Chapter 2

2.0 LITERATURE REVIEW

2.1 Introduction to *Sansevieria* Plant

Sansevieria trifasciata (Figure 2.1) is a species of genus *Sansevieria*, native to tropical West Africa. It is an evergreen herbaceous perennial plant forming dense stands, spreading by its creeping rhizome, which is sometimes above ground and underground. It has stiff, fleshy and sword-shaped leaves grow vertically from a basal rosette. Mature leaves are dark green with light gray-green cross-banding and usually range between 70 – 90 cm in length and 5 – 7 cm in width. Old plants occasionally flower on three-foot long stems bearing small, tubular fragrant, greenish-white flowers (Henley, 1982). It is commonly called the snake plant, because of its leaves resembles some snake skins or mother-in-law's tongue because of their sharpness. It was classified as the following group:

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Liliopsida* – Monocotyledons
Subclass	*Liliidae*
Order	*Liliales*
Family	*Agavaceae* – Century-plant family
Genus	*Sansevieria* Thunb. – sansevieria
Species	*Sansevieria trifasciata* – bowstring hemp

(Source: http://plants.usda.gov/java/profile?symbol=SATRG)

Sansevieria plants can be propagated by leaf cuttings with leaves cut into three inch long segments and then stuck in soil, with the bottom end down. In about three months, the plants will root and send up a new shoot or also propagated by dividing the rhizome. The first method has the disadvantage that the variegation is likely to be lost. If the variegated form is propagated, it will produce a normal green shoot because the plant is a chimera which is a kind of mutation.

Report from Anis and Shahzad, 2005 shows that the regenerated shoots of certain species such *Sansevieria cylindrical* in tissue culture method displayed a unique characteristic of flattened green leaf blade resembles *Sansevieria trifasciata* in the beginning as compared to cylindrical blade in parental plants but acquired normal leaf morphology after 18 months of acclimatization.

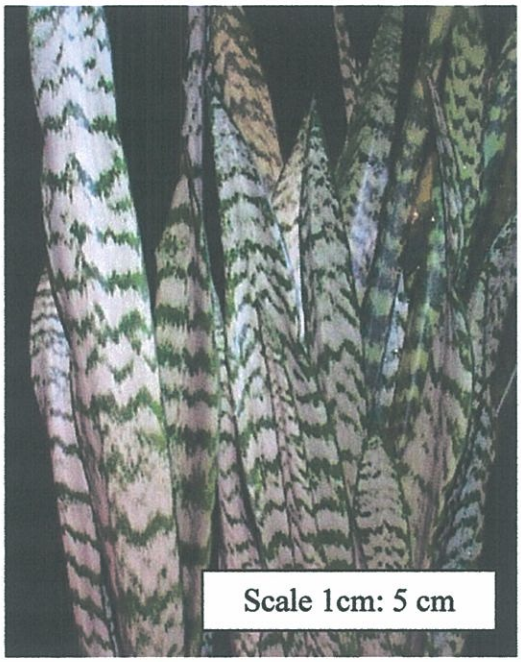

Figure 2.1: *Sansevieria trifasciata* plant.

Source: http://www.plantoftheweek.org/week210.shtml

Like some other members of its genus, *S. trifasciata* yields bowstring hemp, where Africans used the fibers from the plant to produce the strings for their bows. It is popular as house plant as it is tough, tolerant of low light levels and irregular watering as well as durable that can withstand a wide array of conditions. It is now used

predominantly as an ornamental plant, indoors in cooler climates and outdoors in warmer climates. It is also often used as an air purifier because it claims has a tendency to absorb certain poisonous substances (Wolverton *et al*, 1989).

There are two common versions of *Sansevieria* plant. Its variegated counterpart, *Sansevieria trifasciata* var. *Laurentii* (Figure 2.1.1), has a yellow band to the leaves and the dwarf form, birds-nest snakeplant; *Sansevieria trifasciata var. Hahnii* (Figure 2.1.2) grows in a rosette only six inches high (Henley, 1982).

