Chapter – 1

General Introduction

Safflower (*Carthamus tinctorius* L.) has been grown for centuries, primarily for its colorful petals to use as a food coloring and flavoring agent, for vegetable oils and also for preparing textile dye in the Far East, Central and Northern Asia and European Caucasian (Esendal, 2001). It has also received considerable interest recently as forage (Landau *et al.*, 2004, 2005).Safflower is most commonly known as 'kusum' (India, Pakistan), derived from the Sanskrit, 'Kusumbha' and as 'Honghua' (red flower) in China. It is used as a less costly substitute for saffron is indicated by the names false saffron, thistle saffron and dyer's saffron (Weiss, 1983). Safflower (*Carthamus tinctorius* L), which belongs to the *Asteraceae* family, is cultivated in several parts of the world due to its adaptability to different environmental conditions. It has become an increasingly important crop in Turkey and the world due to the rich nutritional value of its edible oil. It is a rich source of oil (35–40 %) and linoleic acid content (75–86 %). Safflower seeds are achenes (fruits) surrounded by a thick fibrous hull. They are smooth, shiny and angular, about 6-9 mm long, white or brownish and white with grey, brown or black stripes. They generally contain 33-60 % hull and 40-67 % kernel. Thin-hulled varieties have been developed (Baumler *et al.*, 2006; Mundel *et al.*, 2004; Ecoport, 2010; Oyen *et al.*, 2007). In India, Pakistan and neighbouring countries, a seed rate of 5-12 Kg/ha is common; the average seed yield of commercially grown safflower has increased steadily to around 2,500 Kg/ha, nearly twice under irrigation (Camas *et al.*, 2007). The world safflower production increased steadily during the 1990's, but decreased from a high of over 930,000 metric tonnes in 1997 to only 6,04,157 metric tonnes in 2004 (Smith and Jimmerson, 2005). The crop is grown in an area of 691000 ha, with a production of about 615000 tonnes in more than 60 countries worldwide. India is the largest producer of safflower in the world, grown in an area of 295000 ha, with a production of 189000 tonnes (2008-09). Currently, the estimated world production of safflower is about 7, 00,000 tonnes in 2011 (FAO, 2011). In India, Maharashtra and Karnataka states account for 72 and 24 % of safflower area and production, respectively. The other safflower producing states are Andhra Pradesh,

Orissa, Madhya Pradesh, Chattisgarh and Bihar. Safflower production in India is mostly confined to rain-fed conditions during winter. It is highly branched, herbaceous, thistle-like annual or winter annual, usually with many long sharp spines on the leaves.

Safflower is generally considered a day length-neutral, long-day plant. Summer crop varieties from temperate regions, sown during shortening days as a winter crop in subtropical or tropical regions, have a very long rosette phase (several months), with greatly delayed maturity. Plants are 30-150 cm tall with globular flower heads (capitula) and, commonly, brilliant yellow, orange or red flowers. The florets are tubular and largely self-pollinating with generally less than 10 % out crossing (Knowles, 1969). The plant has a strong taproot which enables the plant to draw moisture and nutrients from a considerable depth, conferring on safflower the ability to survive in areas with little surface moisture. In India, the crop has traditionally been grown in the 'rabi' or winter dry season in mixtures with other 'rabi' crops, such as wheat and sorghum. True saffron is perhaps the world's most costly spice, and safflower is a common adulterant or substitute. The main constituents of the safflower are carthamin and carthamidin. And other constituents are safflower yellow, arctigenin, tacheloside, N-feruloyl tryptamine, N feruloylserotonin, steroids, flavonoids, polyacetylenes. Carthamin is responsible for to produce water-insoluble red dye and carthamidin for water-soluble yellow colour dye (Kizil *et al.*, 2008)**.** Flower colour is generally considered neutral for seed yield and oil, but when the crop is grown for the florets, colour is important. Selection for high oil content in modern cultivars has reduced the pericarp thickness. Seed mass increases rapidly during the first 15 days after flowering. Maximum dry matter accumulation, oil content, germination and hull percentage was determined to occur 28 days after fertilization of a floret when the seed moisture content was 22-25 % (Leininger and Urie, 1964). Leaves are usually deeply serrated on the lower stem, but short and stiff, ovate to obovate around the inflorescence, where they form the involucral bracts. Lower leaves are generally spineless, but further the stem spines develop in the bud stage and become strong, hard at full flowering. Varieties that are almost completely free of spines have been developed for hand harvest of floral parts and of seeds in certain geographic regions (China, non-traditional areas of India).

