Review Article

In vitro Propagation of Genus *Ceropegia* and Retrosynthesis of Cerpegin - A Review

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ABSTRACT

During the last several years, different approaches have been made for *in vitro* propagation of medicinally important genus *Ceropegia*. The large-scale production of *Ceropegias* requires efficient *in vitro* propagation techniques to avoid the overexploitation of natural populations. Successful propagation of Ceropegia via pre-existing meristems (using apical bud, axillary bud, nodal segments, leaves, cotyledons, embryos) is influenced by several internal and external factors including *ex vitro* and *in vitro* conditions. Specific requirements during stages of micro propagation (establishment, bud induction & shoot multiplication, root induction, acclimatization and field establishment) and requisites for plant regeneration through organogenesis and somatic embryogenesis, as an important step for the implementation of plant improvement programs, were revised. New challenges for refinements of protocols for high rate of shoot multiplication and development of cost effective methods have gained importance in the recent past. Further, the development of new protocols for *in vitro* propagation, *in vitro* flowering, somatic embryogenesis and plant regeneration which is considered the most important step for successful implementation. More over *Ceropegia* possess traditional medicinal properties and play a vital role in the ayurvedic field. On the whole, the present review gives a consolidated account of *in vitro* propagation, traditional uses, chemical constituents and Retrosynthesis of Cerpegin.

Keywords: Alkaloids, Ceropegia, Micro propagation, Organogenesis, Pharmacological uses, Retrosynthesis of cerpegin, Somatic embryogenesis.

INTRODUCTION

Ceropegia L. (commonly known as Lantern Flowers) species are members of the family Asclepiadaceae (Apocynaceae), an old world tropical genus, which are distributed from south-east Asia, India, Madagascar, Tropical Arabia, Canary Islands, Africa except Mediterranean region, New Guinea and northern Australia.[1-4] In India, about 55 species are present and most of them are endemic to Western Ghats, which is one of the centres of diversity of *Ceropegia*[5,6]; 38 species distributed mainly in Western Ghats[7-9] ; and most of them are enlisted under endangered category.[10,11] *Ceropegia* also occurs in tropical and subtropical areas from Western Africa to Eastern China. No recent serious revision of the whole genus *Ceropegia* exists, but a safe estimate of the number of species is around 200.[12] The CITES of India, states: Analysis of field records reveals that they (*Ceropegia*) prefer undisturbed habitat and climate and any sort of disturbance affect the population resulting into quick decline of wild status.[13] *Ceropegia* species are store house of starch, sugars, gum, albuminoid, fats, crude fibre and other valuable phyto-constituents which are routinely used in traditional Indian ayurvedic drugs for the treatment of gastric disorders, diarrhea, dysentery, urinary tract disorders and etc.[14] The pharmacological importance of the genus *Ceropegia* is mainly due to the presence of a pyridine alkaloid called 'Cerpegin'.[15] Some of the *Ceropegia* species are used in folk medicine in India.[16-18] Habit dilapidation; anthropogenic activities are major threats to the survival of this plant. The scarcity of pollinators and the poor seed setting are the major constraints in the natural propagation, leading to a continuous depletion of its natural population. Besides conventional methods of propagation, *in vitro* cultural methods contribute importantly for the propagation of many important and economic plants. Conventional propagation methods are very slow and propagation by tissue culture is potential and effective tissue culture and genetic engineering techniques for propagation and genetic improvement of *Ceropegia*. The present review aims to give overviews of *ex situ* conservation, pharmacological, ornamental and retrosynthesis of *Ceropegia.*

MICROPROPAGATION

Micropropagation has been demonstrated as an excellent method of large- scale production of true-to-type. Several factors were responsible for the success of micropropagation which includes explant types, nutrients, growth regulators and other additives and also culture conditions (temperature, light intensity and duration) are essential for the development of micropropagation protocols. Work on micropropagation of *Ceropegia* is summarized in Table 1.

Stages involved in micropropagation

A successful micropropagation protocol proceeds through a series of stages, each with a specific set of requirements. These are (i) initiation of aseptic cultures,

(ii) shoot multiplication, (iii) rooting of micropropagated plants and (iv) hardening, field transfer and survival of tissue culture raised plants.

Initiation of Aseptic Cultures

Selection of explant

The selection of explant for initiation of aseptic culture is largely dictated by the method to be adopted for *in vitro* propagation. Explants with vegetative meristems are often suitable for enhanced axillary branching (Table 1). The most commonly used explant is nodal stem segment or an axillary bud, wherein the axillary bud is made to proliferate to form multiple shoots. The performance of nodal segments is much better than the shoot tips. Significant differences observed in the rate of shoot multiplication depending upon position of node on the stem in different species of *Ceropegia*.[19]

Sterilization

Even though *in vitro* cultures can be established at any time of the year, success depends on the season for collection of explants. Contamination of explants was found to be less if they were collected during spring or summer compared to explants collected in autumn or winter.[20] For initiation of aseptic cultures, a thorough knowledge of the physiological status and the susceptibility of the plant species to different pathological contaminants are required. Most of the explants, were washed in 1-2 drops of Tween 20(10%) per 100 ml of water followed by rinsing in running water, and surface sterilized in 0.1% mercuric chloride for 5 min followed by 3-4 rinses in sterile water.[19] However, sterilized the nodal segments by washing under running tap water followed by detergent, extran (5% v/v) for 5 min with mercuric chloride (0.5% w/v) solution for 10–14 min followed by washing in sterile distilled water. The disinfection procedure for 6 nodal explants (*C. fantastica*, C. *spiralis*, C.*intermedia*, C. *sahyadrica*, C. *attenuate* and C. *candelabrum*) originating from green house stocks resulted in 70–90% survival of explants.[21-27] The explants were washed with two drops of liquid detergent (1% Tween-20) for 20 min with constant shaking followed by running tap water for half-an-hour, then repeated rinsing with Millipore water, and then subjected to 70% (v/v) ethyl alcohol treatment for 30 s and again washed with sterilized Millipore water followed by 20% hydrogen peroxide (H_2O_2) treatment for 6 min and later rinsed 5 times with sterilized Millipore water.[28] From our study under running tap water for 20 to 30 min and surface sterilized with 70% ethanol for 30 s, rinsed with sterile distilled water and treated with 0.1% $HgCl_2$ for 3 min duration seems to be best in *C. thwaitesii.*[29]

Shoot multiplication

This is the majority decisive stage of micropropagation. The success of a micropropagation protocol, to a large extent, depends on the rate and mode of shoot multiplication. Different factors that influence *in vitro* shoot multiplication in *Ceropegia* are listed below.

Species

Patil[19] observed that the rate of shoot multiplication of different Ceropegia species such as *C. jainii, C. bulbosa var. bulbosa, C. bulbosa var. lushii* varied significantly in different subculture periods. At lower concentrations of BAP (2.2 to 8.8µM), the development of axillary buds was stimulated in *C. bulbosa* var. *bulbosa* and *bulbosa* var. *lushii* not in *C. jainii*. Axillary bud initiation was not occured in higher concentration of any growth regulator. The influence of genotype on shoot proliferation could easily be interpreted by linking it with the recent progress in functional genomics of plants. Current studies indicate that there are genes responsible for increased number of shoot bud initials and proliferation of shoots. Moreover, the possible involvement of the gene in modulating hormone levels has also been reported.[30]

Media

Murashige and Skoog's[31] (MS) medium was found to be the most commonly used for *Ceropegia* propagation. The basal MS medium induced the best rates of shoot induction and proliferation in different *Ceropegia* species also reported.[19] Based on the literature most of the authors used MS medium combined with different PGRs with different concentration and different combination for shoot multiplication. Though, the use of other media has also been reported i.e. B5 medium supplemented with different concentrations of BA and Ads each in combination with 0.05 mg/L NAA for shoot initiation for *Ceropegia.*[32]

Carbohydrates

Commonly 3% sucrose was used as a source of carbohydrate. In general for tissue culture, Murashige and Skoog[31] also stated that the use of 3% sucrose is better than lower or higher concentration. However**,** there are several accounts of the usage of higher concentrations of sucrose both for shoot initiation and proliferation. Nair et al.,[20] reported lower (87mM) and higher amount (233mM) of sucrose was not suitable for *in vitro* flowering of six Ceropegia species. Commonly used sucrose levels obtained flowers were smaller in size, there *in vitro* flowers were morphologically comparable with *in vivo* derived flowers but the average number was more (3.6±0.32).