2.1.1 Characteristics of *Sansevieria trifasciata* var. *Laurentii*

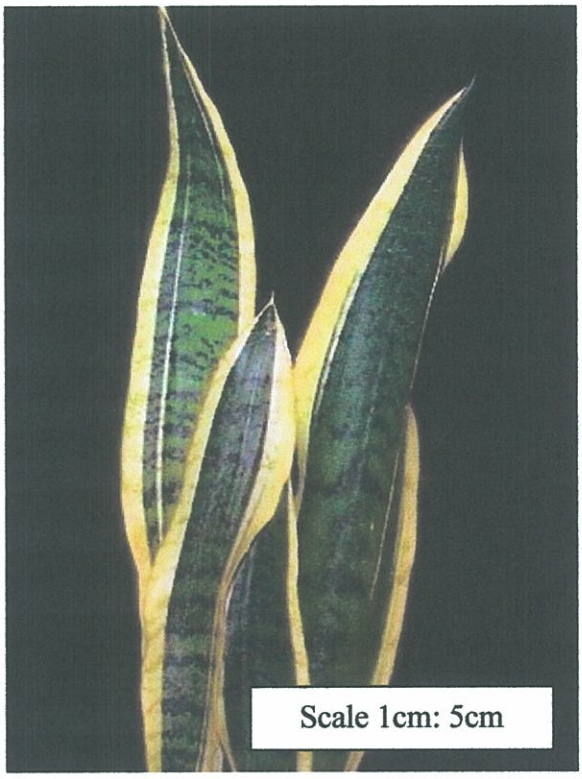

Figure 2.1.1: *Sansevieria trifasciata* var. *Laurentii* plant.
Source: http://www.plantof the week.org/week192.shtml

Sansevieria trifasciata "Laurentii", the goldband *Sansevieria* or Variegated Snake Plant is native to Africa. It has stiff sword-shaped leaves with upright growth up to 4 feet (1.3 m) long by 2.75 inches (8 cm) width. Leaves are banded yellow on either side with a deep green, lightly banded center. It is the leading commercial variety of

Sansevieria. It is grown for the hemp-like fiber in the leaves, which is called bowstring hemp. They are an attractive plant for pot culture and are very durable to a wide range of condition. They tolerate the low light conditions and are very drought tolerant. *Sansevieria trifasciata "Laurentii"* are propagated by division of rhizomes (Henley, 1982).

2.1.2 Characteristics of *Sansevieria trifasciata* var. *Hahnii*

Figure 2.1.2: *Sanseviera trifasciata* var. *Hahnii* plant.
Source: http://www.plantoftheweek.org/week159.shtml

Sanseviera trifasciata 'Hahnii' or Birds-nest *Sanseviera* is a sport of *Sanseviera trifasciata 'Laurentii'*. It was discovered by William W. Smith, Jr. in the Crescent Nursery Company, New Orleans, Louisiana in 1939 and was patented in 1941 (Henley,1982). It has attractive short, dark green, reflexed leaves which form a vase-shaped rosette. They form a low growing, vase-like rosette of broad elliptic spirally-arranged leaves. Plants form clumps growing to 15cm tall with an equal spread, are very robust and sucker freely. Leaves have similar banding patterns as the species, but 'Hahnii' and the other birdnest cultivars of *Sansevieria trifasciata* are not known to flower. They also will survive in a wide range of conditions. They tolerate low light

conditions and are very drought tolerant. *Sanseviera trifasciata 'Hahnii'* are propagated by the removal of suckers (Gilman, 1999).

2.2 Introduction of Plant Tissue Culture

Plant tissue culture is a fascinating and useful technique, which allows the rapid production of plants using relatively small amounts of space, supplies and time. It also has a number of commercial applications include producing huge numbers of identical individual plant and for production of variants. This micropropagation is more rapidly and known as the better way in commercial forestry and in floriculture. It has the advantages of preserving healthy plant material for internal exchange as well as cost reduction by tissue culture practices.

