REVIEW OF LITERATURE

The concept of cell theory of Schleiden (1838) and Schwann (1839) forms the basis of plant cell, tissue and organ culture. Haberlandt (1902) for the first time attempted to cultivate the isolated plant cells *in vitro* and this has led to many important discoveries such as somatic embryogenesis, somaclonal variation, protoplast fusion, genetic manipulation, molecular biology of plant genes (Bajaj 1977) and also has been commercially exploited (Murashige 1974, Reinert and Bajaj 1977, Yeoman 1986). The concept of cell totipotency was reported by Krikorian (1982) which supports the view of Haberlandt (1902).

The success in micropropagation mainly depends on the discovery of auxins (Kogl *et al*. 1934) and cytokinins. The idea of synergistic effects of auxins and cytokinins in promoting cell division in tobacco tissue cultures triggered the imagination of physiologists (Skoog and Miller 1957). The discovery of first auxin IAA and cytokinin, kinetin created the opportunity for the *in vitro* propagation of higher plants. The success of tissue culture techniques with herbaceous taxa has led to apply the same to woody plants (Zimmerman 1986). Woody plants have generally been considered recalcitrant towards micropropagation techniques, despite notable early success with *Populus tremuloides* (Winton 1970). In spite of constraints encountered with woody systems, *in vitro* techniques have been successfully applied for array of perennial woody ornamentals. The following
literature review summarizes the *in vitro* propagation, germplasm preservation of ornamental and woody climbers.

Micropropagation of *Bougainvillea glabra* ‘Magnifica’ has been achieved by Chaturvedi *et al.* (1978) by shoot apex culture. Multiple shoots were induced on MS medium with BA (1.5 mg/l) and IAA (1.5 mg/l). The shoots could root on liquid basal MS medium comprising only the inorganic salts and the regenerated plants were successfully transplanted to sterilized pots containing soil and compost in 3:1 ratio.

Tideman and Hawker (1981) have successfully regenerated shoots from nodal, internodal and leaf explants of *Araujia serecifera*. Direct shoots were formed from internodal explants on MS medium with BAP (2.5 μM), from nodal explants on medium with BAP (10.0 μM) as well as MS half strength medium supplemented with (BAP 2.5 μM) or BAP (10.0 μM). Directs shoots were resulted from seedling leaf explants on MS medium with BAP (5.0 μM) or BAP (10.0 μM). Shoots resulted from all the explants could be rooted on half strength MS medium with NAA (0.5 μM). The rooted shoots were transferred to well drained pots containing course sand, peat and perlite (6:3:1 ratio) mixture for hardening and finally transferred to soil in the glass house.

Regeneration of multiple shoots from shoot tip explants of *Passiflora alato-caerulea* (Muralidhar and Mehta 1986) have been obtained on MS medium
fortified with BA (0.5 mg/l), NAA (0.2 mg/l) and peptone (250.0 mg/l). The shoots were found to root on MS (3/4 strength) medium with IBA (0.5 mg/l).

Micropropagation of *Passiflora edulis* was achieved by Kantharajah and Dodd (1990) by using the seedling nodal explants on MS medium fortified with BAP (2.0 mg/l) and coconut milk (200.0 ml/l). Shoots were rooted on medium fortified with NAA (1.0 mg/l) and successfully hardened in pots containing soil in humid environment before transferring to glass house.

Adventitious shoots from leaf callus have been achieved from hybrid varieties of *Vitis*. Kober 5 BB (*berlandieri × riparia*), SO 4 (*berlandieri × riparia*), 41 B (*vinifera × belandieri*), LN 33 (1613 *Couderc × sultana*) and *Vinifera* (Pinot noir and Chardonnay) by Clog et al. (1990) on MS medium supplemented with BAP (4.43 μM) the adventitious shoots were successfully elongated and rooted on hormone free liquid medium.

Torne et al. (1992) have reported the use of seed cotyledons for the induction of somatic embryos in *Araujia serecifera* on M1 medium (Pecaut et al. 1983) fortified with BA (4.4 μM) NAA (0.5 μM) and adenine sulphate (100 μM). The plants developed from embryos were transferred successfully to pots containing soil.

