Discussion

Direct organogenesis

Shoot tip

Shoot tips appeared to be potentially suitable explants for micropropagation (Kamnoon and Kantamant, 2000). Multiple shoots were induced from shoot tip explants on MS medium supplemented with different concentrations of BAP, Kn with combination of NAA and GA$_3$. Frequency and number of multiple shoots were more on MS medium containing 0.6mg/l BAP in combination of 0.2mg/l NAA when compared with other concentration. BAP has been well documented in many species such as *Piper* sp. (Bhat *et al*., 1995), *Ocimum* sp. (Pattnaik and Chand, 1996), *Withania somnifera* (Manickam *et al*., 2000) *Phyllanthus carociniersis* (Catapan *et al*., 2000) *Phyllanthus amarus* (Bhattacharya and Bhattacharya, 2000) and in *Celastrus paniculatus* (Lakshmi and Seeni 2001). The number of multiple shoots decreased with further increase in cytokinins concentration and it promoted callusing. Similar results were obtained by komalavalli and Rao (2000) in *Gymnema sylvestre* and Delse *et al*. (2002) in *Rotula aquatica*. Combination of auxins and cytokinins on MS medium favoured shoot bud differentiation in many plant species (Sharma *et al*., 1991; Hiraoka and Oyanagi 1998). Many scientists used this characteristic combination of auxin and cytokinin in various ratios from *in vitro* explants (Mukhopadhyay *et al*., 1991; Sharma and Chandel 1992; Upadhyay *et al*., 1992; Sarker *et al*., 1996; Sudha and Seeni 1996; Tiwari *et al*., 2001; Sehrawat *et al*., 2002 and Tiwari and Shah 2003). The shoot tip culture could be a valuable technique for the production of these
secondary metabolites in large scale (Sen and Sharma, 1999). In many medicinal plants shoot tips were found to be the best explants for multiple shoot formation (Sharma et al., 1991 and Hiraoka and Oyanagi 1998). In accordance with this, in the present study also the shoot tip explants were found to be suitable for multiple shoot regeneration in *Wattakaka volubilis*.

**Nodal**

Nodal explants have been used to raise higher rate of shoot multiplication of several plants (Shekawat et al., 1993). In the present investigation, the nodal explants on MS medium supplemented with BAP (0.6mg/l) in combination of NAA (0.2mg/l) showed maximum number of shoots. Similarly Sita (1986), Kumar et al. (1991), Sen et al. (1992), Islam et al. (1994) and Gill et al. (1997) have reported that a combination of BAP and NAA proved suitable for shoot multiplication in woody plants. *In vitro* plant regeneration from nodal explants was reported in *Camellia sinensis* (Phukan and Mitra, 1984), *Prosopis juliflora* (Nandwani and Ramawat, 1991), *Excoecaria agallocha* (Rao et al., 1998), *Hyptis suaveolens* (John Britto et al., 2001), *Acacia catechu* (Rohini and Shrish, 2002), *Zehneria scabra* (Anand and Jeyachandran, 2004), *Momordica charantia* (Mala and Raka, 2004) and *Gloriosa superba* (Sayeed and Shyamal, 2005). Muthukumar et al. (2004) reported that plant regeneration from nodal explants of *Datura metel* with BAP in combination of NAA showed better response than the other concentrations. In the present study, BAP with NAA combination responded better than those on BAP with GA₃ and Kn with NAA. Induction of multiple shoots was achieved from axillary regions with BAP with NAA 4-5 weeks after inoculation. Explants inoculated at higher concentrations of BAP alone or in
combination with NAA produced clumps of highly reduced shoots with smaller leaves. Patnaik and Debata (1996) and Sairam et al. (2004) found a similar response in *Hemidesmus indicus* and *Gymnema sylvestre*. Multiple shoot induction and regeneration of *Ceropegia bulbosa* from nodal explants were reported by John Britto et al. (2003). BAP in combination of NAA was suitable for multiplication. The superior activity of BAP compared to other cytokinins is reported in many plants such as *Gymnema sylvestre* (Komalavalli and Rao., 1997, 2002), *Holostemma annulare* (Sudha et al., 2000), *Hyptis suaveolens* (John Britto et al., 2001a) and *Anisomeles indica* (John Britto et al., 2001b). Elongation of shoots were enhanced by auxin combination with cytokinin. Similar results are obtained in *Cassava* (Bhagwat et al., 1996); Apple (Fasola et al., 1989); *Rhododendron* (Preece and Imel, 1991).

