Medicinal plants have been the subject of man’s curiosity and purpose since time immemorial. The importance of medicinal plants in the treatment of chronic diseases needs no elaboration. In fact, even with the tremendous advancement in the field of synthetic chemistry, almost 50% of the commercial drugs available in the market remain of plant origin. Even with the advent of allopathic system, the age-old system of herbal medicine is being revived due to its long-lasting curative effect, easy availability, natural way of healing and rare or no reported side-effects. Owing to growing world population, increased anthropogenic activities, rapidly eroding natural ecosystem etc., the natural habitat for a great number of plants are dwindling and many of them are facing extinction (Kamboj, 2000). New strategies are, therefore, being formulated for rapid multiplication and conservation of medicinal plants. Besides the conventional methods, biotechnology has proved useful in the improvement of herbs that yield drugs (Nasim et al., 2009a).

The medicinal plant biotechnology has grown from cell technology, specifically plant tissue culture. Regeneration of plants has been achieved with cells and tissues excised from various medicinal herbs. The powerful techniques of plant cell and tissue culture, recombinant DNA and bioprocessing technologies etc. coupled with sophisticated analytical tools such as NMR, HPLC, GC-MS, LC-MS etc. have offered mankind a great opportunity to exploit the totipotent, biosynthetic and biotransformation capabilities of plant cells under in vitro conditions. The intervention of plant tissue culture for accelerating clonal propagation of desired clones and strains (high-yielding) of medicinal plants through micropropagation and their conservation through establishing Tissue Banks or Gene Banks are warranted in the right earnest. This has brightened the scope for in vitro germplasm preservation and large-scale production of plant secondary metabolites (Narula et al., 2004).

2.1 Micropropagation of medicinal plants

In vitro cultured cells and tissues can be induced to differentiate into plants through organogenesis (Sai et al., 2000) or somatic embryogenesis (Jayanthi & Mandal, 2001). The response of any tissue in vitro is attributed to the composition of the medium besides other factors including a balance between growth regulators (Cardell & Frett, 1990). MS medium originally developed for rapid growth of tobacco tissue culture is the most frequently used for majority of the species. Already there are credible reports of in vitro propagation of medicinal plants by using various explants, such as leaf (Malabadi &
Nataraja, 2001), stem (Uander, 1991), shoot buds (Fracaro & Echeverrigaray, 2001),
anthers (Srivastava et al., 1993), roots (Sudha et al., 2000), shoot-tips, nodal segments
(Butiuc-Keul & Deliu, 2001; Selvakumar et al., 2001) and seedlings (Perez-Bermudez et
al., 2002).

There are quite a few medicinal plants which are micropropagated on a moderate
scale. Clonal mass multiplication is imperative in case of such medicinal plants, which
yield precious active principles present in small quantities, but required in huge amounts.

2.2.1 Garlic and its chemistry

Garlic (Allium sativum L.) is an important and widely cultivated plant with both
culinary and medicinal uses owing to its biological activities related to sulphur
compounds, which include anti-biotic, anti-cancer, anti-thrombotic, anti-diabetic and
lipid-lowering cardiovascular effects (Nasim et al., 2009).

Despite the numerous therapeutic effects attributed to garlic, the chemistry behind
its health-promoting effects is still poorly understood. Garlic is a major source of sulphur-
containing compounds, particularly S-alk(en)yl-L-cysteine sulphoxides (ACSOs), alliin
being the major one. Volatiles such as allicin and lipid-soluble sulphur compounds such
as diallyl sulphide, diallyl disulphide, diallyl trisulphide, dithiins, ajoene and others are
originated from ACSOs by different metabolic pathways after tissue damage of garlic by
cutting, crushing or biting. These compounds provide to garlic its characteristic odour and
flavour, as well as most of its biological properties. It has recently been demonstrated that
additional garlic constituents such as organo-selenium compounds, steroid saponins and
sapogenins, vitamins B6 and B12, flavonoids (eg. Allixin), lectins and N-fructosyl-
aminoacids, may contribute, along with organosulfur compounds, to the biological effects
of garlic (Alejandra et al., 2010).
Figure 2.1 Formation of organo-sulphur compounds during metabolic pathways in processed garlic (Corzo-Martinez et al., 2007).

Though such medicinal use of garlic existed for centuries, there was limited scientific support for its therapeutic and pharmacological properties. However, there has been a recent upsurge of research on garlic aiming to understand its exact mechanism(s) of action in each case so that garlic and its products may have more judicious future applications. Since garlic is vegetatively propagated, its improvement for desired traits through conventional means is difficult. The intervention of biotechnological methods such as tissue culture and tissue cultured nutritional enrichment based protocols hold great promise for improving this crop. New innovations in instrumentation and processing...
technologies coupled with more judicious experimental models could raise garlic to a
reputable position in the medicinal world market (Bhagyalakshmi et al., 2005).

2.2.2 Micropropagation of garlic

Plant Tissue Culture methods offer not only high frequency regeneration of
disease-free material but also provide chance for obtaining variants with useful
agronomical traits as well as improvement in nutritional and medicinal properties. Several
reports on organogenesis or embryogenesis in garlic tissues cultured in vitro (Novak,
1981) have been documented. Among the different explants, shoot-tips (Kehr &
Schaeffer, 1976; Nagakubo et al., 1993; Meyers & Simon, 1998), basal plate (Xue et al.,
1991; Barandiarian et al., 1998) and receptacle (Koch et al., 1995), first primordial leaves
(Novak, 1980; Sata et al., 2001) and root-tips (Haque et al., 1997, 1998; Robledo-Pez et
al., 2000) have been found to be useful to various extents in establishing in vitro cultures.

For callus induction up to bulb formation in garlic variety ‘Rouge de la Reunion’, high
frequency of fast-growing callus formed from leaf explants (Haque et al., 1998).
However, young leaves were reportedly endowed with very high embryogenic potential
producing globular somatic embryos within two months on Gamborg’s medium
(Gamborg et al., 1968) supplemented with 0.1 mgL\(^{-1}\) 2,4-D and 0.5 mgL\(^{-1}\) kinetin. Of
these, about 30% regenerated into shoots with normal roots after 8 weeks on another
medium (Dunstan & Short, 1978) containing 0.3 mgL\(^{-1}\) of BAP (Fereol et al., 2002).
Though nearly 15% embryos germinated on basal medium itself, BAP had marked
influence on somatic embryo germination where 0.3 mgL\(^{-1}\) of BAP supported conversion
of nearly 30% into plantlets.

To overcome yield decrease in garlic due to systemic viral infections, production
of virus-free garlic using shoot-tip culture has been widely adopted (Bhojwani, 1980;
Chovelon et al., 1994). However, to replace the conventional seed clove production
system, in vitro bulblet formation from shoot-tip explants has been reported where a
production level of 10–12 bulblets per shoot-tip, i.e., per clove, was achieved (Bhojwani,
1980; Ayabe & Sumi, 1998). For rapid propagation and producing virus-free plants, stem-
disc culture has been found very effective. From 1 disc ca., 30 shoots regenerate in 1
month, of which more than 90% form bulblets in vitro and the in vitro raised plants have
been grown normally under field conditions (Ayabe & Sumi, 1998). For further
enhancement of the overall productivity, root-tips (Nagakubo et al., 1993) have been
cultured on MS-medium supplemented with 1µM NAA and 10µM BAP to initiate shoot cultures. Initiated shoots were proliferated on MS medium with 0.5µM BAP and multiple shoots, thus produced, were transferred to PGR-free MS media with 12% sucrose. The best response with 95% shoot regeneration has been reported in a Japanese cultivar “White Roppen” which produced 285 bulblets from root-tips of a single clove within 4 months. The bulblets produced could form plants when transferred to soil indicating the repetitive use of the protocol for rapid multiplication of virus-free garlic clones (Haque et al., 1997).

2.2.3 Somatic embryogenesis in garlic

Somatic embryogenesis is a valuable tool for achieving a wide range of objectives. One of the main applications of somatic embryogenesis is its employment as an approach to investigate the initial events of zygotic embryogenesis in higher plants (Kiyosue et al., 1993). Also, secondary or recurrent embryogenesis, offers a great potential for in vitro production of embryo metabolites, such as lipids and seed storage proteins. Improvement of crops by transgenic processes by adding traits/genes to superior elite genotypes takes relatively short period of time. In order to be more effective, an efficient and stable plant regeneration system is essential and a primary consideration. Somatic embryogenesis offers this opportunity and thus has been exploited in a wide range of plant groups (Rommens et al., 2004; Walter, 2004). It has several advantages over organogenesis and appears to be the most promising method for large scale clonal propagation of selected elite genotypes (Ignacimuthu, 1996; Lelu-Walter et al., 2008). In addition, embryogenic cells or suspensions can effectively be exploited to conserve genetic resources by in vitro cryopreservation.