For example, the genus *Sansevieria* plant normally is propagated by division of rhizome or leaf cuttings, but it is slow in terms of the time needed to regenerate new plants. The cutting technique also normally will give rise to only one plant. Thus, improvements by tissue culture procedures are useful for in vitro propagation of this plant (Blazich and Novitzky, 1984). The entire tissue culture process for *Sansevieria* plant requires about 4 - 6 months obtaining plants growing on their own roots. A distinct advantage of this procedure as opposed to using leaf cuttings is that tissue culture procedures utilize much smaller pieces of leaf tissue, which individually can give rise to more plants. Besides that, plants that have been propagated by the tissue culture methods also appear to be similar in vegetative characteristics to the parents and are making normal growth.

There are two concepts considered in plant tissue culture and regeneration, which are plasticity and totipotency. When culturing in vitro, all the needs of the plant cells have to provide by the media in culture vessel. Generally, culture media used for the in vitro cultivation of plant cells is composed of three basic components:

i. Essential elements, or mineral ions, supplied as a complex mixture of salts

ii. An organic supplement supplying vitamins and/or amino acids; and

iii. Source of fixed carbon; usually supplied as the sugar sucrose.

Specific media manipulations are used to direct the development of plant cells in culture (Slater *et al*, 2003).

Basically, this technique consists of taking a piece of a plant (such as a stem tip, node, leaf, meristem, embryo, or even a seed) which is called "explants" and placing it in a sterile (usually gel-based) nutrient media where it multiplies. The formulation of the growth media is changed and depending upon the trying to get the plant to produce whether undifferentiated callus tissue, multiply the number of plantlets, grow roots, or multiply embryos for "artificial seed".

2.2.1 Tissue Culture Media

Murashige and Skoog (MS medium) is the most widely used tissue culture media and many variations have been developed. The medium is derived from White's medium and originally developed for the cultivation of *Nicotiana tabacum* calli. Media used in plant tissue culture are composed of several components: salts, vitamins, amino acids, growth regulators, sugars, agar or gelrite and water. All these compounds fulfill one or more functions in the in vitro growth of plants (Murashige and Skoog, 1962).

All minerals present in plant tissue culture media can be used by the plant cell as building blocks for the synthesis of organic molecules or as catalysators in enzymatic reactions. Nitrogen, sulfur and phosphorus are components of proteins and nucleic acids. Magnesium and many microelements form essential parts of enzymes as well as cell organelles and therefore important in the catalyzation of various reactions. Calcium and boric acid mainly found in the cell wall where the calcium has an important task in the stabilization of biomembranes. Potassium and chloride on the contrary, are

important in the osmotic regulation, for maintenance of the electrochemical potential and for the activation of a large number of enzymes (Dirr and Heuser Jr., 1987).

The salts in media can be divided into micro and macro elements. Fe, Cu, Mn, Mo, B, I, Ni, Cl and Al are considered as microelements and Mg, Ca, P, S, N and K as macroelements. Mainly the subdivision in microelements and macroelements are based on plant needs for these elements. The need for microelements is small, reflected by the low concentrations of these elements in the medium. Most microelements are present in micromolar quantities. The need for macroelements is much larger and therefore it present in millimolar concentrations in media (Dirr and Heuser Jr., 1987).

Addition of vitamin to the plants in several forms and concentrations are essential for many biochemical reactions. In almost all media for plant cell and tissue culture, Thiamine (vitamin B1) is included. Inositol is often mentioned as a vitamin which significantly stimulates the growth and development of plants. However, the vitamin is not essential for growth. Concerning other vitamins, it is hard to judge their virtual importance. The effect of vitamins on the development of the cell in vitro differs from species to species or might even be harmful.

2.2.2 Plant Growth Regulators

Plant growth regulators are the critical media components in determining the developmental pathway of the plant cells. The plant growth regulators that used most commonly are plant hormones or their synthetic analogues. Auxins and cytokinins are the most widely used plant growth regulators in plant tissue culture and are usually used together where the ratio of the auxin to the cytokinin determining the type of culture established or regenerated. Other hormones, in particular gibberellins, ethylene, abscisic acid or jasmonates are used occasionally (Smith, 2008).