**Rooting and Hardening for Direct Organogenesis**

Rooting of shoots is the most critical step in the production of complete plantlets and their subsequent survival. Growth regulators and nutrient contents of the medium play a vital role in the rooting process. According to Eliasson (1978) the external application of nutrients is not required during rooting because the nutrients are supplied from the shoot through phloem to the region of root initiation, in the full strength basal medium. For rooting of *in vitro* raised shoots, half strength basal MS medium with growth regulator was used in *Vigna radiata* (Mendoza and Fulshuhara 1990) and *Alstroemeria* (Hakkaart and versluijs (1998). 0.1% IBA for Soybean (Buising et al., 1994) IAA for common bean (Kartha et al., 1981) and NAA for *Cajanus cajan* (Mehta and Mohan Ram 1981).
In the present work IBA is considered to be the most effective growth regulator for the induction of roots. IBA (1.0mg/l) was effective on maximum induction of roots in *Thuja occidentalis* (Kabir *et al.*, 2006), *Emilia zylanica* (Philip *et al.*, 2006), *Musa* sp (Rahman *et al.*, 2006), *Rhinacanthus nasutus* (Sudhakar *et al.*, 2006) *Cryptolepis buchananii* (Prasad *et al.*, 2004), *Ceropegia jainii* (Patil, 1998), *Exoecasia agallocha* (Rao *et al.*, 1998) *Hemidesmus indicus* (Raghu Ramulu *et al.*, 2002), *Bambusa wamin* (Sheeba *et al.*, 2005). IBA is the most widely used auxin to induce *in vitro* rooting in micropropagation studies (Anju and Ananda, 2005). But in the present study 0.6mg/l IBA produced optimum number of healthy roots. Though the number of roots increased with increase in the concentration of IBA, the number of roots decreased and based callus formed. Thiruvengadam *et al.* (2001) reported that the full strength MS nutrient medium induced maximum rooting in *Vitex negundo* but in the present experiment maximum rooting was only in half strength nutrient medium. There are several reports, Muthuvel *et al.* (2005), Mahapatra and Ratha (2005), Alagumanian *et al.* (2004) and Giridhar *et al.* (2004) which suggest that the half strength MS medium achieved best results of root induction.

**Indirect Organogenesis**

In the present study, callus induction response varied with the type of explants and hormones used. Among the five explants used leaf explants showed maximum response followed by cotyledon, petiole, root and internodal. The differential response of callus induction from the explants was also previously reported by Mendoza and Futshuhara (1990), Bharal andRalid (1979) Sounder Raj *et al.* (1991), Gopi and Vatsala (2006), Hazra *et al.* (2002) and Muthuvel *et al.* (2005). Morphology, texture, nature and colour of callus
varied with the nature of hormones used. 2,4-D, NAA and IAA individually produced yellowish white, green, less compact or soft friable calli while cytokinins (BAP/ Kn) induced green, nodular, compact and sometimes friable calli. These results are in accord with the previous findings of Sounder Raj et al. (1991). Combinations of auxin and cytokinin improved the frequency of callusing. Greenish and compact nature of callus increased with the increase in concentration of cytokinin. Similar observation was observed in *Vigna radiata* (Mathews. 1987; Chandra and Paul 1995); *Arachis hypogaea* (Narasimhulu and Reddy 1983); *Hybanthus enneaspermus* (Natarajan et al., 1999) and *Tylophora indica* (Faizal and Anis, 2003, 2005).

Callus produced from the explants when cultured on the MS medium containing strong auxin and cytokinin was highly responsive for callus-mediated regeneration in *Vigna unguiculata, Salvia canariensis* (Sebastiana, 2004; Nguyen et al., 2003). This is in accordance with the present investigation

