INTRODUCTION
AND
REVIEW OF LITERATURE
INTRODUCTION

Mankind today is facing with a number of problems related to the availability and quality of food, fuel, fibre and other crops. These urgently need attention in the areas of agriculture and forestry. Established appropriate tissue culture methods for the clonal propagation of some of the species, have been attempted, however for others serious difficulties still remain. In general, work on economic and food crops is rather limited, as compared with model systems such as carrot or tobbaco. Woody plants of which our knowledge is particularly poor, fall into the neglected category. (Rao and Lee, 1986).

*Ceratonia siliqua* L. (Family - Leguminosae, S.family - Caesalpineae) known as Carob is a handsome evergreen tree. It is a native of mediterranean and southern Europe. It was introduced into India and has become naturalised in Punjab and few other regions. The plant is economically important for its wood, fodder, pods and seeds. Wood is hard, heavy and used for cabinet making. Pods being rich in sugar are used in the manufacture of sugar used in confectionery. Seeds being rich in proteins are used as cattle feed. Seeds yield a valuable gum known as carob gum. It is used in pharmaceuticals as a sweetening agent, thickener, stabiliser and in cosmetic preparations. The trees are known to grow even on dry, stony or calcareous soil. (Wealth of India, vol. II C, 1950). It is one of the tree species widely used in tropical and subtropical agroforestry systems. It is also used in siliopastoral systems, in antierosion hedges and as windbreaks. (Nair, 1993). Plant is dioecious. It is usually grown from seeds. It is difficult to raise the plant from cuttings and requires bottom heat and careful treatment.
REVIEW OF LITERATURE

Tissues from woody plants have been cultured since nineteen thirties. Progress has been made in the culture of tissues, organs, cells and protoplasts of woody plants. However though some woody plants including forest trees have been induced to grow and differentiate in vitro, others still appear to be recalcitrant. By employing juvenile tissues, clonal propagation has been achieved in a large number of woody plant species. On the other hand only in a handful of tree species have mature trees been clonally propagated by tissue culture technology. (Ahuja, 1993).

In the pioneering work of Gautheret (1938) the cambial tissues of tree species were found to be highly responsive, giving rise to callus. Fully organized plantlets of tree species, propagated in vitro, capable of subsequent transfer to soil were first obtained in Populus tremuloides (Winton, 1978).

Direct or indirect rhizogenesis, caulogenesis and embryogenesis have been reported from embryo or very young seedlings or mature tree explants.

Tissue culture of forest trees includes work on Hevea brasiliensis, (H.B.K.) Muell. Arg. (Muzik, 1956); Ilex aquifolium, (Hu and Sussex, 1971); Santalum album, Linn (Rao and Rangaswamy, 1970, Rao and Bapat, 1978, 1993); Bambusa arundinacea, Willd. (Mehta et al., 1982); Acacia koa, Gray (Skoleman and Mapes, 1976); Eucalyptus citriodora, (Muralidharan et al., 1989); Tectona grandis, L. (Lakshmi Sita and Chattopadhyay, 1986), and Populus ciliata, Wall. ex Royle (Mehra and Cheema 1980).

In vitro culture and micropropagation of economically important tree species have been reviewed: Sandal wood and Mulberry (Rao and Bapat, 1993), Teak (Mascarenhas et al., 1993), Eucalyptus (Lakshmi Sita, 1993), Leucaena (Nagmani and Venketeswaran, 1987),
Tamarindus indica L. (Mascarenhas et al., 1987) and legumes (Davey et al., 1994).

Uptil now significant advances have been made in regenerating plants from various tissues of legume trees. Following is a review of reports on in vitro responses of mature tree, seedling - seed explants obtained from tree species of Leguminosae.

Axillary bud proliferation has been reported from nodal segments in Leucaena leucocephala (Lam) de Wit. (Datta and Datta, 1985), Bauhinia variegata, L. and Parkinsonia aculeata, L. (Mathur and Munthakumar, 1992), Caesalpinia pulcherrima, Sw. (Rahman et al., 1993) and Acacia mangium, Wild. (Bhaskar and Subhash, 1996).