The uses of plant growth regulators are to modify the factors that govern all the stages of crop development from seed germination through vegetative growth, maturity, senescence, aging and postharvest preservation has become more frequent in recent years studies (Hudson, 1976). Among the plant hormones and synthetic growth regulators uses are in plant rooting, flowering, fruit set and development, plant size, axillary bud formation, abscission, plant shape and cell metabolism (Wareing, 1976; Pharis, Ross, 1976; Sachs, Hackett, 1972). Some of these possible modes of action were used in practice for improving the yield or control the growth of horticultural crops (Cathey, Meredith, 1983; Soczek, 1979; Jourdan, Oplinger, 1983).

A high auxin to cytokinin ratio generally favour root formation, whereas a high cytokinin to auxin ratio favours shoot formation. An intermediate ratio favours callus production. Auxins promote both cell division and cell growth. The most important naturally occurring auxin is IAA (indole-3-acetic acid), but it is used is limited because it is unstable to both heat and light. Cytokinins promote cell division and naturally occurring cytokinins are a large group of structurally related (purine derivatives) compounds. The synthetic analogues, kinetin and BAP (benzylaminopurine) are also used more frequently.

Plant growth regulator and hormone also have roles in phenomenon known as somaclonal variation. Somaclonal variation is the term used to describe the variation seen in plants that have been produced by plant tissue culture (Larkin and Scowcroft, 1981). Chromosomal rearrangement is an important source of this variation. It is particularly common in plants regenerated from callus. The variations can be genotypic or phenotypic, which in the later case can be either genetic or epigenetic in origin. Typical genetic alterations are changes in chromosome numbers (polyploidy and aneuploidy), chromosome structure (translocations, deletions and duplications) and DNA sequence (base mutations).

The major likely benefit of somaclonal variation is in plant improvement where leads to the creation of additional genetic variability. Characteristics for which somaclonal mutants can be enriched during in vitro culture include tolerance to environmental changes or variation of colour and shape in horticulture. Different steps can be taken to induce spontaneous somaclonal variation, such as increasing numbers of subculture. Another way is to introduce 2,4-D (2-4-Dichlorophenoxyacetic acid) in the culture medium. It is the most commonly used auxin and is extremely effective in most circumstances to introduce variation.

The main difficulty to conduct the experiment using plant growth regulator where there is a great number of species and cultivars of ornamental plants which differ in their reaction to the plant growth regulators. The synthesis of a plant hormone is often not localized in a specific tissue, but may occur in many different tissues. Furthermore, plant hormones may be transported and act in distant tissues, but often they have their action at the site of synthesis. Another property of plant hormones is their lack of specificity where each of them influences a wide range of processes. Auxin, for example, has been found to influence cell elongation, cell division, induction of primary vascular tissue, adventitious root formation, senescence, fruit growth, outgrowth of axillary buds and sex expression (Giafagna, 1987).

Normally, plant hormones add to plant tissue culture media are taken up and increase the level within the tissue. Most of the increase is, however it transient because plant hormones are rapidly inactivated after uptake. Usually only very small amounts of the applied hormones remain in the free form. The effect of hormones not only depends on the rate of uptake from the medium or on the stability in the medium and in the tissue, but also on the sensitivity of the target tissue. Cells in a certain tissue may not recognize the hormonal signal, or may be incapable of carrying out the desirable response.

2.2.3 Tissue Culture Application

The starting point for all tissue cultures is plant tissue; called an explant. It can be initiated from any part of a plant; root, stem, petiole, leaf or flower. Although, the success of any one of these part are varies between species. Moreover, the physiological state of the plant has an influence on its response to attempts to initiate tissue culture. The parent plant or source must be healthy and free from obvious signs of disease or decay. Younger tissue contains a higher proportion of actively dividing cells is more responsive to a callus initiation.

