INTRODUCTION
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Biotechnological tools have revolutionized the entire crop improvement programmes. Many genetically modified fruits and vegetables are already in the market in developed countries. The major areas of biotechnology which can be adopted for improvement are Tissue culture, Genetic Engineering, Molecular markers and development of beneficial microbes.

During the last 20 years there has been rapid growth in the relatively new field of plant biotechnology and its associated techniques. These have applications to a wide range of plant species. Biotechnology is a subject, which was pioneered by US scientist Oswald Theodore Avery in 1943, has finally turned the global spotlight on itself. It is today one of the contenders for the new economic crown with a vast untapped potential. Biotechnology has gradually caught the fancy of even developing countries like India.

Modern Biotechnology now allow researchers to select the gene(s) of choice that encode for a specific trait from unrelated plants, microbes and animals and transfer that gene to a plant of the same species or another species. Using the tools of genetic engineering, a gene for a trait of interest can be removed from DNA of one organism and introduced into the DNA of another. This facilitates introduction of the desired trait without transferring large number of extraneous and at times, confounding genes that can only be eliminated by backcrossing. This capability was first demonstrated in 1984 when recombinant DNA methods were used to introduce an antibiotic resistance gene found in the bacterium *Streptococcus faecalis* into the genome of *Nicotiana plumbaginifolia* (Bevan, 1984). 

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The USA approved the first sale of a food produced from genetically transformed plants in 1994 (Maryanski, 1995; Krimsky and Wrubel, 1996). The underlying technology has been rapidly and widely adapted by the agricultural research and development community and their products have rapidly gained acceptance among farmers. By the end of the 1998 growing season, 22% of the maize and 36% of the soybean grown in USA contained one or more transgenes. Similarly, 50% of the canola produced in Canada and 60% of soybean produced in Argentina was of transgenic origin (James, 1998). Of the transgenic crops in current commercial production, maize, soybean, canola and potato have been transformed with genes conferring herbicide tolerance, insect resistance or both (James, 1997 and 1998).

Crop biotechnology, which broadly includes areas of development of transgenic crops, structural and functional genomics and marker assisted breeding, could provide us with the vital break through to achieve improvements in both quality and quantity in a sustainable manner.

A major achievement of the 20th century has been an increase in crop productivity through conventional breeding methodologies. Revolutionary discoveries in biology in the 1970’s and 1980’s fuelled predictions of dramatic changes in the agriculture and stimulated entrepreneurial excitement and investments. Though conventional plant breeding methods have results in a spectacular improvement in crop production, there are strong pressures for further improvement in crop quality and quantity due to explosion in population, social demands, health requirements, environmental stresses and ecological considerations (Kung, 1993). Conventional plant breeding techniques have limitations as
these depend on sexual compatibility and often take 10-15 years to release a new variety due to extensive backcrossing (Pauls, 1995). These limitations have stimulated the development of more advanced technologies like genetic transformation of plants.

The number of countries in which transgenic crops are being grown has increased markedly over the past several years. During 1996 and 1997, transgenic crops were grown in USA, China, Argentina and Canada, with lesser amounts in Australia and Mexico. By 1998, Spain, France and South Africa were added to the list of countries growing transgenic food plants on a commercial scale (James, 1997 and 1998).

**Plant tissue culture**

Man’s dependence on plant is indispensable. In his dependence for food, shelter and clothing that has led him to explore all possible ways to preserve plants from being lost to the savage of natural or manmade calamites. Accordingly, man has used different methods to overcome their calamite, while doing so, scientists hit upon a technique whereby, plants can not only be stored from being lost, but are able to develop a complete plant from a small plants part. This technique is called “tissue culture”; subsequently proved to be a boon for mankind.

Tissue culture is an important area of biotechnology. The technique can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired trait. The concept of tissue culture dates back to 1878. When a German Botanist Vochting in 1878 has shown that from small plant piece, a whole plant could be regenerated.
This was followed by an important finding by another scientist named White, in 1943, who observed an indefinite growth of tomato roots on a specific nutrient medium (which was later called White’s medium) containing vitamins like pyridoxine, thiamine and nicotinic acid. All attempts to culture plant cells and tissues were unsuccessful till 1930. Around 1939 White, Nobecourt and Gautheret independently reported the possibility of culturing plant tissue for definite period.

Plant tissue culture technique involves maintaining of plant cells in aseptic condition on suitable nutrient medium. The culture can be sustained as a mass of undifferentiated cells for an extended period of time, these cells divide and gradually develop either into unorganized mass of cells called callus or after a few cell division differentiate to form full fledged plants.

