Tissue and organ culture studies in the family Malvaceae centre around cotton, *Gossypium* spp. In cotton six categories of plant tissue culture have been reported (Price and Smith, 1984) - callus, shoot-tip, anther, ovule-embryo, protoplast as well as somatic embryogenesis. The intentions of these investigations were varied. Workers involved with callus cultures ultimately wanted to initiate suspension cultures, obtain somatic embryos and, isolate and fuse protoplast to form somatic hybrids with subsequent plant regeneration. Shoot-tip cultures were studied to develop protocols for virus-free, mass plant propagation. Ovule cultures were examined to gain basic information on fibre development and ultimately a better understanding of fibre-yield and quality. Embryo cultures have been studied to examine embryo rescue techniques to obtain hybrids from embryos which normally abort. Anther cultures was pursued to produce haploid and/or homozygous diploids for breeding purposes and mutagenesis studies.

Besides cotton, there are few reports of favourable responses from other genera like *Abelmoschus*, *Abutilon*, *Althea*, *Hibiscus*, *Malva*, *Malvaviscus*, *Malvastrum* and *Sida*.

**ABELMOSCHUS** Medik.

Normal seedlings were produced from ovule (Bajaj, 1964) and embryo (Patil, 1966) cultures of *Abelmoschus esculentus*. 
Gadwal et al. (1968) obtained interspecific hybrids of four species of *Abelmoschus* through ovule and embryo culture. Somatic embryos produced on callused hypocotyl explants of *A. esculentus* (Blackmon et al., 1981; Reynold et al., 1981) did not show further growth and development. Mangat and Roy (1986) reported plantlet regeneration from in-vitro cultures of *A. esculentus*. Shoots were produced on cotyledon and cotyledonary node explants. The plantlets, after root formation and transfer to soil grew normally.

**ABUTILON MILL.**


**ALTHEA Cav.**

Chopra (1958, 1960) successfully cultured pollinated ovaries of *A. rosea* and produced mature fruits. Reynold et al. (1981) obtained somatic embryos from hypocotyl-derived callus while Lawrence et al. (1982) reported embryoids from callus cultures of *A. rosea*. 
GOSSYPIUM L.

Most of the available literature concerns ovule culture which has been extensively studies by Beasley and his coworkers (Beasley 1971, 1973, 1974; Beasley and Eaks, 1979; Beasley and Ting, 1971, 1973, 1974; Beasley et al., 1971, 1974).

OVULE CULTURE

In vitro studies on cotton ovules have served two purpose. Firstly, they have been valuable in providing information on the physiology of fibre development and second, in obtaining interspecific hybrids.

Callus initiation from culture tissue explants or the micropylar region of cultured ovules from G. hirsutum was first reported by Beasley (1971). This was the beginning of several studies by Beasley to determine the physiological role of growth regulators on fibre development in fertilized (Beasley and Ting, 1974), and unfertilized (Beasley et al., 1974) cotton ovules in culture. Other investigators have also contributed to in-vitro studies on cotton ovules (Joshi 1960; Joshi and Pundir, 1966; Joshi and Johri 1972; Eid et al., 1973; Hsu and Stewart, 1976; Stewart and Hau 1977, 1978, 1979; Pallares, 1984; Gill and Bajaj, 1984a, 1987).
Joshi (1960) could not obtain fiber development in ovules and their growth was for the most part abnormal with callus formation from the outer integument. The proembryos showed very little growth although in some cases cotyledons were differentiated. Joshi and Pundir (1966) reported retarded seed growth leading to abnormalities in crosses between *G. arboreum* and *G. hirsutum*. Joshi and Johri (1972) reported cleavage of the proembryo in cultures of cotton ovule. Eid et al. (1973) obtained normal seedlings from 5 to 10 day-old ovules cultured on MS basal medium without any growth regulators. Friable callus from the micropylar region of *G. hirsutum* ovules was reported by Hsu and Stewart (1976) while studying growth and development of ovules. The presence of 2-chloroethyl phosphonic acid (CEPA) affected cell division and expansion in developing callus. Further studies by Stewart and Hsu (1977) revealed that callus growth in greatly stimulated by the combination of CEPA and GA3. The valuable information provided by Beasley and his co-workers was used by Stewart and Hsu (1977,1978,1979) in studies on in-ovulo embryo culture to obtain interspecific hybrids. Pallares (1984) cultured unfertilized cotton ovules taken along with the ovary excised within 2 days of anthesis. They produced few fibres, but callus developed from their external integument which showed various morphogenetic formations. Gill and Bajaj (1984a,1987) successfully produced interspecific hybrids of different
species of cotton through ovule culture. Song and Shen (1988) produced callus from ovules, of both unfertilized and fertilized, of 21 geneotypes of *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum*. Zhang et al. (1988) obtained plant regeneration from cultured ovules of *G. hirsutum* and of the cross *G. hirsutum* × *G. arboreum*.

