Evaluation of *Gloriosa superba* for Yield Attributing Characters and Quantification of Colchicine Originated from Different Agro Climatic Zones of Tamil Nadu and Andhra Pradesh

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**ABSTRACT**

The study was conducted to evolve *Gloriosa superba* for yield characters and alkaloid content for selecting elite genotypes for commercial exploitation. The genotypes were sown in Vairiyankaval village, Udayarpalayam taluk of Ariyalur district, Tamil Nadu. The highest mean value for fresh and dry seed yield was observed in Chittor local. The genotype Mulanur local has recorded the highest mean value for number of pods per plant and number of seeds per pod and Arupukotai local excelled the general mean for the traits seeds per pod, fresh and dry seed yield and also for tuber characters. An investigation was carried out to quantify the colchicine (alkaloid) present in tubers by High Performance Liquid Chromatography (HPLC) method. The genotypes collected from Arupukotai recorded the highest colchicine content (0.760 mg/g) followed by Chittoor (0.578 mg/g) and Mulanur (0.496 mg/g) and there by these three genotypes were utilized for further crop improvement.

**Keywords:** Gloriosa superb, Yield attributing characters, colchicines, HPLC.

**INTRODUCTION**

Glory lily is one of the modern medicine's most important plants actually facing local extinction*. *Gloriosa superba* derives its name *Gloriosa* from the word ‘glorious’, which means handsome and *superba* from the word ‘superb’ means splendid or majestic kind. This plant has been a source of medicine right from the ancient time. *Gloriosa superb* lily is a native of tropical Asia and Africa. It is found growing throughout tropical India, from the North -West Himalayas to Assam and the Deccan peninsula, extending up to an elevation of 2120 m. In Karnataka, it is commonly found growing all along the Western Ghats; it is also found growing in Madagascar, Sri Lanka, Indo-China and on the adjacent island and *Gloriosa superba* lily is a striking tuberous climbing plant with brilliant wavy-edged yellow and red flowers. There is also a more bushy, yellow-flowered form, *Gloriosa superba* is also known as the national flower of Zimbabwe. Except miscellaneous pharmaceutical product and other therapeutic preparations, it is also a popular plant for providing color in greenhouses and conservatories even immature flowers are beautiful to behold².

**Plant Profile**

**Family:** Liliaceae

**English Name:** Climbing-lily, Creeping-lily, Flame-lily, Glory-lily, Gloriosa lily, Tiger claw

**Sanskrit Names:** Langli, Kalikari, Ailni, Agnisikha, Garbhaghatini, Agnimukhi

**Local Names in India:** Kalihari, Kathari, Kulhari, Languli (Hindi); Bishalanguli, Ulatchandai (Bengali); Duddho, Vacchonag (Gujarati); Indai, Karianag, Khadyanag (Marathi); Karadi, Kanninagadde (Kannada); Adavini, Kalappagadda, Ganjeri (Telugu); Mettoni, Kithonni (Malayalam); Kalappai-Kizhangu, Kannoru (Tamil); Ognisikha, Garbhohghatono, Panjangulia, Meheriapulu (Oriya); Kariari, Mullim (Punjabi).³

**Common Names in World:** Flame lily, Isimiselo, Vlamelie, Riri vavai-moa

**Taxonomic Description**

Erect, perennial, tuberous, climbing herbs; grasp with tendrils formed at the tip of the leaves and stem is Leafy. Leaves sessile, spirally arranged or lanceolate, acuminate, entire, glabrous; the upper ones with cirrhihose tips. Flowers axillary, solitary, large, borne on long, spreading pedicels, actinomorphic, hermaphrodite; perianth segments 6, free, lanceolate, keeled within at base, long-persistent, yellow in lower half, red in upper half; stamens 6, spreading, hypogynous; anthers extrorse, medifixed, versatile, opening by longitudinal slits; ovary superior, 3-celled; ovules numerous; style deflected at base, projecting from the flower more or less horizontally. Capsule 2-3 cm long, oblong. Seeds numerous, subglobeose, black⁴. The fruit is oblong containing about 20 globose red colored seeds in each valve⁵,6,7.

