

Gamma Ray Irradiation for Crop Protection Against Salt Stress

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ABSTRACT

Legumes have tremendous dietary value for human nutrition. However, the productivity of food legumes is always compromised owing to their insufficient ability to tolerate abiotic stresses such as drought or water logging, marginal soil, low/high temperatures and salt stress. Stress induces changes at the morphological, physiological, biochemical and molecular level which are consequently manifested in terms of reduced seed yield and quality. Salt stress is one of the most important constraints to crop production particularly in the arid and semi-arid regions of the world. Low dose of ionising radiation like gamma ray is reported to induce growth and several other physiological attributes in non-legume and legume crops. Relationship between seed gamma irradiation and salinity stress response could be related to favourable maintenance of gas exchange attributes (P_n , g_s and E), ^{14}C partitioning, activity of antioxidative enzymes (SOD, CAT and POX), membrane stability index (MSI) K^+ to Na^+ ratio, proline and glycine betaine content. One or more mechanisms may contribute simultaneously towards salt tolerance response of crop plants. The present review critically analyses the effect of gamma ray irradiation on growth and development of legumes under salt stress and evaluates the contribution of various physiological and biochemical mechanisms towards radiation mediated alleviation of salt stress response.

Keywords: Salt stress; Legumes; Gamma rays; Irradiation; Tolerance mechanisms

1. INTRODUCTION

Radiation can be defined as the energy emitted by a body in the form of rapidly spreading waves or particles as it moves from a higher energy level to a lower energy level. The most common use of the word 'radiation' refers to ionising radiation. The other class of radiation is the non-ionising type that which is relatively less energetic and does not cause removal of the atomic electrons. Sound waves, visible light, and microwaves are some of the common ionising radiations. A radioactive material is a material that emits ionising radiation of any of the three principal types i.e., alpha, beta and gamma radiation, which have enough energy to displace electrons and cause ionisation in matter. The penetrating power of the above particles governs the amount of damage the radiation can cause. Among different types of ionising radiations, gamma rays are most widely studied for their applications in the field of agriculture, medicine and industry¹. Ionising radiation presents immense applications and can be potentially exploited to improve production and quality of agriculture produce. Ionising radiations such as high-speed electrons and gamma rays, produce highly reactive free radicals which can cause denaturation of DNA and RNA, the genetic materials, and thus, influence cell integrity and plant metabolism.

Ionising radiation that encompass high energy gamma ray, presents immense potential application in various fields of agriculture. These are helpful in sterilising and improving shelf

life of agri-produce to benefit their export and import². Among different forms of electromagnetic radiations, gamma rays are more penetrating and potentially the most energetic radiations (10 keV to several hundred kilo electron volt)³. Gamma rays alter the biological and physiological characteristics⁴, by producing ROS on interactions with the water or cellular molecules of the exposed material³. Gamma rays bring about ultrastructural and metabolic changes in the chloroplast and antioxidative defence activity to effect the carboxylation process and dynamics of the source-sink relationship^{3,5-7}. High intensity gamma rays affect the carbon metabolism and pigment system efficiency in the chloroplast⁸ to elevate the photolytic damage and reduce the photosynthetic efficiency. Gamma rays can be exploited to generate favourable changes in plant's morphology, anatomy, biochemistry and physiology to enable abiotic stress tolerance which however, will depend on the plants the dose of irradiation used⁹.

1.1 Gamma Irradiation and Seed Yield and Quality

Every invention have two sides one is positive and other is negative similarly gamma radiations at lower doses shows positive effect and higher doses shows negative effect on growth and development. Mokobia and Anomohanran¹⁰ found the positive effect of gamma irradiation at 0.15 kGy. Using 25 krad dose of radiation Chauhan¹¹, *et al.* identified high yielding barley mutants while at higher doses an inhibitory effect of gamma radiation on yield and yield attributes was recorded by Siddiqi¹², *et al.* In another experiment with variable N

application Subhan¹³, *et al.* observed an increase in radiation effected grain yield in barley at 10 krad dose of gamma ray exposure. Positive effect of low dose radiation (10 krad or below) on plant growth and development have been evidenced by several workers¹⁴⁻¹⁶. Even seed quality is remarkably affected by gamma irradiation. Kattak and Klopfenstein¹⁷ showed that a radiation exposure of 5-15 kR decreased plant height but increased yield and nutritional quality of cereal grains in terms of protein and contents of essential amino acids, which suggests that even such high dose of gamma ray seed irradiation can be used to develop mutants for growth related desirable traits¹⁸. These results find support from the work of Mahdi¹⁹, *et al.* who used gamma ray exposure of seeds to improve nutritional quality of broad bean. Singh and Datta²⁰ also showed an improvement in grain protein, total nucleic acid and total carotenoid content upon gamma ray treatment at low doses of seed irradiation. However, they reported a lower starch content in the irradiated grains than the unirradiated control, which they attributed to a possible poor sucrose to starch conversion. A similar increase in protein content was reported by Mashev²¹, *et al.* and Coksel²², *et al.* at radiation dose upto 0.15 kGy, while a decline in total nucleic acid level was reported by Maity²³, *et al.* at higher doses of radiation exposure i.e., between 1 kGy to 20 kGy.

1.2 Radiation Effects are Dose Dependent

There are innumerable reports describing the physiological effect of gamma ray exposure across a variety of plant species^{3,5,24,25}. These physiological changes can be measured in terms of an enhanced or an inhibited seed germination, seedling vigor and phenotypic changes^{24,25}. While a low level of radiation exposure in dose range of 2.5 Gy - 5 Gy can improve cell growth and development, rate of seed germination rate, activity of rate limiting metabolic enzymes, abiotic and biotic stress tolerance and economic yield²⁶, a higher gamma ray dose above 500 Gy can disrupt the synthesis of proteins and plant growth hormones and disturb the hormone balance, water relations and leaf gas exchange characteristics²⁷. Gamma irradiation at higher dose can be successfully used to develop yield efficient wheat plant types rich in proteins and amino acids of consequence to human nutrition¹⁸. Din²⁸, *et al.* determined inter-varietal variation in radiation response of wheat to gamma ray exposure and found its deleterious effects at dose 30 Krad.