**EMBRYO CULTURE**

Lofland (1950) successfully cultured 20 to 27 day-old excised embryos on White's medium supplemented with various growth adjuvants. 27-day-old embryo produced normal seedlings while younger embryos were slow growing and produced gnarled, twisted seedlings. However, embryos excised earlier than 15 days did not undergo further development. Dure and Jensen (1957) showed that young embryos with fresh weight of 37 mg did not have the capacity to divide or elongate either with or without IAA and GA3. Older embryos (64 mg fresh weight) underwent cell division and elongation. Both these processes were inhibited by IAA and enhanced by GA3 with the combination producing intermediate response. Hybrid embryos of *G. arboreum* × *G. hirsutum* excised 20 days after pollination were successfully cultured to maturity by Weaver (1958). Mauney et al. (1967) cultured young embryos (12-14 day old) at the heart stage of development with upto 75% survival on the Mauney et al. (1967) medium. Brar and Sandhu (1984) obtained
fast growing seedlings from cotton embryos cultured in vitro. Gill and Bajaj (1984b) reported interspecific hybrids among diploid cultivated and wild species of cotton by culturing immature hybrid embryos excised 15 days after pollination. Azizkhodzhaev and Uinarov (1985) observed that normal growth of young embryos (10 to 30 day-old) of cotton was seen only in the presence of 40 mg/l myo-inositol in Beasley and Ting (1973) medium. Gill and Bajaj (1986) obtained hybrid plants through in-vitro culture of embryos from crosses between cultivated and wild Gossypium species.

CALLUS CULTURE

Callus cultures have been successfully obtained from various explants-mesocotyl (Schenk and Hildebrandt, 1972), leaf (Davis et al., 1974; Smith et al., 1977; Finer and Smith 1984; Lev et al., 1986), stem (Rani and Bhojwani 1976; Finer and Smith, 1984; Lev et al., 1986), petiole (Finer and Smith, 1984; Lev et al., 1986), cotyledon (Katterman et al., 1977; Smith et al., 1977; Finer 1988) and hypocotyl (Rani and Bhojwani, 1976; Smith et al., 1977; Price et al., 1977; Price and Smith 1977; Lev et al., 1986; Shoemaker et al., 1986,1987; Trolinder and Goodin, 1987,1988).