Direct organogenesis has been reported from stem segments in Acacia nilotica (Linn.) Willd ex. Delile (Mathur and Chandra, 1983). Indirect organogenesis has been reported from young nodal and internodal explants in Dalbergia latifolia, Roxb. (Lakshmi Sita et al., 1986), juvenile shoot segments of mature tree in D. latifolia, Roxb. (Rai and Chandra, 1988) and Bauhinia purpurea L. (Anjani Kumar, 1992), leaf discs in D. latifolia, Roxb. (Lakshmi Sita and Raghava Swamy, 1993); leaf explants in Sesbania bispinosa (Jacq.) W. F. Wight. (Sinha and Mallick, 1991) and cambial layers in Dalbergia sissoo, Roxb. (Kumar, et al., 1991).

In majority of the legumes, plantlet regeneration has been obtained from seed or seedling explants.

Direct organogenesis from hypocotyl explants has been reported in Tamarindus indica, L. (Mascarenhas et al., 1987), A. richardiana, King. (Tomar and Gupta, 1988a), and Albizia amara, Boivin., A. richardiana, King., A. lucida, Benth., (Tomar and Gupta, 1988b).

Indirect organogenesis from hypocotyl has been reported in Albizzia odoratissima, Benth. (Phukan and Mitra, 1983) and A. lebbek, L. (Rao and De., 1987).
Direct and indirect organogenesis from hypocotyl has been reported in *Sesbania aculeata*, Pers. (Bansal and Pandey, 1993).

Direct organogenesis from cotyledon explants has been reported in *Tamarindus indica*, L. (Jaiwal and Gulati, 1991); *Albizia falcata*, L. (Sinha and Mallick, 1993) and *Sesbania grandiflora*, (L.) Pers. (Detrez et al., 1994).

Indirect organogenesis from cotyledon has been reported in *Leucaena retusa* (Naamani and Venketeswaran, 1987) and *Sesbania bispinosa*, (Jacq.) W. F. Wight. (Sinha and Mallick, 1991).

Direct organogenesis from both hypocotyl and cotyledon explants has been reported in *Sesbania bispinosa*, (Jacq.) W. F. Wight., (Kapoor and Gupta, 1986) and *Prosopis tamarugo*, Phil. (Nandawani and Ramawat, 1992).

Indirect organogenesis from both hypocotyl and cotyledon explants has been reported in *Sesbania grandiflora* (L.) Pers. (Khattar and Mohan Ram, 1983) and *Leucaena leucocephala* (Lam.) de Wit. (Nagmani and Venketeswaran, 1987).

Direct as well as indirect organogenesis from root hypocotyl and cotyledon explants has been reported in *Sesbania bispinosa*, (Jacq.) W. F. Wight., *S. sesban*, Merr., *S. cannabina*, (Retz.) Pers., *S. formosa*, F. Muell., (Zhao et al., 1993) and *Dalbergia lanceolata* (Dwari and Chand, 1996).

From aseptic seedlings, axillary bud proliferation, from nodes in *Acacia auriculiformis*, (A. Cunn.) Benth. (Mittal et al., 1989). *Mimosa tenuiflora*, (Willd.), Poiret. (Villasreal and Rojas, 1996), from cotyledonary node in *Acacia nilotica* subsp. indica Brenan (Dewan et al., 1992), *Swartzia madagascariensis* Desv. (Berger and Schaffner, 1995), and *Bauhinia vahlii*, Wight and Arnott (Upreti and Dhar, 1996); from shoot tip in *Albizia odoratissima*, Benth. (Phukan and Mitra, 1983) and *Tamarindus indica*, L. (Mascarenhas et al., 1987), from nodes and shoot tips in *Prosopis chilensis*, (Mol.) Stuntz. *P. alba* and
P. tamarugo, Phil., (Jordan, 1987); and indirect organogenesis from leaf and stem in Albizzia lebbeck, L. Benth., (Rao and De, 1987), has been reported.

Organogenesis, direct or indirect, has been reported from root explants in Dalbergia sissoo Roxb. (Mukhopadhyay and Mohan Ram, 1981).

Somatic embryogenesis in angiosperms has been reviewed by Raghavan, (1986); Rangaswamy, (1986) and Narayanswamy (1994) and in legumes by Parrott et al., (1995).

Direct somatic embryogenesis has been reported from hypocotyl explants in Albizzia lebbeck, L. (Gharyal and Maheshwari, 1981) and Robinia pseudoacacia L. (Arillaga, et al., 1994), from ovules in Cercis canadensis, L. (Trigiano et al., 1988); and from immature cotyledons in Dalbergia latifolia, Roxb. (Muralidhar Rao and Lakshmi Sita, 1996).