It is essential that the surface of the explant is sterilized to remove all microbial contamination. Plant cell division is slows compared to the growth of bacteria and fungi, and even minor contaminants will easily overgrow the plant tissue culture. The explant is then incubated on a sterile nutrient media to initiate the tissue culture. The composition of the growth medium is designed to sustain the plant cells, encourage cell division, and control development of either an undifferentiated cell mass, or particular plant organs.

The concentration of the growth regulators in the medium, namely auxin and cytokinin would be the critical factor for determining whether a tissue culture is initiated and how it subsequently develops. The first stage of tissue culture initiation is vital for information on what combination of media components will give a friable, fast-growing callus, root or shoot formation (Vinterhalter *et al*, 1989).

There is not much to predict the exact growth medium and growth protocol to generate a particular type of callus. These characteristics have to be determined through a carefully designed and observed experiment for each new plant species and frequently also for each new variety of the species which is taken into tissue culture. The basis of the experiment will be media and protocols that give the desired effect in other plant species, and experience.

Some report showed that the strategy for designing a medium to initiate tissue culture on how growth regulators and other factors modulate development can be demonstrated using the African Violet, a popular house plant. The source of explants obtained from leaf sections. Shoot regenerations in African Violet was affected by factors including leaf age, wounding and leaf disc orientation. Although wound treatment increased shoot organogenesis only when the wound was in direct contact with the medium, possibly by increasing nutrient and hormone uptake (Lo, 1997).

The overall process in tissue culture can be described as following four steps (Dirr and Heuser, 1987):

Step 1: Selection of the leaves

Leaves are cut from healthy plants. They should be selected to each yield several explants of leaf squares with approximately 1 cm sides. The youngest and oldest leaves should be avoided.

Step 2: Surface sterilization and preparation of the explants

This part of the procedure should be carried out in a sterile working area, or with meticulous aseptic technique. The transfer chamber should be ready with the walls and workspace wiped or sprayed with 70% ethanol. There should be a container of 90% ethanol to sterilize and rinse the instruments and gloved hands of the operator. The forceps and scalpel is immersed for 30 seconds or more in the 90% ethanol then rest them on a sterile holder or paper towel to dry for a few seconds. The leaf should be immersed in warm and soapy water and then transferred into 70% ethanol for 15 seconds. Then rinsed into distilled water and immersed into 30% bleach (Sodium hypochlorite) solution for 15 minutes and gently agitating once or twice during this time. They are then washed for free of bleach by immersing in three successive beakers of sterile distilled water for 3 times, leaving them for 2 - 3 minutes in each. Sterile scalpel and forceps are then used to cut squares from the leaf as explants, each with

approximately 1.5 cm sides. Two explants are placed on each universal container or jar of growth medium with the upper epidermis pressed gently against the surface of the agar to make good contact. The jars are sealed with plastic film to prevent moisture loss.

Step 3: Assessment of tissue culture development

The explants are incubated for 4 - 6 weeks, and inspected at weekly or fortnightly intervals. The growth of obvious bacterial or fungal colonies indicates contamination and data from such cultures is obviously suspect. The development of dark brown tissue cultures can also be a consequence of contamination. The media were based on the well-known Murashige and Skoog inorganic medium with additions of hormone combination.

Step 4: Acclimatization

When the plantlets begin to root, it was transplanted to a light artificial soil mix, such as peat, in a pot. The pots were covered with clear plastic and were placed on a lighted shelf or in a shaded greenhouse. After two or three weeks, it begins to leaving the plastic off for a period of time each day. The time the plantlets are left uncovered should get longer each day, until after about a week, then cover can be left off completely.