1.1 Economical Importance

Safflower varieties released for commercial production in India in general possess low oil content of 28 to 32 %, except HUS-305, NARI-6, and nonspiny hybrid NARI-NH-1, each of which contain 35 % oil. The quality of any oil is determined by the fatty acid composition of the oil, and the oils rich in poly-or monounsaturated fatty acids are considered good, as they help in reducing the cholesterol level in blood. In view of the above, safflower oil is considered the best, as it contains very high amounts of polyunsaturated (linoleic acid, 70 to 75 %) or monounsaturated (oleic acid, 70 to 75 %) fatty acids (Knowles, 1989). Standard safflower oil contains about 6 to 8 % palmitic acid, 2 to 3 % stearic acid, 16 to 20 % oleic acid, and 71 to 75 % linoleic acid (Velasco and Fernandez- Martinez, 2001). Young leaves are eaten boiled, as a vegetable side dish with curry or rice in India, Pakistan and Burma. It was reported that the dried flowers of safflower (*Carthamus tinctorius*) had been used in traditional Asian medicine for thousands of years (Gao *et al.*, 2000). Florets were widely used to colour and flavour soups and rice as well as cloth, potions and unguents. In addition to these properties, safflower petals are also used for production of food and fabric colorants (Srinivas *et al.*, 1999). Safflower dyes were particularly important to the carpet-weaving industries of Eastern Europe, the Middle East and the Indian subcontinent. Carthamin dye was used extensively to colour cloth until the 19th century, when cheaper aniline dyes became available. Since the 2nd century AD Hebrew writings have described the use of tablets of carthamin dye for food colouring, rouge and medicine (Weiss, 1983).True saffron is perhaps the world's most costly spice and safflower is a common adulterant or substitute. Addition of safflower florets to foods is a widespread and ancient tradition. Health concerns regarding synthetic food colourings may increase demand for safflower-derived food colouring. China produces carthamin dye for use in food, particularly at a large factory in Kunming in Yunnan Province. Cosmetic rouge can be made from carthamin dye mixed with French chalk and the Japanese cosmetic ('beni') (Weiss, 1983) and lipsticks include safflower colouring (Smith, 1996). Until this century, when cheaper aniline dyes became available, safflower was mainly grown for dye. The water-soluble yellow dye, carthamidin, and a water-insoluble red dye, carthamin, which is readily soluble in alkali, can be obtained from safflower florets (Weiss, 1983). Yellow florets contain little or no red dye (Smith, 1996).

1.2 Pharmaceutical Importance

Safflower has been used in the Middle East, India and Africa for purgative and alexipharmic (antidote) effects, as well as in a medicated oil, to promote sweating and cure fevers (Weiss, 1971). A tea made from safflower foliage is used to prevent abortion and infertility by women in Afghanistan and India (Weiss, 1983). All parts of the plant are sold by herbalists in India and Pakistan as 'pansari' to remedy various ailments and as an aphrodisiac (Knowles, 1965). Kanehira *et al.*, (2003) reported also that kinobeon A, originally isolated from safflower, exhibited stronger effect on the oxidative stresses and could be a useful Cytoprotective reagent. Safflower dilates arteries, reduces hypertension and increases blood flow and hence, oxygenation of tissues. It also inhibits thrombus formation and, over time, dissolves thrombi (Anonymous, 1972). Many prescriptions for invigorating blood circulation, especially those for treatment of heart disease, include safflower along with other herbs and have been used in treatment of many diseases (Guishen, 1985). Cardiovascular disease treatment is the main use of safflower because it invigorates the circulation. In 83 % of patients with coronary disease, blood cholesterol levels have been reduced after 6 weeks of treatment (Guimiao and Yili, 1985). Heart arrythmia and hypertension were reduced by safflower treatment three times a day for 4 weeks (Bingzhang *et al.*, 1978; Guimiao and Yili, 1985). A nasal drip of safflower and other herbs speeded blood flow in the medial cranial artery (Zhenshun *et al.*, 1992). Injections of safflower extract at Fengfu, Yamen, Fengchi and other acupuncture points every 3 days increased blood flow in the coronary artery. Treatment of cerebral thrombosis with safflower improved and lowered blood pressure in over 90 % of patients (Guimiao and Li Yili, 1985; Damao, 1987). Herbal decoctions including safflower were also effective in treatment of cerebral embolism (Zuolin, 1992). Zhoucai (1991) treated hemiplegia with a combination of Western and Chinese medicine including safflower. Safflower decoctions have been used successfully for treatment of male sterility (Yuehao, 1990) and dead sperm excess disease (Chun, 1990). Treatment with safflower resulted in pregnancy in 56 of 77 infertile women who had been infertile for 1.5-10 years (Wenyu, 1986).