Micropropagation of *Tylophora indica* was achieved by Sharma and Chandel (1992) by inducing multiple shoots on axillary meristems of nodal
explants. Shoots developed on MS medium with BAP (5.0 mg/l), NAA (0.5 mg/l) and ascorbic acid (100.0 mg/l) were readily rooted on medium with IAA (1.0 mg/l). The *in vitro* produced plantlets showed 90-100.0 % survival on soilrite, which were later transferred successfully to soil under field condition.

Leifert *et al.* (1992) have reported the use of shoot tip explants of *Clematis* on MS medium fortified with BA (3.6 μM) and NAA (0.54 μM) to regenerate multiple shoots and half strength WPM supplemented with NAA (1.6 μM) was found to be necessary for the induction of roots.

Georges *et al.* (1993) have communicated a unique example of regeneration from the aged calli of *Lonicera japonica* cv. “Hall’s Prolific”. Proliferative callus developed on WPM containing BA (2.7 μM) and NAA (10.7 μM) using stem, leaf and root explants, could regenerate shoots and roots on WPM fortified with BA (4.4-44.4 μM). The plants were successfully acclimatized on peat and perlite medium (1:1 ratio).

Regeneration of *Hemidesmus indicus* (Indian sarasaparilla) through organogenesis and somatic embryogenesis has been achieved by Sarasan *et al.* (1994). Callus from stem and leaf segments on MS medium containing BAP or Kn (0.1-2.0 mg/l), 2.4-D (0.5-4.0 mg/l), NAA (0.5-4.0 mg/l), coconut milk (10.0 % v/v) and sucrose (2.0 % w/v), when subcultured on MS medium containing (Kn 1.0 mg/l) and CM (10 % v/v) developed shoots. The shoots induced roots on half
strength (macro and micronutrients) MS medium. The initial callus developed somatic embryos if cultured on half strength MS or full strength MS or B5 medium (Gamborg et al. 1968). The plants developed from organogenesis and somatic embryogenesis was initially hardened using vermiculite and then transferred to pots containing garden soil and sand.

Plant regeneration was obtained from a diversity of *Passiflora* germplasms (Dornelas and Vieira (1994). Multiple shoots were regenerated directly from either cotyledonary, hypocotyledonary or leaf tissues of *P. amethysina*, *P. giberti*, *P. mollissima*, *P. maliformis*, *P. edulis* var. *flavicarpa* on MS medium containing BAP (2.0 mg/l) and coconut milk (10.0 %), culturing of shoots on half strength basal MS medium could increase the length of shoots and also induce rooting in 40.0 % of shoots. Plants could be hardened in presence of high moisture condition.

Induction of somatic embryogenesis in *Vitis* by phosphinothricin was reported by H’Soule et al. (1995). Embryogenic cell suspension cultures of Chancellor var. Seibel 7053, a complex inter specific hybrid derived from *V. vinifera*, *V. rupestris V. lincecumii*, *V. riparia and V. labrusca* grown on MS medium with BA (1.0 μM), 2,4-D (5.0 μM) and sucrose (2.0 % w/v) when cultured on half strength basal MS medium induced embryos. Addition of varied concentration of phosphinothricin (0.0-10.0 mg/l) in to the medium increased the number of embryos regeneration. However, maximum number of embryos was formed on medium with phosphinothricin (0.5 mg/l).
Micropropagation of *Hemidesmus indicus* through axillary bud culture was achieved by Patnaik and Debata (1996). Multiple shoots regenerated from nodal explants on MS medium fortified with Kn (1.15 μM) and NAA (0.054 μM), could root on MS with Kn (1.15 μM) and IBA (7.35 μM). It was observed that the shoots obtained from fifth and seventh sub-cultures exhibited better rooting response than the shoots derived from the primary cultures.

The embryogenic competence of petal explants of *Araujia serecifera* has been investigated by Torne *et al.* (1997) on M1 medium (Pecaut *et al.*1983) fortified with BA (2×10⁻⁶ M) was effective in the induction of somatic embryos. Increase in the concentration of BA was found to decrease the frequency of embryoid formation while light intensity of 90-100 M cm⁻² S⁻¹ was found to favor somatic embryogenesis.