1.3 Salinity Stress

Soil salinity is a major agricultural challenge and more than twenty per cent of the cultivable soil and half of the irrigated soils globally are challenged by salinity²⁹. In fact, it is a threat to irrigated agriculture in many arid and semiarid areas of the world. Build up of salt challenges production efficiency of a crop even when other conditions of growth are congenial. Plants respond to high salt availability in the soil by effecting changes at the molecular, biochemical and biochemical level³⁰. Soil salinity has an antagonistic influence on the physiological and metabolic processes that consequently cause significant reduction in plant growth and yield³¹. The constituents of aggregate dissolvable salts in soils are normally sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) and

the anions are chloride (Cl^-), sulfate (SO_4^{2-}) and carbonate. However, Na^+ overwhelms the alerts and along with Cl^- and that $NaCl$ constitutes 50–80 per cent of the aggregate solvent salts³². High salt causes water stress in plants by decreasing the osmotic potential of the soil, besides causing severe ion (Na^+) toxicity. Most of the pulse crops such as pigeon pea, lentil, mung bean, black gram and chickpea are highly sensitive to salinity³³. Salt stress may also effect the growth by altering the soil nutrient balance and nutrient availability for plant uptake. However, plants do exhibit inter and intra species variation in salt tolerance which might be related to their ability to prevent or alleviate the salt effected physiological and molecular damage³⁴. Conventional plant breeding approaches have not been able to successfully transfer the salt tolerance traits into the target species.

1.4 General Response of Plants to Salinity

Levitt³⁵ classifies the adverse effects of salts on plants into three categories which included (a) osmotic stress, (b) specific ion effects, and (c) nutritional deficiency. If salt stress lowers the external water potential below that of the cell, it exposes the cell to a secondary water deficit stress. To distinguish this from salt stress, and because it leads to osmotic dehydration, it is called osmotic stress³⁵. It has also been called 'physiological drought'. Salinity related crop growth inhibition, in absence of ionic influence, is majorly related to the osmotic potential of the roots in the soil solution³⁶. Decreasing osmotic potential in the root zone soil has the net effect of reducing the availability of water to plants. Therefore, plants growing on saline soils often appear to be suffering from drought. An excess of specific ions may be toxic to various plant physiological processes including nutritional disorders. Ions contributing appreciably to specific ion effects include Cl , SO_4 , HCO_3 , Na , Ca , Mg . In combination, these ions may contribute to osmotic effects. At equal osmotic concentrations to that of PEG, $NaCl$ depresses the germination of legume seeds much more than does mannitol³⁰. Specific ions may influence respiration as found in pea roots³⁷. High sodium can cause calcium and magnesium nutritional deficiencies³⁸. There have been many reports of salt induced decreases in several metabolic processes such as respiration, protein and nucleic acid synthesis^{30,39-41}.

The changes in nitrogen metabolism are usually accompanied by the accumulation of ammonia, amines, diamines (putrescine, cadavarine), amino acids (hydroxyproline, proline, leucine, isoleucine, alanine, phenylalanine and tyrosine) which can have an adverse effect on the physiological processes of the plant³⁰. The actual toxic substances vary from species to species, depending on the metabolism of each species. Reports have shown that potassium deficiency also leads to an accumulation of putrescine⁴². However, the problem of soil salinity has been addressed superficially so far and most of the research results express it as a cause and effect relationship and do not decipher the underlying mechanisms that effect the salt related retardation of plant growth and development⁴³.

2. SALINITY TOLERANCE IN LEGUMES

Plants ability to survive on highly alkaline/ saline soils is measured in terms of salt tolerance capacity. Salt tolerance of

crop plants is measured as decline in economic yield under salt stress relative to the economic yield under non saline control soil. A yield decrease of 50 per cent is usually considered as the cutoff point for evaluating the relative salt tolerance of crops (ECe 50 per cent)⁴⁴. Pulse legumes are regarded as highly susceptible to soil salinity⁴⁴⁻⁴⁵. However, there exists a huge inter and intra species variability in salt stress tolerance which needs to be deciphered and exploited. Among cultivated legumes, *Sesbania cannabina* is most tolerant⁴⁶ and can tolerate and grow at salinity levels of 13.2 dS/m (ECe SOX), while *Cicer arietinum* is the most sensitive among legumes with tolerance only up to 3.0 dS/m (ECe 50 per cent) salinity level. Crop sensitivity to salinity stress varies depending on the growth stage. In Alfa Alfa, salt tolerance was greater during germination than during subsequent growth⁴⁷, whereas in *P. vulgaris* it was found that germination was more sensitive compared to seedling growth stage⁹. Gamma ray mediated alleviation of salt stress has been reported in cowpea⁴⁸ and mungbean, which appears to have been caused by the induction of novel proteins (*de novo* synthesis) and/or the disappearance of some other proteins and/or the over- expression of a existing proteins. Salinity may stimulate the growth in some species. In *Lupinus luteus*, there was 50 per cent fresh weight stimulation (over its control) at 50 mM NaCl (5 dS/m) salinity level⁴⁹. For a few crops, variation to salinity tolerance within a variety has been reported. In *Medicago sativa*, large variation in salt tolerance within the variety CUF 101 was reported⁵⁰. In *Trifolium alexandrinum* and *T. pratense*³³ also a similar variation has been reported. The adverse effect of salinity was attributed to specific ion toxicity. In lentil⁵¹, germination and growth were severely inhibited by $MgSO_4$, followed by $MgCl_2$, while in chickpea was less affected by Na_2SO_4 salinity than NaCl salinity⁵². Climatic factors may significantly influence plant response to salinity, with temperature and atmospheric humidity being most important. A negative relationship between salt tolerance and growing temperature has been suggested⁵³. Gamma irradiation improved the germination and plant vigor in mungbean under salt stress when compared with respective salt treatment controls.