Plant Growth Regulators

Micropropagation are mostly based on a formulation of media containing different cytokinins and auxin as a major PGR, whereas, in some cases, different formulated media, low concentrations of Additives and GA_3 were also used. Successfully regenerated the plantlets from nodal explants of *Ceropegia bulbosa* var. *bulbosa* and *C. bulbosa* var. *lushii* when the media contain BAP+IBA, BAP alone was reported as a most effective cytokinins.[19] The number of shoots significantly reduced with further increase in the concentration. The axillary bud proliferation on nodal explants was promoted by 8.87 mM BA and 2.46 mM IBA in C. *candelabrum.*[27] Using

apical meristems, investigated the ability of different cytokinins to induce shoot formation in *Ceropegia sahyadrica*.[26] They found that 10mM BAP produced greater and more vigorous shoots than either combination of BA, KIN, IAA, NAA and 2,4-D in the range of 0 to 22 mM fortified in the MS medium. One reason for initiating *in vitro* cultures of *C. juncea* is for the commercial potential to produce cerpegin.[33] Regeneration of plantlets from shoots in B5 vitamins supplemented with BA was observed and it was improved by addition of 20mg/L Adenine sulphate.[32] Shoot formation of some species was very low and almost independent of the BA.[22] The MS medium containing BA 4.44 µM+NAA 0.27 µM gave the best shoot production.[34] Used shoot tips as initial explants and the miniature divisions, which arise from them, were repeatedly separated and recultured in *C.odorata.*[35] Using axillary bud explants and obtained more number of shoot clusters in *C. noorjahani.*[36] Using axillary bud segments as explants and up to 13 numbers of multiple shoots were initiated on MS basal medium supplemented with BA 1.5 mg/L. Axillary nodes remained green and fresh but failed to sprout on MS media without cytokinins.[21] BA was the most efficient cytokinin for the axillary bud initiation and subsequent proliferation. BA alone at 6.66 µM induced a mean of 5.57 shoots per explant with 100 percent response. Subculture was carried out at 4 week intervals, on MS medium supplemented with BA 6.66 µM for shoot bud proliferation.[23] Regenerated apical shoots of C. *attenuata* were cultured on MS salts supplemented with picloram 4.14 µM resulted in effective shoot multiplication and proliferation.[24] Likewise Krishnareddy et al[28] obtained effective shoot multiplication and proliferation of *C.juncea* in MS medium supplemented with BA 8.87 µM + TDZ 4.54 µM**.** Recently, in our laboratory successful regeneration of the plantlets from axillary explants of *C. thwaitesii,* was obtained on MS media contain KIN (13.94 µm) + IAA (28.54µm).[29] Effective shoot proliferation of *C. elegans* was in MS medium supplemented with KIN (23.20 µm) + IAA (5.70 µm). The induction of multiple shoots through axillary bud proliferation is now recognized as a useful technique for propagation, *in vitro* conservation and reestablishment of threatened plant species.[37]

Status of the medium

In plant tissue culture media different gelling agents were used such as, agar, gelrite and phytagel, which are natural polysaccharides with higher capabilities of gelation are commonly used. In general agar is the most frequently used gelling agents in tissue culture, because of its desirable characteristics such as clarity, stability and its inertness.

Physical factors

Light

Light is one of the most important environmental signals, it can affect morphogenesis through light intensity, photoperiod, or spectral wavelength. In tissue culture photoperiod and light intensity plays a different important role for satisfactory shoot growth and multiplication. In culture room usually 16/8 h light regime provided by cool white fluorescent light or a cool source of white light is provided in culture conditions.

Rooting

For any propagation protocol, successful rooting of microshoots is a pre-requisite to facilitate their establishment in soil. Considerable work has been done to enhance rooting efficiency in different *Ceropegia* species. Rooting of microshoots can be accomplished under *in vitro* and *ex vitro* conditions. The *in vitro* rooting ability depends on the interaction of internal and external factors. These are described in Table 2.

Species

According to Patil[19] response of rooting in *Ceropegia* was species dependent and in certain species up to 90% success could be achieved. Optimizing factors for *in vitro* multiplication of ornamental and alkaloids bearing *Ceropegia* reported that rooting of microshoots of the alkaloid *Ceropegia* was difficult as compared to the ornamental varieties.[33]

Media

Optimized the use of MS medium with reduced major elements to half strength for root induction of *in vitro* raised microshoots in *C.attenuata***.**[24] Patil[19] reported that 3 different *Ceropegia* species rooted within 15–20 days on one half-strength of MS medium supplemented with IAA at 5.7 µM. Subsequently, achieved high root induction in half- strength MS medium containing different concentrations of auxins.[23] Beena et al[27] achieved very high percentages (90%) of rooting in *C. candelabrum* on half- strength MS with 0.49 mM IBA.

Inorganic salts

Relatively low levels of salt concentrations in the medium are known to enhance the rooting of microshoots.[38] Enhancing root number and length of *in vitro* grown shoots of three *Ceropegia* species of C.*jainii,C.bulbosa* var.*bulbosa, C.bulbosa* var.*lushii*. also succeeded. [19] Chandore et al[21] demonstrated that without or decrease of $CaCl_2$ was the decisive factor for improving the rooting percentage of *C. fantastica*.

Carbohydrates

Sucrose concentration acts as an enhancer of osmotic potential and also plays a vital role in root induction. Highest rooting percentage was obtained in *C.hirstua* on a medium with high sucrose concentration.[39] Rooting percentage of microshoots producing roots was better in liquid medium than compared to agar half strength and full strength MS medium supplemented with different auxins.[35] On an average, about 88% of shoots were rooted within 2 – 4 weeks of culture. It was further observed that root length was short in solid medium as compared to liquid medium. The retardation in root

length in the solidified medium may be attributed to the relatively lower aeration in agar-gelled medium for good root development as indicated by.[22] Moreover, using gelrite obtained high rooting efficiency in *C. fantastica*.[21] 88% of rooting achieved in *C.hirsuta* by using MS medium devoid of growth regulators but supplemented with 5% sucrose. When a comparison was made on rooting response in both agar-gelled, Gelrite and liquid media, there was only 5% rooting in the former and 85% response in liquid culture medium.[39]

Plant Growth regulators

In most reports, varying concentrations of different auxins (0.1 to 5mg/l) were used for root induction (Table 2). Auxin added exogenously to *in vitro* shoots or cuttings has the ability to promote adventitious root formation. However, auxin is required only at an early stage of the rooting process for the induction of adventitious roots.[34] In the latter phases of root development; it can modify or even inhibit the development of the root system.[35] Rooting of microshoots was also achieved by *ex vitro* rooting using the *in vitro* derived shoots in aqueous solution of 49.2 µM of IBA and 6%of sucrose in three species.[18] He also reported the effect of different concentrations and combinations of auxins on root formation of in *vitro* rooting. Optimum rooting in *C.attenuata* was achieved at a high concentration of IBA with low concentrations of salts or intermediate concentrations of IBA. Excised shoots of *C.spiralis* rooted well on MS supplemented with 10.74 mg/l NAA.[46] The microshoots of *C.hirsuta* cultured in liquid medium without IAA were difficult to root and only microshoots that were maintained on a medium with IAA developed roots. However, they observed that there was no difficulty in rooting of microshoots that are cultured in liquid medium supplemented with IAA.[39] Rooting of shoots was favoured by addition of polyamine 6.0 mg−1 spermine and 5% sucrose.[26] Chavan et al[24] reported rooting with half-strength MS medium was incorporated with 2.46 µM IBA. Effect of IBA in root induction has been reported in other rare plant species.[26] The effect of growth regulator in root induction without $CaCl_2$.[21] The effect of 1 mg/L IBA was found to be superior to any concentration of IAA, exhibiting an average of nine rootlets. *In vitro* shoots were rooted only upon transfer to half- strength MS medium containing auxins, whereas no rooting was noted in hormone free ½ MS medium. Half-strength MS medium supplemented with NAA 5.37 µM was the most effective for root induction. The shoots were cultured on ½ MS fortified with 5.37 µM NAA to improve the overall growth of roots and to reduce the basal callusing and time duration of root induction. In contrast, a drastic inhibitory effect of root induction was observed in ½ MS containing 9.80 µM IBA.[23] Excised shoots of *C.juncea* rooted well on MS supplemented with IBA 4.90 µM + NAA 1.27 µM.[28] Nikam et al[35] reported that microshoots of *C. odorata* and *C. maccannii* cultured in liquid medium with IAA were difficult to root induction and only shoots that were maintained on a medium with IBA developed roots. However, we observed that there was no difficulty in rooting of microshoots that are cultured in solid medium or liquid medium supplemented with IBA. Recently Krishnareddy et al[37] reported *in vitro* shoots developed were rooted best on half strength MS medium with IBA 4.90 µM in *C.elegans*. Likewise Abubacker et al[42] obtained roots on *C. juncea* in MS medium supplemented with IAA 0.5mg+NAA 0.5mg + IBA0.5mg+BA1.0mg. Likewise in our laboratory rhizogenesis was observed on MS medium supplemented with IBA 2.46 µm in *C.thwaitesii.*[29]

