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Effects of Additive supplements in tissue culture of *Gloriosa superba* L. - a medicinally important plant

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Abstract

Gloriosa superba is frequently used in traditional medicine system for treatment of several diseases including infectious ones. Several active phytochemicals are present in this plant and they show remarkable effect on pathogenic bacteria. *In vitro* propogation was carried out for direct regeneration of clone plants with same genotype. Optimized hormonal combination of 5.0 mg/l BAP+1.0 mg/l NAA showed 88% bud initiation and multiplication in *Gloroisa superba* was maintained as control medium. Various supplements like coconut water, GA_3, ABA, and Biotin were added to enhance shoot multiplication in tuber sprouts of *Gloriosa superba*. With addition of 15% coconut water (cw) there was enhancement in shoot multiplication (14.6 ±1.1cm), length of shoot was 5.6 ±0.6 cm and days taken were twenty two. Coconut water improved both the quality and development of shoots in *in-vitro* cultured *Gloriosa superba*. The other additives such as GA_3, Biotin and ABA had less or no supportive effect on multiplication of shoots in *Gloriosa superba* explant.

Medicinal plants constitutes a very important natural resources of India because she has one of the richest plant based ethno-medicinal traditions in the world going back to over 3000 years old medicinal heritage[14]. A high level exploitation over the recent years coupled with habitat loss and degradation as a result of various biotic pressures has led to a noticeable decline in the population levels of many valuable medicinal plant species, particularly those belonging to perennial category.

In vitro techniques are being increasingly used for the multiplication and conservation of the germplasm of medicinally important plants threatened with extinction[6,7]. These techniques are now successfully applied to a range of threatened and endangered medicinal and aromatic plant species[16].

Gloriosa superba L. (Glory lily) is a medicinal plant previously belonging to family Liliaceae, presently included in family Colchicaceae. It is a semi-woody herbaceous, branched tuberous climber Traditionally, many

Habit of Gloriosa superba L. (Glory lily)

tribes use *Gloriosa superba* for curing various ailments. In India, it is used in treatment of blood diseases, swelling, wounds, pain and gonorrhea. It is also used as tonics. The tuber is used in colic, chronic ulcers, hemorrhoids, cancer and impotency treatments[12]. Tubers are also used in the treatment of cancer, malaria, stomach ache, piles and leprosy. Folklore tradition mentions "Kalihari" – as an abortifacient, the plant rhizome is prescribed for abortion[7]. A pale yellow to greenish yellow alkaloid, colchicine is mainly responsible for the toxic effect of *G. superba*. . It also contains another toxic alkaloid Gloriosine.

Source of plant material:- One year old plants of *Gloriosa superba* grown and maintained in the garden at Sri Sathya Sai College for Women, Bhopal were used as the source of explant. Excised terminal shoot tips were used as explants.

Nutrient medium:- Murashige and Skoog media was used as basal medium for induction of shoot bud. Basal media strength, different combinations and concentrations of growth regulators, cytokinins (BAP, Kn, 2ip) and auxins (NAA, IAA, 2,4-D) were taken. Standard procedures were followed for the preparation of media. The media strength, plant growth regulators and other supplements used, stock solutions of major and minor salts, vitamins and growth regulators were prepared by dissolving required quantity of chemical in distilled water.

Explant preparation:- The sterilization of the culture medium was carried out in an autoclave for 15 minutes at 121°C and 15 Lbs pressure. After sterilization, the culture tubes and flasks were stored in an air conditioned culture room until further use. The explants collected from the source plants were coarsely trimmed to a size of 3cm and washed in running tap water for 5 minutes followed by washing in distilled water with a few drops of Labolene.

Inoculation & incubation:- All the inoculation operations were carried out under strict aseptic condition inside a Laminar air flow chamber, which was made sterile by the incessant exposure of germicidal U.V. rays for half an hour before use. All operations were

carried out using pre- sterilized instruments and glassware. Explants were then aseptically introduced into culture vessels. In order to curtail contamination during drying and inoculation, only a few explants were treated at a time.