2.1 Mechanisms of Salinity Tolerance

Under salt stress condition, uptake of electrolytes such as Na and Cl can help in alleviating the negative effects of low external water potential. However, an excess uptake and intracellular concentration of Na and /or Cl, can inhibit plant growth. In this potentially disastrous situation, different species may develop diverse mechanisms of adaptation^{36,54}. Halophytes like vacuolated salt water algae maintain cell turgor by maintaining a favourable Na/Cl ion balance⁵⁵. These halophytes generate turgor by high internal sodium and chloride concentrations. These plants tolerate high Na/Cl concentrations by preferentially storing them in the cell vacuoles and thus maintain their low cytoplasm concentrations. On the other hand, neutral solutes like proline, glycine, betaine and sucrose contribute to the osmotic potential of cytoplasm⁵⁶⁻⁵⁸. Greenway and Munns⁵⁴ suggested that salt susceptible plants exhibit water deficit and accumulate excess concentrations of Na/Cl. These reports give support to the hypothesis that salt sensitivity

is essentially caused by ion excess in the tissues and by the plants inability to secure these excessive ions in a unreactive or inaccessible form. A higher accumulation of Na/Cl ions causes leaf chlorosis to consequently inhibit plant growth leading to its death⁵⁹. Sensitivity towards high chloride and sodium in leaves is much greater for non-halophytes than for halophytes. Extremely salt tolerant halophyte '*Suaeda maritime*' can survive on extremely high concentrations (600 mM - 650 mM) of NaCl and can withstand a Na/Cl concentration of about 300 mM in the mesophyll cell. In non-halophytes, growth is severely reduced when ion concentrations in the leaves are as low as 100 mM chloride⁵⁴. It is mooted that halophytes, maintain a lower concentrations of sodium and chloride in the cytoplasm compared to those in the vacuole, which could be responsible for the ability to tolerate 600 mM sodium and chloride concentrations internally without any disturbance in the metabolism³⁰. In another study by Song⁶⁰, *et al.* two salt-tolerant rice mutants out of 1500 M6 mutants, induced by gamma-irradiation were selected for *in vivo* and *vitro* salinity screening for electrolyte leakage (EL), malondialdehyde (MDA), antioxidant, chlorophyll, total amino acid, and Na⁺/K⁺ contents. It was found that the mutants were showing significant fold change of 2.7 over wild type for K⁺/Na⁺ ratio compared to other traits.

2.2 Role of Cytoplasmic Organic Solutes in Salinity Tolerance

Role of organic solutes in maintaining, osmotic balance, cytoplasmic integrity and function under low electrolyte availability in the cytoplasm is suggested⁶¹. On the other hand, under high electrolyte availability condition, these organic solutes have a protective role on the enzyme proteins²⁹. Organic solutes which increase at high salinity in many species include glycine betaine, proline⁶² and sucrose⁶³. Many amino acids and carbohydrates, at 0.1 M to 1 M, mitigated or prevented the loss of activity of several enzymes⁶⁴. Glycine betaine (500 mM) alleviated the inhibitory effects of 200 mM NaCl on malic enzyme isolated from barley^{62,65}. Sairam and Tyagi²⁹ illustrated osmotic potential of various organic solutes and evidenced that *Plantago maritime*, which does not accumulate proline or glycine betaine, showed a substantial increase in sorbitol concentration under increasing levels of salinity i.e., 0 mM to 400 mM NaCl. Greenway and Munns⁵⁴ showed that another organic solute proline has important role in salt tolerance rather than in maintenance of the plant growth. However, contrary to the above, in three halophytes, salt tolerant and sensitive species accumulated substantial quantities of proline (greater than 214 mol/g FW) only when growth is severely reduced and thus, challenge the role of proline in imparting salt tolerance⁶⁶.

2.3 Regulation of Na and Cl Concentrations in the Shoot

In halophytes, inorganic ions (Na, Cl) are used for turgor maintenance through effective compartmentation into the vacuole³⁰. The above mechanism is coupled with salt excreting mechanisms in the shoot system which could effectively excrete the excess sodium and chloride that could not be compartmentalised in the shoot tissues and would

effectively prevent 'ion excess'. The key factor for the tolerance in the non-halophytes to salinity is a synchronisation of ion compartmentalisation with regulated ion transport to the shoot⁵⁵. Most salt sensitive plants possess an inefficient compartmentalisation of Na/Cl ion and thus, end up with their high cytoplasmic concentrations in the leaves.

3. RESPONSES OF DIFFERENT GENOTYPES

Halophytes that can tolerate high salt stress exhibit a higher rate of electrolyte uptake by root, their translocation to the shoot and their accumulation in the photosynthetic organ i.e., leaf⁶⁷. Comparisons between species with extreme differences in salt tolerance support the view that high electrolyte concentration in leaves is an adaptive trait. Soussi⁶⁸⁻⁶⁹, *et al.* reported negative effect of salt stress on growth and yield of *Cicer arietinum*. A high level of *Na/K* was measured in both salt sensitive pepper and salt tolerant sugarbeet at 0 mM as well as 100 mM NaCl in the external solution. Role of high cellular electrolyte concentration in imparting salt tolerance was also shown in closely related tomato species⁷⁰. Asvathappa and Bachelard⁷¹ showed that a relatively superior Cl tolerance of *Casuarina equisetifolia* (tolerant) than *C. cunninghamiana* (moderately tolerant) was caused by its low root uptake and translocation to the shoot and that the root of the tolerant Casurina did not accumulate high concentrations of chloride. Differences between salt resistant and salt sensitive species of Plantano were located in the ion secretory system which was involved in the ion translocation from the root to the shoot rather than in the primary uptake process through the plasmalemma of the cortical cells³⁷. Relatively better salinity tolerance of the Triticeae species depended on the ability of the plant cell to exclude Na and Cl and maintain high K concentration in the cytoplasm⁷². The salinity tolerance in perennial Triticeae members such as *Leymus sabulosus* and *Elytrigia*, was associated with an ability to tightly control osmotic adjustment by strictly regulating the influx of sodium and chloride. A genetic basis was established for the large differences in chloride concentrations in leaves of chickpea⁷³. The relationship between high leaf *Na*⁺, *Cl*⁻, *Ca*²⁺ and *K*⁺ concentrations and salt susceptibility cannot be generalised as evident from the work of Greenways and Munn⁵⁴, who found no consistent relationship between salt tolerance and *Na* concentration in salt tolerant cultivars of rice (*Oryza sativa*). Varietal differences in salt tolerance, despite similar ion concentrations in the shoots, may be related to differences in :

- (a) Tolerance to low external water potential,
- (b) Differences in ion compartmentation in the leaves,
- (c) Ion compartmentation in roots,
- (d) Ion compartmentation between leaves of different ages⁵⁴.

Tabosa⁷⁴ used a very high range of soil electrical conductivity between 14 dS m⁻¹ to 26 dS m⁻¹ to identify salt tolerant sorghum line, where phenotyping based on physiological characters further helped in classifying the sorghum genotypes into salinity tolerant and susceptible groups.