**Habitat**

The plant grows in sandy-loam soil in the mixed deciduous forests in sunny positions and very tolerant of nutrient-poor soils. It occurs in thickets, forest edges and

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Preparation of colchicine standards.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Colchicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 ug/ml</td>
<td>50</td>
</tr>
<tr>
<td>100 ug/ml</td>
<td>101</td>
</tr>
<tr>
<td>200 ug/ml</td>
<td>199</td>
</tr>
<tr>
<td>400 ug/ml</td>
<td>365</td>
</tr>
<tr>
<td>800 ug/ml</td>
<td>653</td>
</tr>
<tr>
<td>1600 ug/ml</td>
<td>1121</td>
</tr>
</tbody>
</table>

Table 1: Collection of tubers from different Agroclimatic zones of Tamil Nadu and Andhra Pradesh.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Locations</th>
<th>Agroclimatic zones</th>
<th>State</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Arupukotai local</td>
<td>Southern zone</td>
<td>Tamil Nadu</td>
</tr>
<tr>
<td>2.</td>
<td>Dharapuram local</td>
<td>Western zone</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Mulanur local</td>
<td>Western zone</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Vedaranyam local</td>
<td>Cauvery Delta zone</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Chittoor local</td>
<td>South zone</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>Nellore local</td>
<td>South zone</td>
<td>Andhra Pradesh</td>
</tr>
</tbody>
</table>

Figure 1: Sprouted tubers from different Agroclimatic Zones.

 boundaries of cultivated areas in warm countries up to a height of 2530 m. It is also widely grown as an ornamental plant in cool temperate countries under glass or in conservatories.

Distribution
A native to tropical jungles of Africa, is now found growing naturally in many parts of Tropical Asia including India, Burma, Malaysia, Srilanka. In temperate countries, 

G. superba is propagated as an ornamental in conservatories, best suited to greenhouses. In India, it is mainly found in Nasik, Ratnagiri, Savaithwadi (Maharastra); Uttara Kannada, Hassan, Chikmagalur, Coorg, Mysore (Karnataka); Cannanore, Palakkad, Trivandrum (Kerala); Tamil Nadu and Goa.

Medicinal importance
The sap from the leaf tip is used for pimples and skin eruptions. Tribals people apply the powder of rhizome with coconut oil in skin eruptions and related diseases for 5 days. This combination is said to be effective in snake and scorpion bites too. Tribals crush roots of the plant in water and apply on head for curing baldness. To avoid painful delivery, people of Patalkot, use rhizome extracts. It induces labour pain and performs normal delivery. Tribal healers generally prescribe 250 to 500 mg of the rhizome as dosage and this dose may lead to abortion if given to a lady with pregnancy of 1 or 2 months. Since the rhizome is having abortive action, this is prescribed for normal delivery and the abortifacient action of the plant rhizome.

In traditional medicine system, tuber is used for the treatment of bruises and sprains colic, chronic ulcers, hemorrhoids, cancer, impotence, nocturnal seminal emissions and leprosy. Many cultures believe the species to have various magical properties. The plump roots of the plant have been used in the treatment of parasitic skin infections, leprosy, and internal worms.

In Ayurveda and Yunani systems of medicine, the tuber of plant is well known due to its pungent, bitter, acrid, heating, anthemic, laxative, alextic and abortifacient nature. It is widely used in the treatment of ulcers, leprosy, piles, inflammations, abdominal pains, intestinal worms, thirst, bruises, infertility and skin problem. However, ingestion of all parts of the plants is extremely poisonous and can be fatal.

Morning Glory Lily combats parasites and worms on the skin’s surface. As an antipyretic, the herb reduces fever. Gloriosa superba is used to cure arthritis, gout, rheumatism, inflammation, ulcer, skin diseases, leprosy,
snake bite, purgative, gonorrhoea, infertility, itching, abdominal pain, cancer, piles, and scrofula. Gloriosa Superba is used in veterinary medicine to treat cancers in some animals. Paste is antidote in snake bite. Even the
leaves of Glory lily have more medicinal qualities, namely for curing asthma, its juice is effective against lice and also against many skin disorders. It can be administered to a delivered mother along with spirituous drink to give relieve to her postnatal complaints and also if its root paste smeared over the palms and feet of a pregnant woman, delivery of child becomes easier. Leaf extract mixed with sesame oil is applied twice a day on the joints affected with arthritis reduces pain. The fresh juice of the leaves and plant is used as uterine stimulant and ingredient in arrow poisons. Tuberous root are anti inflammatory, alterative, anthelmintic, antileprotic. Used for piles, swollen joints, parasitical affections of skin and its derivatives are present in tubers, seeds and flowers. The seeds are used as raw material for preparing drugs for gout.

Chemical components

Especially the tubers are extremely toxic due to the presence of a highly active alkaloid, Colchicine. The species also contains another toxic alkaloid, Gloriosine. Other compounds such as lumicolchicine, 3-demethyl-N-deformyl-N-deacetylvolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine have been isolated from the plant.