Manjula *et al.* (1997) have reported the *in vitro* plant regeneration of *Aristolochia indica* using shoot tip, nodal and leaf explant cultures. Shoot tips and nodal explants, cultured on MS medium with BA (13.31 μM) and NAA (0.54 μM) produced maximum shoots. The callus from leaf segments on medium with BA (4.44-13.31 μM), NAA (0.54-2.69 μM) and phloroglucinol (1.0 mg/l) resulted in indirect adventitious shoots. Direct shoot regeneration from leaves was achieved using BA (13.31 μM) and activated charcoal (50.0 mg/l). Microshoots from different explants would root on White’s medium (White 1954) with IBA (2.46
μM). The rooted plants were hardened using vermiculite supplemented with White’s medium. 85.0 % of rooted plants were observed to survive in the soil.

Rapid multiplication of *Jasminum officinale* by *in vitro* culture of nodal explants was reported by Bhattacharya and Bhattacharyya (1997). Maximum number of shoots were proliferated on MS medium supplemented with BA (17.76 μM) and NAA (0.53 μM). Best elongation of shoots were obtained on medium with BA (8.89 μM) with NAA (16.0 μM). Shoots were successfully rooted in liquid medium with IBA (4.9 μM).

Karhu (1997) reported root induction in two species of *Lonicera-L. caerulea* and *L. edulis* using auxin pulse treatment followed by *ex vitro* rooting as an ideal method to enhance plantlet survival in those genotypes with low endogenous levels of auxins. The microcuttings grown on modified MS medium fortified with various auxins were found to show high percentage of rooting except when NAA was used. It was also reported that continuous exposure of the microcuttings to the influence of auxins resulted in callusing at the basal part and limited root growth. A seven day pulse treatment for root induction has been reported to facilitate lateral root proliferation coupled with elongation of the roots and suppression of callusing at the basal part in both the species of *Lonicera*. 
In vitro multiplication of Gymnema sylvestre was achieved by Reddy et al. (1998) using nodal explants. Multiple shoots obtained on MS medium with BAP (5.0 mg/l) and NAA (0.2 mg/l) rooted successfully on MS half strength medium.

In vitro regeneration of Bauhinia vahlii through nodal explant culture was reported by Dhar and Upreti (1999). The problem of polyphenols exudation from the nodal explants was circumvented by soaking the sterilized explants in a solution of ascorbic acid (50.0 mg/l) and citric acid (75.0 mg/l) and multiple shoots were induced on MS medium fortified with Kn (2.5 μM) and adenine sulphate (100.0 mg/l). The shoots were successfully rooted on half strength medium without growth regulators after a dip in half strength liquid medium containing NAA and IBA at 10.0 μM. The rooted plants were stabilized in a mixture of soil and vermiculite in a ratio of 1:1 under incubation chamber and then transferred to green house.

Direct adventitious shoot regeneration was obtained from mature leaf explants of Passiflora caerulea (Jasrai et al. 1999). Shoots regenerated on MS medium with BA (12.0 μM) and IAA (20.0 μM) were transferred to medium with IBA (246.0 μM) for successful root induction. Rooted shoots were grown under high humidity on vermiculite and perlite mixture and then transferred to field. 90.0 % of plants were survived in the field.
Plantlets were regenerated via somatic embryogenesis in anther cultures of *Vitis latifolia* by Salunkhe *et al.* (1999). When callus produced on Nitsch and Nitsch (1956) medium supplemented with BAP (9.0 μM) and 2,4-D (20.0 μM), subcultured on medium containing NAA (10.0 μM), somatic embryos were resulted. Embryos were converted into plantlets after subculturing on the basal medium. Of the 400 somatic embryos in one gram of callus, 13.7 % were converted to complete plantlets which were subsequently established in soil.