The primary goal of plant tissue culture research is crop improvement. Tissue culture technique offer advantages over the conventional methods of plant breeding principally because new plant traits can be selected at the culture level in the laboratory instead of the whole plant level in the field. This affords advantages of time in that cellular screening for traits requires weeks or months where as screening of whole plants typically requires entire growing seasons.

Designing a strategy to culture cells from a plant for the first time can still seems like a matter of trial and error and luck; in fact success of many in vitro techniques in higher plants depends on developing protocols for whole plant regeneration. Plants can
be propagated from numerous explants like meristem, cotyledon, stem, leaf, hypocotyl, flowering organs viz., anther, ovule, immature embryo and inflorescence and from isolated single cell and protoplasts. Techniques comprising this area of development can be grouped into micro-propagation, haploids, protoplast cultures and somaclonal variations and mutant selection. The plants derived from these tissue culture techniques are said to be capable of greater uniformity, because they are derived from single cells in laboratory cultures rather than through sexual reproduction.

The *in vitro* techniques were developed initially to demonstrate the totipotency of plant cells predicated by Haberlandt (1902). Totipotency is the ability of a plant cell to develop into a complete plant. In 1902, Haberlandt reported culture of isolated palisade cells from leaves in Knop’s salt solution enriched with sucrose. Cells were able to synthesis starch as well as increase in size and survived for several weeks, but failed to divide. He realized that ‘asepsis’ was necessary to make the culture free from micro contamination. Efforts continued to develop techniques for cultivation of plant cells under defined conditions. Brilliant contributions came from Gautheret in France and White in USA in 1985. Most of the modern tissue culture media have been derived from the work of Skoog and co-workers during 1950-60.

Skoog and Miller (1957) have proposed the concept of hormonal control of organ formation. Their classic experiments on tobacco pith cultures showed that the root and bud initiation were conditioned by a balance between auxins and kinetin. High concentration of auxins promoted rooting whereas proportionately more kinetin initiated bud or shoot formation. Auxins are proved to be essential for establishing successful
culture of plant tissues due to their effects on nucleic acid and protein metabolism (Rao and Swamy, 1972). Cytokinins influence cell growth by promoting nucleic acid metabolism and the synthesis of specific proteins required for cell division (Letham, 1968). Physical environment viz., state of medium, light, temperature, humidity besides source and size of explants are also known to play an important role in vitro organogenesis (Murashige, 1974).

In recent times plant tissue culture in conjunction with genetic engineering and related techniques is a promising and potentially emerging area of plant biotechnology and has generated great interest and speculation for genetic manipulation of crop plants with desirable results.

Those problems, which are not possible through conventional techniques, are now being solved through tissue culture techniques. Such as inter and intra specific crosses by somatic hybridization, micropropagation, somaclonal variation, encapsulated seeds etc. The emphasis was also leads to transgenic plants, improved nutritional quality, nitrogen fixation etc. Thus, the plant tissue culture has become capable tool for biotechnological research in agricultural and horticulture.

The plant tissue culture was exploited both for basic and applied aspects of plant research encompassing haploidy, mutagenesis, somatic embryogenesis, somaclonal variations, protoplast fusion and genetic manipulation and even commercial exploitation. Tissue culture combined with genetic engineering has helped to developed new variety of food crops, cereals, vegetables, oilseeds and plantation crops such as spices, coffee, tea and rubber.
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Later studies stressed the role of various macro and micro nutrients and their importance in the culture media. Subsequent research findings showed that many more important compounds are necessary for the complete regeneration of a plant body. These were identified as growth promoting substances and include compounds like auxins, cytokinins, gibberellins etc, when supplied to the culture medium at a particular time point; these compounds would allow the growing cells to differentiate into root, shoot or embryo.