Although a few reports on embryogenesis in Allium are available lately (Fereol et al., 2002, Tokit et al., 2003), the synchronous proliferation of embryos, the maturation, and efficient germination are proving difficult and the information discussing those events are still not adequate. The basic understanding of the nature of somatic embryo quality and the study involving cultural conditions, which facilitate quality in vitro embryo production are not enough to exploit embryogeny for practical exploitation (Nasim et al., 2010). However, sensitivity of embryogenic development of somatic cells to exogenous PGRs offers the possibility of using in vitro screening and selection procedures to identify resistant plant genotypes (Merkle et al., 1990).
Rate of embryogenesis has been reported to be quite high in *Allium sativum* L. (Pullman et al., 2003; Nasim et al., 2010). Exogenous application of PGRs has been found to be important for callus induction, embryogenic culture establishment as well as proliferation, maturation and germination of embryos into plantlets. 2,4-D is considered to be the most important PGR that regulate somatic embryogenesis *in vitro* (Zhang et al., 2007). Application of 2,4-D increases explant’s endogenous auxin level which is one of the crucial signals that determines cultured cells’ fate to become embryogenic (Victor, 2005). Low levels of 2,4-D in combination with BAP has been reported to be more effective in inducing embryogenic tissue (Becwar et al., 1988). Nasim et al. (2010) reported high embryogenic callus production by the application of BAP and NAA at high concentrations (1.0-2.0 mgL\(^{-1}\)) with a low level of 2,4-D (0.25 mgL\(^{-1}\)) in *Allium sativum* L. cv Yamuna Safed 3. Rate of embryogenesis and regeneration also depend on age of culture. Fereol et al. (2005) reported an increase in number of embryos in culture with increase in the age of suspension culture up to 7 months. For most species, however, the regeneration rate decreases with the age of the culture. Similar results have been obtained on garlic (Myers & Simon, 1998) and onion (Phillips & Luteyn, 1983) in the past.

Embryo maturation and simultaneous germination to obtain plantlets is one of the important steps in *in vitro* embryogenesis, which has been partially depending on embryo quality. A number of compounds like abscisic acid, sugar, sugar-alcohol, polyethylene glycol, activated charcoal, low temperature etc. are reported to influence embryo maturation and germination (Lipaska & Konradova, 2004; Robichaud et al., 2004). ABA has been reported to be ineffective for embryo maturation and germination in garlic (Nasim et al., 2010) and hybrid chestnut (Vieitez, 1995). In contrast, ABA-induced improved embryo maturation with quality embryo was reported in several studies of somatic embryogenesis particularly in conifers (Maruyama et al., 2007). In garlic variety Yamuna Safed 3, 0.5 mgL\(^{-1}\) GA\(_3\) had significant influence on embryo maturation and also favoured fast embryo germination. Also, application of fructose, maltose and sucrose at 3% individually in garlic were extremely effective for somatic embryo maturation (Nasim et al., 2010). Similar observations were observed in other plants with 3% sucrose or maltose (Corredoria et al., 2003). Maturation treatments that produce larger embryos do not always yield maximum plant recovery. The high and consistent embryogenic ability, however, may suggest a stable embryogenic cell line, which could effectively be used in transgenic programme. Similarly, effective regeneration methods established through
somatic embryo germination may be used for fast \textit{in vitro} propagation and cryopreservation purposes.

\section*{2.3.1 Secondary metabolite production}

Medicinal plants are the most exclusive source of bioactive compounds which are used as food additives, pigments, dyes, insecticides, cosmetics, perfumes and fine chemicals (Balandrin & Klocke, 1988). These compounds belong to a group collectively known as secondary metabolites. The evolving commercial importance of the secondary metabolites has in recent years resulted in a great interest in secondary metabolism, and particularly in the possibility to alter the production of bioactive plant metabolites by means of cell culture technology. Tissue culture has been used for commercial synthesis of natural products since 1956. However, a reasonable yield of desired secondary metabolites by cell culture technology was demonstrated only after 1973 (Fowler, 1982). For plant cell culture techniques to become economically viable, it is important to develop methods that would allow for consistent generation of high yields of products from cultured cells (Berlin & Sasse, 1985). Careful selection of productive cells and culture conditions resulted in accumulation of several products in higher levels in cultured cells. Research suggests that the ability of product biosynthesis continues throughout the culture regime and can be detected at various stages of growth and differentiation (Parr et al., 1990).

Yield of secondary metabolites can be enhanced by modifying the chemical milieu and culture conditions. Zenk et al. (1975) observed that the composition of culture medium not only affects growth and production of metabolites but also plays a critical role in initiation of morphogenic events in the culture. Manipulation of physical aspects and nutritional elements in a culture is perhaps the most fundamental approach for optimization of culture productivity.

Sucrose is the most commonly used carbon source in tissue culture media. Glucose, galactose and other complex carbohydrates such as milkwhey and molasses have also been tested to support growth. Increased sugar concentration has been reported to favour shikonin synthesis in cell cultures of \textit{Lithospermum erythrorhizon}, diosgenin synthesis in \textit{Dioscorea}, and anthraquinone production in \textit{Gallium mollugo} (Roja & Rao, 1998). On the contrary, lesser amounts of sucrose favour the production of ubiquinone 10 in \textit{Coleus blumei} (Roja & Rao, 1998).
Manipulating the concentration of microelements in the nutrient media also leads to increased production of secondary metabolites. Increase in concentration of Co$^{2+}$ from 1 to 5 μM resulted in enhanced production of betalains in *Beta vulgaris* (Trejo-Tapia et al., 2001). Similarly, increased concentration of both copper and sulphate has reported to enhance shikonin production in *Lithospermum* sp. cultures (Roja & Rao, 1998).

Plant growth regulators (auxins and cytokinins) are also effective triggers of secondary metabolites. Low concentration of 2,4-D (0.1 mgL$^{-1}$) proved favourable for alkaloid production in cell cultures of *Cinchona ledgeriana* (Zenk et al., 1975). The type of auxin used also exerts a strong influence on the secondary product synthesis. Zenk et al. (1975) have reported a reduced production of anthraquinones in the presence of 2,4-D in *Morinda citrifolia*, however, NAA enhanced anthraquinones accumulation. Similarly, in cytokinins, BAP is reported to enhance shikonin production in *L. erythrorhizon* (Ray & Jha, 2001). Likewise, maximum accumulation of withanolide occurred in the cultures of *Withania somnifera* in the presence of BAP, which then declined with an increase (2.0-5.0 mgL$^{-1}$) in the concentration of BAP (Ray & Jha, 2001). According to Decendit et al. (1992), Zeatin or BAP at 1 μM proved more effective than kinetin in *Catharanthus* cell cultures and the alkaloid production was doubled.

Exogenous supply of a biosynthetic precursor to culture medium may also increase the yield of the desired product. Attempts to induce or increase the production of plant secondary metabolites, by supplying precursor or intermediate compounds, have been effective in many cases (Moreno et al., 1993; Silvestrini et al., 2002). Amino acids have been added to cell suspension culture media for production of tropane alkaloids, indole alkaloids etc. Addition of phenylalanine to *Salvia officinalis* cell suspension cultures stimulated the production of rosmarinic acid (Ellis & Towers, 1970). Feeding ferulic acid to cultures of *Vanilla planifolia* resulted in increase in vanillin accumulation (Romagnoli & Knorr, 1988).

Phytotoxicity due to heavy metals shows reduction in quality and productivity of plants when grown on such soils. Tissue culture techniques have helped in raising the tolerance of plants and also demonstrated that subjecting the cultures of some medicinal plants to abiotic stress can be crucial in increasing the yield of secondary metabolites (Saba et al., 2000). Their useful effect has been reported in plants such as *Ammi majus*, *Lepidium sativum*, *Amaranthus* and many more (Saba et al., 2000).
Use of elicitors (biotic and abiotic) has been one of the most effective strategies for improving the productivity of bioactive secondary metabolites (Roberts & Shuler, 1997). Elicitors are signals triggering the formation of secondary metabolites, thereby reducing the process time to attain high product concentrations (Barz et al., 1988). Production of many valuable secondary metabolites like berberine, shikonin, reserpine, capsaicin etc. using various elicitors has been reported.

Plant cell cultures represent a heterogeneous population in which physiological characteristics of individual plant cells are different. Synthesis of several products in high amounts using selection and screening of plant cell cultures have been described by Berlin and Sasse (1985). Cell cloning methods provide a promising way of selecting high yielding cell lines. A strain of Euphorbia milli accumulated about 7-fold the level of anthocyanins produced by the parent culture after 24 selections (Yamamoto et al., 1982).

Not only the chemical milieu, but even the physical conditions of the medium have proved crucial for secondary metabolite synthesis in cultures. Light, pH of the growth medium etc. are some factors which have been reported to affect the production of secondary metabolites. Production of diosgenin and related compounds seem to be controlled by different media ingredients as well as by light (Sengupta et al., 1989). Similarly, alkaloid synthesis in Lupinus polyphyllus cultures was enhanced with a decrease in pH from 5.5 to more acidic at 3.5 (Sengupta et al., 1989).

2.3.2 Bioreactors scaling up for secondary metabolites synthesis

Using bioreactors is a key step towards commercial production of secondary metabolites by plant biotechnology (Leena & Jaendra, 2003). Plant cells in liquid suspension offer a unique combination of physical and chemical environments that must be accommodated in large-scale bioreactor process. It gives better control for scale up of cell suspension cultures under defined parameters for the production of bioactive compounds (Scragg, 1992). However, there are indications that sufficient oxygen supply and proper mixing in airlift bioreactors may not be suitable for high density plant cell suspension cultures. Some of the well known drawbacks of the cell suspension cultures include the instability of the productive cell lines, the slowness of the cell growth and limited knowledge about the metabolic pathway (Fulzele, 2005). Well known problem shear sensitivity and rapid setting characteristics of plant cell aggregates and cell floating tendencies of the cell cultures have to be solved when bioreactors are designed.
2.3.3 Metabolic engineering and secondary metabolite production

Metabolic engineering involves the targeted and purposeful alteration of metabolic pathways found in an organism to achieve better understanding and use of cellular pathways. In spite of the efforts in the field of in vitro production of phytochemicals, few industrial processes have been developed, involving only a limited number of secondary products, such as shikonin, berberine, ginsenosides and paclitaxel (Ramachandra & Ravishankar, 2002). As in many cases production is too low for commercialization, metabolic engineering can provide various strategies to improve productivity, such as: increasing the number of producing cells, increasing the carbon flux through a biosynthetic pathway by over-expression of genes codifying for rate-limiting enzymes or blocking the mechanism of feedback inhibition and competitive pathways and decreasing catabolism. Several genes in the biosynthetic pathways for scopolamine, nicotine, and berberine have been cloned, making the metabolic engineering of these alkaloids possible (Sato et al., 2001).

