



RESPONSES OF PLANTS TO HEAVY IONS IRRADIATION

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SUMMARY

Heavy ion radiations (HIR), feature of the outer space environment limiting life during space mission was evaluated for responses in algae, fungi and dormant seeds. The results obtained mostly are elusive due to unequivocal irradiation doses and microgravity / weightlessness in space. Hence, HIR simulated experiments on earth are performed to understand precisely growth and developmental changes in plants by the operation of heavy ion accelerators at various institutes around the world. Seeds exposed to various heavy ions have shown inhibition in germination and morphological abnormalities in seedlings. Plants tumorous growth, chromosomal aberration and certain mutations due to DNA break and/or miss-repair or transposition is also observed. These might hamper cellular activity leading to lethality. Additionally, the experiments suggest that prolonged exposure of seed/ seedling to HIR during space flight or during simulated experiments may cause differential biological effects. Plant differential responses could be attributed to heavy ion irradiation dose, plant species and its physiological state. It is tempted to suggest that heavy ions/charged particles with specific dose might activate or modify certain processes which might result into few useful plant characters. The heavy ion potential to generate mutants because of localized deposition and/or hitting the molecule in exposed plant tissues make sense to consider it as an alternative tool either for conventional breeding or for genetic modulation in a way to improve the growth and yield of crop plants or to develop stress tolerant strains for farming, in changed agro-climatic conditions. The bystander effect (induced response in cells other than targeted one) in plants is yet to be examined as in animal system. Our understanding of fundamental mechanisms of changing plant system exposed to HIR is still incomplete. More detailed studies on plant physio-chemical regulation, radiation induced mutation, kinetics of DNA repair under heavy ion exposure and microgravity may help in drawing strategies for terrafarming. The variation in radio-sensitivity of plants further gives strong note to perform extensive experiments at molecular level for requisite focus of character and screening of plants for space mission.

Key words: Heavy ion irradiation, mutations, plant growth, space-flight responses

Abbreviations: HIR = Heavy ion radiations; LET = linear energy transfer; RBE = relative biological effectiveness; RFLP = Restriction-fragment length polymorphism, MeV/n or MeV/u = Total energy per nucleon (n) or mass (u), KeV/ μ m or KeV/ μ m² = Energy transfer at the target per micron or micrometer surface area.

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INTRODUCTION

NASA Life Sciences strategic committee (NASA, 1988) highlighted two major challenges that limit human space flight; first, the biological changes due to exposure to ionizing radiations/ heavy ions radiation (HIR) and second, suitable environmental requirements needed for life support on lengthy space journeys. Plants, being an ultimate source for life sustainability, the radiation-induced changes in plants, especially in their physio-chemical responses require elucidation at the molecular level so that a transgenic approach could be implied for maintaining sustainable growth of the plants there by establishing human life in space.

The heavy ion radiations, a high linear energy transfer (LET) radiation are known to cause more localized, dense ionization within the cells compared to low LET radiation (Kraft *et al.* 1992). Being constituent of the outer space environment, the space flights were considered as one of the methods to study the biological responses to these radiations. Hence, the biological samples were carried during space flight mission to trace the changes caused due to heavy ion irradiation. Intriguing observations from seed experiments from early balloon and satellite flights led to an intensive research interest in the effects of heavy ions (Slater and Tobias 1963, Fujii *et al.* 1966). Seeds exposed to heavy ions during space flights showed growth inhibition, somatic mutations and tumor formation in seedlings (Peterson *et al.* 1977). But in few space flights, seedlings from seeds of onion, radish, cucumber, carrot, pea and wheat were developed but died (Gorkin *et al.* 1980). Further more, flowering did not occur; evaluated in orchid (*Epidendrum*) kept in Salyut 6 for a period of 171 days (Gorkin *et al.* 1980). The lack of floral development and flower abortion was not understood (Halstead and Dutcher 1984). However, Delone *et al.* (1963, 1964, 1966 & 1968) performed study with *Tradescantia paludosa* to understand floral disturbances in various space flights (Vostok-3-6, Voskhod 1, Cosmos 110 and Biosatellite II) and found mitotic disturbances in microspores of experimental samples. The *Tradescantia paludosa* florescence examination conducted in Biosatellite II revealed that increased injury was more at meiosis resulted in more pollen abortion,

frequent pollen grain micronuclei formation and stamen hair stunting etc. (Sparrow *et al.* 1971). Though, such responses were not traced under ground experiments with same space factors (Gorkin *et al.* 1980). The results obtained were mostly not understood fully, possibly due to the presence of microgravity, an additional factor in space and the dynamism of the biological samples. Hoson (1999) suggested the application of gravity substitution factors, as the presence of microgravity in space is known to cause favorable and unfavorable changes.

The results obtained from early space experiments on seed to seedling response, especially in higher plants have been reviewed (Khvostova *et al.* 1962, Gorkin *et al.* 1980, Halstead and Dutcher 1987, NASA 1988) and the challenges for biologist are put forth. In these reviews, the ionizing particle endpoint effects are discussed, for instance germination did not show sensitivity, while mitosis and cytokinesis during seedling development affected precisely. The unequivocal conclusions drawn from early space results were bound to limit the strategies planning for plant development in lack of certainty and control system. The differential response to charged particles, i.e. either activation of specific genes in mammalian cells or inactivation of developmental processes at LET 40-100 KeV/ μm including seeds has been reviewed (Blakely 1992). However, focused radiobiological problem of space and thereby raising questions be addressed at cellular and molecular level is a need of the time (Schimmerling 1992). Limited observations are available from ground based plant studies (Hirono *et al.* 1970, Yang and Tobias 1979). Therefore, simulated experiments on earth are required to study precise response of heavy ion irradiation in plants in view of limited opportunity for space experiments. Some cell lines, known to be radio resistant to X rays, have exhibited greater sensitivity to heavy ions. The heavy ions potential to penetrate precisely in tissues, suggested its possibility to be used as radio therapeutic tool, especially in case of cancer (Ando 2000), make sense to be examined in plants for various responses. The differential responses of plants after exposure to heavy ions / charged-particle radiations are shown in Table 1. Apparently, it appears that heavy ion irradiation dose might be a controlling factor in development of lethal and /or useful mutants.

It is envisaged that the information from detailed studies based on simulated experiments could enable scientists for the desired plants transformation also. Hence, it could be an alternative tool over conventional breeding. It can be also considered for plants mutants development over genetic engineering. Moreover to get some insight for terrafarming, it is necessary to compare the end results of simulated experiments with that of space experiments.

MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN PLANTS

Heavy ion irradiations modulate various physiochemical and morphological processes in plants (Table 1). The changes in morphological characters in maize plants such as yellow stripes on some of the leaves, shortening of plant height, flower anormogenesis, i.e. absence of staminate flower, pistillate flower and visible seed in the tassel, colour of seedling from normal green to yellow green have been observed when the seeds exposed to space radiation (Mei *et al.* 1998). Gao *et al.* (1998) assessed the effect of space environment (weightlessness and HIR) on germination and vegetative growth in medicinal plant, *Carthamus tinctorius* L. The seeds of the plant were carried on board, a retrievable satellite for 15 days and after returning to earth it was observed that seedling heights and branch quantities from seeds kept in weightless condition exposed to heavy ion radiations were higher than those of the seeds kept on earth. This showed that weightlessness and little exposure promote the growth and development of *Carthamus tinctorius*. However, simulated experiment with dry seeds of *Lathyrus sativa* exposed to ^{12}C ions (10^2 - 10^7 particles/cm²) showed a decrease in germination frequency and seedling growth, whereas increased root length was positively correlated with the radiation dose (Wang *et al.* 1993). There are other studies too revealing that spaceflight seedlings exhibited enhanced root production relative to their ground control counterparts (Levine and Krikorian 1996, Levine *et al.* 2000). However, it has not been observed all the times in simulated experiments. For instance, strong inhibition of root elongation and curvature was observed at the root tip in *Arabidopsis thaliana* irradiated by heavy-ion micro beams (Tanaka *et al.* 2002). Studies with rice showed significant decrease in both seedling survival and fertility

of plants with increasing dose of argon ions (Mei *et al.* 1994 a). Experiments performed to evaluate ^7Li ion (45 MeV, fluence 10^7 , 5×10^7 , 10^8 p/cm²) response on mustard seeds, which are small in size and belong to family *Brassicaceae* (*Cruciferae*) showed certain modification in siliques character and increase in yield depending on radiation dose (Mishra *et al.* 2000, Verma 2004).