Indirect somatic embryogenesis has been reported from hypocotyl in A. richardiana, King. (Tomar and Gupta, 1988a).

It has been reported that in B. variegata, L. 13.3 μM BA and in P. aculeata, L. 8.9 μM BA, induced axillary bud proliferation (Mathur and Mukunthakumar, 1992).

It has been reported that multiple shoots developed from nodes on medium with 3.0 mg/l BA and 0.1 mg/l NAA in A. mangium, Wild., (Bhaskar and Subhash, 1996) and on medium with 2.0 mg/l BA, in L. leucocephala (Lam.) de Wit. In the later a single vigorous shoot with a large number of long roots developed on medium with IAA. (Datta and Datta, 1985).

In C. pulcherrima, Sw., it has been reported that, with 2,4-D or NAA only callus developed from nodes where as with 4.5 μM BA and 5.5 μM NAA both callus and shoots developed. (Rahman et al., 1993).

In A. nilotica (Linn.) Willd ex Delile, 0.5 to 1.0 mg/l IAA alone, was reported to induce shoots and roots from opposite ends of stem segments. (Mathur and Chandra, 1983).
In *D. latifolia*, Roxb., inductive effect of auxins 2,4-D or NAA alone or in combination with BA or CM, for callusing from both nodes and internodes has been reported. Best callus formation occurred when 2,4-D, NAA, BA and CM were combined. Shoot buds developed from callus on 3.0 mg/l BA and 1.0 mg/l NAA. (Lakshmi Sita et al., 1986). In the same species, Rai and Chandra, (1988) reported that 1.0 mg/l each BA and NAA induced callusing from shoot segments and shoot buds developed on subculture of callus to 5.0 mg/l NAA.

In *B. purpurea*, L. Anjani Kumar, (1992), reported development of callus from stem segments, with 10uM 2,4-D. On subculture of callus caulogenesis and rhizogenesis could be induced on media with 5uM Kn and 5uM NAA respectively.

In *D. latifolia*, Roxb., 2,4-D, NAA, BA and CM together induced compact, nodular callus from leaf discs. Shoot buds and shoots developed from the callus on three fourth MS or WPM with 5.0 mg/l BA and 0.5 mg/l NAA. (Lakshmi Sita and Raghava Swamy, 1993).

In *S. bispinosa*, (Jacq.) W. F. Wight., 2.0 mg/l 2,4-D and 0.5 mg/l BA, induced callus from leaves. Initiation and development of shoot buds occured from callus on subculture to 2.0 mg/l BA or Kn and 15% CM. BA, being more effective than Kn. (Sinha and Mallick, 1991).

In *D. sissoo*, Roxb., Kumar et al., (1991) have reported that, 2.0 mg/l 2,4-D and 0.1 mg/l BA, induced callus from cambial layers. Shoot buds developed from callus, raised from cell suspension, on 1.0 mg/l BA and roots developed on 1.0 mg/l IAA.

In *I. indica*, L., direct organogenesis from hypocotyl, nodes and shoot tips on the medium with 0.2 mg/l Kn, 0.5 mg/l BA, 0.1 mg/l Biotin and 0.1mg/l Cal p has been reported. Cotyledons and roots produced only callus. (Mascarenhas et al., 1987).

In *A. amara*, Boivin., *A. richardiana*, King, and *A. lucida*, Benth., hypocotyl showed direct organogenesis, on B5 supplemented with BA, NAA, IAA or 2,4-D. BA 1.0uM enhanced differentiation of shoots as
compared to NAA, IAA or 2,4-D. On medium supplemented with IAA, shoot buds and roots differentiated from opposite ends. In *A. richardiana*, King., direct shoot bud development has been reported on B₅ alone or with 10⁻⁶ M BA. (Tomar and Gupta, 1988 a, b).

In *A. odoratissima*, Benth., IAA, IBA, NAA or 2,4-D each with BA or Kn, induced callusing from hypocotyl and cotyledon. Callus, from hypocotyl, on 2.0 mg/l NAA and 1.0 mg/l BA, developed shoot buds on subculture to medium with 0.5 mg/l NAA and 4.0 mg/l BA. On medium with 1.0 mg/l BA or 1.0 - 2.5 mg/l Kn, shoots developed directly from seedling shoot tips. Rhizogenesis could be induced on hypocotyl callus on medium containing 2 mg/l IBA and 1 mg/l Kn. (Phukan and Mitra, 1983).