The hairy root system based on inoculation with Agrobacterium rhizogenes has become popular in the two last decades as a method of producing secondary metabolites synthesized in plant roots (Palazon et al., 1997). The hairy root phenotype is characterized by fast hormone-independent growth, lack of geotropism, lateral branching and genetic stability. The secondary metabolites produced by hairy roots arising from the infection of plant material by A. rhizogenes are the same as those usually synthesized in intact parent roots, with similar or higher yields (Sevon & Oksman-Caldentey, 2002). This feature, together with genetic stability and generally rapid growth in simple media lacking phytohormones, makes them especially suitable for biochemical studies not easily undertaken with root cultures of an intact plant. Moyano et al. (1999) showed that the inoculation of leaf sections of tobacco, Duboisia hybrid and Datura metel plants with the A4 strain of A. rhizogenes induced transformed roots with the capacity to produce putrescine-derived alkaloids such as nicotine, hyoscyamine and scopolamine. Transformed roots provide a promising alternative for the biotechnological exploitation of plant cells, thereby enabling commercial production of pharmaceutically important compounds (Karuppusamy, 2009).
2.3.4 Secondary metabolites in garlic tissue cultures

The medicinal properties of garlic have been attributed primarily to the presence and subsequent breakdown of alliin \([\text{alk(en)}\text{ylcysteine sulfoxide (CSO)}]\) and allicin (Jones et al., 2007). Alliin yields a series of thiosulphinates, allylsulphides, dithiines and ajoenes after the action of alliinase and subsequent chemical decomposition (Jones et al., 2007).

*In vitro* cultures accelerate production of specific medicinal compounds at a rate similar or superior to that of intact plants by affecting the biosynthetic activity of cultured cells. The production of metabolite is tissue specific and also depends on age of plant (Junaid et al., 2008; Mukhtar et al., 2008). According to Nasim et al. (2010), *in vitro* grown garlic leaves and plantlets showed highest alliin production in comparison to other plant tissues and explants tested. Also, the production of alliin from *in vitro* grown garlic plantlet and leaves increased till 8 weeks, beyond which no significant change was noted.

Earlier studies reported that somatic embryos (SE) grown under *in vitro* conditions showed higher alliin yield in comparison to field grown clove (Nasim et al., 2009). This result corroborated with earlier study where a significant increase in alkaloid content was reported in *Narcissus confusus* somatic embryos (Selles et al., 1999). Alliin content has been reported to be globally lower in non-embryogenic than embryogenic callus (Nasim et al., 2009, 2010). Under *in vitro* conditions, differentiation of any tissue/form is generally associated with an increased synthesis of secondary products (Collin, 2001). Amongst the various developmental stages of embryo, highest alliin production was reported in germinated embryos (Nasim et al., 2010).

Metabolite production (or metabolism) is not only restricted to specific tissues or cells, it is also modulated by different cellular and environmental factors (Pasquali et al., 1992; Junaid et al., 2008). Sulphur treatment upto 16 mgL\(^{-1}\) is reported to significantly enhance alliin production in all tissues and explants grown under *in vitro* conditions in garlic (Nasim et al., 2010). Callus exposed to irradiation or to the mutagen EMS (ethyl methane sulfonate) led to substantially improved allicin yields (Malpathak & David, 1986). Presence of sulfate ions in the medium combined with irradiation is an efficient strategy for production of the two major flavour components (allicin and methyl cysteine sulfoxide) of garlic from cultured cells, according to a patent claimed by Ajinomoto, a Japanese company.
The information regarding production of secondary metabolites (alliin) in garlic grown under *in vitro* conditions by altering plant growth regulators or modification in medium composition with nutrients in various tissues and organs is still scanty. Besides, more studies need to be carried out to understand the mechanism involved and evaluate the factors responsible for enhancement in metabolite content in this *Allium* specie.

### 2.4.1 Gamma Radiations

A rapid development of biotechnology in recent years has accelerated the studies on development of modernized improvement methods and increased the genetic variability in plants for the benefit of agriculture and agricultural industries (Ahloowalia, 1998). Plant tissue culture is recognized as a source to generate useful genetic variability (somaclonal variation) for crop improvement (Evans et al., 1989) with the intention of including and exploiting useful and economically valuable characters that may not be readily available within other sources of germplasm (Juned et al., 1991). Another source of generating variations in plants is by mutagenesis (physical and chemical). Radiation induced mutations and chemical mutagenesis have been extensively used for the improvement of crop plants (Shah et al., 2008). Semi-dwarf growth, earlier maturity, higher yields, and resistance to diseases (Mei et al., 1994; Li et al., 2007) are examples of some favourable traits induced after exposures to ionizing radiations. The combination of mutagenesis with *in vitro* techniques offers an efficient method for improving vegetatively propagated plants. These techniques allow induction of variation, selection and multiplication of the desired genotypes in a much shorter duration and smaller space than conventional methods. There are several agricultural crops in which positive mutations have been induced using different mutagens (Jan et al., 2012). Improved crops like high-yielding barley variety with early maturity, high protein contents and stiff straw have been successfully developed using mutation breeding techniques (Rehman et al., 1987; Javed et al., 2000). The cumulative number of officially released mutant varieties in the world indicates that Asia tops the rest of the continents. *In vitro* techniques can be employed in various steps of a breeding programme and offer a number of advantages for mutation breeding. In several crop plants, gamma irradiation is known to affect tissue culture response (callus growth and plant regeneration) and often higher doses result in low or no regeneration frequency (Kulkarni et al., 1997).
Electromagnetic (EM) radiations are usually used as a physical mutagen for inducing mutations in plants. Gamma rays, X-rays, visible light and UV rays are the different EM radiations. The various EM radiations differ in frequency and thus energy. Gamma rays are the most energetic form of such EM radiation, having the energy level from around 10 kiloelectron volts to several hundred kiloelectron volts, and therefore they are more penetrating than other radiations such as alpha and beta rays (Kovacs & Keresztes, 2002).

### 2.4.2 Effects of Gamma Radiations

Gamma rays belong to ionizing radiations and interact to atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry, and physiology of plants depending on the irradiation level. Changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds (Kovacs & Keresztes, 2002; Kim et al., 2004; Wi et al., 2005) are some examples of the deleterious effects of gamma radiations.

The predominant effect of ionizing radiations is water radiolysis which induces the formation of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anion, hydroxyl radicals and singlet oxygen that can lead to cell lethality at high concentrations (Halliwell, 1974; Kovacs & Keresztes, 2002). The treatment with ionizing radiations increases the activities of endogenous scavenging enzymes (POD, CAT, SOD, and APX) in order to cope up with the damages caused by ROS (Kim et al., 2005).

These effects of gamma radiations can vary depending upon the dose rate or dose applied, physiological parameters such as the specie/variety/cultivar considered, the developmental stage at the time of irradiation, and also inter-individual response (Gunckel, 1957; Kim et al., 2009), and can vary even among genotypes of the same specie (Kwon & Im, 1973). It is, therefore, difficult to predict a standard response to gamma irradiation in plants; some patterns, however, do emerge (Jan et al., 2012).

### 2.4.3 Effect of radiations on plant growth

Physiological symptoms in plants exposed to low or high-dose gamma radiations include enhancement or inhibition of germination, seedling growth, and other biological
responses (Jan et al., 2012). In Arabidopsis, a low dose of 1-2 Gy slightly increased the growth of seedlings, while it was noticeably decreased by a high-dose irradiation of 50 Gy. There is no conclusive explanation for the stimulatory effect of low-doses and inhibitory effect of high doses of gamma radiations (Wi et al., 2005). However, studies hypothesise the role of low-dose radiations in inducing change in hormonal signalling network and increase in antioxidative capacity of plant cells so as to overcome daily stress factors (Kim et al., 2004). Whereas, high doses are believed to cause cell cycle arrest at G2/M phase during mitotic cell division and/or damages in the entire genome leading to growth inhibition (Jan et al., 2012).

2.4.4 Radiations-induced morphological changes

The relationship between growth of irradiated plants and dose of gamma irradiation has been manifested by investigating the morphological changes and seedling growth of the irradiated plants.