The differential radiation responses such as more damage in root length than in shoot length in rice with 135 MeV/n of ^{16}O ion, is demonstrated (Nakai *et al.* 1990, Kalimullah *et al.* 2003). Schott *et al.* (1993) observed a typical tumor at the shoot meristem in cotyledons after 14 days after exposure of ^{40}Ar ions (44 MeV/n), hence concluded that single particle radiation response may depend on organ / tissue of different endpoints, as radicle tumourisation was not observed with argon ions. Pickert *et al.* (1990) observed differential response in case of growth of pollen tubes when pollen exposed to different heavy ions with different energies (15 to 16 MeV/n U, Pb; 13.6, 14.7 MeV/n Ar). Kranz and coworkers (1994) have demonstrated that charged-particle exposure of *Arabidopsis* shoot meristem caused abnormalities in growth, while exposing root meristem caused inhibition of seed germination ability. These findings together with ours (Mishra *et al.* 2000, Verma 2004) suggest that plant damage / modifications invariably depend on the type of tissues being exposed to heavy ions. It has been mentioned that irradiation of seed or in an embryo might cause somatic chromosomal aberrations which may lead to generate useful mutants such as semi dwarf, early maturity and large grain size (Liu *et al.* 1991).

These observations demonstrate the unique potential of heavy ions use in crop improvement. In spite of lethality action, the information from detailed study of survived useful mutant(s), may be from non lethal dose, could be exploited for developing useful mutants of more important plants.

BIOCHEMICAL CHANGES IN PLANTS

The changes in plant growth and development are attributed to change in primary metabolites level. It has been reported that ^7Li heavy ion irradiation cause differential effect on carbohydrate, photosynthetic

Table 1. Heavy ion irradiation effect on plant growth and development

Ion (energy, LET or dose)	Plant name & irradiated part	Response	RBE and comments	Reference
⁴ He (10.4 MeV/μ, 74 keV/μm) ⁷ Li (10.4 MeV/μ, 172 keV/μm) ¹² C (10.4 MeV/μ, 409 keV/μm) ¹⁶ O (10.4 MeV/μ, 752 keV/μm) ²⁰ Ne (10.4 MeV/μ, 1030 keV/μm) ⁴⁰ Ar (10.4 MeV/μ, 1890 keV/μm)	<i>Arabidopsis thaliana</i> dry seeds	Growth inhibition, tumor induction, and enhancement in somatic mutations	(i) Growth inhibition—5.8, 5.6, 3.9, 3.2, 1.9, 1.1 (ii) Tumor induction—16.2, 17.6, 9.0, 7.4, 5.9, 2.9 (iii) Mutation—15.8, 11.5, 9.1, 8.4, 0.9, 1.2 for respective ions	Hirono <i>et al.</i> (1970)
²⁰ Ne (19 MeV/μ) ⁴⁰ Ar (19 MeV/μ, 14.7 MeV/μ) ¹³¹ Xe (18 MeV/μ)	<i>Arabidopsis thaliana</i> dry and soaked seeds	Aberrations in developmental endpoints such as survival rate and embryo vitality—soaked seeds were more sensitive than dry seeds	Damage increased with particle density and charge	Bork <i>et al.</i> (1986)
⁴⁰ Ar (14.7 and 20.0 MeV/μ) ⁴⁰ Ca (15.1 MeV/μ) ²³⁸ U (13.7 and 16.3 MeV/μ) ¹³¹ Xe (14.0 and 18.4 MeV/μ)	Embryonic tissue of <i>Arabidopsis thaliana</i> seed	Lethality and tumorization in M ₁ generation and embryonic lethality in M ₂ generation		Bork <i>et al.</i> (1989)
¹⁶ O (135 MeV/n) Dose (0-100 Gy)	<i>Oryza sativa</i> dry seed	Differential damage more in root than shoot	All seedlings were killed by irradiation to a dose exceeding 100 Gy	Nakai <i>et al.</i> (1990)
²⁰ Ne ⁴⁰ Ar ⁵⁶ Fe ⁶⁰ Co-gamma rays	Maize seed	Mutation in Lemon White gene (LW//wl), chloroplast structure, number. in mesophyll cells significantly altered, mutagenic effect.	2, 8.3, 12.5	Qiu <i>et al.</i> (1991)
¹² C (Dose 10 ² -10 ⁷ p/cm ²)	<i>Lathyrus sativa</i> dry seeds	Decreased germination, seedling and root growth, mitotic aberration.	All dose effective in causing aberration	Wang <i>et al.</i> (1993)
⁴⁰ Ar (90 keV/μm) ⁵⁶ Fe (190 keV/μm)	Rice root tip cells	Inhibited seedling growth, reduced plant fertility, chromosomal aberrations and micronuclei in root tip cells and pollen mother cells, dwarf, early ripening & high yield mutant	3.3 for Ar and 4.2 for Fe	Mei <i>et al.</i> (1994 a)

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Ion (energy, LET or dose)	Plant name & irradiated part	Response	RBE and comments	Reference
^1H (0.95 keV/ μm) ^4He (9.34 keV/ μm) ^{14}N (221 keV/ μm)	<i>Pisum sativum</i> Root apex cells	Micronuclei formation -maximum with He ions		Vasilenko and Sidorenko (1995)
^{12}C (46.6 MeV/ μ) Radiation Dose (p/cm ²) 1.43 x10 ³ 4.19 x10 ⁴ 2.6 x10 ⁵ 1.8 x10 ⁶ 1.08 x10 ⁷	<i>Triticum aestivum</i> <i>Cucumis melo</i> var. indorous. <i>Lathrus sativus</i> , <i>T. aestivum</i> > <i>Lycium Lycium</i> > <i>Lathyrus</i> > <i>Cucumis barbarum</i> dry seeds	Germination inhibition, differential chromosomal aberration, order sensitivity	Aberration frequencies 17.98 % at 1.8 x 10 ⁶ in <i>Triticum</i> , 7.15 % at 2.6x10 ⁵ in <i>Lathrus</i> , 4.87 % at 1.08x10 ⁷ in <i>Lycium</i> , 0.16 % at 2.6x10 ⁵ in <i>Cucumis</i>	Wei <i>et al.</i> (1995)
^4He (9.34 KeV/ μm) (Dose 50 and 100 Gy) ^{60}Co -gamma irradiation Dose (5, 20, 50 and 100 Gy)	<i>Pisum sativum</i> <i>N. tabacum</i> cells <i>in vitro</i>	Shrinkage in mitochondrial root apices and membrane as well as decline in ATP/ADP ratio	At 50 & 100 Gy, ATP/ADP ratio increased as early response but not at higher dose where rapid decline in ATP (higher dose response compared with sparsely i.e. germination)	Vasilenko and Sidorenko (1996)
^4He (12.1 MeV/n, 17 keV/ μm) ^4He (4.1 MeV/n, 66 keV/ μm) ^{12}C (17.4 MeV/n, 113 keV/ μm) ^{40}Ar (95 MeV/n, 252 keV/ μm) ^{20}Ne (11 MeV/n, 549 keV/ μm)	<i>Arabidopsis thaliana</i> ecotypes (Col and Ler) dry seeds	Survival reduction and germination inhibition	(i) Survival – 11-12 at 252 keV/ μm (Ar). (ii) Germination – continuous increase, 18 up to 549 keV/ μm	Tanaka <i>et al.</i> (1997)
Space radiation Sample sandwiched between nuclear track detectors aboard Chinese satellite for 15 days	<i>Zea mays</i> dry seeds (heterozygous for Lw1/lw1 alleles)	Morphological changes observed such as yellow strips displayed on leaves, dwarf, anomogenesis of floral organs and yellowish-green seedlings		Mei <i>et al.</i> (1998)
^1H (60 MeV, 1 keV/ μm); ^4He (50 MeV, 15 keV/ μm) ^{12}C (220 MeV, 111 keV/ μm) ^{60}Co -gamma-rays (.2 KeV/ μm)	<i>Nicotiana tabacum</i> dry seeds	Inhibition of survival and germination rate, Mitotic index declined, chromosomal aberrations – more fragments and decrease in bridges. -ions more effective than gamma rays	(i) 8.3, 14.3 for He, C – survival inhibition (ii) 7.8, 7.0, 17.5 for H, He, C – chromosomal aberrations	Hase <i>et al.</i> (1999)
Carbon ion (18 MeV/n) Electrons (2 MeV) (0.2 KeV/ μm)	<i>Nicotiana tabacum</i> root tip cells	(i) Mitotic index reduced to half by 1 Gy of C ion and 15 Gy of e ⁻ radiation (ii) Chromosomal	(i) Mitotic index at 15 Gy (ii) Chromosomal aberrations at 10 Gy	Shimomo <i>et al.</i> (2001)