2.3 Mutagenesis in Plants

Mutation breeding has been introduced into modern plant breeding in the early 1940's (Gottschalk, 1983). It has remained popular over the past 70 years because it is simple, cheap to perform, applicable to all plant species and usable at a small or large scale (Waugh, 2006). Induced mutation either by physical or chemical mutagens is a powerful tool for varietal development of vegetatively propagated plants. Several mutants with useful characteristics have been successfully developed in ornamental plants (Basiran and Arifin, 2003). For various reasons mutation breeding has been especially successful in ornamentals. One of these is that the selection of directly

perceptible characteristics, like colour, form of size, which is generally easy. Another reason is that many cultivars are heterozygous and so have a comparatively high mutation frequency (Broertjes and Van Harten, 1978).

The wide range of variation in colour, size and shape and also the possibilities of inducing change in only one character make irradiation-induced mutation a potential tool for further improvement of ornamental varieties (Gottschalk and Wolff, 1983). The normal procedure is to determine optimal doses in each plant genotype before conducting actual experiments, either in the laboratory or in the field. Difference species have different sensitivity to mutagenic agents. Therefore radio-sensitivity study is a prerequisite in every mutation-breeding of crop plants. Plant parts such as seeds, scions, cuttings and callus can be used as starting material for inducing mutation. They can be exposed to chemical or physical mutagens with a range of concentration or intensity of doses. The optimal doses shall be obtained by evaluating the reduction of growth due to mutagenic effects.

Radiosensitivity varies between plant species and depends mainly on the nuclear volume which is the greater of the DNA content the more sensitive, the number of chromosomes where plants with fewer chromosomes given a certain nuclear volume are more sensitive than plants with more but smaller chromosomes and the ploidy level which the higher it is, the less radiosensitive (Broertjes and Van Harten, 1978). At the same time, genetic factors, climatic and other environmental conditions before and after treatment of the plant part as well as the stage of development of root or shoot seem to be importance (Jones and Wilkins, 1971).

2.3.1 Mutagens

Two classes of mutations are spontaneous mutations (molecular decay) and induced mutations caused by mutagens. Induced mutations can be done by both

physical and chemical means. The physical means is through the exposure of the plant to irradiation and the chemical means is treatment with certain chemical agent. Mutagens can be classified as forms of energy or chemical substances that significantly increase the frequency of mutations in the genomes of exposed organisms. Two general categories of biological effects result from exposure to mutagens are somatic and genetic. Genetic effects are heritable because they are present in the germ line of the affected individual. For including the mutations, the mutagenic agents can affect different plant organs such as freshly cut leaves, leaf stalks, tubers, young rhizomes, bulbs, dormant buds, cuttings and dormant stolons (Skirvin, 1978).

The physical mutagen is through the exposure of the whole plant to ionizing radiation of one of the three classes which are χ-rays, Gamma rays, or neutrons (Green and Roderick, 1966). The chemical mutagen is to inject a mutagenic reagent into the plant such that it passes directly into differentiating germ cells. Under different protocols of exposure, χ-ray irradiation was found to induce mutations at a rate of 13 - 50 X 10^{-5} per locus, which is a 20 to 100-fold increase over the spontaneous frequency, but still not high enough to be used by any but the largest facilities as a routine means for creating mutations (Rinchik, 1991). The frequency with which mutations are detected is directly proportional to the radiation dose but above certain levels that the plants are killed. The optimum dose, for Gamma rays is about 5,000 rad (50 Gy), but cultivars differ in the members of visible sports produced at this level.

Physical mutagens are widely used to induce mutations in all kinds of plant parts. In theory, one can choose between varieties of ionizing radiation types. In practice, only an χ-ray machine and a γ-source are generally available. For practical mutation breeding, acute irradiation with fairly hard χ-rays or γ-rays is recommended using a dose rate of 100-1000 rad/min and short irradiation times. The dose to apply depends on the radiosensitivity of the species in general and that of the plant part as

well as the stage of development in particular. Plant parts which still have to produce new (adventitious) roots and shoots (unrooted cuttings, freshly detached leaves etc) are more sensitive than plant parts with existing root and shoot meristems. By shielding the basal part of a non-rooted bud-stick or cutting, better results are obtained and sometimes a much higher dose can be applied. For example, to apple (Lacey, 1977), black currant (Bauer, 1957), carnation (Sparnaay and Broertjes,1977) or potato (Van Harten,1978).