Production of multiple shoots from cotyledonary node explants of *Bauhinia vahlii* was achieved by Bhatt and Dhar (2000). A combination of TDZ and Kn (1.0 μM), each in MS medium increased the number of shoots significantly up to four successive subculture cycles. Over 83.0 % of shoots were rooted on one fourth strength MS medium with NAA (1.0 μM). 50.0 % of the plantlets successfully acclimatized in 90.0 (w/v) soilrite: sand: soil in 2:1:1 ratio.

Somatic embryogenesis and subsequent regeneration of plants has been reported in *Clematis integrifolia × C. viticella* by Mandegaran and Sieber (2000). Callus from the cut surfaces of nodal explants on MS medium with BAP (2.0 μM) and IBA (0.5 μM) regenerated somatic embryos after eighteen months in culture. Highest frequency of conversions of unipolar embryos to bipolar occurred on medium containing Kn (10.0 μM) and BAP (1.0 μM). Shoots from embryos when inserted in sorbarods saturated with liquid medium containing IBA (0.05 μM) and
NAA (0.05 μM) showed root induction. 88.0 % of plants survived when transferred to compost in green house.

*In vitro* propagation of *Decalepis hamiltonii* through axillary bud culture was reported by Bais *et al.* (2000). The nodal explants have shown maximum multiple shoot regeneration on MS medium containing BAP (2.0 mg/l) and NAA (0.5 mg/l). The shoots could be well rooted on MS fortified with IAA (0.5 mg/l) and AgNO₃ (40.0 μM). The rooted plants were planted successively in sand and compost mixture (1:3 ratio) at about 80.0 % relative humidity.

Indirect shoot regeneration in *Decalepis hamiltonii* has been achieved by George *et al.* (2000), using leaf callus. Combination of BAP (1.0 mg/l) and NAA (0.05 mg/l) has been successfully used for induction of shoot differentiation.

Studies on *in vitro* rooting of *Decalepis hamiltonii* by using auxins and root promoting agents was communicated by Reddy *et al.* (2001). It was found that transfer of *in vitro* derived shoots to MS medium with IBA (8.8 μM) and IAA (1.43 μM) resulted in root induction. Dipping of explants in IBA (4.4 μM) for 30 minutes before inoculation on basal MS medium, and also MS medium with Phloroglucinol (69.0 μg), activated charcoal (25.0 %) and CoCl₂ (5.0 or 10.0 μM) found beneficiary for root induction.

Micropropagation of *Gymnema sylvestre* has been achieved by using seedling axillary node explants (Komalavalli and Rao 2000). Multiple shoots
were regenerated on MS medium containing BA (1.0 mg/l), Kn (0.5 mg/l), NAA (0.1 mg/l), malt extract (100.0 mg/l) and citric acid (100.0 mg/l). The shoots could be rooted on half strength medium with IBA (3.0 mg/l). The plantlets developed were successfully transferred to natural soil. 80-85.0 % of plants survived under field condition.

Synthetic seed production and cryopreservation of germplasms of grapevine in vitro have been achieved by Wang et al. (2000). In vitro grown shoot tips of the LN33 hybrid (Vitis) and cv. “Superior” (Vitis vinifera) were successfully cryopreserved in liquid nitrogen by encapsulation-dehydration. After thawing, an optimal survival of cryopreserved shoot tips was achieved when encapsulated shoot tips were dehydrated to 15.6 and 17.6 % water content for the LN 33 hybrid and cv. ‘Superior’ respectively.