Ion (energy, LET or dose)	Plant name & irradiated part	Response	RBE and comments	Reference
^{12}C (22.7 KeV/ μm) ^{20}Ne (64.2 KeV/ μm)	Rice seeds	aberrations – bridges, fragments and laddering observed Chlorophyll deficient mutants (CDM) segregated in M_2 progeny	Highest rate of CDM with C-ion (7.31 %) at 40 Gy and Ne-ion (11.6%) at 20 Gy	Abe <i>et al.</i> (2002)
^{12}C (113 KeV/ μm) (Dose 150 Gy) Electrons (0.2 KeV/ μm) (Dose 750 Gy)	<i>Arabidopsis</i> dry seeds (ecotype Columbia)	Embryonic lethal and chlorophyll deficient mutants (tt & gl mutants) scored in M_1 & M_2 generation	Mutation rate with carbon ion was 17-fold higher than that of electrons	Shikazono <i>et al.</i> (2003)
^7Li (45 MeV) Radiation Dose (p/cm^2) 1×10^7 5×10^7 1×10^8	<i>Brassica juncea</i> L. cv.RH-30 dry seeds	Metabolites level and nitrate reductase activity differentially altered depending on radiation dose		Verma <i>et al.</i> (2004)

pigments and protein level and nitrate reductase activity in mustard plants (Verma *et al.* 2004). Few studies have been performed to evaluate the effect of space conditions on enzymes and protein content in medicinal plants (Gao *et al.* 1999 a,b). When seeds of *Agastache rugosa* set up in retrievable satellites, peroxidase activity and protein content of the weightless group of seeds were found to be little lower than the earth controlled groups. Further, the electrophoresis patterns of protein of the hit group were different from those of other groups (Gao *et al.* 1999 b). In the same condition with other medicinal plant, *Carthamus tinctorius*, Gao *et al.* (1999 a) found that the protein contents of the weightless seeds and the seeds which were exposed once and for short periods to heavy ion radiations were higher than those of the seeds which were kept on earth; however, the seeds which were allowed to have heavy ion penetration for longer duration showed lower level protein than those of the ground controlled seeds. Interestingly in the simulated experiment, with ^7Li heavy ion irradiation, the activity of peroxidase was found higher in the leaves of seedlings from irradiated (fluence 5×10^7 , $10^8 \text{p}/\text{cm}^2$) seeds compared with that of control seeds and it was found positively correlated with the enhanced crop production (Verma 2004). It is also demonstrated that

the protein level in the leaf tissues had differential response depending on irradiation dose of ^7Li ion (45 MeV, fluence 10^7 , 5×10^7 , $10^8 \text{p}/\text{cm}^2$) compared with that of control (Verma *et al.* 2004).

It is inferred that different plants may have different susceptibility to space conditions and that the weightlessness in the space environment may have an effect mainly on the physiological condition of plants, but the HIR has a stronger effect on the hereditary material of plants.

Heavy ion irradiation also causes structural and functional change in cell organelles (Vesilenko and Siderenko 1995, 1996). ^4He ion irradiation (50, 100 Gy) induced modulation in mitochondrial respiration metabolism due to shrinkage in membrane, which led to change in adenylate ratios (ATP/ADP) in root apices of *Pisum sativum* and in exponentially growing *Nicotiana tabacum* cells (Vesilenko and Siderenko 1996). Heavy ions particles can also interrupt the continuity of membranous regions in the cells. Gaikwad and coworkers (1999) studied the effects of different fluence (10^4 to $10^8 \text{p}/\text{cm}^2$) of ^7Li (45 MeV) and ^{16}O (100 MeV) on the photosynthetic apparatus of spinach leaves and found

irradiation induced alteration in the characteristic trap distribution of the various electron transport components of the chloroplast. It is observed that ^1H ions (24 MeV) radiation affects biosynthesis of chlorophyll and inhibits PSII acceptor site of chloroplast during developmental stages of rice seedlings and simultaneously root/shoot growth inhibition (Kalimullah *et al.* 2003). Gao *et al.* (1999 c) observed some changes in chloroplast grana, starch grains and vesicles in chloroplasts and no obvious change was in nuclei of cells of weightless group and heavy ion radiations hit group of seeds of *Datura innoxia* in space condition. Other electron microscopic studies also revealed alteration in both structure and number of chloroplasts in the mesophyll cells of white yellow stripes, a mutant of lemon, developed after exposure to heavy ions (Qiu *et al.* 1991). However, it appears that the heavy ions by virtue of their deep deposition tendency and direct damaging action on biomolecules through interfering with their interaction, chlorophyll might be a very susceptible target (Roots *et al.* 1985). This might be attributed to generation of free radical species. Roots *et al.* (1985) reported that the contribution of hydroxyl radicals to total radiation damage is about 70% for X-rays and about 40% for carbon ions of 52 KeV/ μm . It is also demonstrated that the yields of the OH radicals increase with the specific energy for the same kind of ion and decrease with the atomic number for different ions used at the same specific energy. The free radicals generation caused structural and functional damage could be also one of the major factors operating under heavy ions irradiation stress, but plants having different threshold rate of free radicals generation would be culminating in varied sensitivity and tolerance depending on species.

CHROMOSOMAL ABERRATIONS AND MUTATIONS IN PLANTS

The mutagenic effects of ion particle irradiation, mainly on somatic mutations, have been investigated using various plant species (Smith 1972). From these studies, it has been demonstrated that ions with LET \sim 100-200 KeV/ μm gave the highest value of RBE. The biological effects like lethality, sterility, chromosome aberrations and mutations have been reported in plant species, after their seeds exposed to charged particles (Smith 1972, Mei *et al.* 1994 b, Tanaka *et al.* 1997, Hase *et al.* 1999).

Such responses have been observed in animal system also (Blakely 1992). But the mutational changes in *Arabidopsis* appeared to be gene specific and dose dependent (Brustad 1962, Hirono *et al.* 1968). Hirono *et al.* (1970) studied the comprehensive effects of different HIR at a wide range of LET (2-1890 keV/ μm) on *Arabidopsis thaliana* seeds (Table 1). They demonstrated the maximum RBE in the range of 72-174 keV/ μm in case of growth inhibition, tumor induction and increment in somatic mutation; and suggested that further increase in LET led to decline in RBE. On the other hand, frequency of mutation increased in maize with dose of heavy ions with high LET compared with that of photons (Qiu *et al.* 1991). Tanaka *et al.* (1997) investigated the effects of heavy ions on the survival of *Arabidopsis* using various kinds of ions with LET of 17-549 KeV/ μm , and found the highest RBE by argon ion irradiation at LET 252 KeV/ μm , which is somewhat higher than the well-known RBE peak at 100-200 KeV/ μm . Moreover, it has also been demonstrated that RBE for lethality peaked at LET around 221 KeV/ μm , 354 KeV/ μm and 396 KeV/ μm for carbon, neon and argon ions respectively, while sterility observed at higher RBE at LET of 354KeV/ μm with neon ions and at LET of 113 KeV/ μm with carbon ions (Shikazono *et al.* 2002). These results suggest that the larger the atomic number of ions larger the LET for the peak RBE, as observed in mammalian cells (Kraft 1987). The differential chromosomal aberration in cells of root tip of plants from ^{12}C ion irradiated seeds of *Triticum aestivum*, *Lathyrus sativus*, *Lycium barbarum*, *Cucumis melo* has been recorded (Wei *et al.* 1995). These differential radiation responses could also be due to differential molecular and cellular damage repair mechanism. However, the repair mechanism responses in dry seeds are found to be more efficient than in hydrated embryo under irradiation stress. This could be implicated to different chemical environment and / or hydration state of DNA in the dry seeds compared with that of imbibed seeds.