Comparison of in vitro and ex vitro rooting

Ex vitro rooting was crucial step in microparopagated plants, a comparison of *in vitro* and *ex vitro* rooting has been made in C. *jainii*, C. *bulbosa* var. *bulbosa* and C. *bulbosa* var. *lushii*. They indicated that after 15 days of treatment, either *in vitro* or *ex vitro* resulted in 80% rooting in three species. Shoots underwent rhizogenesis in ¼ MS with 0.18 µM (60 $g'1^{-1}$) sucrose and 2.4 µM (0.5 mg/l) IBA. There was rooting in other media combinations as well, but at relatively lower frequencies *in vitro* rooting. The mortality rate was about 20%, while there was a higher mortality (45%) for *ex vitro* rooting. Under *in vitro* conditions the best rooting percentage was high in *C. jainii* most of them around 80-90%. Moreover, survival after acclimatization of rooted plantlets from these rooting treatments varied between 40 and 80%. *Ex vitro* rooting was obtained in *C. bulbosa* using 100mg IBA for 3min in micropropgated plants.When shoots were rooted *ex vitro* all rootstocks appeared to be easy-to-root, and microshoots rooted *ex vitro* were superior to those rooted *in vitro*.[43] However, *ex vitro* rooting produced longer and more number of roots with increased root area. *Ex vitro* roots were flexible, branched and had root hairs, whereas *in vitro* roots were brittle, unbranched and without root hairs. Moreover, *ex vitro* rooting is a one-step procedure comprising of both rooting and hardening, facilitating early transplantation to soil.

In vitro tuberization

Tubers are storehouse of starch and other nutrients, and on this the young plant lives until it finds suitable condition in which to grow and continue an independent existence. Tuberization is being suggested that the excessive production of roots and microtubers at the nodes of plantlets was an attempt to perenate in response to stress resulting from culture medium during a period of undisturbed vegetative growth (Table 3). It is possible that a condition of sugar occasioned by the exhaustion of media components triggered cultures to begin to relocate food reserves to perenating bodies rather than vegetative shoots. *In vitro* tuber formation in the three *Ceropegia* genotypes of C. *jainii, C.bulbosa*.var.*blbosa, and C.bulbosa*.var.*lushii* was investigated by culturing both individual and multiple shoot clusters on ½ MS medium containing 4% sucrose with KIN and BA at different concentration levels, individually and in combination.[19] Likewise Murthy[44]

investigated the tuber formation in *C.pusilla* the media contain BA. Recently Murthy et al[41] reported basal microtuberization in same species the media contain BAP 13.32µM and 0.11µM 2,4D with 3% sucrose. He has also reported basal and aerial microtuberization in *C.spiralis*. Basal tuberization will occur in the media contain BAP 13.32µM with NAA 1.34 to 2.68µM.The MS media contain IBA 49.2µM with IAA 11.54 µM and high concentration of Sucrose (6%) produce aerial microtuberization.Tubers are store house of starch and alkaloids and tuber formation is helpful for the accumulation of starch and other metabolites in *Ceropegia* species.

In vitro flowering

Ornamental *Ceropegias* are produced exclusively for their aesthetic values. Thus the improvement for quality attributes such as flower colour, longevity and form, plant shape and the creation of novel variants are important economic goals. Several parts such as follicles, apical, axillary bud and *in vitro* shoots were used for *in vitro* flowering, although to a lesser extent (Table 4). Patil[19] observed the flowering in *C.jainii* in the media that contained half- strength MS with 0.25 µM spermine. Well developed shoots were inoculated on the MS media supplemented with 3% sucrose and 11.54 µM IAA and flowering was observed in the plantlets. Hence, some of the plantlets were transferred to low light intensity as it favors the flowering (Murthy, 2010c). Likewise Chavan et al[44] invested the photoperiod of *C.attenuata*, and obtained more number of *in vitro* flowering in the MS media containing Picloram 4.14 µM. MS medium provided with TDZ 0.5 mg/L induced flowering.[21] Britto et al[32] have reported earlier the same in *C. bulbosa* var.*bulbosa* on 1/2 strength MS medium with GA_3. Floral organs of six spring-flowering *Ceropegia* species were investigated for their competence to produce *in vitro* flowering by,[20] viz., *C. lawii, C. maccannii, C. oculata,* and *C. sahyadrica,* as well as the widely distributed *C. bulbosa* var. *bulbosa* and *C. hirsuta***.** They successfully regenerated *in vitro* flowering when the media contain BAP (4.4 to 26.6 µM). They obtained apical and axillary buds flowered with similar frequency. For all six species, the number of flower-producing shoots was greatest on the MS medium containing 26.6 µM BAP. The percentage of flowering shoots and the number of flowers per explant (3.6±0.3) were enhanced as the sugar concentration increased, and the most number of flowering shoots were obtained at 87-175 mM sucrose, above this concentration their frequency decreased. The highest percentage of flowering shoots in the BAP-varying experiment was observed in C. *lawii* (69.8%), followed by *C. sahyadrica* (61.8%), *C. hirsuta* (56.3%), *C. oculata* (44.9%), *C. maccannii* (34.5%), and *C. bulbosa* Roxb. var. *bulbosa* (29.7%). Similarly, the highest percentage of flowering shoots in the sucrose-varying experiment was recorded for *C. sahyadrica* (99.6%), closely followed by C. *lawii* (98.6%), *C. hirsuta* (95.6%), and C. *maccannii* (95.6%), with the low success rate for *C. oculata* (49.0%) and *C. bulbosa* Roxb. var. *bulbosa* (28.6%).This could have been due to fluctuations in the floral signal receptivity of the shoot buds of those species. Both BAP and sucrose *in vitro* flowers were morphologically comparable with *in vivo* derived flowers and all the six species shows the similar response also. In conclusion, BAP can play an important role in the transition from a vegetative to a floral state but there is certainly other biochemical and environmental factors involved in this process

Acclimatization and field establishment

Acclimatization is a crucial step in micropropagation. The successful acclimatization of propagated plants and their subsequent transfer to the field is a crucial step for *in vitro* technology. However, the acclimatization of micropropagated *Ceropegia* was reported to be a difficult procedure because of rapid desiccation of plantlets or their susceptibility to diseases due to high humidity.[45] Goyal et al[34] achieved 20–80% rooting in plastic cups containing vermiculite. Transplanted young plantlets in vermiculite in pots acclimatized and hardened successfully within 10 days. Karuppusamy et al[23] obtained success in hardening of *C.intermedia*. The regenerated plants did not show any detectable variation in morphological or growth characteristics when compared with the donor plants. Relative humidity (RH) plays a crucial role in acclimatization and survival of tissue culture raised plants. The percentage survival of the micropropagated *Ceropegia* varied from 92 to 98% when transferred to the greenhouse at 60-65% RH and planted in earthen pots containing soil and coco-pit (1:1).[25] Chavan et al[24] described a procedure for *C. attenuata* with developed shoot and roots which were transferred to small plastic pots containing different types of substrates. A combination of autoclaved river sand and coco peat was the most effective substrate for the acclimatization of *in vitro* regenerated plantlets of *Ceropegia*, were revived within 20 days. One month after transferring, the survival rate of the plantlets of C.*hirsuta* was highest 85% response. In rainy season 100% rooted shoots survived when transferred directly to the soil and exposed to natural conditions.[39] Using various hardening and acclimatization stages the survival rate was found to be much higher than as compared with the direct transfer of the plantlets. Relative humidity and equal ratio of soil and coco pit was best for the acclimatization of this genus.

PLANT REGENERATION

For a considerable time now, plant scientists have been fascinated and challenged by the phenomenon of regeneration de nova in a variety of tissue culture systems, including thin cell layers, leaf segments; stem explants and variously derived calluses. It is greatly influenced by the genotype, physiological state of explant, age of explant and *in vitro* environment both light and temperature regimes and the constitution of medium particularly growth regulator concentrations. It is often the most crucial step for successful implementation of diverse biotechnological techniques used for plant

improvement techniques. Plant regeneration is the best demonstration of totipotency in plants. In *Ceropegia*, there are few reports which indicate rapid regeneration and multiplication through organogenesis or somatic embryogenesis (Table 5).

Organogenesis

Indirect organogenesis

There are few reports on regeneration of adventitious buds in *Ceropegia*. Nikam and Savant[26] have successfully induced shoot bud regeneration in *C. sahyadrica* by employing appropriate combinations of auxins and cytokinins. A high frequency of indirect organogenesis and plant establishment protocol was developed for *ex vitro* leaf derived callus with 1/2MS+0.5mg BA+0.25 µM spermine in *C.jainii.*[19] Rathore et al[46] obtained efficient regeneration from epicotyls explants of *C. bulbosa* in MS medium contain BA along with 0.1 mg NAA. Murthy et al[47] induced organogenesis on MS medium containing BA 13.32 µM + 2, 4-D 1.130 µM, whereas the medium with BAP 13.32 µM + 2, 4-D 4.52 µM has the highest callus producing ability in recalcitrants as well as in normal explants of *C.spiralis.* Plant regeneration from *C. bulbosa* using different explant epicotyls explants give more response in the media contain 1mg BA with 0.1mg NAA.[43]

Review of research work belongs to indirect organogenesis with a variety of species and different explants are presented in (Table 5). These reports indicate that organogenesis response was low in the genus of *Ceropegia* from internodes, leaves and thin cell layers.