By varying mutagen dose, the frequency of induced mutations can be regulated and saturation can be readily achieved (Waugh, 2006). Generally, mutations can be readily obtained that affect height, maturity and fertility. They have also been used to develop improved nutritional quality (particularly soybeans) and disease resistance. It should be recognized that many of the early experiments to understand biochemical pathways used mutant's lines that blocked the pathway at different enzymatic activity points.

2.3.2 Mutagen-induced Plants

Ornamental foliage is one group of plants that benefit tremendously with the advancement of vegetative propagation technology. The technology helps to expedite development of new plants when combined with the induced mutation technology. This method accelerates the chance of natural mutation to occur by producing leaf chimeras such as different colour and shape, 'sports' and other mutations. Although many of the mutants would be similar to variants already in existence the possibilities exist for the development of a new variant. The same technology is also applicable to flowering ornamental plants to change flower colour and shapes. The objectives for the application of mutagen in tissue culture of plants is to develop varieties that have better agronomic characteristic such as high yield, rapid growth, better and attractive colour and shape as well as good plant architecture.

Chimeras may be horticultural curiosities or economically important plants. Plants with variegated foliage, as found in *Citrus, Vitis, Pelargonium, Chrysanthemum, Hydrangea, Dahlia, Coleus, Euonymus, Bouvardia* as well as *Sansevieria* and others are examples of chimeras. In these plants the plastids in part of the leaf tissue lack the capacity to produce chlorophyll, whereas other leaf cells are normal. The resulting pattern shows distinct green and white (or yellow) areas in the leaf (Hartmann *et al,* 1990).

There is a large variety of vegetatively propagated pot plants that are ornamentally valuable because of the shape or colour of the plant and foliage. Examples are *Ampelopsis*, *Asparagus*, *Begonia rex*, *Popyrus, Pilea*, *Sansevieria*, palm tree varieties and ferns. Although spontaneous mutations must have played an important role in the development of the cultivars of many of them such as *Begonia rex* and *Hedera*; very little effort has been spent on artificially inducing mutations in such plants (Boertjes *et al*, 1978).

Besides the induced mutation activities, new variants were also obtained through somaclonal variations but the occurrence can be as high as 60% depending on the cultivars. Although most of the variations are undesirable agronomically, the phenomenon can be a source of genetic variation (Boertjes *et al*, 1978).

2.3.3 Mutagenesis Application

A result of induced mutation in plant breeding shows that the small numbers have been produced in this way. One of the special interest to horticulturists are induced fruit colour changes in apples (Bishop, 1959), compact mutants in apple and pear (Visser *et al,* 1971), upright habit in black currant (Bauer, 1974) and flower colour changes in several ornamentals (Broertjes *et al*, 1978).

In some species, which can be propagated sexually as well as vegetatively, the methods of mutation breeding seem to have better chances than the conventional methods. Moreover, many findings exist demonstrating that the mutation frequency obtained by the various rays can be positively influenced by specific kinds of pre-treatment or post-treatment. Recurrent irradiation has been found to be considerably more effective than a single treatment (Loose, 1979). The utilization of radiation-induced mutants for successive use of further irradiations proved to be an especially successful method for obtaining a large number of new mutants. In this way, hundreds of Chrysanthemum mutants were produced in the Netherlands, some of them being of direct floricultural interest (Broetjes, 1978).

Species such as *Duranta, Cordylines, Bougainvillea* and *Dracaena marginata* were found to be able to withstand slightly higher irradiation doses than species with softer stem such as *Dracaena sanderiana* and *Rheodiscolor*. Radiosensitivity test on *Cordylines* cuttings has shown that the LD_{50} is 30 Gy while for *Dracaena* and *Rheodiscolor* it is approximately 15 Gy. However, lower doses are often preferred if 'green' or shoot cuttings of the woody species are used. Following the irradiation treatment, mutations can be observed as early as the first vegetative generation (the regenerated shoots from the irradiated axillary buds). However, these are often chimeric and cutting back is necessary to isolate the mutated sector. Mutants with leave variegations and stunted growth (dwarfs) were successfully selected from mutagenesis of *Cordylines*, *Duranta* species and *Rheodiscolor*. Two mutants with flower color change were obtained in Hibiscus rosa-sinensis after three vegetative generations and the mutant remained stable throughout successive generations (Broetjes, 1978).