Generation of genomic mutations in general has been suggested to be one of the important responses in plants to heavy-ion exposure (Pickert *et al.* 1990). This has been validated by early and late effects of accelerated heavy ions, like the embryonic tissue of *Arabidopsis*

seeds evaluation for endpoints results in terms of lethality and tumorization in M_1 generation for early effects, and embryonic lethality in the M_2 generation for the late effects (Bork *et al.* 1989). *Arabidopsis* dry seeds irradiated with heavy ions of ^{20}Ne (19 MeV/u), ^{40}Ar (19 MeV/u, 14.7 MeV/u) and ^{131}Xe (18 MeV/u) yielded aberrations in varied developmental endpoints where survival rate and embryo vitality and other damages increased with particle density and charge (Bork *et al.* 1986). A significant increase (four fold) of spontaneous mutagenesis was observed in either case of total number of aberrant cells or in a single cell with multiple aberrations, when seeds of *Lactuca sativa* exposed to space factors, cosmic radiation and heavy ions, for prolonged periods (175 days) during space flight. Comparison of different earlier studies shows that the frequency of such aberrations increased with the duration of the flight (Nevzgodina *et al.* 1981).

Wang *et al.* (1993) suggested that appropriate doses of charged particle radiation could induce useful mutants. Most of the studies showed that low and intermediate doses of HIR induce more mitotic aberrations than higher dose. Seeds exposed to ^{40}Ar ions at 90-100 Gy, produced several useful mutant lines like semi dwarf, early ripening and high yield (Mei *et al.* 1994 a). Restriction-fragment length polymorphism (RFLP) analysis of the semi dwarf mutants induced with Ar particles showed the involvement of large DNA alterations. Some more positive mutants like M_2 plants of rice derived from the seeds irradiated with ^{14}N ion was found to be resistant for bacterial leaf blight (Nakai *et al.* 1992)

These studies indicate the potential of heavy ions use in crop improvement. The most effective mutagenic nature of heavy ions could be exploited for the induction of useful mutants with desired character for multiple purposes including plant breeding. Systematic studies with various heavy ions have demonstrated that the frequency of mutation in *Zea mays* increased linearly with dose, and that high-LET were many times more effective than photons (Qiu *et al.* 1991). Qiu and colleagues (1991) have found the induction of lemon white gene mutation in inbred plants in the M_2 generation by ^{20}Ne , ^{40}Ar and ^{56}Fe ion irradiation. The frequency of occurrence of white-yellow stripes on leaves of plants grown from

irradiated maize grains, the Lemon-white gene (Lw1) was used as criterion to compare the mutagenic effects of high and low LET radiation of heavy ion. Mei *et al.* (1998) comparing the earlier data from 69 months exposure of dry seeds of *Zea mays* in space (long duration exposure facility-LDEF) mission with short term (19 days in low earth orbit) and Mei *et al.* (1994a) demonstrated nearly the same level of mutation induction in both the cases. The RAPD analysis of mutant plants further revealed that space radiation could induce inheritable mutagenic effects after seeds being exposed, and verified the changes in genetic material in the mutants (Mei *et al.* 1998). Further, more interesting observation was that the M_2 generation having much higher level of mutations than that of M_1 , indicating there by inheritable nature of the changes due to space exposure (Mei *et al.* 1998). Same trend has been recorded in Indian mustard in this laboratory (Verma 2004).

Heavy ions irradiation caused more lethality, chromosomal mutations, and cell transformation than low-LET radiation has been mentioned (Tobias 1985, Shikazono *et al.* 2003). The molecular reasons for this are not completely understood but attributed largely to fragmentation and reintegration of DNA. Though, cells have immense potential to repair these lesions, but many of the deleterious effects might be arising due to miss repair or miss rejoining of DNA. Molecular analysis of carbon ion induced mutations in *Arabidopsis* revealed that inversions and translocations occurred in these mutants (Shikazono *et al.* 1998). Mei *et al.* (1994 a) using random clones as probes, found that a rearrangement had occurred in the genome of dwarf mutant of rice induced by argon ions. In the *Arabidopsis* plants, half of the mutants showed small mutations, such as base changes and small deletions involving a few bases; while other half showed large DNA alterations, such as inversions, translocation and deletions (Tanaka 1999). The analysis of DNA through Restriction Landmark Genomic Scanning (RLGS) method have revealed the DNA fragment deletion in an albino mutant of rice arose from seeds irradiated with C or Ne ions (Abe *et al.* 2002). It is proposed that high LET would predominantly produced double strand breaks with damaged end groups whose repair ability might be low (Blakely and Kronenberg 1998, Nikjoo *et al.* 1998)

PLANT RADIOSENSITIVITY AND RESPONSES

The studies performed clearly indicate that plant responses depend on the genus radiosensitivity, which varies from genus to genus. Therefore, an important area of investigation emerging with significant new results, deals with cell-line differences in radiosensitivity. The radiosensitivity of a cell is a function of the severity of the lesions produced by radiation and their potential of repair. Comparison of the results of dry and soaked seeds irradiated with ^{40}Ar ions (19 MeV/u) showed that hydrated seeds embryo viability declined significantly, thus considered to be more sensitive (Bork *et al.* 1986). No plant survival occurred above fluences of Li^{3+} 10^8 p/cm² in case of dry seeds, while 5×10^7 p/cm² a lower dose, imparted same response in soaked seeds. We have also observed that germination of mustard (*Brassica juncea*) seeds decreased after dry seeds exposed to fluence at 10^8 p/cm² of $^7\text{Li}^{3+}$ (45 MeV), whereas soaked seeds showed inhibition at 5×10^7 p/cm² (Chopra 1999). Increase in sensitivity by soaking the seeds before irradiation has been observed with sparsely ionizing gamma rays as well (Fujii 1967). It could be suggested that, in case of soaked seeds, the hydrated embryos have more water, so they are exposed to an abundance of reactive oxygen species and hence much more susceptible to ionizing radiations in general. Apparently, in the presence of H₂O the densely ionizing radiation can produce OH⁻ radicals which caused more molecular aberrations in DNA. Obviously, the embryo vitality was found to be higher in dry seeds than for soaked seeds (Bork *et al.* 1986). ^7Li ion irradiation of mustard seeds revealed that response magnitude of soaked and unsoaked seeds were varying in terms of metabolites and nitrate reductase activity in leaf of the seedlings also (Copra *et al.* 1999). However, Wei *et al.* (1995) studied radio sensitivity by irradiating dry seeds of a number of plants like cereal (*Triticum aestivum*), forage (*Lathyrus sativus*), medicinal (*Lycium barbarum*), and economic vegetable (*Cucumis melo*) with 46.6 MeV/u of ^{12}C ion (LET 56 keV/μm). Though, they observed a similar sensitivity pattern in relative inhibition of germination rates and enhancement in chromosomal aberrations in root tip cells of each plant type, yet total frequency of aberrations were found to be 12.65% in *Triticum aestivum*, 4.87% in *Lycium barbarum*, 2.58% in *Lathyrus sativus* and 0.26% in

Cucumis melo at 1.08×10^7 p/cm² indicating *Triticum* as the most radiosensitive and *Cucumis* the radioresistant among the four plants examined. This further suggests that various plants have their own level of radio sensitivities and the state of the DNA may further affect the LET dependence of radio sensitivity (Shikazono *et al.* 2002). This has been further substantiated with a study on dry seeds of Col and Ler ecotypes of *Arabidopsis thaliana* exposed to ^4He , ^{12}C , ^{40}Ar and ^{20}Ne ions with LET in the range of 17-549 KeV/μm and to electrons (LET = 0.2 KeV/μm) (Tanaka *et al.* 1997). The RBE for the survival of both ecotypes showed the same pattern of variations, with a maximum RBE of 11-12 at 252 KeV/μm. Interestingly, for germination, RBE increased with increasing LET in Ler but not in Col, showing different sensitivities between the both ecotypes.

