International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 16-03-2021, Accepted: 17-04-2021, Published: 08-05-2021
www.botanyjournals.com
Volume 6, Issue 3, 2021, Page No. 74-77

Gloriosa superba: Its properties and *in vitro* production methods

Ritbrat Gurung[1], Sakshi Sharma[1], Shivika Sharma[2], Vikas Sharma[3*]

[1] Department of Molecular Biology and Genetic Engineering, Lovely Professional University, Jalandhar, Punjab, India

[2] Department of Biotechnology, DAV University, Jalandhar, Punjab, India

[3] Assistant Professor, Department of Molecular Biology and Genetic Engineering, Lovely Professional University, Jalandhar, Punjab, India

Abstract

Gloriosa superba is a perennial climber which flourish at an altitude of 6000ft. Two major factors that are suitable for its growth are tropical climate and high altitude. It is recognized for its array of important medicinal properties owing to the presence of an alkaloid colchicine. It has various medicinal properties ranging from anti-inflammatory, anti-cancer, anti-fungal, anti-bacterial, anti-malarial and also as an agent to induce abortion. Due to tremendous uses of the plant and presence of various important metabolites it is being exploited by pharmaceutical companies thus making the plant to be one of the important plants in the endangered medicinal plants list. Thus, utilization of Plant Biotechnology as a major field for all the tissue culture- based methods in order to meet industries demand and its regeneration capacity.

Keywords: *Gloriosa superba*, colchicine, medicinal, alkaloids, toxicity, *in-vitro* propagation

Introduction

Gloriosa superba is a perennial herbaceous climber belonging to the family Colchicaceae and order Liliales. It is also known as Flame lily, Glory lily, Fire lily and Climbing lily. In World it is habitats in tropical and subtropical areas of the world ranging from Sri Lanka, Malaysia and in Africa covering from east Senegal to Somalia and Ethiopia. Whereas in India it prevails in the tropic and subtropical area [1]. *Gloriosa superba* is derived from two separate words 'Gloriosus' which means handsome owing to its beautiful and vibrant orange-red leaves and the word 'Superba' which means magical and splendid. A distinct appearance of the plant is the major basis for its attraction in the world. It is well known due to its two major properties, first its role as an anti-inflammatory agent for the treatment of diseases like gout and also its role in decreasing cancer progression which is still being studied and researched worldwide. *Gloriosa superba* has also been used in ancient Ayurvedic medicine centuries ago as a tonic, an anthelmintic and also for treatment against snake bite. Another interesting medicinal property of the plant was its function as an abortifacient produced by taking the rhizomes of the plant reported by the Duke in early 1922. Out of all the *Gloriosa species* like *Gloriosa simplex, Gloriosa grandiflora, Gloriosa lutea, Gloriosa longifolia, Gloriosa sudanica. Gloriosa Superba* is one of the most widely important Gloriosa species due to its huge demand and also increased amount of colchicine content in it as compared to other species [2-3].

Gloriosa superba is regarded as an herbaceous vine which climbs perennially and grows up to length of 3.5 m – 6 m. It is tolerant to the poorly enriched soils and grows well in red loamy and sandy loamy soils [4]. Leaves are dark green and glossy in nature and they are spear shaped, etiolated, sessile, ovate to lanceolate and alternate or simple. Growing in whorls of 3 to 4 coinciding with tendrils which helps in clinging to other plants. They are 6-15cm × 1.5-4cm in size [5-6]. The flower which is the trademark feature of the plant is orange red to yellow in color hence gaining the name flame lily blooming from November to March annually. The perianth of the plant consists of petaloid tepals which looks to resemble a petal having a linear shape and having four color combination from orange to yellow to green to crimson. Stamens are prominent six in number circling around the flower and hypogynous in configuration. Anthers residing on top of stamens are linear and dorsifixed producing yellow pollens. The ovary is situated at the base of the flower protruding outwards which is 1cm×0.5cm [7]. The fruits bearded by the plant is loculicidal in nature meaning that in maturity of the fruit the inner structure of the fruit gets weakened in order to release its seeds and this ripening takes about 6-10 weeks' time. The seeds yield per fruit is about 18-20 seeds and the size of the fruit is 4-6cm×1-2cm. *Gloriosa superba* mostly favors or promotes cross pollination which is mostly done via small birds and butterflies mostly due to the open morphology of the flowers. The most compatible pollinators of the plant are the butterflies precisely the pierid Eronia Cleodora [8-9].