4. INTERACTIVE EFFECT OF GAMMA RADIATION UNDER SALT STRESS

Gamma irradiation at low dose is reported to induce

growth and vigour, while salt stress on the other hand causes a reduction in growth and vigour. Role of gamma irradiation to negate the negative effect of salt stress on plant even if it is partial, has assumed significance⁷⁵. Gamma radiation dose of 450 Gy promotes genetic change in plants of sorghum varieties V1 (467-4-2), V2 (02-03-01) and V3 (Sudan4202), which were assessed using SSR markers while a dose of 50 gray was shown to improve germination index and root length of *Arabidopsis* under salt stress⁷⁵. The interaction between gamma radiation induced salt tolerance response of crop plants may operate at various level through the involvement of multiple attributes²⁹.

4.1 Gamma Radiation and Mineral Nutrient Uptake

Among the essential mineral nutrients, potassium (K) is known to play an important role in maintaining efficient water relations, carbon metabolism and activity of key enzymes regulating several biological pathways and is also regarded as a major production constraint⁷⁶. A significant increase in the concentration of Fe, Mn, Zn, and Cu was measured in wheat upon gamma irradiation treatment when compared to non-irradiated control⁷⁷. Gamma treated plants showed significant increase in potassium, phosphorus, cellulose and total nitrogen¹⁸. Further, a change in rooting characteristics upon gamma ray exposure impacted the total uptake of different macro and micro mineral nutrients.

4.2 Gamma Irradiation and Oxidative Damage

Plants perceive radiation exposure as any other type of abiotic stress and respond by inducing the oxidative stress signal which enhances the production of reactive oxygen species (ROS) which are deleterious and can react with structural and functional organic molecules such as carbohydrates, proteins, fats, nucleic acids to cause cellular disturbance^{7,78-79}. However, plants can quench these damaging ROS by adaptive induction of antioxidative defence system, which removes these free radicals and contains cellular damage. In addition, a large number of defence related genes are triggered to modulate the metabolic pathways favourably.

4.3 Gamma Irradiation and Activity of Functional Enzymes

Gamma radiation affects the activities of various enzymes involved in various metabolic pathways. An increased radio sensitivity of starch to amylase⁸⁰ could cause a decline in starch concentration of irradiated to non-irradiated grains. In wheat, amylolytic degradation of starch increased with an increase in the dose of gamma irradiation, however, the activities of α and β -amylases did not change significantly⁸¹⁻⁸². Ribulose-1,5-bisphosphate carboxylase (RUBISCO) which accounts for more than thirty percent of the total leaf nitrogen⁸³ and catalyses the production of two molecules of 3-phosphoglyceric acid (PGA) by a reaction between carbon dioxide and RUBP⁸⁴, is a sensitive enzyme and declines both in terms of Rubisco proein and Rubisco activity under stressful condition of growth⁸⁵⁻⁸⁶. However, Rubisco activity did not decline in response to gamma irradiation treatment in wheat and that an increase in total carboxylation efficiency was measured in comparison to

the unirradiated control at low doses. Nitrate reductase activity, on the other hand, declined even at low doses⁸⁷.

4.4 Radiation Protection Strategies Under Salt Stress

Proline synthesis, implicated in salt tolerance mechanism across crops⁸⁸ was reported to be higher in the irradiated plants than the unirradiated ones⁷⁸. Accumulation of proline, an osmolyte, is regarded as a protective mechanism against salinity stress, wherein it protects the enzymes against degradation and preserves their structural and functional activity. In vitro experiments have confirmed the involvement of proline in providing radiation protection as well as protection against several other abiotic stresses such as high and low temperature, salinity, alkalinity, etc⁸⁹. A radiation dose dependant increase in tissue proline level has been reported⁹⁰. In another study with chickpea, gamma irradiated plants showed significantly lower Na levels even at highest of NaCl stress (Fig. 1). A lower Na/K ratio under gamma irradiated treatments suggests a lower uptake and root-shoot translocation of Na which can be exploited to impart/ improve salt tolerance capacity of crop plants.

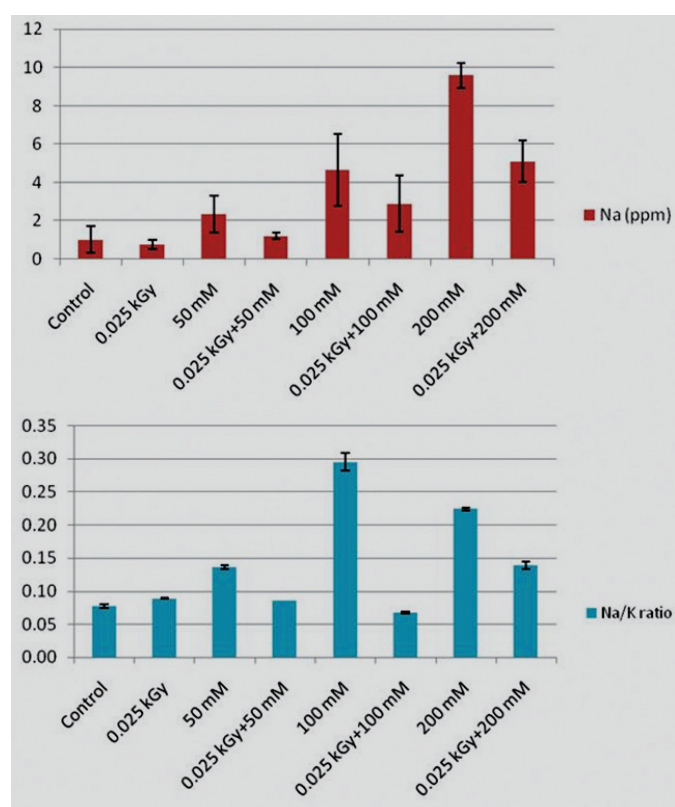


Figure 1. Na and K concentration in stem sap of chickpea exposed to gamma irradiation and salt stress.

4.5 Dissecting the Radiation –Plant Relationship at Molecular Level

Gamma rays have immense application in agriculture from post harvest preservation to development of desirable trait specific promising mutants. However, these applications are dose dependant and it is important to determine LD50 values.