PLANT RESPONSES FOLLOWING FRACTIONATED IRRADIATION

Radio sensitivity depends on various factors like conditions of exposure, factors related to the cellular environment and the kind of cell itself. In terms of survival, it makes a difference if given dose is delivered in a fraction or all in one shot. The fractionation, in certain cases diminished the effectiveness of radiation-exposure (Barendsen 1982). Splitting one single dose into two smaller doses, fractions given over a period of time has been shown to decrease the lethality and frequency of chromosome aberrations, suggesting that some interactions and / or repair processes occur after first exposure (Leenhouts *et al.* 1981). For instance, *Nigella damascene* showed decrease in chromosome aberrations after the fractionated irradiation of gamma-rays, considered as the fast kinetics of the chromosome rejoining process (Gilotdelhaile *et al.* 1973). In *Hordeum distichum* and in *Trillium kamtschaticum*, exposure to X-ray in fraction revealed both fast and slow rejoining processes (Ichikawa *et al.* 1965, Iwabuchi *et al.* 1966). Interestingly, Leenhouts *et al.* (1981) demonstrated that a predose of fission neutron irradiation could reduce the level of lethal damage in *Saintpaulia* also during second dose of neutron, but comparatively to a lower extent than a pre-dose of X-rays. This phenomenon was considered to be due to differences in radiation LET and hence, suggested that the ability of the cells to reduce DNA

damage is low after a pre-dose of high-LET radiation. Experiments with fractionated doses to evaluate the repair process under high LET revealed that fractionated exposure causes comparatively less damage (Shimono *et al.* 2001). Otherwise, high LET tends to cause high dose deposition in a small region, might be resulting into a clustered DNA damages, mostly double strand breaks having lesser degree of repair depending on the system. In tobacco root tip cells, the induction of chromosomal aberrations was measured after exposure to both 18 MeV/n carbon ions and 2 MeV electrons with mean LET 0.2 KeV/ μm (Shimono *et al.* 2001). Splitting of dose into two fractions did not produce any significant effect on the yield of aberrations following carbon-ion exposure, whereas a clear decrease was observed after exposure to electrons. Moreover, this decrease appeared to be independent of the type of aberrations. Therefore, Shimano group (2001) suggested that probably the lack of any significant effect on the yield of aberrations was either due to a lack of error-free-repair or to a less efficient repair of damage caused by exposure to C ion.

Although, repair kinetics of the damage after high-LET irradiation is not well known in plants, there is some evidence for less effective repair after high-LET radiation in mammalian cells (Goodwin *et al.* 1994). It could be suggested that damage by heavy ions is not random and actually it is clustered damage in multiple damaged sites in a closely spaced region (Sachs *et al.* 1998, Terato and Ide 2004). Clustered DNA damage (locally multiple damaged sites) is thought to be a critical lesion caused by ionizing radiation, and high LET radiation of heavy ion particles is believed to produce high yields of such damage (Terato and Ide 2004).

IN VITRO STUDIES OF HEAVY IONS RESPONSES

Generally, *in vitro* studies are performed to understand the precise mechanism of modification in metabolic process at cellular level with a view to extrapolate the results to understand at whole plant level. Though, it does not fit all the time. In the single cell studies, such as bacterium (*Bacillus subtilis*) cell maximum inactivation was at LET of 100 KeV/ μm , while no such peak was observed in *E. coli* at same energy (Yatagai *et al.* 1975). The diploid yeast inactivation has

been also observed with linear energy transfer of 100 KeV/ μm (Kiefer *et al.* 2002). The diploid chromosome number in an organism is considered to be a factor for tolerance in general. It is shown that the RBE of carbon ions (considering active dose to reduce survival about 37%) peaked at LET 230 KeV/ μm in seeds of tobacco (Hase *et al.* 2002), while 221 KeV/ μm in *Arabidopsis* (Shikazono *et al.* 2002). Apparently, it shows that optimum RBE in the higher plants does not require change in LET. Yokota *et al.* (2003) have also observed that ^{12}C ions LET giving maximal RBE in single tobacco cells was similar to that of tobacco seeds. However, it could be suggested that RBE might be peaked at higher LET in case of higher plants compared with that of other organisms, and higher tolerance could be due to either lesser degree of damage and /or efficient repair depending on species..

It will be also pertinent to know the signal transduction for heavy ion irradiation tolerance in plants. Protein Kinase C, a signaling molecule, found to be increased in mammalian transformed cells by heavy ion exposure (Pirolo *et al.* 1993). The *in vitro* studies can provide some insight for drawing strategies for varieties of plants for terrafarming application.

GRAVITATIONAL EFFECTS ON PLANT GROWTH

Gravitational force, a universal factor modifying growth of the organism on earth is considered to be affecting the plant growth during space experimentation. However, the primary processes like seed germination did not change under space environment, and exhibit gravity insensitivity, but the plant cell wall, a first order cell component responded and expressed phenotypically to gravity, which appeared to be very low on earth (1xg) (Hoson *et al.* 2002). The seedling modulation under gravity might be through changing mitosis and cytokinesis leading to decreased growth. Also it has been suggested that lack of much defined growth response is due to lack of directional gravity and force during space mission (Halstead and Dutcher 1987). Various attempts to elucidate the effects of microgravity on plant growth in space have been made and observed that cell wall components decreased under microgravity in space (Halstead and Dutcher 1987, Nedukha 1996). However,

either increase in cell wall elongation in wheat, lettuce hypocotyls (Halstead and Dutcher 1987) or no change or decrease in some cases under microgravity has also been reported (Kiss *et al.* 1998, Levine *et al.* 2001). Hoson *et al.* (2002) observed an increase in elongation in rice coleoptiles due to stimulation of cell wall extension in space.

This physiological process needs further experimentation under simulated condition for precise elucidation. However, few experiments on plant growth under hyper gravity have been performed and demonstrated to be the one directional (Waldron and Brett 1990). Apparently, results obtained from experiments on plant growth responses under gravitational force (microgravity and/or hyper gravity) are varying with different type of plant species. Kuang *et al.* (2000 a) demonstrated that none of the reproductive stage development in *Brassica rapa* L. (cv. Astroplants) was dependent on gravity on space shuttle (STS-87), but lower embryo quality was recorded under microgravity. Hence, gravitational force would be another important factor along with heavy ion irradiation exposure during space mission; therefore, any attempts for terrafarming should be preceded by extensive research on higher plant systems in presence of factors like hyper gravity / microgravity and irradiation exposure with various dosages.

FUTURE PROSPECTS

Building of life support systems in space for a long-term stay of human is one of the major dreams of scientists. Therefore, experiments have been carried out to cultivate auto- and heterotrophic species in the space. Algae were shown to grow normally in the space flight environment and can constitute a part of a photoautotrophic micro ecosystem in space (Sychev *et al.* 2000). However, the characteristic feature of plants to produce oxygen and grain has made it to be considered for detailed experimentation, for permanent life support systems during long-term space missions. A major breakthrough has been made in growing a whole cycle of *Brassica rapa* from seed to seed in space by employing better techniques of proper circulation of CO₂ inside the incubator under microgravity conditions,

regulated moisture transfer and using an ethylene insensitive plant variety (Kuang *et al.* 2000 a,b). It requires more studies for the subsequent identification and development of heavy ion radiation resistant plant varieties to fulfill the desired targets.