Sub culturing & regeneration:- All experiments were conducted in eight replicates twice and date and number of shoots obtained through axillary bud proliferation as well as regeneration of adventitious shoots were analyzed using standard ANOVA procedures and the difference between the means were compared using the Fischer's least significant difference test (LSD). The media were supplemented with different concentrations of the homogenates of organic extracts at 15% of coconut water and were tested to see their effect on direct shoot induction and shoot multiplication in *Gloriosa superba*. All cultures were incubated at 25±1°C and under cool white fluorescent light of 30 µ mol/m2.s for 16 hours per day. Experiments were performed in a completely randomized design. Plant height, fresh weight, number of leaves and number of roots from explants was evaluated after 12 weeks of culture.

Experiments were conducted on micropropagated nodal explant of *Gloriosa superba* L. with additive supplements. Enhancement of shoot induction by additive supplementation. MS medium containing hormonal combination 5mg/l BAP + 1.0mg/l NAA was maintained as the control, as it was proved to be optimum in the given experiment and various supplements like coconut water, Biotin, GA_3 and ABA were added to enhance shoot induction.

Effect of Coconut water: - MS medium containing the optimized hormonal combination when supplemented with 5-25% coconut water (CW), it was observed that at 15% there was enhancement in the number of shoot induction and multiplication. At higher concentration of CW in the medium i.e. above 10%, there was response which varied from 70-80%.

Effect of Biotin: - Addition of Biotin at a concentration of 0.5 to 2.5mg/l when incorporated into the medium, no significant variation in the number of shoots was produced. The number of shoots induced was always less than the control. Days to induce response varied form 25-30, percentage response varied between 60-80%. Length of the shoots were also less than the control.

Table 1: Enhancement of shoot induction by additive supplements on nodal explant of *Gloriosa superba* L.

Additive Supplements (mg/l)	Concentration of additives (mg/l)	% Response	No. of days required for shoot induction	Number of shoots (Mean ± SE)	Shoot length (cms) (Mean ± SE) after 45 days
MS+PGR+CW	**15**	**80**	**22**	**14.6 ± 1.1**	**5.6 ± 0.6**
MS+PGR+Biotin	1.0	80	26	11.0 ± 0.8	4.5 ±0.9
MS+PGR+ GA_3	1.0	80	26	8.6 ± 0.7	6.6 ± 0.8
MS+PGR+ABA	1.0	70	23	11.0 ± 0.9	4.5 ± 0.5

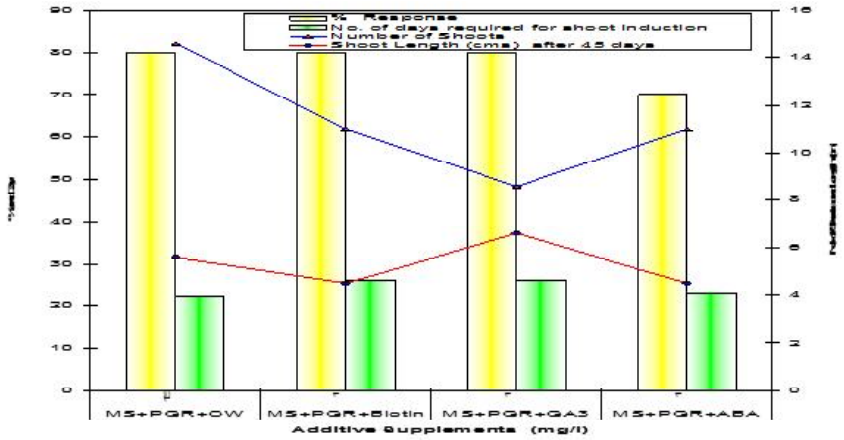

Fig. 1: Effects of shoot induction by additive supplements on nodal explant of *Gloriosa superba* L.

Effect of GA_3:- GA_3 also could not induce any enhanced effect on shoot production. When the concentration ranging from 0.5 mg/l to 2.5mg/l were tried, it was observed that number of shoots produced were less than that of the control. Percentage response varied from 60-80%, length of shoots obtained was greater than that of controlled treatment. Days to induce response varied from 24-28 days (Table1 and Fig.1).