Gamma irradiation can be used to create genetic variability among the segregating population to identify trait positive mutants. Lukanda⁹¹ expanded the genetic base of groundnut using 100 Gy gamma irradiation and then used ISSR markers to differentiate the developed groundnut populations. It would be interesting to record, if the above SSR marker can be used to deduce genetic pathway of leaf size and leaf area increase as observed in irradiated wheat plants in some earlier studies²⁰. Seed gamma irradiation in a dose range of 10 Krad to 35 Krad in wheat, yielded mutants possessing tillers with two ears²⁸. These kind of mutations, observed at 30 Krad - 35 Krad may appear as abnormalities at this moment but can open a new vistas in plant type development to propel the second green revolution. Qi⁷⁵, *et al.* reported an increase in the transcriptional expression of genes regulating the activity of antioxidant enzymes and osmolyte concentrations and components of salt stress signalling pathway were stimulated by low dose of gamma in Arabidopsis grown under salt stress.

5. CONCLUSIONS

A flowchart depicting different physiological and biochemical attributes that are affected upon gamma irradiation treatment at the tissue/cell level to consequently effect salt tolerance at the whole plant level are summarised in Fig 2.

Gamma radiation can complement the conventional plant breeding by increasing the variability base and could confer specific trait improvement without significantly altering crop phenotype. Successful utilisation of gamma rays to generate genetic variability in plant breeding has been reported in different crops. We hypothesise that pre-exposure of the seeds to the gamma radiation could help improve salt stress tolerance of crops. It is, thus, important understand the underlying mechanism determining plant response to gamma

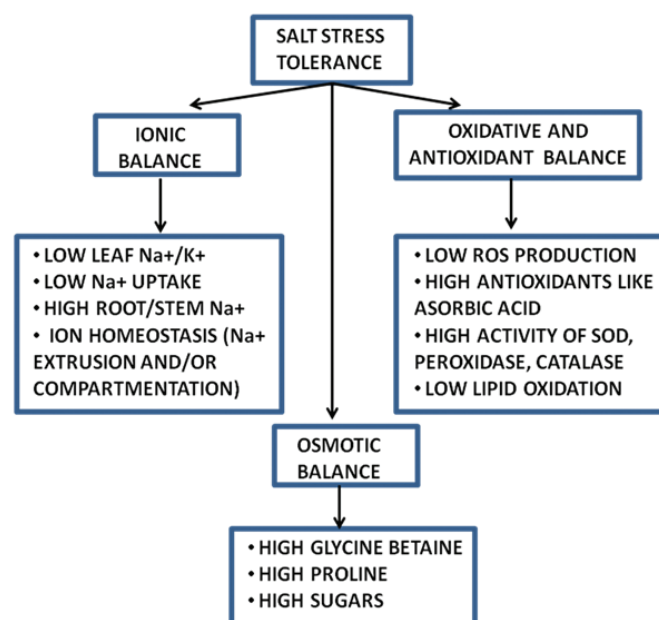


Figure 2. Schematic summary of physiological basis of salt stress tolerance in plants. Gamma ray induced salt stress tolerance operates at the three levels i.e., at the ionic, the antioxidative, and the osmotic level.

radiation. Analysis also reveals that radiations have immense applications, however, several of its potential applications in agriculture in general and stress tolerance in particular are just waiting to be explored and exploited.