From the ultrastructural observations of the irradiated plant cells, the prominent structural changes of chloroplasts after the 50 Gy irradiation revealed that chloroplasts were more sensitive to a high dose of gamma rays than other cell organelles. At high-dose irradiation, there is also accumulation of starch within the chloroplasts accompanied by damage and disorientation of grana and thylakoids indicative of inhibition of carbohydrate transport (Bondada & Oosterhuis, 2003). Plastids were affected by irradiation in two ways: (a) inhibition of senescence, and (b) dedifferentiation into agranal stage (Kim et al., 2004). The developmental regression of chloroplasts can be assumed primarily from destruction of grana (Kovacs & Keresztes, 1989). However, the low-dose irradiation did not cause these changes in the ultra-structure of chloroplasts. Similarly, mitochondria in the control and low-dose-irradiated stems in Arabidopsis possess well-organized cristae, while in the stems exposed to 50 Gy, endoplasmic reticulum (ER) membranes were distended and mitochondria distorted in shape. The size of mitochondria was slightly enlarged after the low-dose irradiation, but not changed after the high-dose irradiation. The low-dose irradiation of 1 or 5 Gy did not affect significantly the ultrastructures of cell organelles. However, even the plasmalemma was separated from the cell wall in the 50 Gy-irradiated plants (Wi et al., 2007).
2.4.5 Radiations-induced biochemical and physiological effects

Based on previous research reports, the total protein and carbohydrate contents decreased with increasingly higher dosage of gamma-irradiation caused by higher metabolic activities and hydrolyzing enzyme activity in germinating seed (Barros et al., 2002; Maity et al., 2004). Radiation resulted in the increased absorption of glucose, pyruvate, and the decreased absorption of acetate and succinate in carrot (*Daucus carota* L.). Total proteins and carbohydrates decreased with increasing high gamma-ray dosage in wheat and rice plants (Hagberg & Persson, 1968; Inoue et al., 1975). Carbohydrates in onion bulbs account for a major portion of their dry matter. The principle components of the non-structural carbohydrates glucose, fructose, sucrose and a series of fructans have an important implication for the physiology during bulb formation, maturation, dormancy and sprouting of the bulb (Suzuki & Cutcliffe, 1989; Brewster, 1990). Glucose and Fructose levels have been reported to increase in the first 5 weeks of storage in the irradiated bulbs in onion followed by return to their initial levels thereafter. However, sucrose content did not show a significant increase or decrease following irradiation (Benkeblia & Varoquaux, 2003). Croci et al. (1995) have also reported a slight increase in the glucose content in both the irradiated (0.05 kGy) and the control bulbs after 3 months of storage at temperatures ranging from 6 to 32°C over 10 months. Similarly, Benkeblia and Selselet-Attou (1994) have reported an increase in sugar concentration following 5-8 weeks of storage in irradiated onion bulbs. Extensive research has shown that proteins, essential amino acids, minerals, trace elements and most vitamins do not represent significant losses during irradiation, even at doses over 10 kGy.

Bourke et al. (1967) reported a decrease in all amino acids upon irradiation except for serine and valine, which were increased at 100 krad (1 Gy). Gamma-irradiation breaks the seed protein and produces more amino acids (Barros et al., 2002; Maity et al., 2004; Kiong et al., 2008). This process may also inhibit protein synthesis. Pradeep et al. (1993) reported that amino acids like lysine and histidine were resistant to gamma-irradiation (5 kGy) in *Ginseng panax* leaf (herb). Al-Jassir (1992) found that the contents of arginine, methionine, lysine, phenylalanine, and leucine of garlic bulbs (*Allium sativum* L.) increased slightly. However, reduction in other amino acids in irradiated samples also occurred, especially at higher doses.
Investigations on the use of ionising radiations for sprout control of onions have been carried out throughout the world. Doses from 0.05 – 0.15 kGy inhibits bulb sprouting, and is more effective when applied during the dormancy period. These treated bulbs can be stored for several months without heavy spoilage, though at optimum storage conditions (Salunkhe & Wu, 1974; Thomas, 1984; Matsuyama & Umeda, 1986; Smierzchalska et al., 1987). Ascorbic acid, a major vitamin in onion bulbs, is degraded rapidly in heat, light and freezing conditions (Lee & Labuza, 1975). However, ascorbic acid content of bulbs irradiated with 0.07 kGy was found to be similar to untreated bulbs even after 5 months of storage at room temperature (Molco & Padova, 1960; Benkeblia & Selselet-Attou, 1999). Similar results have been observed by Ghods et al. (1976) in onions, and Graham and Stevensen (1997) in potatoes.

Irradiation is also known to increase respiration rate (RR), measured 24 hours after treatment. However, the initial RR was restored after less than 2 weeks. This decrease can be attributed to degeneration of meristematic cells and death of the sprout caused by radiation which slows down the complete respiratory pathway including glycolysis (Benkeblia et al., 2002). Low doses of gamma-rays enhance chlorophyll synthesis by activating the enzyme systems (Zeerak et al., 1994; Rascio et al., 2001). Osama (2002) also reported improvement of yield components and chlorophyll parameters in various plants such as tomato (Lycopersicon esculentum L.), maize (Zea mays L.), rice (Oryza sativa L.), and wheat (Triticum aestivum L.) after variable doses of gamma-rays. Higher gamma-irradiation inhibits chlorophyll synthesis in wheat (Kovacs & Keresztes, 2002) and changes in pigmentation (reddening) were observed in the older leaves of Holcus lanatus L. treated with 40, 80, and 160 Gy (Jones et al., 2004).

Many studies (Marchenko et al., 1996) have also shown that irradiated plant cells have a self-protection mechanism. This mechanism works by enhancing the synthesis of substance groups or enzymes containing sulphur such as amino acids (cysteine, cystine), glycation, and superoxide dismutase, which can protect, remove free radicals, and participate in the simultaneous release of protective substances in an organism (Qui et al., 2000).

2.4.6 Radiation and its effect on Anti-oxidative enzyme system

Amongst the various environmental challenges faced by plants, radiation is an important stressor which exerts adverse effects on plant growth and development.
Gamma-irradiation is reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals (O$_2^-$), hydroxyl radicals (OH$^-$) and H$_2$O$_2$ (Apel & Hirt, 2004), which react rapidly with almost all structural and functional organic molecules including proteins, lipids, nucleic acids causing disturbance of cellular metabolism (Salter & Hewitt, 1992). Hydroxyl radicals are generated by ionizing radiation either directly by oxidation of water, or indirectly by the formation of secondary, partially generated ROS.

The amount and rate of hydroxyl radicals generation from ROS is controlled partly by the cellular antioxidant status and partly by the availability of systems capable of reducing (or ‘activating’) superoxide or hydrogen peroxide. It has been shown that the effects of H$_2$O$_2$ resemble those of ionizing radiation (Riley, 1994). Water molecule is the direct target of gamma-irradiation. The primary reactions are excitation and ionization, which produce ionized water molecules (H$_2$O$^+$) and the radicals H$^-$ and ·OH. However, in biological tissue these ionizations are induced all along the path of the radiation and lead to chain reactions, which produce secondary reactive oxygen species (ROS) as a result of H$^-$ and e$_{aq}^-$ becoming trapped. The most important ROS is H$_2$O$_2$; O$_2^-$ is produced to very low extent, depending on the O$_2$ concentration (Tubiana, 2008; Lee et al., 2009). The ·OH radical can react rapidly with various types of macromolecules, including lipids, proteins and, in particular, DNA. However, some of the resulting injuries can be readily repaired and recovered, depending on the dose range.

Superoxide dismutase (SOD) isozymes are compartmentalized in higher plants and play a major role in combating oxygen radical mediated toxicity. Changes in peroxidase (POD) and superoxide dismutase isozyme compositions were seen in Vigna radiata (L.) R. Wilczek (Roy et al., 2006), as well as in calli of two tobacco species (Nicotiana tabacum and Nicotiana debneyi) (Wada et al., 1998) after the irradiation (20-200 Gy) established from leaf discs. Pramanik (1997) correlated morphological damage caused by gamma-rays with the changes in SOD isozyme patterns in callus tissue obtained from irradiated seeds. SOD activity PAGE gels showed an extra band (Rf value – 0.59) in all of the irradiated samples of calli, which were absent in the control. This over expression of SOD is an indication of radioprotection in vitro. Ascorbate peroxidase (APX) and SOD activities in peppers also increased at low doses (from 2 to 8 Gy), whereas glutathione reductase (GR) activity decreased (Kim et al., 2004, 2005). Catalases (CAT) were stimulated at 5.16 C kg$^{-1}$ in safflowers (Singh, 1974). SOD, POD, CAT, and
G6PDH (glucose-6P dehydrogenase) activities are strongly stimulated in the case of new chronic external irradiation. Thus, it has been suggested that these enzymes protect plants more efficiently than the ascorbate glutathione ROS detoxification cycle.

Plant exposure to gamma-irradiation brings about changes in biochemical activity through various metabolites such as peroxyl radicals produced as a function of gamma-irradiation. In plants, gamma irradiation is reported to cause production of free peroxyl radicals through the peroxidation of unsaturated fatty acids (Voisine et al., 1991). Hydrogen peroxide deposits were clearly increased in pumpkin leaves and petioles at the high acute dose of 1 kGy (Wi et al., 2006a, 2006b). These deposits were present in the vessels, in the plasma membrane, in the cell corners of the middle lamella and especially in the parenchyma cells. This stimulation of Peroxidase (POD) activity was also observed in garlic bulbs at low doses (10 Gy) (Croci et al., 1991, 1994), in Saintpaulia petioles at high-doses (0.85 and 1.98 C kg\(^{-1}\); Warfield et al., 1975) and in sweet potato root disks (900 Gy; Ogawa & Uritani, 1970). The radiation protective effect of peroxidase is due to removal of H\(_2\)O\(_2\), peroxides, and especially lipid hydrogen peroxides. Gamma-irradiation of callus culture of Datura innoxia Mill. resulted in stimulation of peroxidase activity and particularly increased the peroxidase enzymes with high electrophoretic mobilities (Jain et al., 1990). Chaomei and Yanlin (1993) reported an increase in the activity of POD and Catalase (CAT) with a corresponding decline in growth of Triticum aestivum L. plants under higher irradiation doses (20, 40, 60, 80 Kr). Singh et al. (1993) reported induction of Ascorbate Peroxidase (APX) activity in two sugarcane varieties grown under gamma-irradiation. The activities of POD, CAT, and SOD in radish (Raphanus sativus L.) leaves were enhanced by gamma-irradiation (10 Gy) treatment. Inhibition of CAT activity was also reported under irradiation stress (Ye et al., 2000; Vandenhove et al., 2009). The present increase in APX activity was reported to compensate for the progressive drop in catalase activity. Peroxidase was considered to be the key enzyme for the decomposition of H\(_2\)O\(_2\), especially under CAT inactivation.