Effect of ABA:- Addition of ABA (0.5-2.5mg/l) to the optimal medium could not enhance the number of shoots. The number of shoots produced was always less than that of control. Time to induce response varied from 23-25 days. Percentage varied form 55-75% least percentage response (55%) was noticed at 2.5 mg/l ABA

Enhancement of shoot initiation and multiplication by additive supplementation:

Coconut water at 15% showed enhancement in shoot induction and multiplication in nodal explants of *Gloriosa superba* L. The numbers of shoots were 14.6 ±1.1 and the length of shoots were 5.6 ± 0.6 and the time taken for induction was 22 days.

A higher concentration of CW (coconut water) causes hormonal imbalance thus decreasing the shoot numbers. According to Jordhan *et al.*,[10] coconut water improved both the quality and development of shoots from *in vitro* cultured nodal segments. Other additives such as GA_3, Biotin and ABA had no supportive effect on multiplication in *Gloriosa superba* explant.

Many undefined additions made to plant tissue culture medium are fluids which nourish immature zygotic embryos[4], juices, pulps and extracts from various fruits; meat, malt extracts and fibrin digest, extracts of seedlings or plant leaves, the extract of boiled potatoes and corn steep liquor. The extensive use of coconut water as a growth-promoting component in tissue culture medium formulation can be traced back to more than

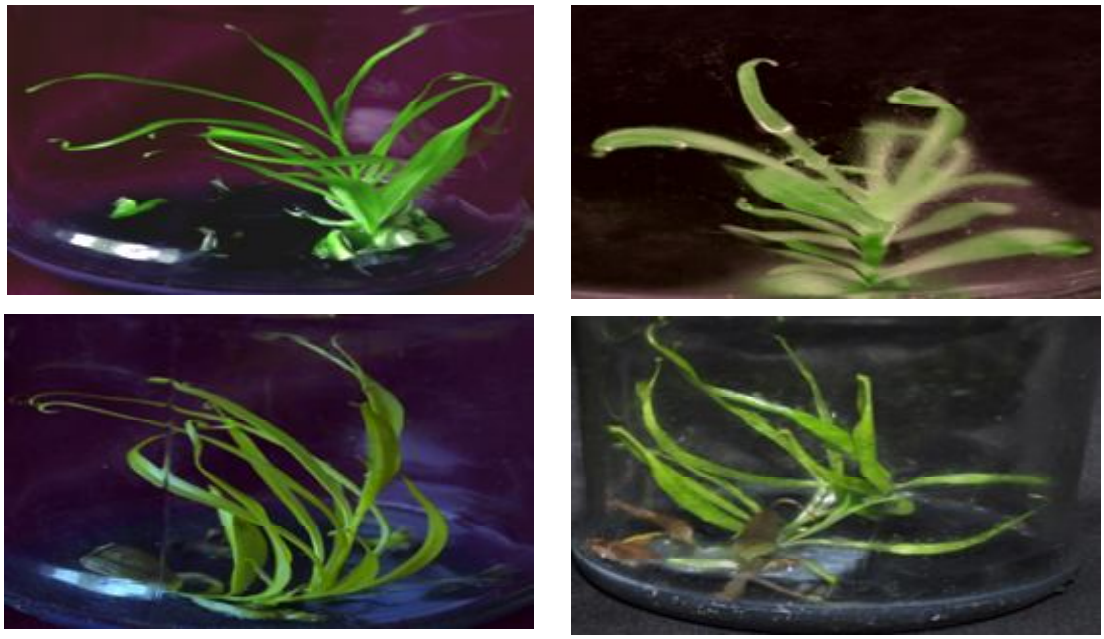

Fig. 2: Elongated multiple shoots of *Gloriosa* superba L. in additive supplements

