INTRODUCTION
Curculigo orchioides Gaertn. is one of the important endangered medicinal herbs in the family Hypoxidaceae. This monocot herb has been regarded as a key member of the Dasapushpa, an ayurvedic formulation used over many centuries in India. This herb is recognized as a highly useful plant in the indigenous system of medicine and consists of approximately over 20 species of exclusively tropical origin (Rudall et al., 1997; Chase et al., 2006). This herb is strictly perennial in nature grows about 30 cm in height with a short or elongated root-stock bearing a number of fleshy shorter lateral roots which are often black in color. The present distribution of this medicinal herb becomes very rare due to over exploitation in addition to indiscriminate removal of this herb in forest. Thus, C. orchioides has been placed under ‘Lower risk near threatened category’ by the International Union for Conservation of Nature (IUCN) as reported by Sharma (2001).

C. orchioides finds its prominent place in the Indian System of Medicine (ISM) since this herb is being used as an important substitute in many ayurvedic formulations, called ‘safed musli’ (Bhattachrjee, 1998). C. orchioides was first introduced in ‘Charak Samhita’ of ‘Agnivesha’, the epic treatise of the medicine school of thought of the Hindu system of medicine. The rhizome of C. orchioides is the most useful part of the plant as described in Ayurveda as a vajikarana and rasayana, alternatively known as virile therapy and rejuvenating effects respectively (Chunekar and Yadav, 2005). Although, C. orchioides has several other synonyms such as Talmuli, Kalimusli, Talusa, Nelatigade, Nelappana and Nelatadi, Golden eye grass is a popular vernacular name being used due to its high esthetic appearance coupled with its valuable medicinal properties.

C. orchioides is predominantly distributed in several parts of Sri Lanka, Japan, Malaysia, Australia, China and India (Pandey et al., 1983). This herb is commonly grows well in moist laterite soil near sea level to 2300 m. In Asian Sub-continent, this herb is characterized by blackish elongated tuberous root with several lateral roots. It often appears with rosette or short petiolate, linear, lanceolate, membranous leaves close to the ground level. C. orchioides is popularly named as ‘Xianmao’ in
Pharmacopoeia of the People’s Republic of China and described as a tonic for its inherent medicinal properties. Another closely related genus is *Chlorophytum* with a similar medicinal property to that of *C. orchioides*. Thus, a common name known as ‘Musali’ was derived from the genus (Singh, 1973). In Chinese Traditional Medicine (CTM), *C. orchioides* is used for the treatment against decline in physical strength (Gudzikiewicz, 1979). In Philippines, *C. orchioides* has been used as medicine for a variety of skin diseases.

The multiple uses of *C. orchioides* have been well documented in ancient literatures (Bhattachrjee, 1998). The various plant parts such as rootstock, rhizome and leaves have wide range of medicinal values. The rhizome as well as tuberous roots of the plant has been extensively used in Indigenous System of Medicine in India, Pakistan and China for the treatment of various health problems, including jaundice, asthma and diarthrosis. In addition, this herb has been regarded as an alternative source of medicine for enhancing the potency (Chopra et al., 1956) and preventing bone loss (Cao et al., 2008). Rhizome has been claimed for the antidiabetic properties in various studies (Parrotta, 2001). The rootstock is considered as a source of medicine for the preparation of herbal formulation for treating muscular and joint pains (Rajagopalan et al., 1995). The herbal formulation of *C. orchioides* is regarded as restorative, rejuvenating and aphrodisiac drugs for maintenance of health (Porwal and Mehta, 1985; Manandhar, 1991; Samanta, 1992). The leaves of *C. orchioides* have been shown to possess anticancer property (Agrawal, 1997).

In India and China, extensive work has been done in *C. orchioides* for eliciting various biochemicals present in different parts of the plant. The whole plant of *C. orchioides* is reported to contain a variety of bioactive compounds, which include flavones, phenolic glycosides, steroids, saponins, terpenoids (Misra et al., 1984; Misra et al., 1990). Higher accumulation of sterols, triterpene and cycloartenene series have been reported in the whole plant of *C. orchioides* (Rao et al., 1978; Garg et al., 1989; Mehta and Gawarikar, 1991). In addition, the other chief biochemical constituents present in the rootstock of *C. orchioides* are glycosides, polysaccharides
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(hemicelluloses, starch, resin, tannins, and mucilage), fat and calcium oxalate. The presence of these chemical constituents was reported to be an indicators for revealing the transition point between the alkaloid bearing species and sapogenin bearing species and thus both types of biogenetic mechanism are reported to be operating in this plant (Rao and Beri, 1951; Kubo et al., 1983; Xu and Xu, 1991; Xu et al., 1992a, b; Tandon and Shukla, 1995).