The degree of success in any technology employing plant cell, tissue or organs culture is related to relatively few factors. A significant factor is the choice of nutritional components and growth regulators. The nutrient media used for most of the cultures were the formulations developed in the early 1930’s by White. A defined nutrient medium consists of inorganic salts, a carbon source, vitamins and growth regulators. Other components such as organic nitrogen compounds, organic acids and plant extracts may be added for specific purposes. The most widely used media for plant tissue culture studies are those of Murashige and Skoog’s (MS) (1962), Linsmaier and Skoog’s (LS) (1965) and Gamborg et al.,(B5) (1968).
Micropropagation

Micropropagation has been defined as *in vitro* regeneration of plants from shoot tips, nodal and internodal region and cotyledonary nodal region (Beversdorf, 1990) and the true to type re-propagation of a selected genotype using *in vitro* culture techniques (Debergh and Read, 1991). True to type propagation has important benefits for highly homozygous plants, such as Australian diocious papaw genotype (Drew, 1988 and 1992), where traditional plant breeding has failed to produce stable lines. It also provides a means of germplasm storage for maintenance of disease free stock, both in controlled environment conditions (Wilkins and Dodds, 1983; Withers, 1989) and long term conservation via cryo-preservation (Kartha, 1985) at very low temperature (-70 °C). However, the ability to propagate plants *in vitro* free of genetic off types is often dependent on the techniques used for micropropagation.

Micropropagation is an inevitable aid in rapid clonal multiplication of superior genotypes having desired traits using tissue culture technology. It can be considered as an extension of the more traditional methods of plant propagation, where in shoot tips and nodal segments of desired elite plant are cultured on appropriate medium to get multiple shoots of true-to-type. These shoots are sub-cultured repeatedly until many plants are produced.

Micropropagation could be used both to rapidly increase new selections and to produce ultimate product. For hybrid seed production rapid increase parental line is required which could be done by micropropagation (Beversdorf, 1987). Commercial groups have recognized applied value of micropropagation and several companies are
involved in micropropagation business around the world (Chu and Kurtz, 1995). Micropropagated plants are genetically uniform, because entire production cycle is maintained under artificial conditions and trees can be produced year round.

**Somatic Embryogenesis**

The initiation and development of embryo from somatic cell in plant culture has been termed as somatic embryogenesis. Embryos can be bipolar in organization with an integrated root, short axis arising in the culture.

Somatic embryogenesis is an *in vitro* tissue culture technique used to clone plants. It is a technique that uses young tree tissue to generate small masses of cells from which many new genetically identical plants and trees may grow. These small masses of cells constitute one of the early developmental stages in plant growth and are called somatic embryos.

The first observation of *in vitro* somatic embryogenesis was made in *Daucus carota* (Reinert, 1959; Steward *et al.*, 1958). Subsequently, somatic embryogenesis has been reported in more than 80 species belonging to 33 different families.
One of the most important achievements in the field of plant tissue culture has been the discovery of the induction of somatic embryogenesis in cell cultures (Steward, 1958 and Reinert, 1959). It demonstrated the persistence of totipotency in cells of higher plants i.e., the regeneration of whole plant. In somatic (asexual) embryogenesis embryo like structures, which can develop into whole plants in way analogous to zygotic embryo are formed from somatic tissues. These somatic embryos can be produced either directly or indirectly (Sondahl and Sharp, 1977). In direct somatic embryogenesis, the embryo is formed directly from a cell or small group of cells without the production of an intervening callus. Though common from some tissues (usually reproductive tissue such as the nucellus, styles or pollen), direct somatic embryogenesis in generally is of rare occurrence in comparison with indirect somatic embryogenesis. In indirect somatic embryogenesis, callus is first produced from the explant. Embryo can be produced from callus tissue; direct as well as indirect somatic embryogenesis has been reported in several species (Mary and Jayabalan, 1997).

The morphogenetic events of somatic cells show a striking resemblance with the development sequences, occurring in the fertilized egg cells and acquiring early bipolarity passing through pro-embryonic, globular, heart shaped and torpedo stages (Rienert, 1959). *In vitro* regeneration of plantlets via somatic embryogenesis has much potential for its use in plant regeneration (Ammirato, 1987; Rao and Chopra, 1989; Sagare *et al.*, 1993 and Venkatachalam, 1997).
Somatic embryogenesis is ideal systems for investigate the entire process of differentiation of plants as well as of the mechanism of expression of totipotency in plant cells, having major advantages as compared to zygotic embryogenesis for example,

- The process of embryogenesis is easily monitored
- The environment of the embryo can be controlled and
- Large number of embryo can be easily obtained (Komamine, 2001)

Somatic embryos are used as a model system in embryological studies. However, the greatest importance of somatic embryos is its practical application in large scale vegetative propagation. In some cases, somatic embryogenesis is favoured over other methods of vegetative propagation because of the possibility to scale up the propagation by using bioreactors. In addition, in most cases the somatic embryos or the embryogenesis cultures can be cryo-preserved, which makes it possible to establish gene banks. Embryogenic cultures are also an attractive target for genetic modification.