In *A. lebbek*, L., on modified MS supplemented with 1.0 mg/l NAA and Kn, callus developed from hypocotyl. On transfer to 1.0 mg/l BA, shoots were induced. Callus developed from both leaf and stem on 0.5 - 1.0 mg/l 2,4-D with 1.0 mg/l Kn. The callus developed shoots on transfer to medium with 5.0 mg/l BA. (Rao and De, 1987).

In *S. aculeata*, Pers. auxins NAA, IBA or 2,4-D induced callus from hypocotyl. Low concentration of NAA and a range of concentration of 2,4-D induced rooting from the callus. Shoots developed from callus on medium with 0.1 mg/l 2,4-D with 1.0 mg/l NAA, 5.0 mg/l IBA and 1.0 - 10.0 mg/l BA. Shoots and roots developed directly from hypocotyl on 0.1 mg/l NAA and 2,4-D with 0.1 - 5.0 mg/l BA and on medium with 0.1 mg/l with 0.1 mg/l NAA or 0.5 mg/l BA respectively. (Bansal and Pandey, 1993).

In *I. indica*, L., Jaiwal and Gulati (1991), reported direct shoot buds differentiation from cotyledon, with 5 x 10⁻⁶ M BA. BA was most effective than Kn or 2-ip or AdS. For root induction 5.7 x 10⁻⁶ M IAA, was reported to be effective.

In *A. falcata*, L. 4.4 - 8.9uM BA with 15% (v/v)CM at 0.4% agar; induced maximum shoot buds from cotyledon (Sinha and Mallick 1993).
In *S. grandiflora* (L.) Pers., direct shoot bud induction was reported from cotyledon, with 1.0 mg/l NAA and BA. These elongated on addition of GA3. (Detrez et al., 1994).

In *L. retusa*, 0.5 mg/l NAA and 1.0-2.0 mg/l BA, induced callus on cotyledon. Shoots developed from callus on transfer to 1.0 mg/l 2-ip, Nagmani and Venketeswaran, (1987).

In *S. bispinosa* (Jacq.) W.F. Wight., 2,4-D and /or BA, induced callus on cotyledon. 2.0 mg/l BA with 15\% (v/v) CM, induced shoot buds from callus. Shoots rooted with 2.0 mg/l IBA. (Sinha and Mallick, 1991). In the same species, hypocotyl and cotyledon showed direct differentiation of multiple shoots, on B5 alone or with 10^{-7} to 10^{-4} M BA, and with 10^{-5} M BA, respectively. (Kapoor and Gupta, 1986).

In *P. tamarugo*, Phil., hypocotyl and cotyledon, developed callus on medium with NAA and BA. 5.0 mg/l BA, induced shoot buds on hypocotyl and embryonal axis. (Nandawani and Ramawat, 1992).

In *S. grandiflora* (L.) Pers., on B5 with 10^{-7} to 10^{-5} M BA, alone or with 10^{-7} to 10^{-8} M NAA and / or GA3, callus and shoot buds were induced from hypocotyl and cotyledon. On B5 alone shoot buds elongated (Khattar and Mohan Ram, 1983).

In *L. leucocephala* (Lam.) de Wit., it has been reported by Nagmani and Venketeswaran, (1987) that, with 0.5 mg/l NAA and 0.5 to 5.0 mg/l BA, callus developed on hypocotyl and cotyledon. Shoot buds developed on transfer to medium containing 2.0 mg/l BA.

In *S. bispinosa* (Jacq.) W.F. Wight, *S. sesban*, Merr., *S. cannabina*, (Retz.) Pers. And *S. formosa*, F. Muell., 2.22 to 8.8\mu M BA along with 2.2 to 9.05\mu M 2,4-D or 0.25 to 4.92\mu M IBA or 2.69 to 10.74 \mu M NAA induced callus on root, hypocotyl and cotyledon. 4.44 to 8.88 \mu M BA with 0.25 to 4.92 \mu M IBA induced shoot buds from hypocotyl and cotyledon, both directly and via callus. (Zhao et al. 1993).

In *A. auriculiformis*, (A.Cunn.) Benth., callus was formed on B5 with 10\%(v/v) CM, from hypocotyl and cotyledon. With 5-10\%(v/v)
CM and $10^{-6}$ M BA, axillary bud of seedling proliferated into multiple shoots. (Mittal et al., 1989).

In *D. lanceolaria*, L. Dwari and Chand (1996) reported direct and indirect organogenesis from both hypocotyl and cotyledon, on 0.5 mg/l NAA and 2.0 mg/l BA.