Direct organogenesis

There are few reports on direct regeneration of adventitious shoots in *Ceropegia* (Table 5). These reports designate that shoot regeneration response in the genus *Ceropegia* has been obtained from different explants (internode, thin cell layers and leaf).[33] Further, studies on direct organogenesis in the genus *Ceropegia* show the preponderance of use of TDZ in inducing shoot morphogenesis on internode explants. On perusal of literature, it is also deduced that spermine has enhanced the regenerative potential of the internode explants.[19] However, all these observations have been on ornamental *Ceropegia* species. Similar regeneration response can be evinced on internode for *C.pusilla* wherein 2, 4-D was effective in inducing shoot morphogenesis and casein hastened the regeneration response by at least a week. It also highlights the importance of *in vitro* pruning for obtaining uniform explants after 4 weeks of pruning. Another feature of this study is the determination of hypodermal origin of the regeneration response from histological sections.[40] It was also found that addition of casein to the induction medium advanced the emergence of shoot buds by 2 weeks. Kondamudi et al[48] took up the study of the regeneration response to evaluate the suitable concentration of plant growth regulators and perfect explant (node, internode and thin cell layer explants-TCLs) for callus induction and subsequent organogenesis in an endangered medicinal plant *C. pusilla*. The best callus induction was found on the MS medium supplemented with BAP 13.32 µM +2,4-D 0.45 µM TCLs. After the initiation of the callus, it was immediately transferred to MS medium supplemented with BA along with other auxins like 2,4-D, IAA, IBA, NAA. The regenerative calli were raised on the MS medium supplemented with 1.13 µM of 2, 4-D. Whereas, the organogenic calli was raised on the medium containing 22.7, µM TDZ induced shoots. The calli could be maintained on the parent medium over a period of 18 months. Adventitious shoot formation is a reliable technique for clonal propagation as it prevents somaclonal variations in the cultures. The type of tissue or explant used for clonal multiplication also influences the chances of genetic variation. Because of the non uniform nature of callus tissue, genetic mutations are more frequent in shoots regenerated from callus, particularly with prolonged subculturing, than from other types of tissues.

Somatic embryogenesis

Somatic embryogenesis is a process of single cell or a group of cells initiating the developmental pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form complete plants. Suspension cultures are generally initiated from friable callus and during the regeneration phase of somatic embryogenesis is easily observed. In tissue cultures somatic embryogenesis occurs most frequently as an alternative to organogenesis for regeneration of whole plants. Adherence to this pattern of morphogenesis depends on coordinated behavior of a cell or cells to establish polarity as a unit and thereby initiate gene action sequentially specific to emerging tissue region. Different explants such as leaf stem and thin cell layers have been used for somatic embryo induction in *Ceropegia* and the medium was supplemented with varied concentrations of PGRs (Table 5). However, in *C. spiralis* direct embryogenic callus was initiated from node, internodes and thin cell layers (TCLs) in BA 13.32µM with 2,4-D.4.52µM. Highest growth rate and cell division occurred in medium containing KIN 4.56µM. Regeneration of callus into somatic embryo on solid media was improved by addition of casein hydrolysate.[40] It was also found that addition of casein to the induction medium advanced the emergence of shoot buds by 2 weeks. The above response could be attributed to the inhibition of ethylene synthesis by casein. The production of non-embryogenic and embryogenic callus for the purpose of protoplast formation, the non-embryogenic callus formed best on media containing MS + 1 µM 2, 4-D+5 µM BA. Patil[19] obtained somatic embryos from shoots with the media containing half-strength MS + 0.5mg BA and 0.25 mg/l spermine. Leaf and internode explants produced callus that regenerated somatic embryos and plantlets when cultured on MS medium with lower levels of 2,4-D (0.23 or 0.45mM) induced the highest number of somatic embryos, which developed up

to the torpedo stage. The embryogenic callus transferred to solid or liquid medium developed somatic embryos at different frequencies. Where high- frequency embryo formation occurred in suspension cultures compared to a lower frequency in static cultures. In the present study, a 100 mg callus induced more than 500 somatic embryos, while liquid media was found better than solid media. Somatic embryogenesis was asynchronous with the dominance of globular embryos was observed. 50% of the somatic embryos underwent maturation and developed into plantlets.[49] Embryogenic cell suspension offers the possibility for large-scale clonal propagation and provides an excellent tool for theoretical and practical applications. Selection scheme with embryogenic cultures gives rise to variants for various abiotic and biotic stresses. Only a few attempts have been made to regenerate *Ceropegia* through embryo-like structures produced through somatic embryogenesis. These results could be used in future to obtain transgenic plants for breeding and cytological studies.

Traditional use of selected species

Ceropegia species have been used by the tribal in various parts of Asia and Africa. Different species, their parts and mode of application/administration in various diseases are presented in (Table 6).

C.juncea was reported to be the source of 'Soma', a plant drug of the Ayurvedic system of medicine with a wide variety of uses.[50] Likewise Jain[51] reported that in *C. juncea* the fleshy stem is used as a raw material for traditional and folk medicines for the treatments of stomach and gastric disorders. A decoction is used to stop haemorrhage and is applied to malignant ulcers and also used for after child birth. The whole plant extract were used as Kidney stone and urinary tract disorders.[52] *C. ciliata* whole plant juice is used for treating fever.[53] Moreover the whole plant is used as astringent and their stem is used as hypoglycaemic and haemostatic. Tuberous root is used for diarrhea and dysentery. The bitter principle of the root is an alkaloid Cerpegin, a naturally occurring 2-pyridone, its functions as an analgesic, anti-ulcer, tranquilizer and anti-inflammatory in *C.hirsuta.*[54,18]

In Namibia C. *nilotica* fleshy roots were eaten and *C.lugardiae* whole plant powder is used in back & other ailments and also rubbed into skin incisions on the force head.[55] *C. juncea* is an edible species contains steroids, polyphenols, sugars and potassium. It is reported to be a tranquilizing, anti-inflammatory, analgesic, and antiulcer. Stem of *C.juncea* crushed with milk taken orally for ulcer in tribes of Sirumalai hills of southern India.[15] Leaf extract of *C. distincta* used as an antiseptic for wounds and *C. hirsute* used by the tribes of northwest Maharashtra for the treatment of gastrointestinal disorders and also their root infusion is used to cure stomachache.[9] *C.tuberose* used as antidote, *C.bulbosa* used in ear-ailment and kidney stone, the tuber of *C. candelabrum* used as headache in India.[56] A tuber paste of *C.spiralis* was used for indigestion.[57]

Edible species

C. papillata tuberous roots and the raw leaves are eaten in the Kota Hills of Malawi and also used as ornamental plant. *C. multiflorais* cultivated in Tanzania for its edible tuber, its tubers has containing per 100 g: water 95 g, energy 65 kJ (15 kcal), protein 0.8 g, fat 0.03 g, carbohydrate 3 g, fibre 0.3 g. *C. dichotoma* and C. *multiflora* subsp. *tentaculata* tubers contain much water and are eaten against thirst in dry regions.[55] Some species were cultivated for their edible tubers such as *C. panchganiensis C. sahayadrica* and *C.hirsuta.* The tuber of *C. purpurascens* and *stenoloba* backed in ashes before they are eaten in Namibia. *C. bulbosa*.var.*bulbosa, C.bulbosa*.var.*lushii,* the fresh tubers are usually boiled before they are eaten, to remove the bitterness.[19] (Table 7).

PHARMACOLOGICAL STUDIES

Ceropegia species have numerous pharmacological activities reported by several workers.