An auxin compound has usually been found to be critical for somatic embryo induction (Ranchi, *et al.*, 1985 and Rani and Padmaja, 1997). Somatic embryos are believed to originate from a single cell (Haccius, 1978; Tomar and Gupta, 1998). While organogenesis is thorough collective organization of cells. Therefore, plants derived from somatic embryo are bipolar structure with root and shoot apices; they can easily develop into complete plants.

**Genetic transformation**
It took more than 2000 years to detect the causal principle of the crown gall disease after it was first described by Aristotle and Theophrastus (Siemens and Schieder, 1996). Smith and Townsend (1907) were the first to report that \textit{Agrobacterium tumefaciens} is the causative agent of the widespread neoplastic plant disease crown gall. The soil bacterium \textit{A. tumefaciens and A. rhizogenes} are considered as natural genetic engineers due to their ability to transfer and integrate DNA into plant genomes through a unique integrative gene transfer mechanism (Jouanin \textit{et al.}, 1993).

Though conventional plant breeding methods have results in a spectacular improvement in crop production, there are strong pressures for further improvement in crop quality and quantity due to explosion in population, social demands, health requirements, environmental stresses and ecological considerations (Kung, 1993). Conventional plant breeding techniques have limitations as these depend on sexual compatibility and often take 10-15 years to release a new variety due to extensive backcrossing (Pauls, 1995). These limitations have stimulated the development of more advanced technologies like genetic transformation of plants.

Genetic transformation can be defined as the transfer of foreign genes isolated from plants, viruses, bacteria or animals into a new genetic background. In plants, successful genetic transformation requires the production of normal, fertile plants, which express the newly inserted genes. The process of genetic transformation involves several distinct stages, namely insertion, integration, expression and inheritance of the new DNA. Process of gene insertion can involve the use of bacterial (\textit{Agrobacterium} species) or viral vectors or direct gene transfer methods (Webb & Morris, 1992).
Genetic engineering has allowed explosive expansion of our understanding in the field of plant biology and provides us with the technology to modify and improve crop plants. A remarkable progress has been made in the development of gene transfer technologies (Gasser and Fraley, 1989) which ultimately have resulted in production of a large number of transgenic plants in both dicots and monocots. Potential benefits from these transgenic plants include higher yield, enhancement of nutritional values and most important of all resistance to biotic and abiotic stress.

Genetic transformation of plants can be transient or stable. While, it is usually desirable to produce uniformly transformed plants, regeneration of transformed cells can be difficult, especially in the case of recalcitrant species (Spolaore et al., 2001). Therefore, efforts are being made to develop plant transformation methods that avoid tissue culture or regeneration. In many cases these methods have targeted meristems or other tissues that will ultimately give rise to gametes (Chee and Slighton, 1995; Birch, 1997).

Choice of explants having competence for transformation and regeneration is a crucial factor. At this point in time efficient tissue culture techniques become the foundation for genetic transformation studies. In addition to regeneration through organogenesis, somatic embryogenesis definitely offers the advantage of single cell regeneration and currently appears to be the most promising approach to introduce new genes into woody tree species.

Transient expression systems have the advantage of being rapid, without the need to regenerate transformed cells, and are therefore being used commonly for functional
analysis of gene regulation (Tucker et al., 2002; Synek et al., 2004; Hoffmann et al., 2004; Luu et al., 2004; Barcenas et al., 2000). Jefferson et al., (1987) recommended the establishment of optimal conditions for gene transfer through preliminary experiments of transient gene expression using reporter genes. Therefore, transient GUS expression based studies will be helpful for optimizing conditions affecting the transgene(s) expression and transformation process. Among several techniques used for transformation Agrobacterium is the most widely used transformation tool, accounting for 80% of the transgenic plants produced so far (Wang and Fang, 1998; Broothaerts et al., 2005). However, Agrobacterium-mediated gene transfer is a complex process (Riva et al., 1998) involving many poorly understood genetic determinants (Gelvin, 2000).

The commercialization of many genetically engineered plants and plant products is currently being actively pursued by biotechnology and seed companies and many genetically engineered plants are presently field-tested to determine their potential for commercialization.

It was only in 1983 that scientist inserted the first foreign genes into Petunia and Tobacco (Kung, 1984). Agrobacterium-mediated gene transfer becomes the method of choice due to convenience and high probability of single copy integration. This changed the situation dramatically in the 80’s and early 90’s and resulted in transformation of a wide range of plants for agronomically important traits (Songstand et al., 1993).