Bio actives of Gloriosa Superba

Gloriosa superba contains vast number of bioactive compounds namely the Alkaloids, Glycosides, Steroids, Tannin and Terpenoids [10]. Out of all these bioactive compounds the types and amounts of alkaloids present in *Gloriosa superba* is the reason why it is so sought out by pharmaceutical companies everywhere. The two most medicinally important alkaloid present in the plant are Colchicine and Colchicoside. A single plant contains up to 0.9% colchicine and over 0.8% of colchicoside and the part of the plant which contains the most of these alkaloids are the stem tubers. The other compounds present in the plant which also possesses some types of medicinal properties are Lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, superbine, Gloriosine, N-formyl

deacetyl colchicine, 3-demethyl colchicine, 1,2-didemethyl colchicine and the latest alkaloid isolated from the plant was 3-O-demethylcolchicine-3-O-alpla-D-glucopyranoside discovered as early as 2001. As alkaloids being a type of heterogenous secondary class biomolecules are composed from the five types of amino acids lysine, phenylalanine, tyrosine, ornithine and tryptophan and the alkaloid. Majority of the medicinal importance of the plant relies onto Colchicine which is regarded as an alkaloid drug (N-[(7S)-1, 2, 3,10-tetramethoxy-9- oxo- 5,6,7,9 - tetrahydrobenzo [a]heptalen-7-ylis) produced or derived from phenylalanine, methionine and tyrosine. The two precursors of phenylalanine and tyrosine, Trans cinnamic acid and p-courmic acid has found to increase the formation of Colchicine. The concentration of colchicine in other parts of the plant are, stem 0.33-0.41%, ovary 0.08%, corms 0.3% and the flower contains 1.18%. The compounds found in the tubers of the plant are colchicine, benzoic acid, salicylic acid, 1,2-dimethyl colchicine, 2,3-didemethyl colchicine, N-deacetyl colchicine, colchicoside, gloriosine and superbine. As in the leaves the compounds that are present are colchicine, gloriosine, superbine, stigmasterin, gloriosol and phytosterils. The colchicine content in new young leaves is 2.35 and in adult leaves is 0.87, hence as the leaves gets older the colchicine content also starts decreasing progressively [11-13].

Pharmacological Properties

The most sought out alkaloid derived from the plant is Colchicine which is an anti-gout agent which works by decreasing the uric acid level in the body and also for the treatment of diseases like the familial Mediterranean fever. *Gloriosa superba* or *Langali* as it is called in Ayurveda has been used in ancient Ayurvedic medicine centuries before the pharmaceutical companies started exploiting it for its vast nature of medicinal properties. In those times primarily it was used as an abortifacient or as an agent to stimulate abortion. The other uses of the plant that the people utilized was for treating wounds, treating loss of appetite, irritable Bowl syndrome, for cough, leprosy, piles etc. Judging from this we can understand that the people from those times had revealed if not all but most of the medicinal properties of the plant [14-15]. Another important property of the plant that was later discovered was its anti-inflammatory effect which was proven helpful in treatment of diseases such as gout which is mainly due to the anti-mitotic effect of the alkaloid colchicine. Another property of the plant is its anti-helminthic property which helps treatment against parasitic worms such as *Eisonia fatida* and also possesses anti-microbial effect which is more affective for gram positive bacteria than for gram negative bacteria. *Gloriosa superba* also contains rich source of anti- oxidants in the leaves, seeds and tubers. *Gloriosa superba* also shows resistance against venom due to its anti-coagulant property by inhibiting clotting induced by thrombin. The other pharmacological properties of the plant are useful in treating ulcers, hemorrhoids and even impotence. It is also used to treat diseases related to the skin and also some respiratory disorders. One of the other unique and important pharmacological properties of the plant is its role as an anti-cancer agent. This role is due to the alkaloid colchicine and its anti-mitotic activity as it binds to the α and β-tubulin heterodimer and is also used a drug for chemotherapy [16].