REFERENCES

- Singh, B.; Anand, A. & Koundal, K.R. Training manual of ICAR winter school on training and capacity building on applications of ionizing and non ionizing energy in agriculture. IARI, New Delhi, 2009, 208.
- Melki, M. & Salami, D. Studies the effects of low dose of gamma rays on the behavior of Chickpea under various conditions. *Pak. J. Biol. Sci.*, 2008, **11**(19), 2326-2330. doi:10.3923/pjbs.2008.2326.2330
- Kovacs, E. & Keresztes, A. Effect of gamma and UV radiation on wheat yield. *Micron.*, 2002, **33**(2), 199–210. doi:10.1016/S0968-4328(01)00012-9
- Kiong, A.; Ling Pick, A.; Grace Lai, S.H. & Harun, A.R. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. *Am. Eurasian. J. Sustain Agric.*, 2008, **2**(2), 135-149.
- Kim, J.H.; Baek, M.H.; Chung, B.Y.; Wi, S.G. & Kim, J.S. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biochem. Biotech.*, 2004, **47**(2), 314-321. doi:10.1007/BF03030546
- Wi, S.G.; Chung, B.Y. & Kim, J.S. Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron.*, 2007, **38**, 553–564. doi:10.1016/j.micron.2006.11.002
- Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech Adv.*, 2009, **27**, 84-93. doi:10.1016/j.biotechadv.2008.09.003
- Sosedov, N.I. & Vakar, A.B. Effect of gamma-rays on biochemical properties of wheat. In Proceedings of the Fifth International Congress of Biochemistry, vol. 8, 10–16 August, Moscow, Pergamon Press, 1961, 133.
- Ashraf, M. & Bashir, A. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. *Flora*, 2003, **198**, 486– 498. doi:10.1078/0367-2530-00121
- Mokobia, C.E. & Anomohanran, O. The effect of gamma irradiation on the germination and growth of certain Nigerian agricultural crops. *J. Rad. Prot.*, 2005, **25**, 181-188. doi:10.1088/0952-4746/25/2/006
- Chauhan, S.V.S.; Kumar, R. & Kindoshita, T. Protein and malt quality in some gamma rays induced high yielding mutant in barley (*Hordeum vulgare*). *Wht. Barl. Trit. Abst.*, 1985, **2**, 356.
- Siddiqi, S.H.; Iqbal, M.; Muhaammad, T. & Jan, M.T. Variation in genetic parameters of barley and triticales after seed irradiation. *Sarhad. J. Agric.*, 1985, **1**, 339–345.
- Subhan, F.; Anwar, M.; Ahmad, N.; Gulzar, A.; Siddiqi, A.M.; Rahman, S.; Ahmad, I. & Rauf, M. Effect of gamma radiation on growth and yield of barley under different nitrogen levels. *Pak. J. Biosci.*, 2004, **7**(6), 981–983. doi:10.3923/pjbs.2004.981.983
- Al-Ouadat, M.; Razzouk, A.K. & Chang Kum. Effect of low doses of gamma radiation on the growth and yield of tomato variety *Caramello* in green house, AECS Feb 1990–1991. *Plant Cultivation Breed.*, 1994, 19.
- Chang Kum, S. Present status of radiation utilization in Thailand. Proceedings of the 21st Japan conference on radiation and radioisotopes, Comar CL, Zscheile FP 1942. 1994, 596–599.
- El-Sayed, H.H.; Abd El-Tawab, F.M.; El-Souedy, A.; Sharabash, M.T. & Asmahan, A.M. Effect of gamma irradiation on growth, yield and chemical constituents for three tomato varieties and their crosses. In Second Arab conference on the peaceful uses energy 5–9 Nov Cairo, 913–923. *Environ. Safety*, 1994, **60**, 324–349.
- Khattak, A.B. & Klopfenstein, C.F. Effects of gamma irradiation on the nutritional quality of grain and legumes and Stability of niacin, thiamin, and riboflavin. *Cereal Chem.*, 1989, **63**(3), 169–170.
- Mashev, N.; Vassilev, G. & Ivanov, K. A study of gamma irradiation treatment on growth yield and quality of peas and wheat. *Physiol Plant.*, 2006, **21**(4), 56–63.
- Mahdi, T.A.; Abdul-Kader, H.A.; Manal, H.M. & Amjed, H.S. Effect of gamma irradiation on antinutritional factors in broad bean. *Rad. Phys. Chem.*, 2003, **67**(3-4), 493-496. doi:10.1016/S0969-806X(03)00091-4
- Singh, B. & Datta, P.S. Gamma irradiation to improve plant vigour, grain development, and yield attributes of wheat. *Rad. Phys. Chem.*, 2010, **79**, 139–143. doi:10.1016/j.radphyschem.2009.05.025
- Mashev, N.; Vassilev, G. & Ivanov, K. A study of N-allyl N-2 pyridyl thiourea and gamma radiation treatment on growth and quality of peas and wheat. *Bulg. J. Plant Physiol.*, 1995, **21** (4), 56-63.
- Coksel, H.; Celik, S. & Tuncher, T. Effects of gamma irradiation on durum wheat quality. *J. Cereal. Sci.*, 1996,**73**(4), 506-509.
- Maity, J.P.; Sukalyan, C.; Sandeep, K; Subrata, P.; Jiin-Shuh, J.; Alok, C.S.; Anindita, C. & Subhas, C.S. Effects of gamma irradiation on edible seed protein, amino acids and genomic DNA during sterilization. *J. Agric. Food Chem.*, 2009, **114**, 1237–1244. doi:10.1016/j.foodchem.2008.11.001
- Kim, J.S.; Lee, E.K.; Back, M.H.; Kim, D.H. & Lee, Y.B. Influence of low dose gamma radiation on the physiology of germinative seed of vegetable crops. *Kor. J. Environ. Agric.*, 2000, **19**, 58–61.
- Wi, S.G.; Chung, B.Y.; Kim, J.H.; Baek, M.H.; Yang, D.H.; Lee, J.H. & Kim, J.S. Ultra structural changes of cell organelles in Arabidopsis stem after gamma irradiation. *J. Plant Biol.*, 2005, **48**(2), 195–200. doi:10.1007/BF03030408
- Chakravarty, B. & Sen, S. Enhancement of Regeneration Potential and Variability by γ -Irradiation in Cultured Cells of *Scilla Indica*. *Biologica. Plantarum.*, 2001, **44**(2), 189-