Glutathione Reductase (GR) activity in shoots and roots of the three Trigonella L. genus significantly increased above the control values when exposed to different doses of gamma-rays. There was slight increase in GR activity in Stipa capillata L. when exposed to gamma-irradiation, probably due to enhancement of the transcription rate of encoding genes (Foyer et al., 1991). The hypothesis of GR radio-induction in Stipa is supported by the fact that a similar response was reported by other workers (Zaka et al., 2002; Moussa,
2006). The role of GR in \( \text{H}_2\text{O}_2 \) scavenging mechanism in plant cells has been well established in Halliwell–Asada enzyme pathways (Yamane et al., 2009).

*Allium* species are known to possess compounds which are responsible for strong antioxidative property of these plants. Their antioxidant capacity has been found to be equal or sometimes even more than that of synthetic antioxidants like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) that are used in the food industry (Barlow, 1990). Oxidation of unsaturated fatty acids leads to the formation of compounds that are undesired from the point of view of both taste and toxicity (Frankel, 1984). Free radicals are among the main products of lipid oxidation and have been implicated in playing a role in over 100 diseases including cancer, atherosclerosis and arthritis (Thomas, 1995).

The high antioxidant activity of *Alliums* and especially high Radical Scavenging Activity (RAS) of garlic were reported by numerous investigators (Velioglu et al., 1998; Miller et al., 2000). However, RAS activity depended on both phenolics and sulfur compounds of *Alliums*. On the other hand, Nuutila et al. (2003) reported that the lowest antioxidant activity was detected in garlic. However, according to some authors (Imai et al., 1994, Amagase et al., 2001; Yin et al. 2002), non-enzymatic antioxidant activity was more attributed to the organosulfur compounds.

2.4.7 Effect of radiations on secondary metabolites and oil components

Many of the plant-derived phenolic compounds (flavonoids, isoflavonoids, coumarins, and lignans) are secondary products of phenylpropanoids metabolism (Dixon & Paiva, 1995). In higher plants, phenylpropanoids provide a UV-A and UV-B screen. Gamma-radiation has different effects on contents and components of volatile oils mainly due to the species of the plant and the dose applied. Koseki et al. (2002) studied the effect of radiation doses (0, 10, 20, and 30 kGy) on the flavonoids, essential oils, and phenolic compounds of Brazil medicinal herbs. The test reports suggested that irradiation of traditional medicines and herbal products do not result in any negative chemical changes or important losses of active components. After irradiation up to 17.8 kGy, the content of the main biologically active substances of two medicinal herbs (*Ginkgo* and *Guarana*) were not modified (Soriani et al., 2005). Lee et al. (2005) showed that the pungency and red colour caused by capsanoids and capsanthin, were not altered when irradiated (3, 7 and 10 kGy) in red pepper powder. However, Mishra et al. (2004) reported that the dose...
of 5 kGy led to a decrease in 6-gingerol, the compound responsible for the pungency of ginger.

Irradiation with 15 kGy caused slight increase in phenol content in *Brassica nigra* L. Koch (0.1%) followed by *Cassia senna* L. (pods) (1.3%) (Musa, 2009). However, the maximum increase was about 70% and was observed in *Cassia senna* L. (leaves) followed by *Lepidium sativum* L. (25.6%) and *Cymbopogon schoenanthus* L. (24.9%) (Musa, 2009). On the other hand, irradiation with 15 kGy reduced the phenol content of *Trigonella foenumgraecum* L. by 4.1% followed by *Hibiscus sabdariffa* L. (5.1%) and *Acacia nilotica* L. (14%). The maximum reduction was 33% and was observed in *Cymbopogon citrates* L. (Musa, 2009).

In a study on nutmeg, Variyar et al. (1998) found that the essential oil constituents showed clear quantitative differences upon gamma-irradiation. Thus, the content of α-terpineol, 1-terpinene-4-ol, and myristicin was increased in irradiated nutmeg, while that of sabinene, α-pinene, and elimicin decreased in comparison to the control sample. Less significant changes in the content of some of the essential oil constituents between the control and the irradiated samples were noted in clove and cardamom. In Welsh onions (*Allium fistulosum* L.), Gyawali et al. (2006) reported enhancement in total concentration of volatile compounds by 31.60 % and 24.85 % in bulbs upon irradiation with 10 and 20 kGy respectively. On the other hand, Kwon et al. (1989) found no influence on the amount of total sulphur and thiosulfinate of stored garlic for 10 months upon gamma irradiation at 0.1 kGy. However, storage period brought about a significant reduction in the content of both components.

γ-Glutamyl transpeptidase (GTP) is the first enzyme in the catabolism of γ-Glutamyl peptides. GTP activity and control pyruvate (CP) content increased in garlic bulbs cv Red irradiated with 50 Gy due to radioinduced metabolic respiratory activation. From 90-180 days of storage, the GTP and enzyme pyruvate (EP) increased in both irradiated and control garlic bulbs. After 180 days of storage, EP and primary sulphur compounds decreased in irradiated garlic and in controls, while GTP, cumulative weight loss and CP kept increasing in both samples with the rate of increase lower in irradiated garlic. This suggests irradiated garlic to be better in quality (Ceci et al., 1992).

Elevation of some compounds by gamma-irradiation may be attributed to their higher extractability. Some reports indicate that irradiation decreases tannins in bean
seeds (Villavicencio et al., 2000). Such differences may be attributed to the differential response and variability in the genetic constituents (strains and varieties). Doses of 7 and 10 kGy significantly reduced the tannin content of Shahalla sorghum variety but not that of Hemaira variety (Sidduraju et al., 2002).

Figure 2.2 Effect of gamma irradiation on plant growth, development and metabolism (Jan et al., 2012).

2.4.8 Gamma Radiations in Food Preservation

The use of ionising radiations as a method of preserving food is fast gaining importance. Nowadays, gamma radiations have also found a potential use in ensuring food safety during storage and upgrading the nutritional quality of food products across the world. Moreover, with the ban on the use of methyl bromide (or ethyl dibromide) and other disinfectants in dates, and maleic hydrazide (MH), carbamate isopropyl N-phenyl (CIP) and CIP-chlore-(CIPC) for sprout inhibition in potatoes, onions and garlic suggest a high potential of the ionising radiation as a replacement treatment that could also complement temperature controlled storage (Benkeblia, 2000). However, similar to other food processing techniques, irradiation can induce certain alterations that can modify the
chemical composition and nutritive values of food (Wiendl, 1984). These changes depend on the factors such as irradiation dose, food composition, packaging, and processing conditions such as temperature and atmospheric oxygen saturation (Giroux & Lacroix, 1998).

While some vitamins such as riboflavin (B2), pyridoxine (B6) and biotin are usually stable, others such as Thiamin (B1) and vitamins A, C and E are relatively labile (Giroux & Lacroix, 1998). Although radiation is one of the conservation techniques that cause fewer damages to food nutrients, vitamin losses resulting from the food irradiation can be substantial. Among the fat soluble vitamins, the most sensitive to irradiation is γ-tocopherol (vitamin E). Ionizing irradiation significantly affected the γ-tocopherol contents of garlic samples only when the doses reached 0.2 to 0.25 kGy (Rios & Penteado, 2003). However, doses used to inhibit the sprouting and extend storage period of garlic was much lower than those that caused vitamin loss to the product (Pellegrini et al., 2000). Regarding vitamin C, no alterations were registered in the garlic and onions receiving irradiation doses enough for sprouting inhibition (Kilcast, 1994). Garlic irradiated at 0.05 kGy gamma radiation and stored for 300 days, presented decrease in vitamin C contents similar to that registered for non-irradiated garlic (Croci et al., 1995). Several chemical parameters were investigated in red variety garlic, irradiated to inhibit sprouting, with doses of 30 Gy and kept under warehouse conditions. The studies were conducted between 210 and 270 days post harvest (critical marketing periods) when this variety was not normally available for raw consumption. During the storage, the irradiated garlic showed a significant increase in ascorbic acid content but no change in dry matter content compared with non-irradiated garlic. Compared with non-irradiated garlic, at 270 days' storage, the irradiated garlic had a higher index of flavour, measured as enzymatic pyruvate, a higher acidity and a lower content of water soluble carbohydrates. From these observations, the irradiated garlic should be suitable for prolonged storage with the object of marketing it during the critical periods (Curzio et al., 1986). Another study evaluated the effects of different radiation doses applied in both dormant and postdormant onions (Allium cepa L.). Elevated irradiation dosages (0.03 to 0.15 kGy) lead to complete inhibition of sprouting and mitosis of onion, independent of the application moment, while low doses (0.002 to 0.01kGy) did not result in any desirable effects on food (Pellegrini et al., 2000).
In addition, studies with dehydrated garlic and onions, seeking product sanitation, showed that doses between 5-10 kGy were enough to reduce the micro flora to desirable levels, and making these products viable to use in the hospitals and in immuno-impaired people diets (Pezzutti et al., 2005).