In *M. tenuiflora* (Willd.) Poiret., on medium with 0.1 mg/l IAA and 3.0 mg/l Kn, axillary bud of seedling developed multiple shoots. 0.1 mg/l Kn, alone induced rooting. (Villarreal and Rojas, 1996).

In *A. nilotica*, subsp. Indica Brenan., B5 with 0.1 to 3.0 mg/l BA or Kn or zeatin or 2-ip, induced multiple shoots from cotyledonary node, of which 1.5 mg/l BA was best. (Dewan et al., 1992).

In *D. sissoo*, Roxb., shoot buds developed from seedling root explants, on B5 with $10^{-7}$ M NAA, or with BA and NAA or IAA either directly or after slight callusing. (Mukhopadhyay and Mohan Ram, 1981).

In *P. chilensis*, (Mol.) Stuntz. *P. alba*, and *P. tamarugo*, Phil., both nodes and shoot tips produced shoots on MS with 1.0-10.0 mg/l NAA with 0.1 to 1.0 mg/l Kn, directly and via callus. (Jordan, 1987).

In *A. lebbeck*, L. direct somatic embryogenesis took place from hypocotyl on B5 basal medium. Embryos germinated into plantlets on same medium. (Gharyal and Maheshwari, 1981).

In *A. richardiana*, King., callus was induced on B5 with $10^{-7}$ to $10^{-5}$ M BA, from hypocotyl. Somatic embryos developed on transfer to MS with $10^{-5}$ M BA. Embryos germinated on MS with 2% sucrose. (Tomar and Gupta, 1988a).

In *C. canadensis*, L. somatic embryos developed from seed embryo on modified Schenk and Hildebrandt (SH) medium with 2.0-3.0 mg/l 2,4-D. These developed into shoots on 1.0 mg/l NAA with 0.1 mg/l Kn. NAA alone or with BA, was reported to be ineffective. (Trigiano et al., 1988).

In *R. pseudoacacia*, somatic embryos were induced from hypocotyl and cotyledon on modified Finer and Nagasawa medium containing
45 μM 2,4-D and 2.2 μM BA. Embryos developed on transfer to same medium without growth regulators. (Arillaga et al., 1994).

In D. latifolia, Roxb., somatic embryos developed directly on transfer of immature cotyledons from high (10.0 mg/l) 2,4-D medium to low (0.5 mg/l) 2,4-D medium with 10% sucrose. Embryos matured and developed into plantlets on medium with BA. (Muralidhar and Lakshmi Sita, 1996)

Ceratonia siliqua, L. is a dioecious plant. There is no obvious morphological difference in male and female plants in vegetative condition. Plants take about fifteen years to mature, flower and fruit. When the carob plants are raised through seeds, the sexes can be differentiated only after plants mature and start producing flowers. As both male and female plants have economic importance, it is advantageous to selectively multiply plants of either sex through micropropagation which involves direct or indirect organogenesis and or embryogenesis.

Martins - Loucao and Rodriguez - Barrueco (1981) first successfully established the carob callus from seedling explants. Attempts to obtain callus from mature plant organs were unsuccessful. Thomas and Mehta (1983) and Mehta and Thomas (1989), reported the effect of phenolic substances on growth and morphogenic responses of carob cultures. Micropropagation technique has been applied by Sebastian and McComb (1986) as an attempt to multiply and propagate carob plants, using seedling explants and mature tree nodes on the medium containing zeatin.

Martins - Loucao (1990), has reviewed tissue culture work on Ceratonia. Shoots developed from shoot tips on medium containing MS salts with 2.0 mg/l BA and 1.0 mg/l NAA. Best callus initiation and shoot formation occurred on hypocotyl explants on medium with 2.0 mg/l BA and 1.0 mg/l NAA. Roots differentiated from cotyledon on medium with 2.0 mg/l IAA.
OBJECTIVES OF OUR WORK WERE:

a) to study the morphogenetic potential of the various explants obtained from mature tree(s), in vitro plants, seeds and seedlings of C. siliqua L.
b) to induce organogenesis and/or embryogenesis on these explants
c) to correlate the response(s) with respect to morphology, age, developmental and biochemical status of the explant, season of explant collection and media composition.

The work presented in the thesis is divided into following chapters:

1. Introduction and review of literature
2. Materials and methods
3. Observations and Results
4. Summary and Conclusion
5. Discussion