Safflower prescriptions have been very effective treatments for rheumatoid arthritis (Yue and Luqiu, 1990; Zhaoming *et al.*, 1985). Safflower, along with other herbs, has been used to treat respiratory diseases including pertussis (whooping cough) and chronic bronchitis (Guimiao and Yili, 1985). Safflower eye drops reduce myopia, especially in children (Genyu, 1990; Guimiao and Yili, 1985). Trachoma has been successfully treated with safflower combined with other herbs (Jialou, 1986). Invigoration of the blood circulation by safflower has also reduced senile cataracts (Qiuyuan, 1992). Clinical improvements due to safflower treatment have been reported for leukemia (Youan, 1988), leucocytopenia and erythrocytosis (Kuijie, 1985), allergic purpura, lupus erythematosus (Zhongying, 1989; Guimiao and Yili, 1985), goitre (Shulin, 1992), anal fissure (Yunshan, 1986), jaundice and viral hepatitis and migraine headaches (Guimiao and Yili, 1985). Climacteric syrup for reduction of menopausal flushing includes safflower (Qiuping, 1989). Knowles (1965) reported that flowers were soaked overnight and applied wet to reduce allergy rashes in Egypt. Around the world, safflower is mainly grown for its edible oil for cooking, salad oil and margarine. In affluent countries, research linking health and diet has increased the demand for the oil, which has the highest polyunsaturated/saturated ratios of any oil available. It is nutritionally similar to olive oil, with high levels of linoleic or oleic acid, but much less costly. Polyunsaturated fats are associated with lowering of blood cholesterol. Also, mono-unsaturates such as oleic safflower oil tend to lower blood levels of LDL ('bad' cholesterol) without affecting HDL ('good' cholesterol) (Smith, 1996). There is a considerable health food market for safflower oil, especially in North America, Germany (Smith, 1996) and Japan. Safflower oil is an effective nonallergenic dispersant for injectable medications, but not widely used (Smith, 1996). In Iran, the oil is used in treatment of liver and heart ailments (Knowles, 1965). The oil is nonallergenic, making it ideal for cosmetics; it is used in Macassar hair oil and Bombay sweet oil (Weiss, 1971). Charred safflower oil has been used to treat sores and rheumatism in India (Weiss, 1971). Many investigations have found that safflower yellow pigment had a lot of pharmacological effects. It could inhibit the conglomeration of haematoblast efficaciously and exhibit anti-inflammatory, anti-allergic, anti-cancer activities and a protective effect on cardiovascular diseases.

Safflower was successfully used as sole food for late-pregnant dairy cows (Landau *et al.*, 2004). Safflower cropped at the budding stage can be ensiled (Weinberg *et al.*, 2002), and safflower silage is substituted for cereal silage in the diet of high-yielding dairy cows without affecting dairy performance (Landau *et al.*, 2004).

1.3 Safflower Disease Resistance

Safflower is attacked by many diseases caused by fungi, bacteria, viruses, or physiological disorders due to abiotic stresses. (Patil *et al.*, 1993) reported that safflower is recorded to be infested around the world by 57 pathogens, including 40 fungi, 2 bacteria, 14 viruses, and 1 mycoplasma. Foliar diseases have been particularly serious in areas where rainfall occurs between the late bud stage and near maturity. Most serious and widespread is leaf blight caused by *Alternaria carthami.* Other foliar diseases of more localized concern include those caused by *Botrytis cinerea*, *Cercospora carthami, Pseudomonas syringae, Puccinia carthami* and *Ramularia carthami.* Safflower rust (*Puccinia carthami)* is widespread in all areas of commercial production. The rust fungus has a complex life cycle and produces different types of spores, depending on the stage of the fungal life cycle present. Black teliospores appear at the end of the crop growth cycle and can infest seeds or persist in the soil. Many races of the causal fungi are endemic in California soils and attack the roots and lower stem under favourable environmental conditions. Plants are susceptible at all stages of growth but the visible symptoms become more apparent from flowering onward. Infected plants become light coloured, wilt and die. In the early stages of infection, roots may show a reddening of tissue. Infected roots and lower stems later become darkly discoloured. *Alternaria carthami* Chowdhury (1944) is an important seed-borne pathogen of safflower (*Carthamus tinctorius* L.) in Western Canada, U.S.A, Australia and India. Mortensen *et al.*, (1983) found that *A. carthami* was pathogenic on safflower at all growth stages in Montana, causing up to 50 % seed rot and seedling blight in susceptible cultivars. In Canada, (Petrie 1974) reported that *A. carthami* occurred upto 95 % in safflower seeds. A seed produced in Alberta, Monilotba, Saskatchewan and Montana is often heavily contaminated with this pathogen, resulting in reduced germination and seedling vigor (Mortensen *et al.*, 1983). In years of frequent rains and high humidity, the disease may cause severe damage to leaves, flower bracts, capitula