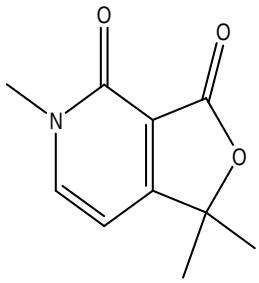
Chemical constituents in *Ceropegia*

The investigation for the phytochemistry of genus *Ceropegia* possibly began with Sivakumar et al.[58] So far, a variety of interesting but limited compounds have been isolated and identified from the plant of this genus which are alkaloids. Cerpegin, a new pyridine alkaloid, has been isolated and identified as pyridine type alkaloid from *C. juncea* together with lupeol, which are relatively rare in nature. Based on spectroscopic methods the structure of this alkaloid has been elucidated as 3, 4-dioxo-1,1,5-trimethyl-1,3,4,5-tetrahydrofuro-[3,4-*c*]-pyridine. The total alkaloid fraction exhibited promising hepatoprotective, antipyretic, analgesic, local anesthetic, anti-ulcer, and mast-cell stabilizing, tranquilsing and hypotensive activities and was devoid of side effects as noted out by the sub-acute toxicity studies.[59] Cerpegin, a novel pyridine alkaloid isolated from *C. juncea* has shown an analgesic effect (not involving the opioid pathway) against acetic acid induced writhing in mice. The chloroform extract of *C. juncea* was found to be toxic at doses above 400 mg/kg and the mice showed excitation, irritability, convulsions and respiratory paralysis.[15] Sekar and Francis[60] reported it was used as alternative source for renewable energy due to presence of polyphenol, oil and hydrocarbon.

Ceropegia the name derives from the Greek words 'keros', a wax candle and 'pegnynai', assemble or unite; for the chandelier like flower structures of some of the species. Alkaloids, triterpenes, phenolics, flavonoids, tannins, saponins, and carbohydrates were identified from Ceropegia. Only one alkaloid was identified, 3, 4-dioxo-1,1,5-trimethyl-1,3,4,5-tetrahydrofuro-[3,4-*c*]-pyridine called Cerpegin.[59] Cerpegin is a rare naturally occurring pyridinone alkaloid isolated from *C.juncea* and it is used in traditional Indian medicine as tranquillizer,

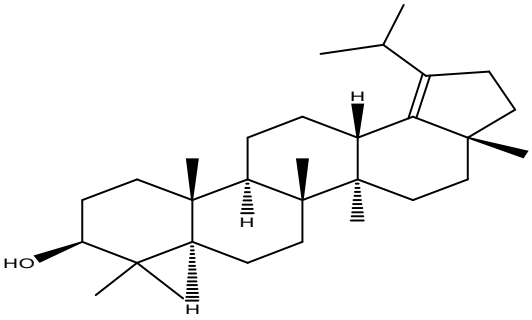
anti-inflammatory, analgesic and antiulcer.[61] Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. The basic unit in the biogenesis of the true alkaloids is amino acids. The non-nitrogen containing rings or side chains are derived from terpene units and / or acetate, while methionine is responsible for the addition of methyl groups to nitrogen atoms. Alkaloids are highly reactive substances with biological activity in low doses. Triterpenes seems to be rare in *Ceropegia*. Only two triterpenes were identified, 2Guimarenol and lup-18-en-3beta-ol from *C. dichotoma.*[62] Glycosides also seem to be rare in *Ceropegia*. Steroidal glycosides and their derivatives were identified from aerial parts of *C.fusca.*[63] They were classified on the basis of Carbohydrate residue is attached by an acetal linkage at carbon atom 1 to a noncarbohydrate residue. *Ceropegia spp* has revealed the presence of volatile oil and terpenes, in *C. woodii*, 41 peaks were isolated, of which 24 compounds were identified. Of the total volatile matter, 70.73% was terpenes, 5.82% was taxanes, and 1.52% was ketones.[64]

Alkaloid

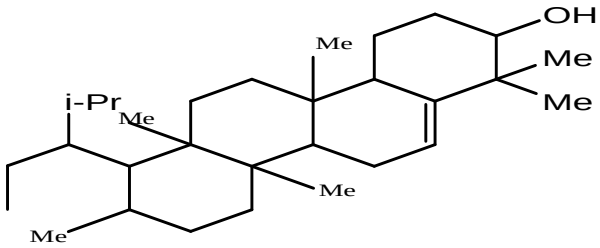


3, 4-dioxo-1, 1, 5-trimethyl-1, 3, 4, 5-tetrahydrofuro-[3,4-c]-pyridine

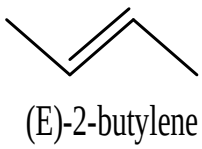
Triterpenes



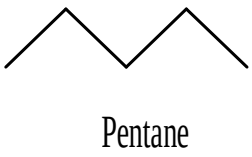
lup-18-en-3beta-ol



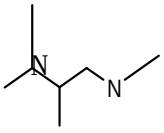
7-(1-Ethyl-2-methyl-propyl)-1,1,4b,6a,8,10a-hexamethyl-1-4a,4b,5-6a,7-11-hexadecahydro-chrysen-2-ol (2-Guimarenol)