Colchicine Toxicity

Apart from the medicinal properties of these alkaloids these make the plant very poisonous in nature and ingesting even a milligram more than the required amount can prove to be fatal even resulting in death. Colchicine also has a poisonous nature capable of causing fatality when ingested in higher quantities. In Ayurvedic medicine *Gloriosa superba* comes under the seven Upavisha or the seven poisonous plants. When more than normal amount is ingested or taken the toxins of the plant creates inhibition of cell division resulting in various symptoms such as diarrhea, alopecia and even burning sensation in the stomach, vomiting and stomach pain [17]. Apart from being poisonous to humans when experimentation was done by treating colchicine to monkeys and rats the effect caused seizures and also induced formation of epileptic foci in the brain eventually causing death in both the animals. Since the maximum amount of colchicine content is present in the tubers and the seeds the majority of the recorded deaths is due to the inhalation of the tubers or the seeds [18]. Some other symptoms following colchicine toxicity are acute renal failure (ARF), gastroenteritis followed by sweating and convulsions sometime after ingestion. Due to its very poisonous nature, it has also been used as an agent for attempting suicides in various parts of India and Africa. In cases when suicide was attempted by ingesting the tubers of *Gloriosa superba* about 3.5-4 hours after ingestion the first symptoms produced were immense thirst, burning sensation in the throat and the mouth followed by nausea and vomiting. Twenty-four hours after admission the urine output becomes less than 400-500 ml in adults meaning the person becomes oliguric. The amount of colchicine content required to cause fatality has been estimated to be as less as 7-8mg or in some cases even as much as 60mg. Of all the symptoms cause by colchicine poisoning the most common diagnosis is severe gastroenteritis and colchicine has seen to mostly affect the central nervous system (CNS) [19].

***In vitro* Micropropagation:** As the plant consists of about various important metabolites and is holding a important position in the useful medicinal plants list but is being exploited and is on the verge of extinction. Invitro culturing of this is very much needed in order to conserve the plant and for its regeneration purposes. Various different *in vitro* micropropagation methods have been followed in order to regenerate the plant.

(Through Nodal Segments)

Due to the risk of endangerment of *Gloriosa superba* and the importance of the vast medicinal properties it contains, experiments of *in vitro* micropropagation were done to produce the plant in vast amount and without altering its genetic content. One such method is Somatic embryogenesis. Firstly, the nodal segments of the plant were taken and were first washed with distilled water 4-5 times. Arter which sterilization of the surface of the explant was done by using disinfectant such as Mercuric chloride taking its concentration of 0.2% for 2.5-3 minutes [20-21]. Fungicides like Bavistin and Mancozeb were also used on the explant. The explant was then transferred to a basal M.S medium containing various auxins and cytokinin's like 2,4-D (Dichlorophenoxy acid), BAP (benzyl amino purine), NAA (naphthalene acetic acid), IAA (indole acetic acid), casein hydrolysate (CH), and Kinetin. Now for callus induction

through the nodal segments 2,4-D and Kinetin were used in combination and in concentrations of 1-5mg/l 2,4D and 1-5mg/l Kinetin. In the M.S media supplements like coconut water 20% was also used which showed to increase the frequency of callus formation and somatic embryo development [22]. The culture was then maintained at a Ph of 5.8 at a temperature of 25 °C-26 °C under 16/8-hour period of light and dark. The best result of callus formation was observed when 2,4-D and Kinetin were used in combination and in concentration of 4mg/l 2,4-D and 5mg/l of Kinetin. After callus has been developed the callus is subjected for somatic embryo development. The callus was then transferred to a M.S media containing CH, kinetin and 2,4-D in concentrations of 10mg/l CH, 5mg/l kinetin and 4mg/l of 2,4-D and coconut water 20% w/v was also used. The effect of these Plant growth regulators and supplement caused globular embryo formation and then finally the embryoids which then can be made to develop into the roots or the tubers. Thus, the final survival rate of the explants was calculated at 85% [23].