Since the initial successes in the Agrobacterium mediated transformation were mostly confined to dicotyledonous plants, concerted efforts were made to look alternative methods of gene transfer. A method of direct gene delivery into protoplast was the next
development in genetic transformation (Draper et al., 1982). Further many more techniques such as macroinjection (Zhou et al., 1983 and 1988), particle bombardment (Klein et al., 1988; Sanford 1988), microinjection (Neuhaus and Spangenberg, 1990), electroporation (Griesbach and Hammond, 1993) and laser mediated gene transfer (Guo et al., 1995) have been developed. However, none of these approaches has, so far, been developed into a reproducible universal gene transfer techniques (Potrykus, 1995).

*Agrobacterium* genetically transforms its host by transferring a well defined DNA segment from its tumour inducing (Ti) plasmid to the host cell genome (Gelvin, 1998). Recombinant *Agrobacterium* strains in which the native TDNA has been replaced with genes of interest, are the most efficient vehicles used today for the introduction of foreign genes into plants and for the production of transgenic plant species (Draper et al., 1988). Moreover the DNA transferred to the plant genome is defined, it doesn’t normally undergo any major rearrangements and it integrates into the genome as a single copy (Walden and Wingender, 1995). The *Agrobacterium* biology and biotechnology have been the subject of numerous studies over the past few decades (Nester et al., 2005) resulting in the establishment of many *Agrobacterium* strains, plasmids and protocols uniquely adopted for the genetic transformation of various plants species (Draper et al., 1988). 25 years ago the concept of using *Agrobacterium tumefaciens* as a vector to create a transgenic plants was viewed as a proposed and “wish”. Today, many agronomically and horticulturally important species are routinely transformed using this bacterium and the list of species that is susceptible to *Agrobacterium* mediated transformation seems to grow daily (Gelvin, 2000).
Plants are usually transferred with relatively simple constructs, in which the gene of interest is coupled to promoters of plant, viral or bacterial origin. Some promoters confer constitutive expression while others may be selected to permit tissue-specific expression.

**Salinity stress**

Salinity is a significant environmental stress for crop plants. Soil salinization may arise from intrinsic soil components, use of low quality of water for irrigation and excessive use of fertilizers. Salinity in the soil is a major interacting factor for crop production both in arid and semi-arid regions of the world (Epstein, 1978). Today 20% of world cultivated land is affected by salinity. High concentrations of salt cause stress in plants. Hence there is an immediate need to have varieties tolerant to salinity stress and genetic engineering coupled with tissue culture offer potential to develop salinity tolerant varieties. Excess amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world’s cultivated area and nearly half of the world’s irrigated lands are affected by salinity (Zhu, 2001). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce.

Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within next 25 years and up to 50% by the middle 21st century (Wang et al., 2003). High salinity causes both hyperionic and hyperosmotic stress and can lead to plant demise. Sea water contains approximately 3% of NaCl and in
terms of molarities of different ions, Na\(^+\) is about 460mM, Mg\(^{2+}\) is 50mM and Cl\(^-\) around 540mM along with smaller quantities of other ions. The basic physiology of high salt stress and drought stress overlaps with each other. High salt deposition in the soil generates a low water potential zone in the soil making it increasingly difficult for the plant to acquire both water as well as nutrients. Therefore, salt stress essentially resulted in a water deficient condition in the plant and takes the form of a physiological drought. The major ions involved in salt stress signalling, include Na\(^+\), K\(^+\) and Ca\(^{2+}\). It is the interplay of these ions, which brings homeostasis in the cell.

Water deficit, more commonly referred to as “Drought”, causes major economic losses in crop production throughout the world. Drought has been, and continues to be, the single most devastating factor that is menacing food production and food security, especially in areas with inadequate agricultural water resources. Consequently, with the global shortage of water, reducing water consumption in crop production has now been generally recognized as an essential strategy for sustainable agriculture.

In recent years, the idea of developing drought tolerance crops has been well recognized as the most promising and effective strategy for food security against drought and water shortage. However, drought tolerance is a complex trait that involves numerous aspects of developmental, physiological, biochemical and molecular adjustments. These includes, for examples, changes in root growth, guard cell regulation, osmotic adjustments, alterations in photosynthesis and synthesis of protective proteins and antioxidants. The regulatory pathways leading to these adjustments are poorly understood and remain a focal point of research. Under drought stress, plant accumulate ABA, which
is critical for stomatal closure leading to reduced transpirational water loss, induces the expression of many gene with presumed protective roles (Zhu, 2002).