It could be inferred here that research must be focused on assessing functionality of survivors out of heavy ions exposed plants to understand the detailed molecular mechanism of the responses. It is implied that heavy ion radiations are deleterious only when their dosage exceed the life resonance in the plants, which depends upon plant species. Abe *et al.* (2003) observed that irradiation with ¹⁴N (31KeV/μm) or ²⁰Ne (63KeV/μm) ion at dose 5-10 Gy did not change survival percentage of *Nicotiana tabacum* strains Xanthi and BY-4, while morphological abnormalities appeared just after irradiation with lower dose but abnormalities increased with increasing dose up to 10 Gy in case of Xanthi. At the same time, BY-4 did not respond with both ions. Further, this group has recorded their observation of floral mutants of two different plants with dosage (5-10 Gy) of N and the preliminary experiments with cultured plant tissues have shown some developmental effects. Due to the excitement generated by these kinds of results with plants, which are not being observed previously with low-LET radiations, both basic and applied heavy ion research with seeds and plants are required. It seems that heavy-ion irradiations could be employed to produce novel mutants for desired genetic characters, which have been attempted in few cases (Abe *et al.* 2003, Shikazono *et al.* 2003). The scientific curiosity for understanding biological systems variation in radiosensitive or resistant plants could be accomplished by evaluating possible variations in genetic properties of individual cell lines. Although, there are evidences for the existence of induced activation of repair of genes associated with ionizing radiation damage, the identification of these genes at the present time remains elusive. This area of research may surely yield exciting new information (Hagen 1990). Study of radiation sensitivity may also provide insight for understanding of delayed senescence or quick anti senescence properties of plant species, under changed agro climatic conditions. It is envisaged here that precise dosages of heavy ions could be used as a tool, alternative to time consuming

traditional breeding methods and/or costly biotechnological methods for transgenic approaches also. The detailed physiological and biochemical study of plants thus may help to draw precise strategies for transformation of a number of important plant species for human kind. The study of bystander effects (late changes in other than the targets) due to heavy ion irradiation is to be also examined simultaneously, as some consequential effects are being noticed in animal system (Nagasawa *et al.* 2003). The bystander effect of HIR could be possibly explained by examining the virtual epigenetic control. This may also elucidate the molecular responses as well. Because methylation of cytosine, acetylation of protein(s), transposition of certain sequences within DNA chain under epigenetic control during heavy ion exposure of plants and plants part, may lead to subtle changes in the way of genetic information processing and expression. The magnitude of epigenetic markers might be attributing in differential responses of plants with HIR exposure. Because of these potentials of heavy ion, response study may also throw some light on inducing regenerability by a change in genome in recalcitrant plants. Terrafarming is a challenging goal of space science, which could be achieved with detailed studies of plant species after the screening of tolerant strains. Adding to the future prospects of HIR application, it will be also interesting to evaluate HIR induced change in quantity and quality of biomolecules in medicinally important plants.

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REFERENCES

- Abe, T., Matsuyama, T., Sekido, S., Yamaguchi, I., Yoshida, S. and Kameya, T. (2002). Chlorophyll-deficient mutants of rice demonstrated the deletion of a DNA fragment by heavy ion irradiation. *J. Radiat. Res.* **43**: S157-S161.
- Abe, T., Miyazawa, Y., Saito, H., Yoshida, S., Fukunishi, N., Ryuto, H. and Yano, Y. (2003). Utilization of heavy ion beam as a mutagen in plants. The 14th Symposium on Accelerator Science and Technology, Tsukuba, RIKEN, Wako, Saitama, Japan.
- Ando, K. (2000). High LET radiobiology at NIRS: Current status and future plan. *Phys. Med.* **17** (suppl 1): 292-295.
- Barendsen, G.W. (1982). Dose fraction, dose rate and iso-effect relationships for normal tissue responses. *Int. J. Radiat. Biol. Phys.* **8**: 1981-1997.
- Blakely, E.A. (1992). Cell inactivation by heavy charged particles. *Radiant. Environ. Biophys.* **31**: 181-196.
- Blakely, E.A. and Kronenberg, A. (1998). Heavy ion radiobiology: new approaches to delineate mechanisms underlying enhanced biological effectiveness. *Radiat. Res.* **150**: 126-145.
- Bork, U., Gartenbach, K., Koch, C. and Kranz, A.R. (1986). Biological effects of heavy ions in *Arabidopsis* seeds. *Adv. Space Res.* **6**: 149-152.
- Bork, U., Gartenbach, K.E. and Kranz, A.R. (1989). Early and late damage induced by heavy charged particle irradiation in embryonic tissues of *Arabidopsis* seeds. *Adv. Space Res.* **9**: 117-121.
- Brustad, T. (1962). Heavy ions and some aspects of their use in molecular and cellular radiobiology. *Advan. Biol. Med. Phys.* **8**: 161-224.
- Chopra, G. (1999). Biochemical changes in seedlings of *Brassica juncea* irradiated with heavy ion ⁷Li⁺, M.Sc. thesis, M.D. Univ., Rohtak
- Chopra, G., Choudhary, D. and Mishra, S.N. (1999). High LET radiation induced changes in growth and metabolites in Indian mustard (*Brassica juncea*) seedlings. Annual Report, pp. 131-132. Nuclear Science Centre, New Delhi.
- Delone, N.L., Bykovskii, V. F., Antipov, V. V., Parfenov, G. P., Vysotskii, V. G. and Rudneva, N. A. (1964). Effect of space flight factors on *Tradescantia paludosa* microspores on board the satellite spaceships Vostok 5 and 6. *Cosmic Res.* **2**: 268-277.
- Delone, N.L., Egorov, B. B. and Antipov, V. V. (1966). Effect of the factors of cosmic flight of the satellite ship Voskhod on the microspore *Tradescantia paludosa*. *Cosmic Res.* **4**:139- 143.