(Through Auxillary Buds)

The auxiliary buds of *Gloriosa superba* were collected to be used as explants. Then after the collection the explant was subjected to sterilization which was first done by washing the explant with distilled water 4-5 times after which the disinfectant mercuric chloride was used and since the explant was delicate or fragile only 0.2% concentration of mercuric chloride was used [24]. Now after the sterilization process was complete the explant was moved to a M.S media at a pH of 5.8 and the temperature was maintained at 25 °C to 26 °C [25]. For the initiation of callus development through the auxiliary buds these auxins and cytokinin's were used in combination with each other which gave the best result, 2,4-D, IAA, IBA, BAP and BA. Coconut water supplement was also used 20% w/v which showed to increase the frequency of callus development. Callus formation was observed when the auxin 2,4-D was used alone and also in combination with kinetin and coconut water 20% w/v taking the concentrations in 18.06 µM 2,4-D and 23.18 µM Kinetin. Still while conducting the experiment it was observed that maximum callus induction and proliferation was observed when 2,4-D was used in combination with BAP (benzyl amino purine) instead of Kinetin taking the concentrations of 4.52 µM 2,4-D and 13.28 µM BAP. Maximum shoot formation was also seen when 2,4-D and BAP were used in combination and in concentrations of 4.50 µM 2,4-D and 17.80 µM BAP. Eight weeks after the callus has been developed it was transferred to another M.S media containing BAP and 2,4-D in combination to induce somatic embryo formation. The concentration of 2,4-D and BAP used were 2.0mg/l-3.0mg/l of 2,4-D and 0.5mg/l-2mg/l of BAP. When BAP and 2,4-D were taken in more than these concentrations it proved to have a degenerative effect on the callus. After this globular embryo development was seen which later proliferated to produce somatic embryos [26-27].

(Through Corm Bud Explants)

Gloriosa superba plant, it's seeds and rhizomes were collected from Nathapalayam in Tamil Nadu. Surface sterilization of the seeds under tap water for two minutes followed by Tween 1% v/v for a time period of five minutes. Further using 70% mercuric chloride. Culturing seeds on MS medium supplemented with 0.8% agar and 30% sucrose. After 4 to 6 weeks, 0.5 to 1cm corn bud was isolated and cultured in MS medium supplemented with 2,4-D (1 to 10 mg/l) and IAA (0.5 to 5 mg/l). For shoot multiplication BAP +Kinetin was used and for root multiplication BAP+ Zen +NAA and acetone was preferred. When highest concentration of 2,4-D (10mg/l) and IAA (5mg/l) was used within 25 to 30 days percentage of yellow callus found to be was 39 +/- 3.48. Suitable and best shoot multiplication media for corm buds was MS medium further supplemented with 1.5mg/l BAP, 0.2mg/l NAA, 15% coconut water and 2 g/l acetone. After 25 to 30 days shoot explants initiated multiple roots. If activated charcoals were used here for initiation a 16% induction was observed. When BAP 8 mg, GA_2 1 mg, Zen 0.5 mg, NAA 1 mg and 2 g acetone was used a root initiation percentage rate of 96.2% was observed [28].