High levels of salt in the soil can often cause serious limitations to agricultural production and land development. The main factors that contributed to this problem are the arid and semi arid climates and the salt load in the water used for irrigation. The soil salinity may cause several deleterious effects on growth and development of plants at physiological and biochemical levels (Gorham et al., 1985; Munns, 2002). These effects can be due to low osmotic potential of soil solution, specific ion effect; nutritional imbalance is a combined effect of all these factors (Ashraf, 1994 and Marschner, 1995).

Drought and soil salinity are among the most damaging abiotic stresses affecting today’s agriculture, it is understandable that plants are under periodic water stress because of the unpredictable nature of rain fall. Salt stress may also occur in areas where soils are naturally high in salts and/or where irrigation, hydraulic lifting of salty underground water or invasion of sea water in coastal areas brings salt to the surface soil that plants inhibit. Plants have evolved mechanisms that allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with them (Zhang et al., 2006). A good example of such a feed-forward mechanism is the ability of pants to regulate their water loss through partial closure of stomata and/or reduced leaf development, long before there is a substantial loss of their leaf turgor or some irreversible damage to inner membrane system (Jones, 1980; Cown, 1982; Davis and Zhang, 1991).

**Botanical description of *Sesamum indicum* L.**
Sesamum indicum L. (Plate: 1) is an annual erect or prostrate herbs growing to 50-100cm, largely cultivated throughout India for the oil furnished by its seeds. It is probably a native to Tropical Africa, but its original habitat is not known with certainty. It has been cultivated in India from a very remote period and is grown in many of the tropical regions of the world. The plant is usually about 2ft height; leaves opposite below (5-6 Inches), alternate above, entire, toothed and lobed. Stem erect with long ascending branches from the base. Upper leaves lanceolate, entire, the lower often cut at the base into two or more serrate segments, glabrous above, puberulous beneath. Flowers pinkish-purple, axillary, solitary or few and fascicled, shortly pedicillate. Calyx small, five-partite. Corolla two lipped; tube ventricose; lobes rounded, those of the upper lip usually rather smaller than the others. Stamens four di-dynamous, included; anthers sagitate, the cells sub parallel, distinct. Ovary two celled, the cells each soon divided into two chambers by the intrusion of a false dissepiment between the placentas; ovules numerous, 1-serrate in each chamber; style filiform; stigma 2-lobed. Capsules oblong, usually four angled and four grooved, loculicidallt, two chambered, blunty quadrangular, shortly beaked, pubescent; walls two, separating half way down, gaping. Seeds many, obliquely oblong, slightly compressed, glabrous, black or white in colour. Flowering Augst-September (Cooke, 1905).

Systematic position

Class : Dicotyledonae

Subclass : Gamopetalae
Series : Heteromerae

Order : Personales

Family : Pedaliaceae

Genus : Sesamum

Species : S. indicum L.

Bentham and Hooker system of classification (1862-1883).

History

Origin

Majority of the species of the genus Sesamum are native to sub-saharan Africa, Africa is considered to be the primary centre of origin of this crop because of the presence of its diverse wild species in that continent. India is the secondary centre of origin and another secondary centre is Japan (www.krishworld.com/html/sesamum.html) with respect to India, archaeological evidence have been found to conclude that it was cultivated at Harappa in the Indus valley between 2250 and 1750 BC. The plant has a special mention in Hindu legends and beliefs, that sesame seeds represent a symbol of immortality and the God Maha Vishnu’s consort Maha Sri Devi herself representing the properties of the sesame seed, as such it is considered as the most auspicious oil next to Ghee used in Hindu rituals and prayers (en.wikipedia.org/wiki/sesame).

Distribution
Sesamum is one of the important genus of family Pedaliaceae consists of 40 species, of these 7 are distributed in India (Subramanian, 2003) and has been cultivated in both tropical and temperate areas from ancient times (Kwon et al., 1993). Geographically sesame species are distributed in Tropical Africa, Madagascar, Tropical Australia and few of the Eastern Islands of Malaysian Archipelago.

**Production**

India, China and Myanmar are the important sesame producing countries (Table-1). India ranks first, both in the area and production of sesame in the world (Table-2). The annual area put under its cultivation in India is about 2-5 million hectares (45% of the world area) and the total production is nearly 52 thousand tones. Sesamum is grown in India (Table-3) mainly in eight states (Table-4) viz., Rajasthan (422,100 Ha), Gujarat (364,000 Ha), Madhya Pradesh (150,100 Ha), West Bengal (146,000 Ha), Andhra Pradesh (116,000 Ha), Tamil Nadu (116,000 Ha), Maharashtra (107,000 Ha), among other states only Karnataka has a sizable area (103,000 Ha) under sesame cultivation.