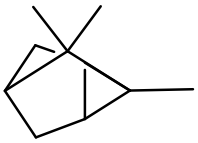
(E)-2-butylene



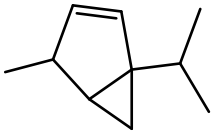
Pentane



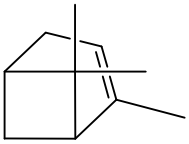
(2,3-Dimethyl-butyl)-methyl-amine



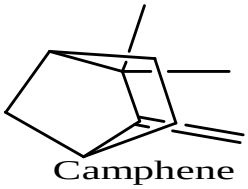
Tricyclene



Thujene

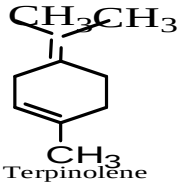


alpha-pinene

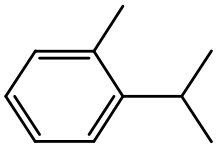


Camphene

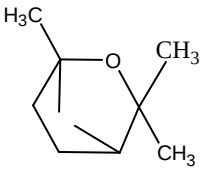
Sabinene



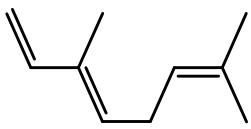
Terpinolene



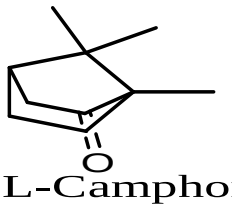
O-cymene



Eucalyptol

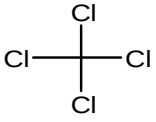


Ocimene

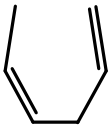


L-Camphor

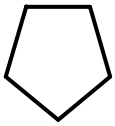
Taxanos



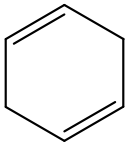
Tetrachloro-methane



1,3,5-cycloheptatriene



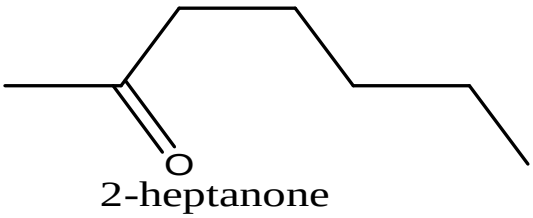
Cyclopentane



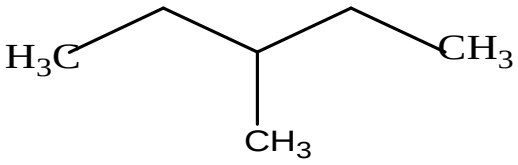
Cyclohexa-1,4-diene

Undecane

Ketones

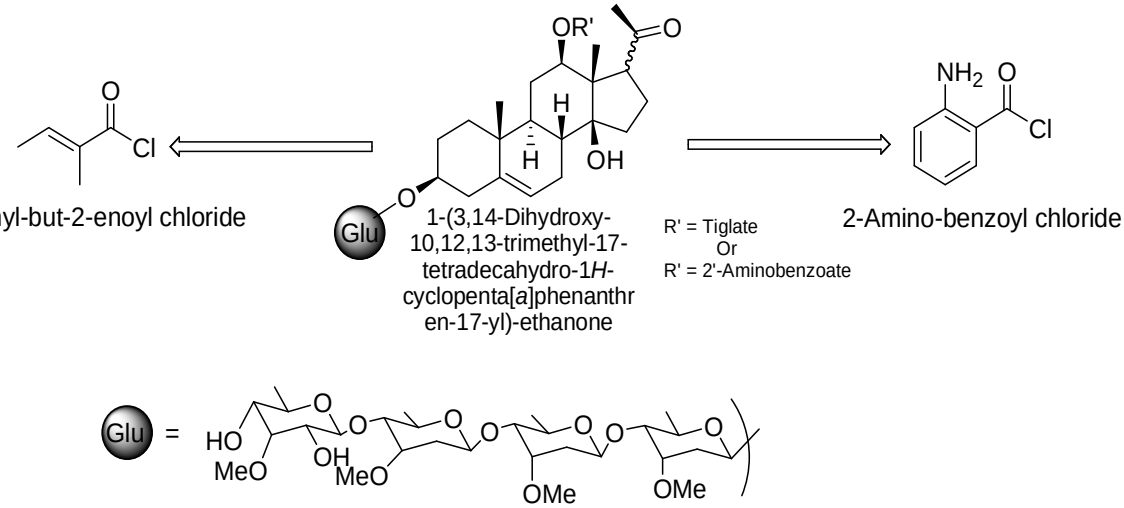


2-heptanone



3-methylpentane

Steroidal glycosides and their derivatives



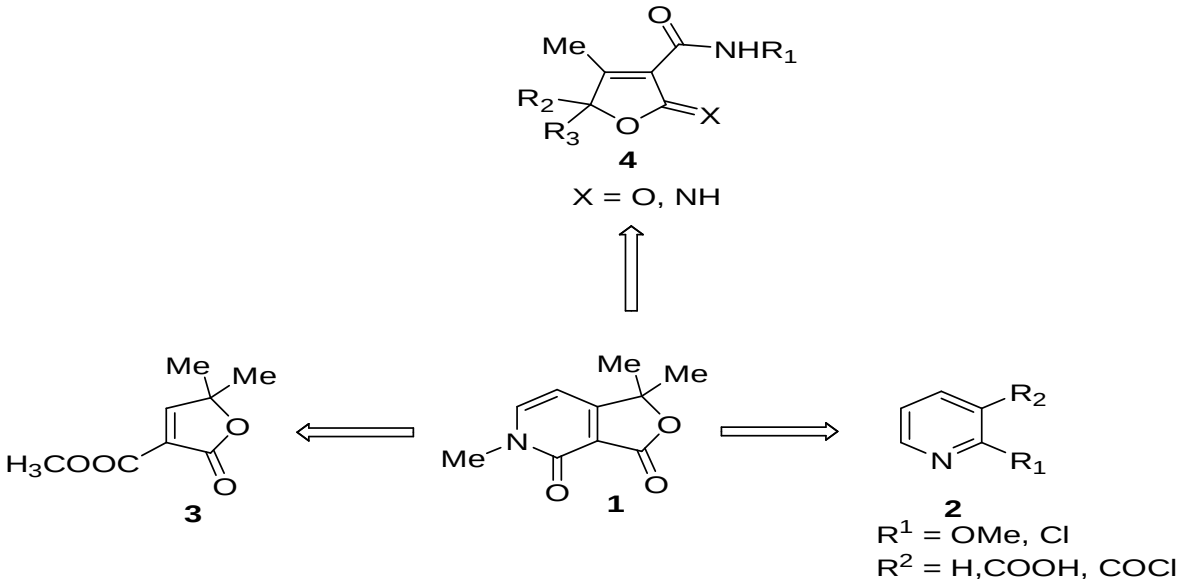
2-Methyl-but-2-enoyl chloride

1-(3,14-Dihydroxy-10,12,13-trimethyl-17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-ethanone

R' = Tiglate
Or
R' = 2'-Aminobenzoate

2-Amino-benzoyl chloride

Scheme: Retrosynthesis of cerpegin



Uses

Ceropegia are always cited as antimicrobial agents used to treat several infections. The plant extract of *C.fusca* active against the bacteria *Klebsiella pneumonia, Pseudomonas aerugimzosa, Staphylococcus aures, Staphylococcus epidermidis, Bordetella bronchiseptica, Micrococcus luteus, Bacillus cereus var. mycoidcs, Bacillus subtilis, Escherichia Coli*. The methanol extract of *C. fusca* active against the fungi *Candida albicans, C. tropicales, C. guillermondi, Saccharomyces cerevisa, Cryptococcus albidus.*[65,66] Hydroalcoholic extract from leaves of *C. bulbosa* possess significant antiurolithic activity in rats.[67] *Ceropegia spp*. have revealed the furopyridine alkaloid. Pyridine alkaloid from *C.juncea* has shown an analgesic effect (not involving the opioid pathway) against acetic acid induced writhing in mice. It potentiated pentobarbitone hypnosis and exhibited analgesic and diuretic activities. It also antagonized histamine-induced asphyxia in guinea pigs. Pavan Kumar et al[68] reported the

hydro-alcoholic extract of *C. juncea* whole plant possesses hepatoprotective activity against paracetamol induced hepatic damage and significant antioxidant activity in rats. The antioxidant and antibacetrial activity of stem extracts of this plant showed against some bacteria.[69] The plant extract from *C.rupicola* has been inhibiting the enzymatic activity of aminopeptidase N activity and leaf extract inhibit the activity of human neutrophil elastase.[70] Eucalyptole volatile oil from *C.woodii* has been shown active against *Micrococcus luteus*, and ocimene inhibit the saprophytic *staphylococcus.*[64] The root extract of *C. macrantha* is active against *Bacillus cereus*.

The extract of dichloromethane exhibited significant cytostatic activity against HL-60, A-431 and SK-MEL-1 cells, human leukemic, epidermoid carcinoma and melanoma cells, respectively in *C.fusca*. It has been used in traditional medicine as a cicatrizant, vulnerary and disinfectant in Canary Islands.[63] Darias et al[71] confirm the cicatrizant, haemostatic and chemotherapic properties that are traditionally attributed in this species.

Chemical synthesis of cerpegin

The cerpegin (**1**) have gained considerable interest in recent years, as they exhibit important pharmacological activities. Consequently a variety of synthetic approaches for the synthesis of cerpegin, have been reported in past two decade and recent literature. In which, the most prominent synthetic route of the alkaloids cerpegin, the reactions involve pyridine derivative **2** underwent ortho-lithiated product, was quenched with acetone and further acid-catalyzed manipulation of the certain product followed by methylation to afforded the cerpegin in moderate yields.[72-74] The general and very straightforward method of the synthesis of (**1**), in the four step procedure involved one of the most efficient carbon-carbon bond-forming reactions of Michael reaction between phenylthioacetonitrile and the butenolide **3**. Following oxidation of thioether and further structural transformation occurred to yield nitrile spontaneously, which could be reduced and cyclised to yield (**1**).[75,76] Likewise Tarasov et al[77] reported intramolecular HeteroDiels-Alder Cycloaddition Reaction of an Acetylene Tethered Pyrimidine for synthesis of cerpegin. In addition the conventional cyclization methods used by[78] and other methods such as thermal rearrangements of enamine **4** has also been reported for the synthesis of (**1**) is well documented.[79,80]

Table 1: *In vitro* propagation of *Ceropegia* using different explants

Species/cultivars	Explant	Culture medium	Response	Reference
C.sahyadrica Ans. & Kulk.	Axillary shoot	MS+10µM BA	Shoot multiplication	Nikam and Savant, 2007
C.elegans Wall	Axillary shoot	MS+KIN 23.20 µM+IAA 5.71 µM	Axillary bud proliferation	Krishnareddy et al., 2012
C.candelabrum L.	Axillary bud	MS+8.87 µM BA+ +2.46 µM IBA	Shoot proliferation	Beena et al., 2003a
C.bulbosa.var.*bulbosa* Roxb.	Nodal	B_5+3mg/L+0.05mg/L+30 mg Ads MS+BA0.5mg+IBA1mg	Shoot multiplication, Shoot culture	Britto et al., 2003; Patil, 1998
C.attenuata Hook.f.	Nodal	MS+13.31mM BA	Shoot multiplication	Chavan et al.,2011a
C.pusilla Wight & Arn.	Shoot tip Multiple explants	MS+ 1mg TDZ MS+0.5mg BA	Shoot multiplication propagation	Kondamudi and Rama Murthy, 2004 Ananthan, 2003
C.bulbosa Roxb.	Nodal	MS+4.44 µM+0.58 µM GA_3+0.27 µM NAA	Shoot multiplication	Goyal et al., 2006
C.jainii Ansari & Kulk	Nodal	MS+BA 0.1 µM +IBA 1 µM	Shoot culture	Patil, 1998
C.juncea Roxb.	Nodal	MS+BA 8.87 µM + TDZ 4.54 µM, MS+BAP+IAA	Shoot multiplication, Multiple shoot induction	Krishnareddy et al., 2011; Nikam and Savanth, 2009;
C.intermedia Wight var. wightii	Axillary shoot	MS+6.66 µM BA	Shoot multiplication	Karuppusamy et al., 2009
C.spiralis L.	Nodal, axillary bud	MS+2.22 µM BA	Shoot multiplication	Murthy et al., 2010a Chandore et al.,2011b
C.thwaitesii Hook.	Nodal	MS+KIN (13.94 µm) + IAA (28.54 µm).	Shoot multiplication	Muthukrishnan et al., (2012)
C.bulbosa var.*lushii* (Graham) Hook. f.	Nodal	MS+BA+IBA	Shoot culture	Patil, 1998
C.hirsuta Wt.& Arn.	Nodal	MS+7.5 µM BA	Shoot multiplication	Nikam et al., 2008
C.fantastica Sedgw.	Nodal	MS+1.5mg BA	Shoot multiplication	Chandore et al., 2010
C.mahabalei Hemadri et Ansari	Tubers	MS+ 0.1mg BAP	Shoot multiplication	Deshmukh, 2010
C. noorjahani Ansari	Axillary bud	MS+0.5mg BA+0.3mg KIN	Multiple shoot	Kedage et al., 2006