2.5.1 Salinity

Abiotic stresses elicit complex cellular responses. Drought stress, Anaerobic stress, Salinity stress, Temperature stress, Heavy-metal stress etc. are some common abiotic stresses affecting the vegetation adversely throughout the globe. The response and degree of susceptibility of different plant species to these abiotic stresses is often varied. The tolerance of plants to these stresses can be understood and manipulated by understanding the plant abiotic stress responses at whole-plant, physiological, biochemical, cellular and molecular levels.

Among the abiotic stresses, salinity is one of the major environmental constraints affecting more than 800 million hectares of arable land throughout the world (Munns & Tester, 2008) and, therefore limits the production capabilities of agricultural soils in large areas of the world. Low rainfall, high evaporation, native rocks, saline irrigation water, and poor water management increasingly cause salinity problems in agricultural areas. It has been estimated that the world is losing at least 3 hectares of arable land every minute because of soil salinity (FAO, 2008). Also, improving irrigation techniques and reclamation of salinized land are prohibitively expensive and short-term methods of controlling salinity (Singh & Singh, 2000). Therefore, to maintain global food production, enhancement of the salt tolerance of crops through various plant breeding programs is an increasingly important aspect.

2.5.2 Effects of Salinity

Salinity limits vegetative and reproductive growth of plants by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects, even at low salt concentrations (Shannon et al., 1994). Tissue injury is induced not only by the osmotic effects of salts but also by specific toxic effects resulting from the accumulation of Cl− and Na+ (Hasegawa et al., 2000). Crop productivity is affected by salinity due to decrease in water potential of the root medium, ion toxicity due to
excessive sodium and chloride uptake, and nutrient ion imbalance by the disturbance of essential intracellular ion concentrations (Hasegawa et al., 2000).

Modifications in different morphological, physiological and biochemical processes and anatomical changes are responsible for adversely affecting plant growth (Tester & Davenport, 2003). Salt stress-induced modifications may lead to the accumulation of certain metabolites and alterations in the level (increase or decrease) or the presence (appearance or disappearance) of some cellular proteins (Kong-Negren et al., 2005). Several studies have shown that salt stress may induce not only osmotic stress and oxidative stress but may also cause protein denaturation in plants, which lead to cellular adaptive responses and accumulation of compatible organic solutes such as soluble carbohydrates, amino acids, proline, betaines, etc., (Hasegawa et al., 2000; Munns, 2002). Gonzalez et al. (1995) reported that NaCl stress reduces cell survival rate in four sugarcane genotypes using cell suspensions and found that genotypes respond differently when confronted with NaCl in their culture medium.

At high salinity levels, progressive reduction in plant height and increase in leaf tip necrosis has been reported (Francois, 1994). The yield and quality aspects are significantly reduced at high salinity levels (~ >3.9 dSm⁻¹). Several aspects of plant metabolism, including lipid metabolism are also known to be affected by salinity in many species (Erdei et al., 1980). Salinity impact on the yield and the fatty acid composition of oil has been previously reported in different plant species, such as *Carthamus tinctorius* L. (Bassil & Kaffka, 2002), *Oenothera biennis* (Heuer et al., 2002), *Brassica juncea* L. seeds (Parti et al., 2003) and *Helianthus annuus* L. (Flagella et al., 2004). Neffati and Marzouk (2008) indicated a significant reduction of the total fatty acid amount from coriander leaf under saline conditions.

However, all salinity effects may not be negative; salinity may have some favourable effects on yield, quality, and disease resistance (Shannon & Grieve, 1999). Numerous reports also suggest the significant role of medium salt strength in improving biomass production and secondary metabolite accumulation such as essential oil compounds (Dow et al., 1981) in cell cultures of several plant species such as in adventitious roots of *Echinacea angustifolia* (Wu et al., 2006). In spinach, yields may initially increase at low to moderate salinity (Osawa, 1963). Sugar contents increase in carrot and starch content decreases in potatoes as salinity increases (Bernstein, 1959);
cabbage heads are more solid at low salinity levels, but are less compact as salinity increases (Osawa, 1961). Celery has been reported to be both more resistant and more susceptible to blackheart (Osawa, 1963; Aloni & Pressman, 1987).

In addition, salt stress can induce oxidative stress, as common plant responses to different abiotic and biotic stresses are accelerated, such as generation and/or accumulation of reactive oxygen species, including hydrogen peroxide ($\text{H}_2\text{O}_2$), superoxide anion, and hydroxyl radicals (Kovtun et al., 2000; Azevedo-Neto et al., 2006; Ashraf, 2009). $\text{H}_2\text{O}_2$ is an active signaling molecule, and its accumulation (oxidative stress) leads to a variety of cellular responses which are dose-dependent. Plants employ antioxidant compounds (e.g., ascorbate, glutathione, $\alpha$-tocopherol, and carotenoids) and detoxifying enzymes (e.g., superoxide dismutase, catalase, and enzymes of ascorbate-glutathione cycle) to combat oxidative stress (Ashraf et al., 2010). The activity and expression levels of the genes encoding detoxifying enzymes are probably enhanced by ROS under abiotic stresses. Transgenic plants overexpressing ROS scavenging enzymes, such as superoxide dismutase (Alscher et al., 2002), ascorbate peroxidase (Wang et al., 1999), and glutathione S-transferase/glutathione peroxidase (Roxas et al., 2000) showed increased tolerance to osmotic, temperature, and oxidative stresses. However, plant tolerance to oxidative stress alone is unlikely to be a major determinant of overall salt tolerance (Fadzilla et al., 1997). Therefore, understanding the biochemical and molecular basis of salt-stress signaling and tolerance mechanisms is essential for breeding and genetic engineering of salt tolerance in crop plants (Zhu, 2001).

It is also important to understand that plant growth and development can be affected by salinity stress at any time during the crop life cycle, but the extent and nature of damage and the impact on yield depend on the developmental stage at which a crop encounters the stress. In general, cereal crops are most sensitive to salinity during the vegetative and early reproductive stages and less sensitive during flowering and the grain filling periods (Maas & Poss, 1989). However, plant genotypes may respond differently to salt stress at different growth stages. Differences in salt tolerance responses among rice genotypes at different growth stages as observed by Zeng et al. (2002) supports this view.

2.5.3 Salinity tolerance in crops

Different crops have different sensitivity to salinity. Plant genotypes may also respond differently to varying levels of salt stress and the response may still vary with the
developmental stages. The figure below shows the salt tolerance of different crops in terms of decline in their yield.

![Salt Tolerance Chart](image_url)

**Figure 2.3** Salt tolerance of several vegetable species as rated by the salinity threshold and percent yield decline (Shannon & Grieve, 1999).

Asparagus is considered to be the most salt-tolerant vegetable crop commercially available but it grows better in sandy, well-drained soils than in heavy textured soils. In vitro studies of asparagus tissues found that tolerance was directly related to cellular organization and organogenesis with rooted and unrooted plantlets showing similar levels of tolerance (Mills, 1989). In these studies, both Na\(^+\) and Cl\(^-\) increased with salinity treatment in tissues of friable and compact callus cells, and in roots, shoots, and rhizomes of plantlets.

Based on yield decline, onion and garlic are considered to be salt-sensitive crops. Though onions are relative excluders of Na\(^+\) and Cl\(^-\), they are sensitive to sulphate. Increase in salinity results in decrease in bulb diameter, bulb weight, root growth, plant height, and number of leaves per plant. Garlic (*A. sativum* L.) is one of the important crops grown on 1 lakh 20 thousand hectare area in arid and semiarid regions of India for its bulbs which is an important spice/condiment. It shows similar effects as onion where all yield components (bulb weight and diameter, and plants per unit area and percent solids) are reduced with increasing salinity, thus effecting the bulb quality (Francois, 1994). The leaf tissues accumulate significantly higher Cl\(^-\), Na\(^+\), and Ca\(^{2+}\) concentrations than bulbs (Francois, 1994). No or little research has been conducted to determine whether rooting systems can be genetically modified to improve salt tolerance of these
crops and if variability can be introduced without affecting the commercial quality of the bulb (Pasternak et al., 1984). Moreover, according to some reports, garlic tends to be slightly more salt tolerant than most vegetable crops. Still, there is a need to maintain low soil salinity levels so as to obtain maximum yield.

2.5.4 Biotechnological strategies in improvement of salt tolerance

The understanding of the mechanisms that enable plants to adapt to salt stress is necessary for exploiting saline soils. Salt tolerance in higher plants is the result of numerous physiological and biochemical processes. In several plant species, salt tolerance may operate at cellular level. Plant tissue culture techniques provide a unique platform to study these changes at the cellular level of plant development and have been employed for obtaining salt tolerant lines. Firstly, mutant cell lines are selected from the cultured cells and it also provides a rapid and reliable in vitro screening procedure for germplasm evaluation and thus improvement of salt tolerance amongst plants. Moreover, field selection for salinity tolerance is a laborious task. Several screening and selection schemes have been proposed for salt tolerance improvement in wheat and other crop species (Munns & James, 2003). Field screening procedures in saline soils are confronted by spatial heterogeneity of soil chemical and physical properties as well as seasonal fluctuations in rainfall (Munns & James, 2003). Hence, many screening experiments for salt tolerant genotypes were conducted either under in vitro (Dasgupta et al., 2008) or under controlled environmental conditions (Munns et al., 2000; Mohammadi-Nejad et al., 2008).