**Active ingredients**

Sesame seed is also a traditional toothache remedy and contains at least seven pain-relieving compounds.

**Sesame seed oil contents**

Palmitic acid (7-12%); Stearic acid (3.5-6.0%); Oleic acid (Omega-9 monosaturated fats) (35-50%); Linolic acid (Omega-6) (35-50%); Phytosterols
(117.64mg/tbsp); Phenylalanine (255mg/oz); Glutamine (3.8% or 1.1g/oz) and Vitamin E (4% or 0.556mg/tbsp). (1tbsp of sesame consists of 45 calories of energy, 1g of Carbohydrate, 2g of Protein, 0.0mg of cholesterol, 8g of weight, 4g of fat and 0.6g of saturated fat). (1 tbsp 13.6g) sesame seed meal (partially defatted used in preparation of cattle feed) contains: Water 5%; protein 17% or 4.8g/oz; total lipid (fat) 48% or 13.6g/oz; Carbohydrate, by difference 26% or 7.4g/oz; Ash 4% or 1.13g/oz; Minerals including Calcium 43.3mg/oz; Iron 4.1mg/oz; Magnesium 98.1mg/oz; Phosphorus 219.4mg/oz; Potassium 115.1mg/oz; Sodium 11.0mg/oz; Zinc 2.9mg/oz; Copper 0.41mg/oz; and Manganese 0.40mg/oz.

**Vitamins**

Thiamin, Riboflavin, Niacin, Pantothenic acid, Vitamin B-6; Floate and Vitamin A, Vitamin B1 (thiamine) and Vitamine E (tochopherol).

**Amino acids**

Tryptophan, Threonine, Isoleucine, Leucine, Lysine, Methionine, Cystine, Phenylalanine 255mg/oz, Tyrosine, Valine, Arginine 2.5% or 713mg/oz, Histidine, Alanine, Glutamic acid 3.8% or 1.1g/oz, Glycine, Proline and Serine. (1 oz = 28.35g). (USDA Nutrient Database. www.nal.usda.gov).

The seeds are rich in manganese, copper and calcium (90mg per tablespoon for unhulled seeds, 10mg for hulled) and contain powerful antioxidants called lignans (sesamins), sesamolin and Phytosterol which impart a high degree of resistance against oxidative rancidity (Kato et al., 1998 and Sirato et al., 2001).
Sesame seed contains lecithin which has antioxidant and hepatoprotective activity and ranges from 58ppm to 395ppm (Beckstrom et al., 1994a). Sesame seeds contain phytosterols associated with reduced level of blood cholesterol, but do not contain caffeine. (Source: en.wikipedia.org/wiki/sesame).

**Cultivation**

In India, *sesamum* is grown in three seasons, viz. kharif, semi-rabi and summer. The kharif crop occupies over 70% of the area, whereas the semi-rabi and summer crops occupy 20% and 10% area respectively. The kharif, *sesamum* is sown in June-July with the onset of the monsoon and is harvested in December-January (Table-5). The kharif and semi-rabi crops are entirely rainfed, whereas summer crop is grown under irrigation. The yield of the kharif crop is poor, whereas those of the semi-rabi and summer crops are high, as they are grown in rich soils and under better management. The preparatory cultivation for the kharif crop is usually not thorough. For the semi-rabi and summer crops, the land is ploughed and harrowed repeatedly to secure a clean and fine seedbed. Except in Maharashtra and Gujarat, where line-sowing is practised, in all other states it is sown broadcast. The recommended spacings for the line-sown crop in different regions, however, range from 25 to 35cm between the rows and 10 to 20cm between the plants in the row. The seed being small, it is often mixed with sand to ensure its even distribution and the drill is operated rather shallow to avoid deep sowing. After sowing, the seed is covered lightly with a brush harrow. The seed-rate varies from 3 to 5kg per hectare. The seed should be treated with Captan or Thiram at the rate of 3g per kg of seed to control seed-borne diseases.
The rainfed crop is weeded and hoed once or twice and the irrigated crop is weeded often. Wherever the weed control with mechanical means is a problem, chemical weed control with pre-emergence application of Lasso at the rate of 3litres/ha is recommended. The crop is generally recommended for different regions as 25-30kg of weight, 20-30kg of P$_2$O$_5$ and 0-20kg of K$_2$O per hectare. The crop is harvested when the leaves, stems and capsules begin to turn yellow and the lower leaves start shedding. To prevent the shedding of grains, the crop should not be allowed to become a drip in the field. The ripe plants are cut, carried to the threshing-yard, stacked for a week in the sun with the cut-ends downwards and well shaken or beaten to take out the grains from the dry capsules. Winnowing and cleaning completes the process (www.krishiwold.com/html/sesamum.html).