Table 2: *In vitro* rooting of *Ceropegia* using different growth regulator

Species/cultivars	Explant	Culture medium and growth regulators	Response	Reference
C.sahyadrica Ans. & Kulk.	*In vitro* shoot	MS+6mg/L spermine+5%sucrose	Rooting	Nikam and Savant., 2007
*C.spiralis*L.	*In vitro* stem *In vitro* shoot	MS+9.04 µM 2,4-D+5.37 µM NAA ½MS +10.74 µM NAA	Rooting Rooting	Murthy et al., 2010a Murthy et al, 2012
C.candelabrum L.	*In vitro* shoot	½MS 0.49 µM IBA	Rooting	Beena et al., 2003
C.bulbosa var.*bulbosa* Roxb.	*In vitro* shoot	MS+2mg/L IBA. ¼ MS+0.18mM BA+6% sucrose	Rooting	Britto et al., 2003. Patil, 1998
C.jainii Ansari & Kulk	*In vitro* plant	½MS+0.18mM IBA+6% sucrose	Rooting	Patil.,1998
C.juncea Roxb.	*In vitro* plant *In vitro* plant	MS+ IBA 4.90 µM + NAA 1.27 µM MS+IAA0.5mg+NAA0.5mg+IBA0.5mg+BA1.0mg	Rooting Rooting	Krishnareddy et al., 2011 ıubacker et al., 2012
C.pusilla Wight & Arn.	*In vitro* shoot *In vitro* shoot	MS+13.32 µM BA+1.23 µM IBA MS+KIN+IBA	Rooting Rooting	Murthy et al., 2010c Kondamudi and Murthy, 2004
C.attenuata Hook.f.	*In vitro* culture	½MS+2.46 µM IBA	Rooting	Chavan et al., 2011a
C.bulbosa var.*lushii* Hook.f.		½MS+0.18mM IBA+6% sucrose	Rooting	Patil., 1998
C.bulbosa Roxb.	*In vitro* shoot	MS+11.42 µM+3.55 µM IAA	Rooting	Goyal et al., 2006
C.intermedia wight	*In vitro* shoot	½MS+5.32 µM NAA	Rooting	Karuppusamy et al., 2009
C.hirsuta Wight & Arn.	*In vitro* shoot	½MS+2 µM IAA+5% sucrose	Rooting	Nikam et al., 2008
C.fantastica Sedgw.	*In vitro* shoot	MS+1mg/L IBA	Rooting	Chandore et al., 2010
C.thwaitesii Hook.	*In vitro* shoot	MS+ 0.5mg/l IBA	Rooting	Muthukrishnan et al., 2012

Table 3: *In vitro* tuberaization of *Ceropegia* using different growth regulator

Species/Cultivars	Explant	Culture medium and growth regulators	Response	Reference
C.jainii Ansari & B.G. Kulk.	*In vitro* shoots	½ MS+46.5µM KIN+22.2 µM BA +4% Sucrose	Tuber formation	Patil, 1998
C.bulbosa var.*lushii* (Graham) Hook. f.	*In vitro* shoots	½ MS+46.5µM KIN+22.2 µM BA +4% Sucrose	Tuber formation	Patil, 1998
C.pusilla Wight & Arnott	*In vitro* shoots *In vitro* raised shoots	MS+BA MS+BAP 13.32 µM+ 2,4D 0.11 µM+3sucrose	Tuber formation Microtuberization	Kondamudi and Murthy, 2011; Murthy et al.,2012
C.bulbosa.var.*bulbosa* Roxb.	*In vitro* shoots	½ MS+46.5µM KIN+22.2 µM BA +4% Sucrose	Tuber formation	Patil, 1998
C.hirusta Wight & Arnott	seeds	MS+BA+KIN	Tuber formation	Pandit et al., 2008
C.maccannii Ans	Seeds	½ MS+BA	Micro tuber formation	Pandit et al., 2008
C.sahyadrica Ans. & Kulk.	Seeds	MS+BA	Microtuberization	Pandit et al., 2008
C.spiralis Wight	*In vitro* raised shoots	MS+BA13.32 µM µM+NAA1.34to2.68µM	Microtuberization	Murthy et al., 2012

Table 4: *In vitro* flowering of *Ceropegia* using different growth regulator

Species/cultivars	Explant	Culture medium	Response	Reference
C.sahyadrica Ans. & Kulk.	Follicles	MS+BA 0.5 mg+1%sucrose	Flowering	Nair et al., 2007
C.pusilla Wight & Arn.	Axillary shoot	MS+0.5BAP+IBA	Flowering	Kondamudi et al., 2011
C.attenuata Hook.f.	*In vitro* shoot	MS+4.14Pic µM	Flowering	Chavan et al.,2011a
C.lawii Hook.f.	Apical shoot	MS+87mM Sucrose+4.4µM BA	Flowering	Nair et al., 2007
C.bulbosa var. *bulbosa* Roxb.	*In vitro* shoot Apical bud and axillary bud	½ MS+1mg GA_3 MS+87mM Sucrose+26.6 µM BAP	Flowering Flowering	Britto et al., 2003; Nair et al., 2007
C.spiralis L.	*In vitro* shoot	½ MS+11.54µM IAA+3% sucrose	Flowering	Murthy et al., 2010a
C.maccannii Ansari	Axillary bud	MS+87mM Sucrose+4.4µM BA	Flowering	Nair et al., 2007
C.jainii Ansari & B.G. Kulk.	Axillary shoot	½ MS+0.25µM spermine	Flowering	Patil., 1998
C.fantastica Sedgw.	*In vitro* axillary shoot	MS+0.5mg TDZ	Flowering	Chandore et al., 2010
C.oculata R.A. Dyer	Apical bud and axillary bud	MS+87mM Sucrose+26.6 µM BAP	Flowering	Nair et al., 2007
C.hirsuta Wight & Arnott	Apical bud and axillary bud	MS+87mM Sucrose+26.6 µM BAP	Flowering	Nair et al., 2007

Table 5: *In vitro* regeneration of *Ceropegia* using different explants

Species/cultivars	Explant	Culture medium	Response	Reference
C.sahyadrica Ans. and Kulk.	Internode	MS+1 µM 2,4-D + 5 µM BAP	Indirect organognesis	Nikam and Savant, 2007
C.pusilla Wight & Arnott	Node,internode, thin cell layers	MS+13.32 µM BA+0.45 µM2,4-D +45.4 µM TDZ + casein hydrolate	Indirect Organogenesis	Kondamudi et al., 2010
C.juncea Roxb.	Internode	MS+1 µM2,4-D + 5 µM BA	Organogenesis	Nikam and Savant, 2009
C.candelabrum L.	Leaf and internode	MS+4.52 µM 2,4-D	Indirect somatic embryogenesis	Beena and Martin, 2003a
C.spiralis Wight	Node, internode,	MS+4.52 µM 2,4-D+ 4.56 KIN +250mg/L casein MS+13.32 µM BA+0.53 µM NAA	Direct somatic embryogenesis Organogenesis	Murthy et al, 2010b,a
C.jainii Ansari & B.G. Kulk.	Nodes	½ MS+0.5mg + 2mg 2,4-D BA+0.25 µM spermine ½ MS+0.5 mg BA	Indirect organogenesis Indirect Somatic	Patil, 1998 Patil, 1998
C.bulbosa Roxb.	Epicotyl, hypocotyl, root, cotyledonary node Epicotyl	MS+1mg BA+0.1mg NAA MS+BA+0.1mg NAA	Indirect organogenesis Indirect organogensis	Phulwaria et al., 2013; Rathore et al.,2010

Table 6: Traditional uses of *Ceropegia* species

Species / Cultivars	Part	Mode	Indications	Country	References
C.ciliata Wight	Whole plant	Juice	Fever	India	Rajan et al., (2005)
C.oculata	Tubers	Raw tubers	Child fever	India	Jagtap et al., (2006)
C.odorata R.A. Dyer	Leaves, tubers	Raw leaves, tubers	Stomach ache, tuber juice is dropped in eye to cure opacity. Tubers are used as vegetable	India	Jagtap et al., (2006)
C.spiralis Wight	Tubers	A teaspoon of tuber paste taken daily once for 3 days	Indigestion	India	Reddy et al., (2009)

Table 6: Traditional uses of *Ceropegia* species (Continued.....)