and seeds. Fungicidal seed treatments have only partly reduced the incidence of seed-borne *A. carthami,* with the pathogen internal to the seed coat not being killed (Irwin, 1976; Mortensen *et al.*, 1983). Infection by fungal pathogens results in a change of host plant physiology and in mechanical and biochemical disruptions. Intercellular penetration of tissues results in direct effects on the middle lamellae between associated plant cells followed by invasion of plant cells, membranes and organelles, increased nutrient leakage and host cell death, which results in visible disease symptoms (Elad, 1997). Chemical control measures for diseases and pests are expensive and ineffective. Breeding safflower for disease resistance is the most economical and convenient method for controlling major diseases in safflower. Mundel and Huang (2003) described in detail how to control major diseases of safflower by breeding and using cultural practices. The genetics and the mode of inheritance of disease resistance and tolerance in safflower have not been studied for most diseases (Li and Mundel, 1996). Though germplasm lines or cultivars showing partial or full resistance to some of the major diseases have been identified, the genetics has been determined only for a few. Moreover, the increasing use of chemicals in agriculture affects the quality of the products as well as presenting problems to ecosystems. Most of the resistant lines identified earlier were able to maintain the same level of resistance over the time. An understanding of the inheritance of resistance in the host and of virulence and non-virulence of different physiological races of pathogens are needed.

1.4. Chitinase

Chitinase is a type of pathogenesis-related proteins, are ubiquitous enzymes of bacteria, fungi, animals and plants. Chitinases are hydrolytic enzymes produced by plants in response to fungal infection (Punja and Zhang 1993). They are proposed to have a role in plant defense by degrading the fungal cell walls. They hydrolyze the β-1,4-linkage between *N*-acetylgucosamine residues of chitin, a structural polysaccharide of the cell wall of many fungi and of the exoskeleton of invertebrates (Sami *et al.*, 2001). Plant chitinases that hydrolyse fungal cell wall chitin, thereby inhibit the growth of fungi and also generate chitin oligosaccharides acting as elicitors. These enzymes probably play a role in the generation of signal molecules not only those involved in plant resistance to external environmental factors, but also in plant

growth and development. It is supposed that chitinases detach these molecules from larger precursors, i.e. polysaccharides or glycoproteins (Zhong *et al.*, 2002). Additionally, the regulatory role of chitinases may involve oligosaccharide degradation. This was proved in the case of regulation of the nodulation process in legume plants where chitinases hydrolysed bacterial lipochitooligosaccharides (Cullimore *et al.*, 2001). It is one of the most abundant naturally occurring polysaccharides and has attracted tremendous attention in the field of Agriculture, Pharmacology and Biotechnology (Antranikian *et al.*, 2005; Muzzarelli *et al.*, 2005). Chitinases can be used to treat fungal infections (Liu *et al.*, 2002). Chitin also has multiple applications mainly in the bioremediation of environmental pollutants like Pb^{2+}, Zn^{2+} and Bi^{2+} (Rae and Gibb, 2005). The development of research in plant defence mechanisms has led to a rapid and steady interest in Chitinase, since this is the first pathogen induced proteins whose function was identified. Its substrate is present in the cell wall of many fungi, as well as in insects and nematodes, which are major pathogens of crop plants. Several studies have been made on transgenic plants integrated with chitinase genes. Transgenic tobacco and canola which have been engineered with bean endochitinase gene were shown to exhibit resistance to *Rhizoctonia solani* (Broglie *et al.*, 1991). Transgenic rice integrated with rice endochitinase driven by the 35S promoter showed enhanced resistance to sheath blight (Lin *et al.*, 1995). The activation of the anti-fungal activity of chitinase, *in vitro* and in transgenic plants, may be defensive against fungal pathogens (Nishizawa *et al.*, 1999; Yamamoto *et al.*, 2000). Transgenic plants and biological control agents offer many opportunities to manipulate genes from a variety of sources for the enhancement of host plant resistance to pathogens, respectively, against insects and other types of pests (Herrera-Estrella *et al.*, 1999; Estruch *et al.*, 1997). Whereas transgenic tobacco harboring rice endochitinase gene also possessed increased resistance against powdery mildew, *Erysiphe cichoracearum* (Nishizawa *et al.*, 1993). Chitinase enzyme possesses added value in food and pharmaceutical industries, anti-bacterial agents, elicitors, lysozyme inducers and immunoenhancers (Kato *et al.*, 2003, Muzzarelli *et al.*, 2005). Due to the massive biological applications, the characterization and synthesis of chitinase is highly anticipated in biotechnology industry.