RESPONSES OF PLANTS TO HEAVY IONS IRRADIATION

- Delone, N.L., Popovich, P. R., Antipov, V. V. and Vysotskii, V. G. (1963). Effect of Space-flight Factors on *Tradescantia paludosa* microspores on board the satellite space-ships 'Vostok-3' and 'Vostok-4'. *Cosmic Res.* **1**: 257-268.
- Delone, N.L., Trusova, A. S., Morozova, E. M., Antipov, V. V. and Parfenov, G. P. (1968). The effect of space flight on Cosmos-110 on the microspores of *Tradescantia paludosa*. *Cosmic Res.* **6**: 250-253.
- Fujii, T. (1967). Comparison of the killing effect of gamma-rays and thermal neutrons. *Arabid. Inf. Ser. (Göttingen)* **4**: 59.
- Fujii, T., Ikenaga, M., Lyman, J.T. (1966). Radiation effect on *Arabidopsis thaliana*-II. *Radiat. Bot.* **6**: 297-306.
- Gaikwad, J., Thomas, S., Kamble, S., Vidyasagar, P.B. and Sarma, A. (1999). Effect of ^7Li (45MeV) ions on spinach leaves studied by thermoluminescence technique. *Nuclear Instruments and Methods in Physics Research B.* **156**: 231-235.
- Gao, W., Zhao, S., Xue, L., Xiao, P., Li, X. and Qi, Z. (1998). Effect of space environment on germination and vegetation growth in *Carthamus tinctorius* L. *Zhongguo Zhong Yao Za Zhi* **23**: 712-713.
- Gao, W., Zhao, S., Xiao, P., Li, X. and Qi, Z. (1999 a). Effect of space environment on physiological state in *Carthamus tinctorius* L. *Zhongguo Zhong Yao Za Zhi* **24**: 77-79.
- Gao, W., Zhao, S., Xue, L., Quan, L. and Xiao, P. (1999 b). Effects of space flight on medicinal plant *Agastache rugosa* (Fisch. et Mey.) O. Ktze. *Zhongguo Zhong Yao Za Zhi* **24**: 138-140.
- Gao, W., Zhao, S., Xue, L., Quan, L., Fu, R. and Xiao, P. (1999 c). Effect of space flight on ultrastructure in medicinal plant *Datura innoxia* Mill. *Zhongguo Zhong Yao Za Zhi* **24**: 332-334.
- Gilot-Delhaile, J., Thakare, R. and Moutschen, J. (1973). Fast rejoining processes in *Nigella damascene* chromosomes revealed by fractionated ^{60}Co γ -rays exposure. *Radiat. Bot.* **13**: 229-242.
- Goodwin, E.H., Blakely, E.A. and Tobias, C.A. (1994). Chromosomal damage and repair in G_1 -phase Chinese hamster ovary cells exposed to charged-particle beams. *Radiat. Res.* **138**: 343-351.
- Gorkin, Y., Mashinskiy, A. and Yazdovskiy, V. (1980). Birthday flowers: 'Salyut-6', our commentary. October, 27, pp. 3. Pravda.
- Hagen, U. (1990). Molecular radiation biology: Future aspects. *Radiat. Environ. Biophys.* **29**: 315-322.
- Halstead, T.W. and Dutcher, F.R. (1984). Experiments on plants grown in space: Status and prospects. *Ann. Bot.* **54**: 3-18.
- Halstead, T.W. and Dutcher, F.R. (1987). Plants in space. *Annu. Rev. Plant Physiol.* **38**: 317-345.
- Hase, Y., Shimono, K., Inoue, M., Tanaka, A. and Watanabe, H. (1999). Biological effects of ion beams in *Nicotiana tabacum* L. *Radiat. Environ. Biophys.* **38**: 111-115.
- Hase, Y., Yamaguchi, M., Inoue, M. and Tanaka, A. (2002). Reduction of survival and induction of chromosome aberrations in tobacco irradiated by carbon ions with different linear energy transfers. *Int. J. Radiat. Biol.* **78**: 799-806.
- Hirono, Y., Smith, H.H. and Lyman, J.T. (1968). Tumor induction by heavy ionizing particles and X-rays in *Arabidopsis*. *Radiat. Bot.* **8**: 449-456.
- Hirono, Y., Smith, V., Lyman, J.T., Thompson, K.H. and Baum, J.W. (1970). Relative biological effectiveness of heavy ions in producing mutations, tumors and growth inhibition in the crucifer plant, *Arabidopsis*. *Radiat. Res.* **44**: 204-223.
- Hoson, T. (1999). Interaction of gravity with other environmental factors in growth and Development: An Introduction. *Adv. Space Res.* **23**: 1971-1974.
- Hoson, T., Soga, K., Mori, R., Saiki, M., Nakamura, Y., Wakabayashi, K. and Kamisaka, S. (2002). Stimulation of elongation, growth and cell wall loosening in rice coleoptiles under microgravity conditions in space. *Plant Cell Physiol.* **43**: 1067-1071.
- Ichikawa, S., Ikushima, T. and Nishiyama, I. (1965). Two kinds of chromosome rejoinings in X-rayed two-rowed barley. *Radiat. Bot.* **5**: 513-523.
- Iwabuchi, M., Tanifuji, S. and Ochiai, H. (1966). Production of two different types of chromosome breaks by X-rays. *Jpn. J. Genet.* **41**: 395-402.

- Kalimullah, M., Gaikwad, J.U., Thomas, S. Sarma, A. and Vidyasagar, P.B. (2003). Assessment of ¹H heavy ion irradiation induced effects in the development of rice (*Oryza sativa* L.) seedlings. *Plant Sci.* **165**: 447-454.
- Khvostova, V.V., Prokof'Yeva-Bel'Govskaya, A.A., Sidorov, B.N. and Sokolov, N.N. (1962). The effect of space flight conditions on the seeds of higher plants and Actinomycetes. In: N.M. Sisakyan, V.I. Yazdovskiy (eds.), *Problemy Kosmicheskoy Biologii*, Vol. II, pp.153-162. Academy of Sciences, Moscow.
- Kiefer, J., Genolf, R. and Ikpeme, S. (2002). Heavy ion-induced DNA double-strand breaks in yeast. *Radiat. Res.* **157**: 141-148.
- Kiss, J.Z., Katembe, W.J. and Edelmann, R.E. (1998). Gravitropism and development of Wild type and starch-deficient mutants of *Arabidopsis* during space flight. *Physiol. Plant.* **102**: 493-502.
- Kraft, G. (1987). Radiobiological effects of very heavy ions: Inactivation, induction of chromosome aberrations and strand breaks. *Nucl. Sci. Appl.* **3**: 1-28.
- Kraft, G., Kramer, M. and Schulz, M. (1992). LET, track structure and models. *Radiat. Environ. Biophys.* **31**: 161-180.
- Kranz, A.R., Gartenbach, K.E., Shevchenko, V.V., Schopper, E., Baican, B., Schott, J.U., Setz, R., Heilmann, C., Dudkin, D., Nefedov, N. and Potapov, Y. (1994). Recent results of the joint ESA-DARA / IBMP experiments Biokosmos 'seeds'. *Acta. Astronaut.* **32**: 761-766.
- Kuang, A., Popova, A., Xiao, Y. and Musgrave, M.E. (2000 a). Pollination and embryo development in *Brassica rapa* L. in microgravity. *Int. J. Plant Sci.* **161**: 203-211.
- Kuang, A., Xiao, Y., McClure, G. and Musgrave, M.E. (2000 b). Influence of microgravity on ultrastructure and storage reserves in seeds of *Brassica rapa* L. *Ann. Bot.* **85**: 851-859.
- Leenhouts, H.P., Sijsma, M.J., Litwiniszyn, M. and Chadwick, K.H. (1981). The repair of sub lethal damage and the stimulated repair of potentially lethal damage in *Saintpaulia*. *Int. J. Radiat. Biol.* **40**: 413-425.
- Levine, H.G. and Krikorian, A.D. (1996). Enhanced root production in *Haplopappus gracilis* grown under space flight conditions. *J. Grav. Phys.* **3**: 17-27.
- Levine, H.G., Sharek, J.A., Johnson, K.M., Stryjewski, E.C., Prima, V.I., Martynenko, O.I. and Piastuch, W.C. (2000). Growth protocols for etiolated soybeans germinated within BRIC-60 canisters under space flight conditions. *Adv. Space Res.* **26**: 311-314.
- Levine, L.H., Heyenga, A.G., Levine, H.G., Choi, J.W., Devin, L.B., Krikorian, A.D. and Lewis, N.G. (2001). Cell-wall architecture and lignin composition of wheat developed in a microgravity environment. *Phytochemistry* **57**: 835-846.
- Liu, Z., Qiu, Q., Huang, W., Mei, M. and Yang, J. (1991). Study of biological effects of accelerated heavy ion radiation on rice seeds at the first generation. *J. Radiat. Res. Radiat. Process.* **9**: 139-144.
- Mei, M., Deng, H., Lu, Y., Zhang, C., Liu, Z., Qiu, Q., Qiu, Y. and Yang, T.C. (1994 a). Mutagenic effects of heavy ion radiation in plants. *Adv. Space Res.* **14**: 363-372.
- Mei, M., Qiu, Y., He, Y., Bucker, H. and Yang, C.H. (1994 b). Mutational effects of space flight on *Zea mays* seeds. *Adv. Space Res.* **14**: 33-39.
- Mei, M., Qiu, Y., Sun, V., Huang, R., Yao, J., Zhang, Q., Hong, M. and J. Ye (1998). Morphological and molecular changes of maize plants after seeds flown on Recoverable satellite. *Adv. Space Res.* **22**: 1691-1697.
- Mishra, S.N., Verma, S. and Sarma, A. (2000). Heavy ion irradiation of seeds: Induced plant growth and yield in Indian mustard. Ann. Rep. pp. 192-197. Nuclear Science Center, New Delhi.
- Nagasawa, H., Huo, L. and Little, J.B. (2003). Increased bystander mutagenic effect in DNA double-strand breaks repair-deficient mammalian cells. *Int. J. Radiat. Biol.* **79**: 35-41.
- Nakai, H., Watanabe, H., Kobayashi, Y., Asai, T. and Kitayama, S. (1990). III-4-4. Studies on induced mutations by ion beams in plants. *RIKEN Accel Prog. Rep.* **24**: 91.
- Nakai, H., Watanabe, H., Kobayashi, Y., Kitayama, S., Takahashi, T. and Asai, T. (1992) III-4-9. Studies on induced mutations by ion beam in plants. *RIKEN Accel Prog. Rep.* **26**: 109.
- NASA (1988). Life Sciences Strategic Planning Study Committee, Exploring the Living Universe: A Strategy for Space Life Science. NASA, USA.