Schenk and Hildebrandt (1972) were successful in establishing friable callus cultures from G.hirsutum mesocotyl explants. Similarly suspension cultures have been
established from leaf callus by Davis et al. (1974). Rani and Bhojwani (1976) obtained slow-growing friable callus from stem and hypocotyl explants. Katterman et al. (1977) used a strong antioxidant, dithiothreitol (DDT) and high concentrations of NAA to initiate a compact callus from cotyledons of \textit{G. barbadense}. They observed root formation and regeneration of one plantlet. Smith et al. (1977) defined conditions for the initiation and sub-culture of hypocotyl derived callus of \textit{G. arboreum}. These workers regenerated one plantlet from hypocotyl explants of six species of cotton. Price and Smith (1979) obtained vigorously growing friable callus from hypocotyl of \textit{G. klotzschianum}. Finer and Smith (1984) obtained rapidly growing, friable, green callus from stem and petiole segments of \textit{G. klotzschianum}. Lev et al., (1986) cultured vegetative explants of twelve species of cotton and reported embryogenic callus in three species. In \textit{G. hirsutum}, the embryogenic potential was retained over a long period of culture. Lui et al. (1986) obtained embryogenic callus from different seedling parts of three species of cotton. Shoemaker et al. (1986) induced embryogenic callus from hypocotyl explants of \textit{G. hirsutum}. Abdukarimov et al. (1987) regenerated plantlets for the first time from callus cultures of the wild cotton, \textit{G. klotzschianum}. Zimmerman and Rubaker (1988) obtained more callus formation on media modified with gelrite than with agar. They observed that the best medium for callus
initiation and proliferation, in the diverse group of varieties they used, was MS and LS with 30g/l glucose, 0.1 mg/l BA and 2,4-D and solidified with 2g/l gelrite.

SHOOT TIP CULTURE

Shoot tip cultures were initiated by Chappell and Mauney (1967) to determine the nutritional requirements for normal growth and development of apical meristems of G.hirsutum. New leaves, were obtained from the meristematic tissues of the culture apical meristems without further growth and development. Smith (unpublished cited in Price and Smith 1984) cultured axillary buds from mature cotton, which developed into shoots at a frequency of 20-40%.

ANTHER CULTURE

Callus formation in anther cultures of cotton has been reported by a number of workers (Iyer et al., 1974; Barrow et al., 1978; Bajaj, 1982; Barrow, 1986; Shamina et al., 1986; Taraev and Shamina, 1986). Barrow (1978) obtained a mixture of haploid and diploid callus from anther culture of *G. barbadense* and *G. hirsutum*. Roots differentiated from the callus with increase in light intensity.

PROTOPLAST CULTURE

Bhojwani et al. (1977) isolated protoplasts from hypocotyl derived callus cells. The protoplasts regenerated walls, divided and subsequently formed colonies. Khasanov and Butenko (1979) used cotyledonary tissue of *G. hirsutum* for protoplast isolation. Though cells, were regenerated from the isolated protoplasts, no further growth was observed. Finer and Smith (1982) isolated and cultured protoplasts, from hypocotyl-derived callus of *G. klotzschianum*. The protoplasts regenerated cell walls and formed macroscopic colonies. Thomas and Katterman (1984) could obtain macroscopic callus colonies from anther callus derived protoplasts of *G. hirsutum*. Firoozabady and De Buer (1986) observed cell division in protoplasts isolated from cotyledons of *G. hirsutum* and *G. barbadense*. Gould et al. (1986) obtained cell wall formation in cultured protoplasts isolated from epidermal cells of cotton ovule. Saka et al. (1987) isolated protoplasts from 12-day old sub-
cultured phytohormone habituated callus tissue of G. hirsutum. Cell wall regeneration and callus formation was observed after four weeks.

SOMATIC EMBRYOGENESIS

Price and Smith (1979) reported somatic embryogenesis in suspension cultures of G. klotzschianum. Embryogenesis occurred after a precultured of callus on a medium containing 10mg/l of the cytokinin 2ip. Somatic embryoids differentiated in suspension cultures after 3-4 weeks of culture in a liquid medium containing glutamine (10-15 M). Davidonis and Hamilton (1983) described for the first time somatic embryogenesis and plant regeneration in cotyledon-derived cultures of G. hirsutum. Finer and Smith (1984) obtained somatic embryos in suspension from stem and petiole-derived callus of G. klotzschianum. Most embryos did not show further maturation while some developed abnormal leaves and shoots. Since the first reports there have been numerous reports on cotton regeneration via somatic embryos. These include induction of embryogenesis from leaf (Lui et al., 1986), hypocotyl (Voo et al., 1987) leaf and petiole (Gawel et al., 1986), hypocotyl, leaf, petiole and stem (Lev et al., 1986); development of a liquid suspension culture (Trolinder and Goodin, 1987); optimization of initiation of embryogenesis (Trolinder and Goodin, 1988a,b); characterization of embryogenesis (Shoemaker et al., 1986); initiation, proliferation and development of somatic embryo
(Finer 1988). Trolinder and Chen (1989) studied the genotypic specificity of the somatic embryogenesis response in cotton. 38 genotypes form 3 species (36 of G. barbadense, 1 each of G. arboreum and G. hirsutum) were screened for embryogenesis under four induction systems. Screening of individual seedlings with a cultivar indicated that genotype variation of embryogenises existed. Highly embryogenic individuals were selected from the cultivars for use as germplasm sources for transfer of the embryogenic trait to other cultivars and genetic stocks.