Toxic effect

A pale yellow to greenish yellow alkaloid Colchicine is mainly responsible for the toxic effect. The toxins in G. superba have an inhibitory action on cellular division resulting in diarrhoea, depressant action on the bone marrow and alopecia. After ingestion of tubers, initial symptoms develop within two to six hours. Intense vomiting, numbness and tingling around the mouth, burning and rawness of the throat, nausea, abdominal pain and bloody diarrhoea leading to dehydration etc. are some of the primary symptoms developed initially in the victim. The other important complications include respiratory depression, shock, hypotension, marked leucopenia, thrombocytopenia, coagulation disorders, oliguria, haematuria, confusion, seizures, coma and ascending polynuropathy.

MATERIALS AND METHODS

Planting Materials

Medicinally important plant species Gloriosa superba L. (Family: Liliaceae) was generally propagated through its tubers which are usually ‘V’ and ‘L’ shaped. Tubers were collected from 6 locations; 4 from Tamilnadu under 3 agroclimatic zones and 2 from Andrapradesh under an agroclimatic zone (Table 1).

Evaluation Block

The plants were raised in field at Vairyanakaval, Udayarpalayam taluk of Ariyalur district, Tamil Nadu. Sprouted tubers of uniform size weighing 50-60g were selected as planting materials. The tubers were sown during August 2014 in randomized block design with three replications for evaluation of different genotypes. The experimental area was tilled and planting furrows (30 cm deep) was made at a distance of 1.5 m, 20 days before planting. Each plot consisted of 5 m long rows with inter and intra row spacing of 150 cm and 30 cm respectively. The plots were irrigated at weekly intervals. Recommended agronomic and plant protection practices were adopted. Agro morphological observations were recorded on ten randomly selected plants on each accession per replication for plant height (cm), days

Table 2: Evaluation of different genotypes for vegetative, floral, and yield characters.

<table>
<thead>
<tr>
<th>GENOTYPES</th>
<th>Plant ht(cm)</th>
<th>50% flowering</th>
<th>No. of Pods plant</th>
<th>No. of Seeds per pod</th>
<th>Fresh yield per plant(g)</th>
<th>Dry seed yield per plant(g)</th>
<th>Dry seed content (mg/g)</th>
<th>Retention time (min)</th>
<th>Colchicine content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARUPUKOTAI</td>
<td>137.40</td>
<td>103.84</td>
<td>24.70</td>
<td>58.73</td>
<td>162.56</td>
<td>30.42</td>
<td>16.64</td>
<td>1.05</td>
<td>3.08</td>
</tr>
<tr>
<td>CHITTOR</td>
<td>143.12</td>
<td>106.75</td>
<td>26.50</td>
<td>64.96</td>
<td>203.10</td>
<td>61.28</td>
<td>18.77</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>DHARAPURAM</td>
<td>116.67</td>
<td>101.80</td>
<td>20.11</td>
<td>41.54</td>
<td>131.44</td>
<td>40.73</td>
<td>16.26</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>MULANUR</td>
<td>141.62</td>
<td>109.86</td>
<td>32.95</td>
<td>65.65</td>
<td>171.40</td>
<td>56.91</td>
<td>20.72</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>NELLORE</td>
<td>126.76</td>
<td>105.59</td>
<td>23.09</td>
<td>55.12</td>
<td>135.46</td>
<td>42.36</td>
<td>20.35</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>VEDARANYAM</td>
<td>113.73</td>
<td>106.18</td>
<td>21.42</td>
<td>47.59</td>
<td>82.97</td>
<td>26.41</td>
<td>16.93</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>General Mean</td>
<td>129.88</td>
<td>105.67</td>
<td>24.79</td>
<td>55.60</td>
<td>147.82</td>
<td>46.35</td>
<td>18.28</td>
<td>1.03</td>
<td>3.08</td>
</tr>
<tr>
<td>CV</td>
<td>8.19</td>
<td>4.59</td>
<td>15.99</td>
<td>24.11</td>
<td>22.12</td>
<td>28.60</td>
<td>5.28</td>
<td>27.54</td>
<td>3.07</td>
</tr>
<tr>
<td>SE</td>
<td>6.14</td>
<td>2.80</td>
<td>2.29</td>
<td>7.74</td>
<td>18.88</td>
<td>7.65</td>
<td>0.56</td>
<td>12.54</td>
<td>3.07</td>
</tr>
<tr>
<td>SED</td>
<td>8.69</td>
<td>3.96</td>
<td>3.24</td>
<td>10.94</td>
<td>26.70</td>
<td>10.82</td>
<td>0.79</td>
<td>21.59</td>
<td>3.07</td>
</tr>
<tr>
<td>CD (5%)</td>
<td>19.36</td>
<td>8.82</td>
<td>7.21</td>
<td>24.39</td>
<td>59.49</td>
<td>24.12</td>
<td>1.76</td>
<td>14.30</td>
<td>3.07</td>
</tr>
<tr>
<td>CD (1%)</td>
<td>27.54</td>
<td>12.54</td>
<td>10.26</td>
<td>34.69</td>
<td>84.61</td>
<td>34.30</td>
<td>2.50</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3: Estimation of colchicine for six different genotypes of Gloriosa superba by using HPLC method.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Genotypes</th>
<th>Retention time (min)</th>
<th>Colchicine content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Arupukotai local</td>
<td>3.090</td>
<td>0.760</td>
</tr>
<tr>
<td>2.</td>
<td>Chittoor local</td>
<td>3.090</td>
<td>0.578</td>
</tr>
<tr>
<td>3.</td>
<td>Dharapuram local</td>
<td>3.083</td>
<td>0.370</td>
</tr>
<tr>
<td>4.</td>
<td>Mulanur local</td>
<td>3.080</td>
<td>0.496</td>
</tr>
<tr>
<td>5.</td>
<td>Nellore local</td>
<td>3.075</td>
<td>0.384</td>
</tr>
<tr>
<td>6.</td>
<td>Vedaranyam local</td>
<td>3.073</td>
<td>0.144</td>
</tr>
<tr>
<td>Mean value</td>
<td></td>
<td></td>
<td>0.455</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td></td>
<td>45.944</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td>0.209</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td></td>
<td>0.085</td>
</tr>
</tbody>
</table>
Figure 8: Performance of genotypes for various morphological traits.