1.5. Safflower Biotechnology

Safflower has attracted very little attention as far as tissue culture and genetic transformation are concerned. Initial efforts in safflower were directed to develop suitable culture conditions for whole plant regeneration. It has been demonstrated that regeneration frequencies are very high and regeneration is possible through embryogenesis and organogenesis pathways. The mode of regeneration in general is through direct or indirect organogenesis (George and Rao, 1982; Tejovathi and Anwar, 1987; 1993; Sujatha and Suganya, 1996; Nikam and Shitole, 1999; Walia *et al.*, 2005). The tissue culture of safflower has been the subject of several studies focusing on establishment of *in vitro* propagation. It offers a method to increase valuable genotypes rapidly and expedite release of large number of plantlets. It is well advanced and regularly used for the conservation, multiplication and distribution of elite germplasm. The alternative method to select or detect resistance is the use of *in vitro* selection methods. In comparison with *in vivo* selection, which is commonly applied in plant breeding programs, *in vitro* selection methods have several advantages. It is possible to screen a large number of individuals within a short time under controlled conditions in the lab and, therefore help to advance breeding progress. It is possible to exclude interactions with the environment, which might cover certain traits. Instead of whole plants, organs and small amounts of tissues or even single cells can be tested (Ahmed and Sagi, 1993). *In vitro* selection allows the specific use of genetic variability which is induced from the *in vitro* culture of plant cells or tissue. It is a clean and rapid way in genetic engineering by which the materials can be grown for identification and manipulation of genes or transfer of characters from one plant to another.

Genetic improvement in safflower through conventional breeding is limited to the development of new cultivars with widely varying oil content and quality (Knowles, 1982). Genetic improvement of this crop for agronomical attributes is constrained by the modest levels of variability available in the cultivar germplasm. Development of *in vitro* shoot regeneration techniques is essential for introgression of desirable traits from alien sources. In earlier studies of safflower, there are few reports demonstrating anther culture (Prasad *et al.*, 1991), *in vitro* regeneration (George and Rao, 1982; Tejovathi and Anwar, 1984; 1987; Ying *et al.*, 1992, Orlikowska and Dyer,

1993, Sujatha and Suganya, 1996, Tejovathi and Anwar, 1993; Nikam and Shitole, 1999, Vijaya Kumar and Ranjitha Kumari, 2005; Radhika *et al.,* 2006) and somatic embryogenesis have only been briefly documented (Mandal *et al.*, 1995; Mandal *et al.,* 2001; Mandal and Gupta, 2002; 2003). The addition of toxic compounds to the culture media or the application of abiotic stress results in a defined selection pressure. Together with the *in vitro* selection for disease resistance, there are number of other possible applications for *in vitro* selections such as selection for herbicide tolerance, selection for salt tolerance, selection for tolerance to metals, selection for tolerance to high or low temperatures and selection for tolerance to water stress (Haines, 1993). Evaluation of NaCl tolerance in safflower callus cultures by the repeated transfer of selected clones to the NaCl-rich medium enabled identification of salt-tolerant cell lines (Nikam and Shitole, 1997). Induction of high variability for qualitative and quantitative traits through tissue culture indicated that tissue culture can be suitably utilized to detect spontaneous somaclonal variants with improved tolerance to abiotic stresses. *In vitro* selection for resistance to a pathogen can be realised when *in vitro* cultures are exposed to toxins produced by the pathogen, synthetic toxin analogues, to a pathogen filtrate, to extracts of the pathogen or to the pathogen itself (Daub, 1986). Numerous studies have demonstrated the utility of tissue culture-based testing of agrochemicals, microbial toxins or allelochemicals on plant tissues. These studies have also contributed useful data on physiological and biochemical processes affected by induced stress (Smeda and Weller, 1991). Matern and Kneusel (1993) attempted to use genetic engineering techniques for safflower to introduce resistance to leaf blight caused by *Alternaria* species. This group used molecular methods to identify the macrolide brefeldin A, as the phytotoxin from *A. carthami* which suppresses the plant's defense response and is thus identified as a virulence factor of the fungal pathogen.