Umbeck et al. (1987) have reported regeneration of genetically transformed cotton plants.

HIBISCUS L.

Davis et al. (1974) first reported callus initiation and cell suspension cultures of Hibiscus esculentus. Kuwada and Mabuchi (1976) cultured embryos and ovules of H. cannabinus and H. sabdariffa which differentiated into plantlets. Adamson and O'Bryan (1981) regenerated plantlets from embryo derived callus of H. hiernianus but could not repeat their experiments successfully. Blackmon and Reynolds (1982) obtained shoot regeneration from cotyledon and primary leaf explants of H. acetosella. Although a little root formation was observed in some cases complete plantlet regeneration was not obtained. Thulajappa (1982) reported
organogenetic callus from vegetative explants of *Hibiscus "Benazeer"*. The callus produced roots, early stages of embryoids and shoot buds.

Somatic embryos have been reported from callus, derived from various explants of *H.acetosella* (Reynolds et al. 1980; Blackmon et al., 1981; Reynolds et al., 1981; Reynolds and Blackmon, 1983). Reynolds and Blackmon (1983) regenerated plantlets by germinating somatic embryos obtained from leaf and root-derived callus of *H.acetosella*. They have developed an in-vitro system for induction, proliferation, maintenance, and germination of somatic embryos of *H.acetosella* into plantlets in large numbers.

**MALVA L.**

Yano et al. (1976) obtained callus from cultured cotyledons of *M.parviflora* and *M.sylvestris*. Orisini (1987) has reported somatic embryogenesis and plantlet regeneration in *M.parviflora*.

**MALVASTRUM A.Gray**

Callus cultures of *M.coromandelianum* was generated from stem explants by Minakshi Sethi (1976).
MALAVISCUS Adams.

Thulajappa (1982) obtained organogenetic callus from different explants of *M. arboreus*. There was no shoot regeneration, but root formation was seen in some cases.

SIDA L.

Nataraja and Patil (1980) produced organogenetic callus from cotyledon, of *S. acuta* and stem and leaf explants of *S. rhombifolia*. Minakshi Sethi (1976) reported callus from stem explants, while Rangaswamy et al. (1980) reported callus from the nucellus of *S. rhombifolia*. Organogenetic callus derived from various vegetative explants of *S. cordifolia* was reported by Thulajappa (1982).

PRESENT INVESTIGATION

Kenaf and roselle being important crop plants, some of their traits deserve genetic improvement. The application of biotechnological approach for plant improvement being easier in crops that are amenable to in vitro regeneration, the present investigation is an attempt to develop a protocol for in vitro regeneration of kenaf and roselle. In addition to kenaf and roselle, five ornamental varieties of *Hibiscus rosa-sinensis* were also studied in vitro. In this species stem cuttings can maintain the genotype but the rate at
which new plants can be produced in slow and pathological infection is often a problem. Since tissue culture methodologies can be used to avoid these problems the development of an organ tissue culture technique to mass produce superior plants is necessary.

With a view of developing a protocol for in vitro plantlet regeneration and micropropagation, tissue culture studies were conducted using the available methods viz., callus, suspension, embryo, anther, shoot-tip and nodal cultures.