Climate and condition

Sesame grows in the plains and at elevations up to 1,200m. It cannot stand frost, continuous heavy rain. It is grown in sandy-loam to heavy black soils. Most of the crop is confined to lighter soils and its cultivation in heavy soils is limited to certain regions. The pH of sesame growing soils ranges from 5.5 to 8.2.

Uses and properties of sesamum seeds and oil
1. The seeds are recommended by Chinese herbalists for nursing mothers to stimulate milk flow and for men to prevent hair loss.

2. The seed is still today recommended for treating tinnitus, blurred vision and dizziness.

3. Sesame seed meal is an excellent source of both calcium and antioxidants. The seed is also rich in the amino acid Arginine and Phenylamine (225mg/oz) which according to naturopaths is important for countering low sperm counts, and for relieving pain respectively.

4. The oil also contains two other powerful phytochemicals, sesamin and episesamin, lignans known to stimulate cellular regeneration, detoxification and cleansing.

5. Phytosterol, such as beta sitosterol, found in sesame to be as effective as conventional drugs for relieving frequent urination in aging men suffering from prostate conditions and quickly lower cholesterol. (www.nal.usda.gov)

6. Both sesamin and sesamolin were reported to increase both the hepatic mitochondrial and the peroxisomal fatty acid oxidation rate (Bradley, 2002).

7. Sesame seed consumption appears to increase plasma gamma-tocopherol and enhanced vitamin E activity which is believed to prevent cancer and heart disease (Cooney et al., 2001).
8. Lecithin present in sesamum seed is reported to be effective for reducing hepatic steatosis in long term parenteral patients and a successful treatment for dermatitis and dry skin (Jellin et al., 2000).

9. Myristic acid also has cancer preventive capability and is found in sesame seed ranging from 328 to 1,728ppm (Beckstrom et al., 1994a).

10. Sesame oil is a pharmaceutical aid as a solvent for intramuscular injections and has nutritive, demulcent and emollient properties (Tyler et al., 1976) and has been used as a laxative (Dark, 1998). The Indians have used sesame oil as an antibacterial mouthwash, to relieve anxiety and insomnia (Anrussek, 2001).

11. A recent clinical trial proved that sesame oil was significantly more effective for treating nasal mucosa dryness due to a dry winter climate than isotonic sodium chloride solution (Johnson, et al., 2001). In addition, sesame oil contains large amounts of linoleate in triglyceride which selectively inhibits malignant melanoma growth (Smith and Salerno, 2001).

12. Sesame seeds (approximately 50% oil and 25% protein) are used in baking, candy making and other food industries. Oil from the seed is used in cooking and salad oils and margarine. Foods fried in sesame oil have a long shelf life because the oil contains an antioxidant called sesamol. The oil can be used in the manufacture of soaps, paints, perfumes, pharmaceutical and insecticides. Sesame meal, left after the oil is pressed from the seed is an excellent high (34 to 50%) protein feed, for poultry and livestock (Oplinger, et al., 1990).
13. Sesame seeds are used in many salads and baked snacks in Japan. The seeds are also eaten on bread in Sicily and France (called “ficelle sesme”, sesame thread).


Aim and objectives of the thesis

- Keeping in view of the work done in India and elsewhere, the programme on *Sesamum indicum* L. CV. E-8 has been investigated with the following objectives,

  ✓ To standardize the cultural conditions and nutritional requirements to induce callus by using different explants of *Sesamum indicum* L.

  ✓ To find out the frequency and number of multiple shoots from different explants using different concentrations of cytokinin alone or in combination with auxins.

  ✓ To standardize protocol for somatic embryogenesis from different explants by using auxins alone or in combination with cytokinins.

  ✓ To develop the protocol for *Agrobacterium tumifaciens* mediated genetic transformation, for salt and drought tolerance using AP37 gene.

  ✓ To confirm the transgenic nature of the *Sesamum indicum* plants by using PCR and Southern blotting techniques.