RESPONSES OF PLANTS TO HEAVY IONS IRRADIATION

- Nedukha, E.M. (1996). Possible mechanisms of plant cell wall changes at microgravity. *Adv. Space Res.* **17**: 37-45.
- Nevzgodina, L.V., Maximova, E.N. and Akatov Yu, A. (1981). Effects of prolonged exposure to space flight factors for 175 days on lettuce seeds. *Adv Space Res.* **1**: 83-85.
- Nikjoo, H., Uehara, S., Wilson, W.E., Hoshi, M. and Goohead, D.T. (1998). Track structure in radiation biology: Theory and applications. *Int. J. Radiat. Biol.* **73**: 355-364.
- Peterson, D.D., Benton, E.V., Tran, M., Yang, T., Freeling, M., Craise, L. and Tobias, C.A. (1977). Biological effects of high-LET particles on corn seed embryos in the Apollo-Soyuz-Test-Projects: Biostack-III Experiment. In: R. Holmquist, A.D. Stickland (eds.), *COSPAR Life Sciences and Space Research*, Vol.15, pp.151-155. Pergmon Press, Oxford, New York.
- Pickert, M., Kranz, A.R. and Somer, S. (1990). *Arabidopsis thaliana* (Hyynh) Pollen: A new tool in heavy ion radiation. Proc. IV European Symposium Life Sciences Research in Space, pp.581-582. Italy.
- Pirollo, K.F., Tong, Y.A., Villegas, Z., Chen, Y. and Chang, E.H. (1993). Oncogene transformed NIH-3T3 cells display radiation resistance levels indicative of a signal transduction pathway leading to the radiation-resistant phenotype. *Radiat. Res.* **135**: 234-243.
- Qiu, Y., Mei, H., He, Y. and Lu, Y. (1991). Mutagenic effects of accelerated heavy ion irradiation on *Zea mays*. *J. South China Agri. Univ.* **12**: 48-54.
- Roots, R., Chatterjee, A., Chang, P., Lommel, L. and Blakely, E.A. (1985). Characterization of hydroxyl radical-induced damage after sparsely and densely ionizing irradiation. *Int. J. Radiat. Biol.* **47**: 157-166.
- Sachs, R.K., Brenner, D.J., Hahnfeldt, P. J. and Hlatkys, L.R. (1998). A formalism for analyzing large-scale clustering of radiation-induced breaks along chromosomes. *Int. J. Radiat. Biol.* **74**: 185-206.
- Schimmerling, W. (1992). Radiobiological problems in space: An overview. *Radiat. Environ. Biophys.* **31**: 197-203.
- Schott, J.U., Kranz, A.R., Gartenbach, K. and Zimmermann, M. (1993). Particle radiation mapping in growing organisms with charge coupled devices (CCDs). *J. Photog. Sci.* **41**: 115-116.
- Shikazono, N., Tanaka, A., Kitayama, S., Watanabe, H. and Tano, S. (2002). LET dependence of lethality in *Arabidopsis thaliana* irradiated by heavy ions, *Radiat. Environ. Biophys.* **41**: 159-162
- Shikazono, N., Yokota, Y., Kitamura, S., Suzuki, C., Watanabe, H., Tano, S. and Tanaka, A. (2003). Mutation rate and Novel *tt* mutants of *Arabidopsis thaliana* induced by carbon ions. *Genetics* **163**: 1449-1455.
- Shikazono, N., Yokota, Y., Tanaka, A., Watanabe, H. and Tano, S. (1998). Molecular analysis of carbon ion-induced mutations in *Arabidopsis thaliana*. *Genes Genet. Syst.* **73**: 173-179.
- Shimono, K., Shikazono, N., Inoue, M., Tanaka, A. and Watanabe, H. (2001). Effect of fractionated exposure to carbon ions on the frequency of chromosome aberrations in tobacco root cells. *Radiat. Environ. Biophys.* **40**: 221-225.
- Slater, J.V., Tobias, C.A. (1963). Effects of cosmic radiation on seed differentiation and Development. *Radiat. Res.* **19**: 218-220.
- Smith, H.H. (1972). Comparative genetic effects of different physical mutagens in higher plants, In: IAEA (ed.), *Induced mutations and plant improvement*, pp.75-93. International Atomic Energy Agency, Vienna.
- Sparrow, A.H., Schairer, L. A. and Marimuthu, K. M. (1971). Experiment P-I 123: Radiobiologic studies of *Tradescantia* plants orbited in Biosatellite II. In: J.F. Saunder (ed), *The experiments of Biosatellite-II*. Special Publication pp. 99-122. NASA, USA.
- Sychev, V.N., Shepelev, Ye. Ya., Meleshko, G. I. and Levinskikh, M.A. (2000). The effect of microgravity on active cultures of unicellular algae. *Fiziol. Rast.* **47**: 774-781.
- Tanaka, A., Shikazono, N., Yokota, Y., Watanabe, H. and Tano, S. (1997). Effects of heavy ions on the germination and survival of *Arabidopsis thaliana*. *Int. J. Radiat. Biol.* **72**: 121-127.
- Tanaka, A. (1999). Mutation induction by ion beams in *Arabidopsis*. *Gamma Field* **38**: 19-28.
- Tanaka, A., Kobayashi, Y., Hase, Y. and Watanabe, H. (2002). Positional effect of cell inactivation on root gravitropism using heavy-ion micro beams. *J. Exp. Bot.* **53**: 683-687.

- Terato, H. and Ide, H. (2004). Clustered DNA damage induced by heavy ion particles. *Biol. Sci. Space* **18**: 206-215.
- Tobias, C.A. (1985). Failla Memorial lectures. The future of heavy-ion science in biology And medicine. *Radiat. Res.* **103**: 1-33.
- Vasilenko, A. and Sidorenko, G. (1996). Alterations in adenylate ratios in plant cells after accelerated ion irradiation. *Adv. Space Res.* **18**: 59-62.
- Vasilenko, A. and Sidorenko, P.G. (1995). Induction of micronuclei in plant cells after exposure to accelerated ion irradiation. *Radiat. Environ. Biophys.* **34**:107-112.
- Verma, S., Mishra, S.N., Sarma, A. and Phogat, S.B. (2004). Growth responses and biochemical changes in Indian mustard after irradiation of seeds with ${}^7\text{Li}^{+++}$ heavy ion. *Brassica* **6**: 35-40.
- Verma, S. (2004). Growth responses in Indian mustard seedlings exposed to salinity and heavy ion irradiation: Antioxidant and nitrogen assimilating enzymes. Ph.D. thesis, M.D. Univ., Rohtak.
- Waldron, K.W. and Brett, C.T. (1990). Effects of extreme acceleration on the germination, growth and cell wall composition of pea epicotyls. *J. Expt. Bot.* **41**: 71-77.
- Wang, C.Y., Yang, H.M., Wang, Y.F., Wei, Z.Q., Liu, Y.Y. and Wang, G.L. (1993). The mutation effect of ${}^6\text{C}^+$ heavy ions on seeds of *Lathyrus sativa*. *Hereditas-Beijing* **15**: 28-31.
- Wei, Z., Liu, Y., Wang, G., Chen, X., Li, H., Yang, H., Wang, L., Gao, Q., Wang, C. and Wang, Y. (1995). Biological effects of carbon ions with medium energy on plant seeds. *Radiat. Res.* **141**: 342-344.
- Yang, T.C. and Tobias, C.A. (1979). Potential use of heavy-ion radiation in crop improvement. *Gamma-field Symposia* **18**: 141-154.
- Yatagai, F., Takahashi, T. and Matsuyama, A. (1975). Inactivation of bacterial cells by cyclotron beam. *J. Radiat. Res.* **16**: 99-112.
- Yokota, Y., Hase, Y., Shikazono, N., Tanaka, A. and Inoue, M. (2003). LET dependence of lethality of carbon ion irradiation to single tobacco cells. *Int. J. Radiat. Biol.* **79**: 681-685.