Currently*Agrobacterium*-mediated transformation of safflower and the efficient recovery of transgenic plants via grafting (Belide *et al.*, 2011). *Agrobacterium* based vectors have been successfully employed to transfer genes into a number of dicotyledonous plants (Gelvin, 2003). *Agrobacterium*-mediated genetic transformation is still the most widely used method of producing transgenic plants (Dunwell *et al.*, 2000). Besides being cheaper and simpler than most direct gene transfer methods, it

allows little rearrangement of transgenes and efficient integration of the transgene into the plant genome (Ingelbrecht *et al.*, 1991). However, the most limiting factor for efficient transformation is the absence of high-yielding regeneration protocols. Transformation of plant cells results from transfer of a segment of the tumour-inducing (Ti) plasmid (T-DNA) and its subsequent integration into plant chromosomal DNA (Tzfira and Citovsky, 2006). It has been suggested that *Agrobacterium tumefaciens*-mediated transformation may offer a better alternative than the direct method for delivery of transgenes into plants. A better understanding of this process will facilitate manipulation of the plant host, both to increase transformation efficiency and to achieve the important strategic objective of targeted T-DNA integration into plant chromosomes (Gelvin, 2003; Tzfira and Citovsky, 2006). This gene delivery system results in a greater proportion of stable, low-copy number transgene events than the Biolistic gun does and offers the possibility of transferring larger DNA segments into recipient cells (Hiei *et al.*, 1994; Ishida *et al.*, 1996; Shibata and Liu, 2000). In earlier studies, regeneration of whole plant transformants of saffower (*Carthamus tinctorius* L.) following *Agrobacterium tumefaciens*-mediated transformation has largely been unsuccessful (Ying *et al.*, 1992; Orlikowska *et al.*, 1995; Sankara Rao and Rohini, 1999). The successful reports on plant regeneration of safflower through *Agrobacterium tumefaciens* mediated transformation was reported limitedly in few cultivars (Rohini and Sankara Rao, 2000; Sujatha, 2006). Another important and interesting use of safflower seed has recently emerged by means of its genetic modification to produce high-value proteins as pharmaceuticals and industrial enzymes. SemBioSys a Calgary-based (Canada) company—transforms safflower tissue genetically in order to get the proteins of interest to accumulate in the seed of the mature transgenic plant (Mundel *et al.*, 2004). The process of transformation of safflower tissues follows the patented Stratosome Biologics system, which facilitates the genetic attachment of target proteins of interest to oleosin, the primary protein coating the oil-containing vesicles (oil bodies) of the seed. Such attachment permits the target protein to be purified along with the oil body fraction, which upon centrifugation floats to the surface of ground seeds/water slurry (Van Rooijen *et al.*, 1992). The purification process of the Stratosome system makes it more efficient than the other transgenic systems. The attachment of proteins to the oil bodies of safflower in the

Stratosome Biologics system is expected to stabilize intracellular accumulation of foreign proteins, and also provide a useful attachment matrix and deliver benefits for useful applications. The commercialization of genetically modified safflower will further increase the acreage and production of this crop in the world. Therefore, genetic modification of safflower would be of enormous importance in improving productivity, production and remuneration per unit area from the crop, which in turn would certainly help in increasing safflower area in the world.

In view of these, the present study was undertaken in safflower with the following objectives.

- ✓ To standardize techniques for *in vitro* plant regeneration from direct and indirect Organogenesis.
- ✓ To construct the *Agrobacterium* strain LBA 4404 harbouring the binary plasmid pCAMBIA-Bar-Ubi-Chi11 Rice Chitinase.
- ✓ To infect safflower explants with *Agrobacterium tumerfecians* containing chitinase gene for transformation.
- ✓ To standardize *in vitro* plant regeneration of transgenic explants from direct and indirect organogenesis.
- ✓ To standardize a protocol for somatic embryogenesis from transgenic immature leaf explants.
- ✓ To analyse chitinase enzyme in control and transgenic Safflower leaves.
- ✓ To confirm the putative transgenic by PCR and Southern Blotting Techniques.
- ✓ Field testing of the transgenic plants for the disease resistance by using the challenging inoculum.