193.
doi:10.1023/A:1010282805522
27. Hameed, M.; Naz, N.; Ahmad, M.S.A. & Islam-ud-Din, R.A. Morphological adaptations of some grasses from the salt range, Pakistan. *Pak. J. Bot.*, 2008, **40**, 1571–1578.
 28. Din, R.; Khan, M.M.; Qasim, M.; Jehan, S. & Khan, M.M. I. Induced mutability studies in three wheat (*L.*) varieties for some morphological and agronomic characteristics. *Asian J. Plant Sci.*, 2003, **17**(2), 1179-1182.
 29. Sairam, R.K. & Tyagi, A. Physiology and molecular biology of salinity stress tolerance in Plants. *Curr. Sci.*, 2004, **86**(3), 407-421.
 30. Manchanda, G & Garg, N. Salinity and its effects on the functional biology of legumes. *Acta. Physiol. Plant.*, 2008, **30**(5), 595-618.
doi:10.1007/s11738-008-0173-3
 31. Ashraf, M. & Harris, P.J.C. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, 2004, **166**, 3–16.
doi:10.1016/j.plantsci.2003.10.024
 32. Rengasamy, P. World salinization with emphasis on Australia. *J. Exp Bot.*, 2010, **57**(5), 1017–1023.
doi.org/10.1093/jxb/erj108
 33. Ashraf, M.; Mcneilly, T. & Bradshaw, A.D. Selection and Heritability of tolerance to NaCl in four species. *Crop Sci.*, 1987, **27**, 232-234.
doi:10.2135/cropsci1987.0011183X002700020021x
 34. Zhu, J-K. Plant salt tolerance. *Trends Plant Sci.*, 2001, **61** (1), 66–71.
doi.org/10.1016/S1360-1385(00)01838-0
 35. Levitt, J. Responses of plants to environmental stresses. 2nd Ed. Academic Press, New York, 1972.
doi:10.2307/3899731
 36. Munns, R. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 2002, **25**, 239–250.
doi:10.1046/j.0016-8025.2001.00808.x
 37. Sanders, D.; Brownlee, C. & Harper, J.F. Communicating with calcium. *Plant Cell*, 1999, **11**, 691-706.
doi:10.1105/tpc.11.4.691
 38. Zhu, J.K. Salt and drought stress signal transduction in plants. *Ann. Rev. Plant Biol.*, 2002, **53**, 247–273.
doi:10.1146/annurev.arplant.53.091401.143329
 39. Gilmour, D.J.; Hipkins, A.N.; Wibber, A.N.; Baker, N.R. & Boney, A.D. The affect of ionic stress on photosynthesis in *Dunaliella Tertiolecta*. *Planta.*, 1985, **163**, 250-256.
doi:10.1007/BF00393515
 40. Jain, M.; Mathur, G.; Koul, S. & Sarin, N.B. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). *Plant Cell Rep.*, 2001, **20**, 463–468.
doi:10.1007/s002990100353
 41. Zhu, J.K. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.*, 2003, **6**, 441–445.
doi:10.1016/S1369-5266(03)00085-2
 42. Cerezo, M.; Agustin, P.G.; Serna, M.D. & PrimoMillo, E. Kinetics of nitrate uptake by citrus seedlings and inhibitory effects of salinity. *Plant Sci.*, 1997, **126**, 105–112.
doi:10.1016/S0168-9452(97)00095-2
 43. Munns, R.; Greenway, H. & Kirst, G.O. Halotolerant *Eukaryotes*. 1983, 59-135 O.L.
doi:10.1007/978-3-642-68153-0_4
 44. Maas, E.V., & Hoffman, G.J. Crop salt tolerance – current assessment. *Am. Soc. Chl. Eng. J. Irr. Drainage Div.*, 1977, **103**, 115-134.
 45. Patil, D.A.; Patil, A.G.; Phatak, A.V. & Chandra N. Effect of salinity on germination and some metabolic changes in *Dolichos lablab* (L.) var. konkan val –2. *Elect. J. Env. Sci.*, 2008, **1**, 15-18.
 46. Keating, B.A. & Fisher, M.J. Comparative tolerance of tropical grain legumes to salinity. *Aust. J. Agric. Res.*, 1985, **36**, 373-383.
doi: 10.1071/AR9850373
 47. Djilianov, D.; Prinsen, E.; Oden, S.; van Onckelen, H. & Muller, J. Nodulation under salt stress of alfalfa lines obtained after in vitro selection for osmotic tolerance. *Plant Sci.*, 2003, **165**(4), 887–894.
doi:10.1016/S0168-9452(03)00291-7
 48. Abdel Haleem, M.; Mohammed, A.; Mohammed, H.I.; Zaki, L.M. & Mogazy, A.M. Pre-exposure to gamma ray eliminates the harmful effect of salinity on cowpea plants. *J. Stress Physiol. Biochem.*, 2012, **8**, 199-217.
 49. Steveninck, R.F.M.V.; Steveninck, M.E.V.; Stelzer, R. & Lauchli, A. Studies on the distribution of sodium and chloride species of lupin (*Lupinus luteus* & *L. angustifolius*) differing in salt tolerance. *Physiol. Plant.*, 1982, **56**, 465-473.
doi:10.1111/j.1399-3054.1982.tb04541.x
 50. Noble, C.L.; Halloran, G.M. & West, D.W. Identification and selection for salt tolerance in lucerne (*Medicago sativa* L.). *Aust. J. Agric. Res.*, 1984, **35**, 239-252.
doi:10.1071/AR9840239
 51. Jana, M.K. Crop improvement in relation to environmental stresses. Salt stress in legumes. *Harvester*, 1979, **21**, 32-38.
 52. Lauter, D.J. & Munns, D.N. Salt resistance of chickpea genotypes in solutions salinized with NaCl and Na₂SO₄. *Plant Soil*, 1986, **95**, 271-279.
doi:10.1007/BF02375078
 53. Uma, S.; Prasad, T.G. & Kumar, M.U. Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in finger millet. *Ann. Bot.*, 1995, **76**, 43–49.
doi:10.1006/anbo.1995.1076
 54. Greenway, H. & Munns, R. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.*, 1980, **31**, 149–190.
doi:10.1146/annurev.pp.31.060180.001053
 55. Hu, Y. & Schmidhalter, U. Limitation of salt stress to plant growth. In *Plant toxicology*. Edited by Hock B, Elstner CF. Marcel Dekker Inc., New York, 2002, 91–224.
doi: 10.1201/9780203023884.ch5_
 56. Parida, A.K. & Das, A.B. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.*, 2005, **60**(3), 324-49.
doi:10.1016/j.ecoenv.2004.06.010
 57. Munns, R. & Teste, R.M. Mechanisms of Salinity