**Leaf**

Leaf callus proliferation in the present study has been achieved in the presence of Kn alone or in combination with NAA or IAA, and BAP with NAA. However maximum callus was observed in MS medium with Kn. Gopi and Vatsala (2006) reported callus formation in *Gymnema sylvestre* on MS medium supplemented with 2.5 mg/l Kn. On the contrary in the present investigation profuse callusing was achieved in 1.0 mg/l Kn. Among various concentration of cytokinins and auxins tested, the highest shoot regeneration frequency and highest number of shoots were recorded at 0.6 mg/l BAP with combination of 2.0 mg/l NAA. It was in contrast to the earlier reports on *Echimua parpongea* (Percira et al., 2000), *Pothomorphe umbellata* and (Pretto
and Santarem, 2000) *Hypericum perforatum*. Synergistic effect of shoot regeneration was found to be more effective in BAP with NAA. Similar response was also observed in other plants (Madhumita *et al.*, 2002). However, BAP and 2,4-D combination was highly effective for adventitious buds differentiation, (Heng *et al.*, 1998) BAP when combined with IBA promoted long shoot regeneration (Choi *et al.*, 1998).

**Internode**

Callus regeneration from internodal explants were reported in *Hybanthus enneaspermus* (Natarajan *et al.*, 1999) *Tylophora indica* (Faisal and Anis, 2005), *Piper colubrinum* (Kelkar and Krishnamurthy, 1998) *Salvia canariensis* (Sebastiana, 2004) *Euphorbia tirucalli* (Hindenobu *et al.*, 2004) and *Alstoemeria* sp. (Martha *et al.*, 2006). Similar results were observed in internode on MS medium supplemented with BAP (0.6 mg/l) and GA$_3$ (0.3mg/l). In the present investigation the internode callus explants on MS medium supplemented with BAP (0.6 mg/l) and GA$_3$ (0.6 mg/l) showed maximum number of shoots. The highest shoots regeneration frequency and highest number of shoots were recorded at 0.6 mg/l BAP supplemented with 0.3 mg/l GA$_3$.

**GC-MS Analysis**

Arunkumar and Muthuselvam (2009) reported in the GC-MS analysis 26 bioactive phytochemical compounds were identified in the ethanolic extract of *Aloe vera*. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. J. Sitosterol (C$_{29}$H$_{50}$O) with RT 38.78 has peak area 13.19%, Oleic acid (C$_{18}$H$_{34}$O$_2$) with RT (21.85)
and 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) (C_{19}H_{33}O_{2}) with RT 22.6 ranks next having peak area 11.74% and 11.36% respectively.

Ivanka Kostova *et al.* (2002) investigated fourteen aromatic and 24 aliphatic acids were determined by GC-MS analysis of acidic fractions obtained from *Paronia peregrine* and *Paeonia tenuifolia* roots. Benzoic acid and its monohydroxy-dihydroxy-and tri-hydroxy derivatives are the main acid compounds of both Paronia species. Some fractions could serve as a source of benzoic, 4-hydroxy benzoic, vanillic and gallic acids as well as of ethyl gallate.

Ahmed Al-Harrasi and Salim Al-Saidi (2008) reported phytochemically centrified oleogum resin of *Boswellia sacra* essential oil revealed the presence of 34 monoterpenes and 16 sesquiterpenes.

**Antidiabetic Activity**

The hypoglycemic ethanol effect of *W. volubilis* leaf was found to be inducing insulin release from pancreatic cells of diabetic rats (Sharma and Garg, 2009). Earlier many plants have been studied for their hypoglycemic and insulin release stimulatory effects (Morrison *et al.*, 1987).

Glycosylated haemoglobin determinations are self monitoring of blood glucose therefore play an important complementary role for the management of diabetes mellitus (Thai *et al.*, 1983). The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study (Preethi and Kultan, 2009).
In addition to the assessment of SGPT and SGOT levels during diabetes the measurement of enzymatic activities of phosphatases such as acid phosphatases (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants (Singh et al., 2001).

The lipolipidemic effect may be due to inhibition of fatty acid synthesis (Chi and Koh, 1982). Earlier studies have reported that there was an increased lipid peroxidation in liver. Kidney and brain of diabetic rats (Ananthan et al., 2003). This could be correlated with previous study with Cassia auriculata flower (Pari and Latha, 2002) and Scoparia dulcis (Latha and Pari, 2003).

Several authors reported that flavanoids sterols / terpenoids, phenolic acids are known to be bioactivative antidiabetic principles (Oliver Bever, 1986; Rheman and Zaman, 1989). Flavonoids are known to regenerate the damaged beta cells in the alloxan diabetic rats (Chakravarthy et al., 1980). Phenolics are found to be effective antihyperglycemic agents (Manickam and Ramanathan, 1997).