in *et al.,* 2011).Plants do not have a predetermined germline, germ cells are produced during plant development from somatic cells, so mutations occurring during somatic development can be inherited (Walbot, 1985).The role of microevolutionary processes in a population'sresponse to low-level chronic exposure is still not clearly understood.Natural populations can respond to radioactive contaminationnot only by enhanced level of genetic alterations and reductionin reproduction ability but also by radio-adaptation, which means physiological acclimation or changes in sexual, age or geneticstructure of populations.Therefore, man-made pollution mayresult in improved resistance to pollution. However, there arera dioecological situations where enhanced radio resistance has not evolved or has not persisted. To more accurately predict the probability of local extinctions and shifts in vegetation distributions, it is important to consider a plant species capacity for radio-adaptation.Revelation of these limits should be a major researchpriority (Geraskin *et al.,* 2011).

6. Conclusion

The effects of chronic exposure on livingorganisms and populations to different increasing levels of anthropogenic impactremain poorly explored. There is

vital importanceto understand the inherited responses to the combine effects of different pollutions and environmental stressors present nowadays in the nature, such as low dose ionizing radiation, chemical pollution, high level UV-B and etc., so further research on that field under different controlled conditions should be done and after that check it in the nature.

It is important for the prevention of DNA changes caused by environment to understand the biological consequences of DNA damages and their molecular modes of action that lead to repair or alterations ofthe genetic material. Obtaining good dose-response models, clarifying the suitable endpoint for estimation of the harmful environmental factors and their genetic and epigenetic expression in the plants, are crucial for correct predictions, as well as and applying the knowledge's to the practice. Developing sustainable agriculture production of artificial ecosystem, depended from the basic knowledge in mechanisms of adaptation, acquire tolerance and resistance in plants.

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