- Tolerance. *Annual Review Plant Biol.*, 2008, **59**, 651–8.
doi:10.1146/annurev.arplant.59.032607.092911
58. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G. & Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends Plant Sci.*, 2014, **19**, 371–379.
doi:10.1016/j.tplants.2014.02.001
 59. Sibole, J.V.; Cabot, C.; Michalke, W.; Poschenrieder, C. & Barcelo J. Relationship between expression of the PM H⁺-ATPase, growth and ion partitioning in the leaves of salt-treated *Medicago* species. *Planta.*, 2005, **221**(4), 557–566.
doi.org/10.1007/s00425-004-1456-6
 60. Song, J.Y.; Kim, D.S.; Lee, M. C.; Lee, K.J.; Kim, J.B.; Kim, S.H.; Ha, B.K.; Yun S.J. & Kang S.Y. Physiological characterization of gamma-ray induced salt tolerant rice mutants. *A J. Crop. Sci.*, 2012, **6**(3), 421-429.
 61. Stewart, G.R. & Lee, J.A. Role of proline accumulation in halophytes. *Planta.*, 1974, **120**, 279-289.
doi:10.1007/BF00390296
 62. Rhodes, D.; Rich, P.J.; Brunk, D.G.; Ju, G.C.; Rhodes, J.C.; Pauly, M.H. & Hansen, L.A. Development of two isogenic sweet corn hybrids differing for glycine betaine content. *Plant Physiol.*, 1989, **91**, 1112–1121.
doi:10.1104/pp.91.3.1112
 63. Arakawa, T. & Timasheff, S. N. The stabilization of proteins by osmolytes. *Biophys. J.*, 1985, **47**, 411-414.
doi:10.1016/S0006-3495(85)83932-1
 64. Lin, C.C. & Kao, C. H. Levels of endogenous polyamines and NaCl inhibited growth of rice seedlings. *Plant Growth Reg.*, 1995, **17**, 15–20.
 65. Grumet, R. & Hanson, A.D. Glycine-betaine accumulation in barley. *Aust. J. Plant Physiol.*, 1986, **13**, 353–364.
doi:10.1071/PP9860353
 66. Zhu, J.K.; Liu, J. & Xiong, L. Genetic analysis of salt tolerance in *Arabidopsis thaliana*: Evidence of a critical rate for potassium nutrition. *Plant Cell*, 1998, **10**, 1181–1192.
doi:10.1105/tpc.10.7.1181
 67. Singh, K.N. & Chatrath, R. Salinity Tolerance. In Application of physiology in wheat breeding. Edited by Reynolds MP, Monasterio JIO, McNab A. CIMMYT, Mexico, DF, 2001, 101–110
 68. Soussi, M.; Lluch, C. & Ocaña, A. Comparative study of nitrogen fixation and carbon metabolism in two chick-pea (*Cicer arietinum* L.) cultivars under salt stress. *J. Exp. Bot.*, 1999, **50**, 1701-1708.
doi:10.1093/jxb/50.340.1701
 69. Soussi, M.; Ocaña, A. & C. Lluch. Effect of salt stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicer arietinum* L.). *J. Exp. Bot.*, 1998, **49**, 1329-1337.
doi:10.1093/jxb/49.325.1329
 70. Dehan, K. & Tal, M. Salt tolerance in the wild relatives of the cultivated tomato: Response of *Solanum pennellii* to high salinity. *Irrig. Sci.*, 1978, **1**, 71-76.
doi:10.1007/BF00269009
 71. Asvathappa, N. & Bachelard E.P. Ion regulation in the organs of Casuarina species differing in salt tolerance. *Aust. J. Plant Physiol.*, 1986, **13**, 533-545.
doi:10.1071/PP9860533
 72. Gorham, J.; Budrevicz, E.; McDonnell, E. & Wyn Jones R.G. Salt tolerance in the Triticeae. Salinity induced changes in the leaf solute composition of some perennial Triticeae. *J. Exp. Bot.*, 1986, **37**, 1114-1128.
doi:10.1093/jxb/37.8.1114
 73. Sekeroglu, N.; Kara, M.S.; Dede, O. & Askin, T. Effect of salinity on germination, early seedling growth, Na and K constituents of chickpea. *Turk. J. Field Crops.*, 1999, **4**, 79–84.
 74. Tabosa, J.N.; Colaco, W.; Reis, O.V.; Simplicio, J.B. & Dias, F.M. Sorghum genotypes tolerant to soil salinity-progenies developed under gamma ray doses. *E. J. Icrisat. Org.*, 2007, **5**(1), 1-5.
doi:10.18512/1980-6477/rbms.v6n3p339-350
 75. Qi, W.; Zhang, L.; Xu, H.; Wang, L. & Jiao, Z. Physiological and molecular characterization of the enhanced salt tolerance induced by low-dose gamma irradiation in *Arabidopsis* seedlings. *Biochem. Biophys. Res. Commun.*, 2014, **25**(2), 1010-1015.
doi:10.1016/j.bbrc.2014.06.086
 76. Pettigrew, W.T. Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiol. Plant*, 2008, **28**(4), 212–217.
doi:10.1111/j.1399-3054.2008.01073.x
 77. Ozakaya, B.; Ozakaya, H.; Eren, N. & Koxsel, H. Effects of wheat maturation stage and cooking method on physical and chemical properties of firiks. *J. Food Engg.*, 1999, **15**(4), 110–115.
doi:10.1016/s0308-8146(98)00249-0
 78. Al-Rumaih, M.M., & Al-Rumaih, M.M. Influence of ionizing radiation on antioxidant enzymes in three species of *Trigonella*. *Am. J. Environ. Sc.*, 2008, **22** (2), 151-156.
doi:10.3844/ajessp.2008.151.156.
 79. Noreen, Z. & Ashraf, M. Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *J. Environ. Exp. Bot.*, 2009, **67**, 395–402.
doi:10.1016/j.envexpbot.2009.05.011
 80. Haber, A.H. & Luippold, H.J. Effects of gibberellin on gamma irradiated wheat plants. *Am. J. Bot.*, 1961, **47**(2), 140–144.
doi:10.2307/2439048
 81. Srinivas, H.; Ananthswamy, H.N.; Vakili, A. & Shrinivasan, A. Effect of gamma irradiation on wheat proteins. *J. Food Sci. Technol.*, 1972, **115**(1), 59–66.
 82. Ahuja, S.; Kumar, M.; Kumar, P.; Gupta, V. K.; Singhal, R. K.; Yadav, A. & Singh, B. Metabolic and biochemical changes caused by gamma irradiation in plants. *J. Radioanalytical Nuclear Chem.*, 2014, **300**(1), 199-212.
doi: 10.1007/s10967-014-2969-5
 83. Miller, B.L. & Huffaker, R.C. Differential induction of endo proteinases during senescence of attached and detached barley leaves. *Plant Physiol.*, 1985, **78**, 442–446.
doi.org/10.1104/pp.78.3.442
 84. Ellis, R.J. The most abundant protein in the world. *Trends*

- Biochem. Sci.*, 1979, **4**: 241–244.
doi:10.1016/0968-0004(79)90212-3
85. Thoenen, M.; Herrmann, S. & Feller, U. Senescence in wheat leaves: Is a cysteine endopeptidase involved in the degradation of the large subunit of Rubisco. *Acta. Physiol. Plant.*, 2007, **29**, 339-350.
doi:10.1007/s11738-007-0043-4
86. Feller, U.; Anders, I. & Mae, T. Rubiscolytics: Fate of Rubisco after 1st enzymatic function in a cell is terminated. *J. Exp. Bot.*, 2008, **34**, 254-259.
87. Singh, B.; Ahuja, S.; Singhal, R.K. & Venu Babu, P. Effect of gamma radiation on wheat plant growth due to impact on gas exchange characteristics and mineral nutrient uptake and utilization. *J. Radioanal. Nucl. Chem.*, 2013, **298**(1), 249-257.
doi:10.1007/s10967-012-2342-5
88. Esfandiari, E.; Shakiba, M.R.; Mahboob, S.A.; Alyari, H. & Shahabivand, S. The effect of water stress on antioxidant content, protective enzyme activities, proline content and lipid peroxidation in seedling wheat. *Pak. J. Biol. Sci.*, 2008, **11**(15), 1916–1922.
doi:10.3923/pjbs.2008.1916.1922
89. Kishor, K.; Sangam, P.B. & Amrutha, S.R.N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and a biotic stress tolerance. *Curr. Sci.*, 2005, **88**, 424–438.
90. Ashraf, M. & Foolad, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *J. Environ Exp Bot.*, 2007, **59**(2), 206–216.
doi:10.1016/j.envexpbot.2005.12.006
91. Lukanda, L.T.; Nkongolo, K.K.C.; Narendrula, R.; Kalonji-Mbuyi, K. & Kizungu, R.V. Molecular characterization of groundnut (*Arachis hypogaea L.*) accessions from a gene pool: application of Gamma ray radiations. *J. Plant Breed Crop. Sci.*, 2012, **4**(11), 175-183.

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