(Through Root Explants)

Collection of Gloriosa superba plant and seeds from the Glorious Endangered Medicinal Plant Conservation Centre in Alagappa University, Tamil Nadu. Following the complete procedure of surface sterilization utilizing tween 20 and mercuric chloride. Then culturing the sterilized seeds onto MS medium containing 30% sucrose and solidified on 0.8% agar. After four to six weeks of culturing root segments of 0.5 to 1cm were excised from it. A 94.40% of callus initiation from root explants was observed when 2,4-D was 2 mg/l, IAA was 1 mg/l and NAA was 0.75 mg/l. A 93.80% of greenish roots was observed from the root induction medium containing MS with GA_3 8mg/l, IAA 4 mg/l and BAP 2 mg/l. A shoot initiation % of 90.60% was observed from rooted callus when cultured on half strength MS medium supplemented with BAP 3mg/l, IBA 1mg/l and IPA 0.75 mg/l. It was seen that the shoot initiation percentage was reduced when concentrations of BAP and IPA were decreased. Thus, we can utilize this procedure for rapid multiplication and for crop conservation [29].

Conclusion

They say almost all plants or event plant ever discovered possesses some form of medicinal properties in minute or immense quantity and *Gloriosa superba* possesses a vast amount of them due to the presence of its precious alkaloids which can be used to treat a wide range of diseases and even used to suppress diseases like cancer. This being the case it is sought out by major pharmaceutical companies for its Colchicine content leading to the decrease in its habitat putting it in the list of endangered plants in the under the IUCN. People have been using this plant to treat various diseases and disorders since centuries ago and even in ancient ayurvedic medicine where primarily it was used as an abortifacient and was recognized as the seven most poisonous plants. It is also recognized by its poisonous nature mainly due to the alkaloid colchicine which possesses anti mitotic properties too and even a small amount of its tuber of seeds when ingested can prove to be fatal making it a suitable agent for attempting suicides. Hence due to its endangerment methods have been developed to mass propagate it invitro without any change in its genetic content one such methods being somatic embryogenesis which can be conducted using the seeds, leaves, nodal segments, tubers and auxiliary buds of the plant. This has been a powerful tool for its conservation and

hopefully removing its name from the list of endangered plants.