χ-ray induction technique is well proven and effective for several vegetatively propagated florists crops for flower colour mutants. It has been used successfully with *Chrysanthemum, Saintpaulia, Begonia* etc. The effect of irradiation is generally to cause

a mutation of the gene or genes controlling flower colour to more recessive alleles. It has therefore been suggested that should concentrate attempt to obtain the desired flower form and plant habit with flowers of the most dominant colour type. When this has been achieved, a series of cultivars of different flower colours can be produced by radiation and all with the other desirable characters (Broetjes, 1978).

The optimal dose of χ-rays depends on the species and the type of material being treated, but 10 Gy – 15 Gy is most likely to be effective with pieces of stem, root and tuber. Higher doses may be optional with seeds. Rate of application is generally thought to be less important than total Gray (Gy) treatment and about 3 Gy per min is usual. However, some report suggests that lower application rates may reduce the number of lethal mutations most induced mutations are recessive and since they often involve a change to one chromosome only of a pair they may not be observed directly.

Radiation treatment does not affect every cell of the material in the same way, so that only parts of the plant may be mutated and a sectorial chimaera is then produced. Tissues which form adventitious shoots arising from single cells are especially suitable for radiation treatment as entire shoots may then grow from one mutated cell (Broertjes *et al*, 1978).

Chemical mutagens are much less effective than radiation treatments though they are cheap and simple to apply. The most commonly tried chemical is Ethyl methane sulphonate (EMS). Although the mutagenic chemicals are easily available, rays are preferred by many geneticists. Their main advantages lies in the fact that the seeds are irradiated in a dry condition and that they can be handled for sowing like untreated material. If they have been treated with soluble solutions of chemical compound, however, they are very susceptible and need much more care than dry seeds. Another disadvantage following application of some of the most potent chemical mutagens is that their negative effects on the vitality and fertility of the irradiated plants are

considerably stronger than the corresponding effects of mutagenic rays. This is a very important problem in the practical performance of applied mutagenesis, which has not yet been solved (Gottschalk and Wolff, 1983).

2.3.4 In vitro Mutagenesis

There are a number of reasons why the combination of mutagenesis with tissue culture can be advantageous:

i. Tissue culture plants are generally very small making it easier to treat a lot of material.

ii. Propagation via tissue culture is fast and not dependent on seasons. Therefore, the preparation of plant material for mutagenesis treatment, propagation or regeneration of plants can take place quickly and all year round.

iii. Tissue culture takes place in special conditioned areas making the whole process easier to control and measure.

iv. Tissue culture takes place in well equipped laboratories, ensuring the safe use of chemicals required for mutagenesis.

In general, well-established parts of the plants will be treated with mutagenic irradiation or chemicals. This treatment will cause damage to the cell's DNA. Because this occurs entirely at random, the scale of damage will differ in each cell. Further, the DNA repair mechanism within the cell will result in a real change in the DNA sequence. In the treated part of the plants each cell might have a different DNA sequence due to the damage and repair process of the DNA.

The improved characters of the vegetatively propagated plants controlled by the mutant genes were cover a range of traits. An increase of the genetic variability was obtained with regard to:

i. Flower colour and flower shape in many ornamentals

ii. Earliness, sometimes also lateness in almost all the crops treated

iii. Shortening of internodes in fruit trees and ornamentals

iv. Alterations of the plant type in ornamentals

v. Improvement of the resistance behavior

vi. Desirable biochemical alterations in some fruits trees, in the plants and in other crops (Gottschalk and Wolff, 1983).