There are several phenolic compounds isolated and characterized from the rhizomes of C. orchioides. These include curculigoside, curculigine A, orcinol glucoside, corchioside A, and flavanone glycoside-I (Tiwari and Misra, 1976; Kubo et al., 1983; Oru and Kogyo, 1983; Chen and Chen, 1989; Xu and Xu 1991; Buckingham, 1994; Mamta et al., 1995). In addition, several other fatty acids such as palmitic, oleic, linoleic, arachidic and behenic acid have been isolated and characterized from root oil of C. orchioides by gas liquid chromatography (Mehta et al., 1980). It was documented that C. orchioides is one of the plant species that appears early in the forest after the first shower of rains and also the last to disappear on completion of monsoon. Thus, in other seasons, the plants are generally hidden inside the soil as the leaves produced by the plant dry and decompose after the monsoon. Although, propagation of C. orchioides was recommended through seeds and rhizome, such method is not practicable owing to poor seed set and viability (Suri et al., 1999). In addition, cloning of C. orchioides requires large quantity of rhizome for which, large number of wild plants have to be sacrificed.

In India, C. orchioides has been included under the category of endangered (Anonymous, 2000). The reason for the endangered status of C. orchioides was reported to be primarily due to over exploitation of this precious herb without any initiative of conservation and large-scale multiplication. In addition, indiscriminate removal of wild plants coupled with deforestation lead to destruction of natural habitat. The other contributory factors include extensive denudation of the forest floor, intensive cattle grazing and collection of leaf litter (Jasrai and Wala, 2000), complete
removal of tuberous roots due to high price in the market for its metabolic – enhancing principles and aphrodisiac formulations become the other possible reason for its threat (Ramawat et al., 1998; Subramonium and Gayathri, 2002). It was reported that high incidence of viral and bacterial diseases affecting rhizomes, cause serious problem for its existence (Dhenuka et al., 1999).

The rapid decline in the population of *C. orchioides* demands systematic conservation strategies to ensure the availability of this precious medicinal herb for future use as well as to establish a large population through cloning for balancing the cycle of harvest and renewal as already stressed (Odum, 1971). As conventional method of propagation of *C. orchioides* has several limitations, large scale propagation through direct bulbils formation from leaf explants in shake flask culture was suggested for establishment of genetic stocks to meet the ever increasing demands by various pharmaceutical industries (Suri et al., 2000). Methods for rapid multiplication of *C. orchioides* are highly advantageous to meet the commercial demand in addition to conserve the valuable endangered plants (Rout et al., 2000; Ramawat et al., 2004). Thus, *in vitro* culture technique is the only way to increase the number of plants within a short time as reported by several workers.

Theoretically, a single cell or piece of plant tissue can produce an infinite number of new plants. The main industrial goal of *in vitro* culture is to produce a large number of plants within a short period by optimizing the growth and development of *in vitro* grown tissues (Haque et al., 2009). However, the underground parts of the plant generally pose serious problem of contamination when used as an explant and aerial parts such as leaf, stem of monocots are difficult to regenerate. In spite of these problems, regeneration of plants from leaf explants has been reported in several monocots such as *Curculigo* (Suri et al., 1998), *Agapanthus* (Suzuki et al., 2002), *Lilium* (Bacchetta et al., 2003), *Curcuma* (Prakash et al., 2004) and *Chlorophytum* (Arora et al., 2006). However, no systematic studies have been carried out to optimize the protocol for micropropagation of *C. orchioides*. 

The major bottlenecks in developing clonal propagation protocols for *C. orchioides* through leaf and rhizome explants of field grown plants are low recovery of explants due to high percentage of microbial contamination, loss of initial cultures due to phenolic oxidation and very slow *in vitro* response of explants. Hence, basic investigations are necessary to overcome these problems for standardization of micropropagation techniques for *C. orchioides*. According to Debergh and Read (1991) micropropagation of plant species through direct regeneration involves sequential stages viz., explant preparation (stage 0), initiation of culture (stage 1), shoot proliferation and multiplication (stage 2), rooting of microshoots (stage 3) and *ex vitro* establishment (stage 4). Each stage has its own specific problems and *C. orchioides* has no exception. Considering the medicinal values of *C. orchioides* and its present status towards extinction, the present study was undertaken in the following lines to standardize micropropagation techniques using leaf and rhizome explants. Although considerable number of reports on micropropagation of *C. orchioides* are available, there is no report on genetic and phytochemical aspects of micropropagated plants of *C. orchioides* to ensure the genetic fidelity as well as the medicinal properties of *in vitro* regenerated plants.

The present study is carried out systematically in various aspects for developing reliable *in vitro* culture methods for propagation of *C. orchioides* for conservation as well as for propagation of this precious medicinal plant on a large scale. In addition, systematic experiments were undertaken to evaluate the micropropagated plants of *C. orchioides* for its phytochemical properties. Genetic fidelity of plantlets regenerated through *in vitro* culture also becomes an important part of the present investigation. Thus, the objectives of the present investigation include the following.

- Standardization of *in vitro* technique for cloning of *C. orchioides*
- Determination of genetic fidelity of micropropagated plants of *C. orchioides*
- Evaluation of phytochemical constituents of *in vitro* propagated plants