half a century ago, when, Van Overbeek *et al.*,[18] first introduced coconut water as a new component of the nutrient medium for callus cultures in 1941 .Besides its nutritional role, coconut water also appears to have growth regulatory properties, *e.g.*, cytokinin-type activity[8]. Some of the most significant and useful components in coconut water are cytokinins, which are a class of phytohormones[11]. Other components found in coconut water include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and Auxin Activity Coconut water contains indole-3-acetic acid (IAA), the primary auxin in plants[19]. IAA is a weak acid (pKa = 4.75) that is synthesized in the meristematic regions located at the shoot apex and subsequently transported to the root tip in plants[5]. 4.2.3. Cytokinin Activity Coconut water is an important additive in the tissue culture media of several plants, including orchids and traditional medicinal herbs. The cytokinins found in coconut water support cell division, and thus promote rapid growth. Cytokinins are a class of phytohormones that exert various roles in the different aspects of plant growth and development, *e.g.*, cell division, formation and activity of shoot meristems, induction of photosynthesis gene expression, leaf senescence, nutrient mobilization, seed germination, root growth and stress response[2]. One advantage of coconut water is that it results in considerable plant cell proliferation without increasing the number of undesirable mutations[3]. Protein efficient plant regeneration is the primary objective of many studies in plant tissue culture

Among the different natural supplements tested, coconut water found to be most suitable when compared to other organic additives used

in the media. In 15% coconut water enriched medium, the highest number of shoot per explant (14.6+1.1) was observed and also there was an accelerated developmental process leading to healthy plantlets. Similarly use of 15% coconut water was found to be most effective in increasing frequency of response and multiplication in many plants like *Cynbidium pendulum*[14], *Phalaenopsis violacea*[6] and *Paphiopedillum villosum.*

Similarly the use of coconut water in increasing the number of shoot, shoot length and the number of nodes has also been reported[13]. Though other supplements (Biotin, ABA, GA3) exhibited positive effect, the response percentage and other physiological parameters were significantly lesser (Table-1). The increased morphogenetic effect of coconut water when compared to other organic supplements can be attributed for its chemical composition (glucose, fructose, different amino acids and minerals).[5] The presence of diphenyl urea in coconut water acts as cytokinin and induces the growth and cell division as reported by Santoso *et al.*,[15]. Agampodi and Jayawardena[1] also reported that the effective growth and regeneration in plants of coconut water supplemented media is due to the natural content of cytokinin and auxin. We observed that higher concentration of coconut water (20%) reduced the percentage of response, number of multiple shoot, shoot length.

This observation is supported by the findings of Gnasekaran *et al.*,[9] who stated that use of coconut water at 20% to 30% showed inhibitory effect on shoot regeneration. Observation is further supported by the study of Baque[4] in Calanthe hybrids produced abnormal plants with retardation in growth and morphological characteristic at higher concentration of coconut water.

Therefore it is speculated that coconut water at 15% could be used as a potential organic additive in the culture medium for propagation of *Gloriosa superba* which can be comparable with other synthetic growth hormones. So use of coconut water can practically reduce the cost of media for commercial production of *Gloriosa superba*.

Micropropagation system provides a method for rapid regeneration of various medicinal crops of high economic value. The improved *in vitro* plant culture system has the potential for commercial production of medicinal crops on large scale. During the past decade remarkable progress resulted in plant biotechnology has been witnessed with a constant flow of improved transformation regeneration protocols for many medicinal crops. A good regeneration protocol is always needed for genetic transformation studies for up-regulation of secondary compounds. In such instances usage of natural organic extracts in culture medium rejuvenates the *in vitro* plant system resulted with good regeneration frequencies and enhanced shoot multiplication. Supplementation of natural organic extracts as additives for standardizing regeneration protocols of commercially important medicinal crops has been increased significantly. These promissory organic extracts described in this review would certainly be of increasing importance in near future in the field of medicinal plants research, such as genetic transformation studies and scale-up of secondary compounds through cell suspension cultures in bioreactors.

It is concluded from the above study that carbon source plays important role in growth and morphogenesis of *G.superba*. In the present study natural additives proved to be efficient in induction of multiple shoots.

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