Plant tissue culture studies, thus, facilitate to unravel the plants’ salt tolerance mechanism at the unorganised cellular or organised tissue level, and may provide information on the potential for physiological, biochemical, and growth responses to salt stress at different levels of tissue organisation. Besides, in vitro studies allow relatively faster responses, shorter generation time and controlled environment as compared to ex vitro conditions (Zhang et al., 2004; Perez-Tornero et al., 2009) and the inferences obtained from in vitro cultures under salt stress may be directly applicable at the whole-plant level. Several studies have shown that in vitro culture is a reliable method for testing salt tolerance in many plants using both shoot apex and callus cultures (Basu et al., 2002; Bracci et al., 2008). Shoots in comparison to callus cultures are easy to propagate in vitro
and plant material selected from salt-stressed cultures can be used to establish plantation in saline soils.

Also, genetic manipulation (genetic transformation in particular) and selection of favourable somaclonal variant strains from callus culture are supplementary tools to traditional breeding for production of stress-resistant plants (Ashraf, 1994). *In vitro* selection of salt tolerant cell lines has been reported for several species (Tal, 1994). Recent reports on production of fertile and genetically stable salt-tolerant regenerants in durum and bread wheats (Barakat & Abdel-Latif, 1996) and other species (Tal, 1994) also suggest that tissue culture selection can be useful in improving salt tolerance of plants.

2.6.1 Selenium

Discovered by Swedish chemists J.J. Berzelius and J.G. Gahan in 1817, Selenium is a group VIB metalloid (Atomic Weight 78.96) with its chemical and physical properties lying between those of metals and non-metals. Selenium has been recognized as an essential trace element for humans and animals based on its presence in antioxidative defence systems (Rotruck et al., 1973) and in hormone balance (Pallud et al., 1997). It is a complex trace element which is unevenly distributed in the earth's crust. Its concentration in the soil varies markedly between geographic regions. In India, although a small dimension in terms of area affected, the seleniferous region of Punjab, India bordering Nawanshahr and Hoshiarpur region, has been of serious concern to the research community (Srivastava et al., 2006).

Se can exist in five valence states namely selenide (2⁻), elemental Se (0), thioselenate (2⁺), selenite (4⁺), and selenate (6⁺) (Lauchli, 1993), however, the most frequently found in soils are selenite and selenate (Dhillon & Dhillon, 2003). The speciation of Se depends on redox conditions and pH of the environment. Selenium also exists in volatile forms. Though essential as a trace element, extremely low and extremely high Se concentrations are detrimental to human and animal health (Combs, 2001). It has three levels of biological activity: (a) trace concentrations are required for normal growth and development; (b) moderate concentrations can be stored to maintain homeostatic functions; and (c) elevated concentrations can result in toxic effects (Hamilton, 2004). It has a catalytic role in variety of enzymes that contain selenocysteine residues as a part of their active site (Birringer, 2002). Se is important in antioxidation in humans and may play a role in antioxidative mechanisms in plants (Ekelund & Danilov, 2001).
In plants, Se can be found both in inorganic and organic Se forms, including selenoamino acids and methylated compounds. Current interest in Se is focused on the health benefits of high-Se plants as a source of cancer preventative Se compounds (Finley et al., 2000, 2001), and the metabolism of Se in plant species that accumulate Se and are able to remediate Se-polluted soils thereby preventing Se from entering into the food chain (Berken et al., 2002).

Although Se is not classified as a micronutrient for higher plants, numerous studies have shown that at low concentrations, Se exerts a beneficial effect on growth and stress tolerance of plants by enhancing their antioxidative capacity (Xue et al., 2001; Kong et al., 2005). Similarly, as in human and animal cells, Se increases plant resistance against oxidative stress caused by free oxygen radicals. However, agricultural crops are sensitive to high tissue Se concentrations, but sensitivity varies among plant species (Hartikainen et al., 2001; Lyons et al., 2005). In *Eichhornia crassipes*, selenium is known to inhibit chlorophyll-a, chlorophyll-b, total chlorophyll, starch and carbohydrate at a high concentration of 20 mgL^{-1} (Mane et al., 2011). While the proteins and free amino acids, with lesser sensitivity to Se were inhibited at a higher concentration of 50 mgL^{-1} (Mane et al., 2011). However, up to a low concentration of 10 mgL^{-1}, all these biochemical parameters showed a significant increase (Mane et al., 2011).

### 2.6.2 Selenium- Beneficial or Harmful?

Though an essential trace element, selenium is detrimental to normal growth at extremely low or extremely high concentrations. The recommended daily intake of Se for human adults is 50–70 µg and for children 20–30 µg. The minimum Se dietary requirement is approximately 20 µg Se per day for adults (RDA, Recommended Dietary Allowances, 1989). Its deficiency may result in symptoms including muscle pain, weakness, and loss of pigment in hair and skin, and whitening of nail beds (Subcommittee of Selenium, Committee on Animal Nutrition, Board of Agriculture National Research Council, 1983; Haygarth, 1994).

The Food and Nutrition Board has set the Tolerance Upper Intake Levels (UL) for Se at 400 µg per day for adults (Food and Nutrition Board and Institute of Medicine, 2005). However, Se intakes greater than those set by RDA may be beneficial to human. Clark et al. (1996) demonstrated that dietary supplementation of 200 µg of Se per day significantly reduced lung, prostate, and colorectal cancer in humans. Specific methylated
forms of Se, such as Se-methylselenocysteine have also been shown to provide chemoprotective effects against certain types of cancer in humans (Ellis & Salt, 2003). Se is an integral component of GSH-Px (Rotruck et al., 1973), an important antioxidative enzyme that catalyzes the destruction of hydrogen peroxide generated during oxidative metabolism in human and animal cell. Se has, therefore, been identified as an essential component of thioredoxin reductase (Tamura & Stadman, 1996), and for a number of selenoproteins (Kryukov et al., 2003).

On the other hand, at Se concentrations of 3–15 mg kg\(^{-1}\) DW in tissues, some plants can cause toxic symptoms in animals. Chronic Se toxicity is caused by intakes of 2–4 mg per day or prolonged intakes of 1 mg per day. Chronic symptoms of excessive consumption of Se include morphological changes in fingernails, nail brittleness and loss of hair, as well as nausea, vomiting and skin lesions. Gupta et al. (1982) have given preliminary reports on chronic Se toxicity as a cause of hoof and horn disease in livestock in seleniferous regions of Punjab. The malformations appearing in livestock were attributed to feeding of fodder grown in seleniferous soils (Dhillon & Dhillon, 1991). Limited observations on human population in selenium laden regions of Punjab state has also shown signs of selenium toxicity with chronic selenosis in humans mainly due to consumption of grains and vegetables harvested from selenium-rich agricultural soils (Hira et al., 2003). In such regions, plants with selenium accumulating potential may be used as a remedial measure for controlling the selenium levels in soils.

### 2.6.3 Plants as Se delivery system

Plants play a unique role in recycling and delivering Se from the soil to the food chain. The concentration of Se in agricultural products and fodder depends on the content of Se in the soil and its bioavailability (Ylaranta, 1985). Low availability of Se in soil leads to low Se intake in humans, thereby increasing risk of cardiovascular disease, coronary heart disease and cancer and results in nutritional disorders in animals. Selenium intake of the human population and domestic livestock may be increased by supplementing multinutrient fertilizers with sodium selenate (Terry et al., 2000). However, in most countries the regulations governing organic farming do not allow Se to be added to organic fertilizers. As a result, in organically-cultivated cereals the Se concentration is low, 0.01 to 0.02 mg kg\(^{-1}\) DW (Eurola et al., 2005). Also, Se-supplemented fertilizers are not yet allowed in greenhouse cultivation. Moreover, plants
that can accumulate Se may also be useful as a “Se-delivery system” to supplement the mammalian diet in many areas that are deficient in Se.

2.6.4 Selenium biochemistry in animals

In animals, Se is covalently bound to various different compounds and the metabolic function of selenium in the body depends on the specific chemical compound consumed. It is essential for the function of multiple selenoproteins, including thioredoxin reductase and glutathione peroxidase (Burk & Hill, 1993; Gladyshev & Hatfield, 1999). In selenoproteins, Se is at the active site as selenocysteine (SeCys) (Gladyshev & Hatfield, 1999) and a unique biochemical pathway is employed to synthesize and insert SeCys into proteins.

Selenide is a primary intermediate that may either be used for selenoprotein synthesis or excreted (Ganther, 1986). Selenium is commonly found in inorganic salt forms that are easily converted to selenide and incorporated into selenoproteins (Ganther, 1986). Selenomethionine (SeMet), the Se analog of methionine, may be used by the body interchangeably with methionine (Met), thus, accumulating in protein masses such as muscle, and being released when the protein pool turns over (Beilstein & Whanger, 1986). Consequently Se consumed as SeMet is excellent for restoring Se concentrations in Se depleted tissues (Xia et al., 2005). Se consumed as SeMet may be transformed through the trans-sulfuration pathway, to selenocysteine (SeCys), the Se analog of cysteine. SeCys also may enter the body from dietary sources, primarily from animal proteins; SeCys is readily cleaved by a specific lyase to form hydrogen selenide (Deagen et al., 1987). The dietary SeCys, however, is not used as the source of SeCys in selenoproteins, rather SeCys is formed de novo by the reaction of a selenide metabolite with serine already joined to a specific tRNA (Driscoll & Copeland, 2003).