References

1. Sonali J, Gyan S. Critical review on medicinally potent plant species: *Gloriosa superba*. Fitoterapia,2011:82(3):293-301.
2. Sivakumar G, Krishnamurthy KV. *Gloriosa superba* L. — a very useful medicinal plant. Recent Progress in Medicinal Plants, Ethnomedicine and Pharmacognosy,2002:7:465-82.
3. Ravindra A, Mahendra KR. Current advances in *Gloriosa superba* L. Biodiversitas Journal of Biological Diversity,2009:10(4):210-214.
4. Ritu M. *Gloriosa superba*: An Endangered Medicinal Plant. Hort Flora Research Spectrum,2015;4(2):168-171.
5. Padmapriya, S, Rajamani K, Sathiyamurthy VA. Glory lily (*Gloriosa superba* L.) A review. International Journal of Current Pharmaceutical Review and Research,2015;7(1):43-49.
6. Haroon K, Murad Ali K, Tahira M, Muhammad IC. Antimicrobial activities of *Gloriosa superba* Linn extracts. Journal of Enzyme Inhibition and Medicinal Chemistry,2008;23(6):855-859.
7. Sonali J, Gya SS. Plant growth regulators, adenine sulfate and carbohydrates regulate organogenesis and *in vitro* flowering of *Anethum graveolens*. Acta Physiol Planta,2010;33:305-311.
8. Anandhi S, Rajamani K. Studies on seed germination and growth in *Gloriosa superba* L., Global J Res. Med. Plants & Indigen. Med,2012;1(10): 524–528.
9. Animesh B, Muntaha SN, Rahman MM. Comparative karyotype analysis in two lifeforms of *Gloriosa superba* L. Journal of Pharmaceutical Biology 2014;4(2):77-80. 77.
10. Chaudhuri, Prabir K, Raghunath S. Thakur. 1, 2-didemethylcolchicine: a new alkaloid from *Gloriosa superba*. Journal of natural products,1993;56(7):1174-1176.
11. Ghosh S, Ghosh B, Jha S. Aluminium chloride enhances colchicine production in root cultures of *Gloriosa superba*. Biotechnology Letters,2006;28:497-503.
12. Maroyi, A, Van der Maesen LJG. *Gloriosa superba* L. (Family Colchicaceae): Remedy or poison?. Journal of Medicinal Plants Research,2011;5(26):6112-6121.
13. Padmapriya S, Rajamani K, Sathiyamurthy VA. Glory Lily (*Gloriosa superba* L.) - A Review. International Journal of Current Pharmaceutical Review and Research, 2015;7(1):43-49.
14. Shah Kautuk Kumar, Vidya Sagar G. Phytochemical and pharmacological evaluation of *Gloriosa superba*. Journal of Drug Delivery and Therapeutics, 2015, 27-42.
15. Ashokkumar, Kaliyaperumal. *Gloriosa superba* (L.): A brief review of its phytochemical properties and pharmacology. International Journal of Pharmacognosy & Phytochemical Research,2015;7(6):1190-1193.
16. Sivakumar G. Colchicine semisynthetics: chemotherapeutics for cancer?. Current medicinal chemistry,2013;20(7):892-898.
17. Mendis, Shanthi. Colchicine cardiotoxicity following ingestion of *Gloriosa superba* tubers. Postgraduate medical journal,1989;65(768):752-755.
18. Gooneratne, Inuka Kishara, *et al*. Toxic encephalopathy due to colchicine—*Gloriosa superba* poisoning. Practical neurology,2014;14(5):357-359.
19. Lal, Hari Shankar, Mishra PK. *Gloriosa superba*-an endangered plant spotted for the first time from forest of Tpchanchi, Hazaribag (Jharkhand) India. Science Research Reporter,2011;1(2):61-64.
20. Ade, Ravindra, Mahendra Rai. Multiple shoot formation in *Gloriosa superba*: A rare and endangered Indian medicinal plant Nusantara Bioscience,2011, 3(2).
21. Ritu M, Nisha K, Pallavi B. Callus proliferation and *in vitro* organogenesis of *Gloriosa superba*: An endangered medicinal plant. Annual Plant Science, 2016;5:1466-1471.
22. Ritu M. Gloriosa superba L.: An endangered medicinal plant. Hort Flora Research Spectrum,2015;4:168-171.
23. Rajagopal C, Kandhasamy R. Genetic variability of kazhappai kizhangu (*Gloriosa superba* L.) in Tamil Nadu. J Trop Agric,2009;47(1-2):77-79
24. Ritu M, Nisha K, Iqbal S. Somatic embryogenesis and callus proliferation in *Picrorhiza kurroa* Royle ex. Benth. Journal of Experimental Biology and Agricultural Sciences,2016;4(2):201-209.
25. Chandrawanshi NK. *In vitro* tuberization and colchicine content analysis of *Gloriosa superba* L. Biotechnology,2015;14(3):142.
26. Sara B, Roberto D, Maurizio L. Somatic plant regeneration from selected common cypress (*Cupressus sempervirens* L.) clones resistant to the bark canker disease. Plant Cell, Tissue and Organ Culture,2016:124:393-403.
27. Kuldeep Y, Ashok A, Narender S. Actions for ex situ conservation of *Gloriosa superba* L.-an endangered ornamental cum medicinal plant. Journal of Crop Science and Biotechnology,2012;15:297-303.
28. Arumugam A, Gopinath K. *In vitro* micropropagation using corm bud explants: An endangered medicinal plant of *Gloriosa superba*. Asian Journal of Biotechnology,2012;4(3):120-128.
29. Gopinath K, Karthika V, Gowri S, Arumugam A. *In vitro* Morphogenetic Regeneration from Root Explant of *Gloriosa superba* L. for Enhanced Crop Production. The Scitech Journal,2014;1(8):27-30.