Somatic embryogenesis and long term maintenance of embryogenic lines from Vitis × Labruscana ‘Niagara’ and ‘Fredonia’ were reported by Motoike et al. (2001). Embryogenic cultures from two cultivars were induced in an embryogenesis establishment medium from ovaries. The cultures could be maintained stable for more than 2 years through repeated subcultures on long term maintenance medium consisting of Nitsch and Nitsch salts (Nitsch and Nitsch 1956) supplemented with 2,4-D (2.0 μM), sucrose (30.0 g/l), myo-inositol (100.0 mg/l), casein hydrolysate (0.8 g/l) along with indole-3-acetyl-L- aspartic acid, dissolved in dimethyl sulfoxide (17.0 μM).
Plant regeneration through somatic embryogenesis has been achieved in *Gymnema sylvestre* (Kumar *et al.* 2002) using callus cultures derived from hypocotyl, cotyledon and leaf explants from seedlings. Embryogenic callus was induced on MS medium fortified with BA (0.5–2.0 μM), 2,4-D (0.5–5.0 μM) along with sucrose (2.0 % w/v). Embryoids were developed on subculturing of callus on medium containing MS salts, B5 vitamins (Gamborg 1968) with BA (0.5 μM) and sucrose (2.0 % w/v). Germination of embryoids was achieved on medium containing MS salts, B5 vitamins supplemented with BA (0.5 μM) and sucrose (2.0 % w/v). The plantlets were successfully transplanted to pots containing vermiculite in presence of 70.0 % relative humidity with an adequate irrigation along with N:P:K nutrients in the ratio of 17:17:17. The stabilized plants were successfully transferred to pots with soil, sand and farmyard manure at 1:1:1 ratio under green house.

Faisal and Anis (2003) have developed an efficient protocol for rapid mass propagation of *Tylophora indica* from leaf derived callus. Callus on MS medium with 2,4,5,-trichlorophenoxy acetic acid (10.0 μM) could regenerate adventitious shoots on MS medium with Kn (5.0 μM). The shoots were rooted on half strength MS medium with IBA (0.5 μM). The regenerated plantlets were transferred to pots containing sterile vermiculite with an adequate supply of half strength MS salts under high humidity and later the plants were successfully transferred to field conditions.
Shoot tip cryopreservation and maximum recovery of *Holostemma annulare* was reported by Decruse *et al.* (2004) by preparing sodium alginate beads using MS medium containing $\text{NO}_3^{-}$ at 18.8 mM, sucrose (0.75 M) and dimethyl sulfoxide (3.0 %). The beads after dehydration in sterile air, successfully stored in liquid nitrogen with a maximum recovery of 55.0 %.

High frequency regeneration of plants has been achieved in *Tylophora indica* using root explants (Chaudhuri *et al.* 2004) on MS medium with BA (5.36–26.80 μM)) and 2-isopentyl adenine (4.92–24.62 μM). The roots produced organogenic nodular meristemoids. Meristemoids directed into shoots on MS medium with BA (10.7–26.80 μM) and these shoots would be rooted in presence of IBA (28.54 μM). The meristemoids on MS with BA (10.72 μM), dedifferentiated into friable callus and induced somatic embryos. The complete plantlets were obtained on MS basal medium. It was found that survival rate of hardened potted plants derived from somatic embryos was 88.0 % and those obtained via shoot organogenesis was 96.0 %.

A standardized protocol has been established by Vidya *et al.* (2004) for micropropagation of *Entada pursaetha* using cotyledonary node explants. Adventitious shoots were developed on MS medium fortified with BAP (5.0 mg/l) and NAA (0.5 mg/l). The microshoots would be rooted on MS half strength medium with IBA (2.0 mg/l). 70.0 % of hardened plants were successfully transferred to the soil.
Faisal et al. (2005) have reported high frequency regeneration and plant establishment of *Tylophora indica* from petiole derived callus. Callus on MS medium with TDZ (2.5 μM) and 2,4-D (10.0 μM), when subcultured on medium with TDZ (2.5 μM), adventitious shoots were developed and the shoots would root on half strength medium with IBA (0.5 μM). 100.0% plant survival was observed upon transferring *in vitro* raised plantlets to pots containing garden soil in green house.

Thomas et al. (2005) have successfully regenerated plants using leaf derived callus of *Tylophora indica*. Organogenic callus on MS medium with BAP (1.5 μM) and 2,4-D (7.0 μM), when subcultured on medium with TDZ (8.0 μM), adventitious shoots were regenerated. The shoots were rooted on half strength MS medium with IBA (3.0 μM). 92.0% plants survived upon transfer to soil.

The perusal of literature review clearly reveals that meager work has been done with respect to *in vitro* studies on woody ornamental climbers and especially no work has been done on the three ornamental climbers selected for the present investigation.