The inorganic salts of Se, selenite and selenate may be reduced non-enzymatically by glutathione to form hydrogen selenide (Ganther, 1986). This result in bifurcation of the metabolic pathway, whereby, Se may either be sequentially methylated in preparation for excretion, or it may be metabolized into selenoproteins. At low to moderate intake of Se, there is formation and subsequent methylation of seleno-sugars involving S-adenosyl methionine (SAM). The other route involves initial methylation of Se to form mono-methyl selenol which has been suggested as the most important anti-cancer metabolite by limited in-vitro studies (Ip & Ganther, 1990). This undergoes further methylation and is
eventually excreted out as tri-methyl selenonium ion alongwith seleno-sugars (Kobayashi et al., 2002). The anti-cancer metabolite, monomethyl selenol is directly generated by plant sources of Se-methylselenocysteine (Ip & Lisk, 1996). This suggests that such plants may be used as efficacious sources of Se in treatment or reduction of cancer risk.

The selenide intermediate may also be incorporated into seleno-proteins in which the Se is present at the active site in the form of SeCys, where it functions as a redox catalytic centre. The selenoproteins utilize the ability of Se as SeCys to sequentially get oxidised and reduced, thus catalysing redox reactions. The metabolism of Se in animals or humans is greatly dependent on the Se status, and the metabolism may be quite different in adequate versus deficient populations (Combs, 1999).

Figure 2.4 Generalised schematic of Se metabolism in animals, also showing the possible metabolism of some food forms of Se (Finley, 2007).

2.6.5 Selenoproteins: Protection against ROS

Reactive oxygen species are derived from cellular oxygen metabolism and from exogenous sources, and include free radicals such as superoxide anion, hydroxyl and lipid radicals as well as oxidizing non-radical species such as hydrogen peroxide, peroxynitrite and singlet oxygen. At higher concentrations, ROS can damage cellular macromolecules including DNA, proteins and lipids. Thus, cells possess antioxidative systems for detoxification of ROS and repair of deleterious oxidative modifications on cellular
structures. Among the dietary supplements, ingested by many individuals to improve their state of health, the essential micronutrient selenium has received attention for its antioxidant properties. Usually, humans take up selenium with their diet, predominantly from cereals, fish and meat. Selenium-enriched yeast and garlic are two natural products containing selenium mostly as highly bioavailable selenomethionine or gamma-glutamyl-Se-methylselenocysteine (Ip et al., 2000).

The antioxidant capacity of selenium relies on the ROS-degrading selenoenzymes that contain selenocysteine at its catalytic centre. Unlike other metal ions which are attached to their respective apoproteins as cofactors, selenium is cotranslationally incorporated into selenoproteins as selenocysteine (Berry et al., 2001), the selenium analogue of cysteine. Selenoenzymes are more efficient in catalysing redox reactions mainly due to two biochemical properties of selenocysteine. Since the selenol group in selenocysteine (pKa≈5.2) is more acidic than the thiol group in cysteine (pKa≈8.5), it is deprotonated at physiological pH values and is more reactive in nucleophilic reactions (Stadtman, 1996). In addition, selenocysteine is more readily oxidized than cysteine (Nauser et al., 2006). Apart from its function in the catalytic center of selenoenzymes, selenocysteine also has the potential to repair oxidative damage by reducing tyrosyl radicals in proteins (Steinmann et al., 2008).

Selenomethionine, the selenium analogue of methionine, can also act as ROS scavenger. Free selenomethionine is rapidly oxidized by peroxynitrite to methionine selenoxide (Padmaja et al., 1996), which can be reduced back to selenomethionine in a non-enzymatic reaction maintained by glutathione (Assmann et al., 2000).

### 2.6.6 Selenium biochemistry in plants

Most researchers believe that Se is present in plants as a by-product of normal metabolism, or as a result of metabolism directed towards compartmentalization and/or detoxification. However, a small amount of evidence suggests that some plants may require Se as an essential nutrient.

Plants absorb Se from soil primarily as selenate, translocate it to the chloroplast and then metabolize it by the sulfur assimilation pathway. ATP sulfurylase forms the activated moiety adenosine-5’-phosphoselenate, which is reduced to selenide and then reacts with serine to form SeCys (Wang et al., 1999). SeCys can be further metabolized to
SeMet and methylated to form products such as Se-methyl selenomethionine. A specific enzyme, selenocysteine-specific methyltransferase, methylates specific forms of Se, resulting in products such as SeMSC; this may be the factor that allows many plants to accumulate extraordinarily large amounts of Se (Wang et al., 1999). There is limited support for the theory that plants, like animals, may contain Se in the form of SeCys that is incorporated into true selenoproteins (Fu et al., 2002; Rodrigo et al., 2002).

### 2.6.7 Food sources of selenium

The concentrating of essential minerals, vitamins, and bioactive phytochemicals into human foodstuffs is gaining importance in our rapidly expanding world. Plant foods may be divided into two general groups: (a) high-Se plants that accumulate Se in response to the amount of Se available from the soil; and (b) plants that actively accumulate Se well in excess of the amounts in the soil (often called ‘Se hyper-accumulators’) (Ip et al., 2000). Plants that accumulate elements such as selenium may be used as a natural source of mineral supplements for both animals and human beings, especially in areas that are mineral deficient (Moreno et al., 2001). Some plants display the ability to accumulate large amounts of selenium without showing symptoms of toxicity (Terry et al., 2000). These species have been applied for the bioremediation of Se-laden soils and water involving phytoextraction and phytovolatilization processes (Banuelos et al., 2002). Se in food sources, especially in plants, is more readily accumulated in animal tissues and proteins.

Wheat is known to be a good source of bioavailable selenium and further enrichment of selenium in wheat by selenium fertilization has been attempted through several experiments (Terry et al., 2000). Furthermore, broccoli, onions and lettuce have been found to be good accumulators of selenium (Shane et al., 1988). Several reports (Ip & Lisk, 1994; Whanger et al., 2000) suggest hyperaccumulating effect of *Alliums*, though they have not been envisaged as phytoremediating crops. Although Ip and Lisk (1995) and other researchers showed the implications of using Se hyperaccumulating crops like *Allium* for prevention of cancer, the concept of using metal hyperaccumulating crops for nutraceutical applications is still a point of discussion. Different alliaceous species may respond in their unique way to selenium fertilization and the biological benefits of selenium enrichment may vary depending on the species (Ip et al., 2000). The various organosulfur compounds present in different *Allium* species like onion and garlic possess
various medicinal properties chiefly chemopreventive, cardiopreventive and other health-related benefits. Sulphur and selenium are nutrients with very similar chemical properties and their uptake and assimilation proceed through common pathways. Se is also thought to play a role in reducing the growth of cancerous tumors in animal systems (Axley et al., 1991) and lung, colorectal, and prostate cancers in humans (Clark et al., 1996). Se-enriched garlic has higher anticarcinogenic activity than the common plant (Ip et al., 1992). This increased effect of cancer prevention is achieved at least partly by S substitution with Se, since pure Se-compounds have proved to be superior anticancer agent than their corresponding S-analogues. Se-methyl selenocysteine, the major organo-Se-compound in garlic bulb has been shown to be the most effective Se-compound so far in the reduction of tumors (Whanger, 2004). By controlling the intensity and frequency of crop fertilization with water-soluble selenite salts it is possible to cultivate Se enriched garlic with 100-1355 µg g⁻¹ of Se (Ip & Lisk, 1995). This Se-enriched garlic and onion when fed to cancer-induced rats reduced total tumor yield but did not cause excessive Se accumulation in animal tissues (Ip & Lisk, 1994). This suggests that Se-enriched vegetables may be a better delivery source for organoselenium analogs than the commonly used selenite or selenomethionine. Yadav et al. (2007) envisaged that Allium species could be introduced as commercially viable crops in seleniferous region for the selenium mobilization through fortification. Since Allium cepa is an established selenium hyperaccumulator with definite anti-oxidant and other health related properties, research in selenium biofortifying potential of other Allium species that can be used as additives in selenium deficient foods could also be of tremendous benefit.

2.7.1 Biotechnological Approaches in Garlic- Past, Present and Future

The Allium crop species are probably the most economically important vegetable species consumed throughout the world. Amongst the Alliums, garlic (Allium sativum L.) is one of the most important and prominent vegetable crops grown throughout the world. The historical, medicinal and cuisinal value of garlic far exceeds that of any other domesticated vegetable crop (Lawson, 1996). India ranks second by area (0.96 lakh ha) after China and ranks third by production (4.10 lakh tonnes) in the world. Despite such importance, garlic has a low rate of multiplication and low diversity being an asexually propagated crop. This makes it susceptible to viral diseases (Nasim et al., 2010a). Leek yellow stripe virus, onion yellow dwarf virus, shallow latent virus, garlic common latent virus and certain rod-shaped virus are known to infect almost all the commercially grown
garlic cultivars. The asexual nature also makes it difficult to develop new forms of garlic by using the conventional methods of breeding. Moreover, flowering is limited and the flowers produced are sterile (Raghavan, 1983). This is a serious impediment in developing new varieties of garlic. Biotechnological approaches are thus required for the improvement of such plants where sexual mating and production of true seed is not possible. Though a lot of work has been done on tissue culture, mostly meristem culture and embryogenesis; and also on genetic transformation, isozyme studies and molecular mapping of garlic, yet more research needs to be done to understand and tap the nutritional importance of this crop to the fullest.