Species / Cultivars	Part	Mode	Indications	Country	References
C.juncea Roxb.	Fleshy stem	Raw stem	Treatments of stomach and gastric disorders	India	Jain (1991)
	Tubers	Boiled	Consumed after cooking	India	Mabberley, 1997;
C.bulbosa Roxb.	Tubers	Boiled tubers	Food and Stomach ache, Urinary bladder stone	India	Jain and Defillips (1991), Sharma et al., (2011)
C.candelabrum L.	Tubers	Raw tubers	Stomach pain	India	Jain and Defillips (1991)
C. mahabalei	Tubers	Raw tubers	Indigestion	India	Deshmukh 2010
C.*lugardiae* N.E. Br.	Whole plant	powder	Success of hunting	Namibia	David Aiyambo, 2010
C.pusilla Wight & Arnott	Leaf&Flower	Raw extracts	Nervous weakness	India	Rajan et al.,(2005)

Table 7: Selected edible species from *Ceropegia*

Species/ Cultivars	Species Part	Mode	Indications	Country	References
C.vincaefolia Hook.	Tubers	Cooked	Used as a vegetable	India	Jagtap et al., (2006)
C.multiflora subsp. *tentaculata* (N.E. Br.) H. Huber	Tubers	Raw tubers	Nourishment	Namibia	David Aiyambo, 2010
C.nilotica Kotschy	Roots	Raw root	Diet	Namibia	David Aiyambo, 2010
C.purpurascens K.Schum.	Tubers	Dry tuber	Eaten	Namibia	David Aiyambo, 2010
C.stenoloba Hochst. ex Chiov.	Tubers	Raw	Edible	Namibia	David Aiyambo, 2010
C.bulbosa var.*bulbosa* Roxb.	Fresh tubers	Boiled	Boiled before eaten	India	Patil (1998)
C.bulbosa.var.lushii (Graham) Hook. f.	Fresh tubers	Boiled	Boiled for food	India	Patil (1998)

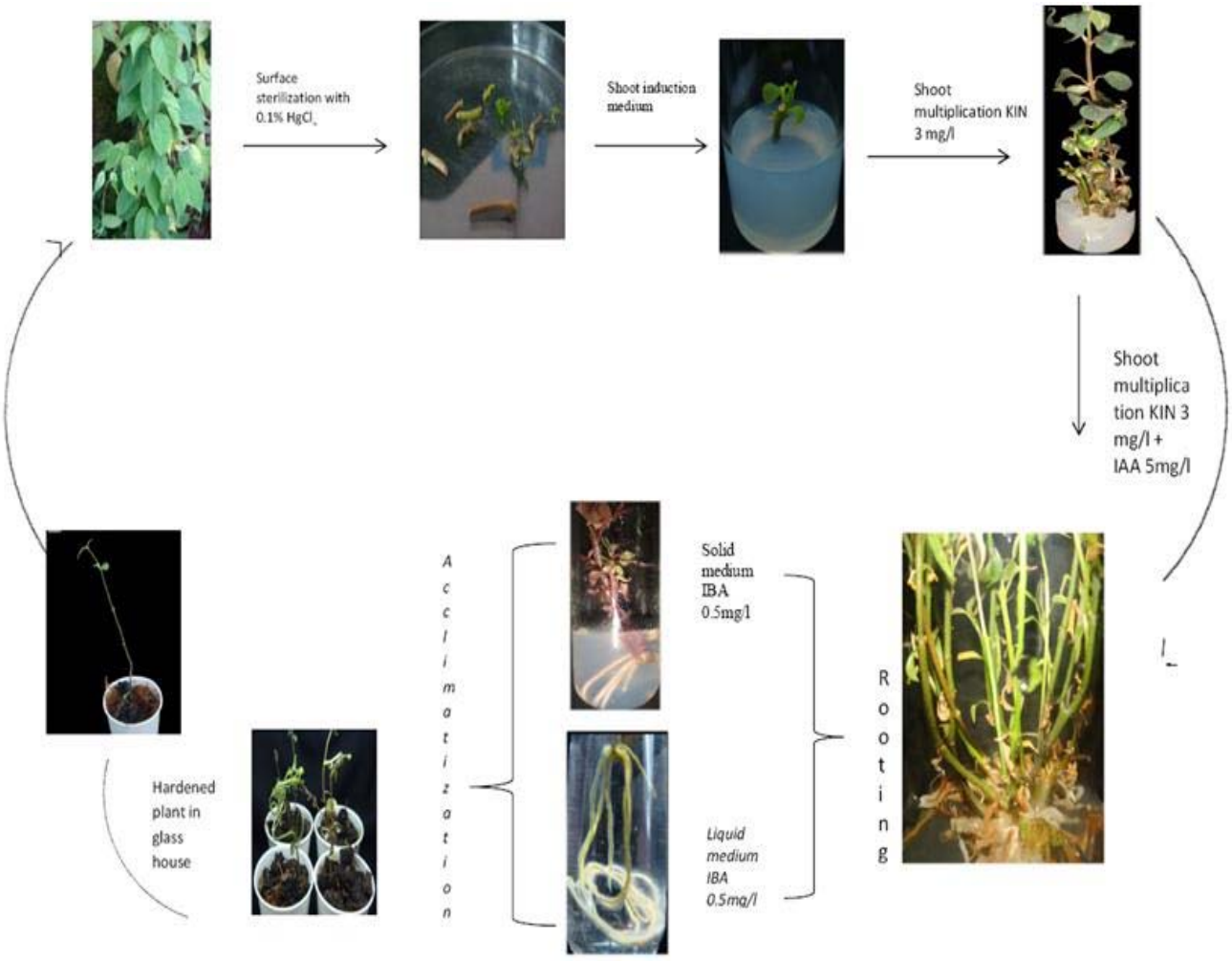
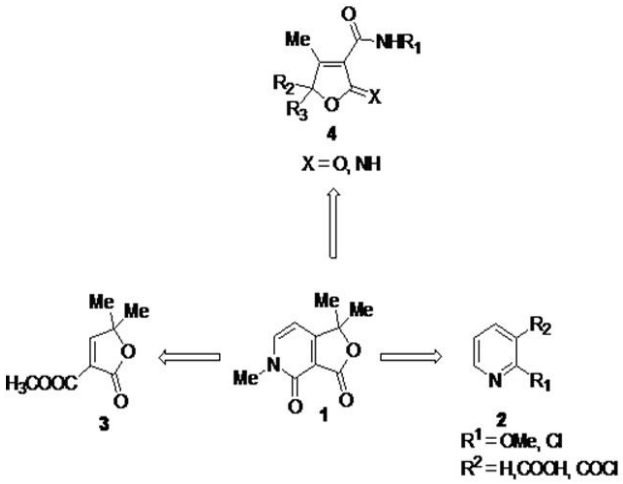


Figure 1: Micropropagation protocol of *C.thwaitesii*

Scheme: Retrosynthesis of cerpegin



SUMMARY AND FUTURE PROSPECTS

The following manifestations can be made on the basis of this comprehensive perusal of literature. Based on the literature 17 species were studied in *in vitro*, among these species *C.bulbosa* and *C.juncea* were suitable for *in vitro* propagation due to the presence of alkaloid Cerpegin most of researches focus on their work in this species. Attempt must be made to increase the content of cerpegin by various treatments and need to quantify the cerpegin in tissue cultured raised plants. Among the different explant used axillary bud explant was suitable for multiplication and epicotyls explant was suitable for regeneration studies. *Ceropegia* spp are being used traditionally, due to their immense therapeutic potential to treat/cure various diseases. Alkaloids present in plants and exhibit significant biological activity. Cerpegin is a key compound of the genus *Ceropegia*. The clonal propagation of *Ceropegia* via axillary bud culture, shoot tip culture and rooting of microshoots from *in vitro* plants are very useful in conserving *Ceropegia* species. *In vitro* propagation of *Ceropegia* via somatic embryogenesis offers a great potential for rapid propagation and improvement of *Ceropegia*. Thin cell layer tissue of *Ceropegia* is amenable to somatic embryogenesis in the presence of 2, 4-D in combination with other phytohormones and additives. Germination of somatic embryos and plantlet survival has been achieved, although at a low frequency. If maturation and germination techniques for somatic embryos could be improved and the number of plantlets increased, the potential exists for genetic improvement and multiplication of *Ceropegia*. Rapid multiplication of elite clones, production of healthy variety and disease-free plants and faster introduction of novel cultivars with desirable traits are urgently needed in *Ceropegia* improvement programmes. In this regard, *in vitro* propagation techniques are likely to play a vital role. At present, there are many reproducible protocols for *in vitro* propagation of *Ceropegia*. The studies carried out during the last few decades on different stages involved in *Ceropegia* micropropagation has led to considerable improvement of protocols and methods. Shifting of medium status from gelled to liquid medium during shoot multiplication and rooting would be a strategic step for automation and cost efficiency in micropropagation.

However, the new challenges that are faced today by the tissue culture industry include cost efficiency, automation, control and optimization of the micro environment. The recent trend to move from agar gelled media to liquid media is a strategic step in this direction. A big challenge in *Ceropegia* studies focuses on genetic improvement of elite species with desirable treats by transformation systems and identification by molecular markers.

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