Figure 9

Figure 10
The statistical parameters like mean, standard error and critical difference for all the characters were worked out by adopting the standard methods of the analysis\textsuperscript{23}. Variation among different genotypes for all the vegetative, floral and yield characters were observed.

**Quantification of Colchicine**

**Extraction Method**

In the extraction process, 0.5 g of powdered tuber material was extracted twice with 25 ml of petroleum ether with frequent shaking for 1 hr. followed each time by filtration. The solid residues were air dried and then extracted with 10 ml of dichloromethane at room temperature for 30 min with frequent shaking. Then 10% solution of ammonia (0.5 ml) was added to the mixture with vigorous shaking for 10 min; the mixture was left undisturbed for 30 min and then filtered. The residue was washed twice with 10 ml of dichloromethane and then combined with the filtrate. The organic phase was evaporated to dryness and then dissolved in 1 ml of 70% ethanol to yield the test sample\textsuperscript{24}. Tubers of all the six genotypes were used to prepare extracts as described above.

**Standards preparations**

Pure colchicine from SIGMA, supplied by Lakshmi Scientific Company, Chidambaram was used as reference substance. Accurately weighed six different colchicine standards viz. 50, 100, 200, 400, 800 and 1600 µg/ml was run for getting retention time and peak area (Fig. 2 - 7). Then, the tuber samples of different genotypes were run in HPLC to quantify the amount of colchicine.

**HPLC Analysis**

Quantitative determination of colchicine was carried out by comparing the retention time of the sample with that of the standard. Shimadzu HPLC system equipped with a binary pump 1525 (Max. Pressure: 6000 psi.) and a porous silica with 5µm diameter C18 4.6 × 150 mm column was used for separation. The mobile phase consisted of Acetonitrile: 3% Acetic acid (60:40), at a flow rate of 1ml/min and an injection volume of 20 µl. The peaks eluted were detected at 245 nm and identified with authentic standards.

Amount of colchicine present in dry weight of sample was calculated using the following formula, given by scott, 1996 and expressed in per cent dry weight.

\[
Cp(s) = \frac{Ap(s)}{Ap(st)} \times Cp(st)
\]

*Cp (s)* is the concentration of the solute in the mixture.

*Ap (s)* is the area of the peak for the sample in HPLC chromatogram.

*Ap (st)* is the area of the peak for the standard in HPLC chromatogram.

*Cp (st)* is the concentration of standard used for injecting in HPLC.

**RESULTS AND DISCUSSION**

The results of variability in the morphological and quality traits of *G. superba* indicate the variations among the genotypes studied. The genotype Vedaranyam local has registered lowest mean value for plant height (113.73cm), followed by Dharapuram local (116.67cm). The minimum mean value for days to 50% flowering was recorded by genotype Dharapuram local (101.80 days), followed by Arupukotai local (103.86 days). The genotype Mulanur local was recorded the highest mean value for number of pods per plant (32.95), followed by Chittor local (26.50) and Arupukotai local (24.70). The genotype Mulanur local occupied the highest mean value for number of seeds per pod (65.65), followed by Chittor local (64.96) and Arupukotai local (58.73). The highest mean value for fresh seed yield per plant was observed in chittor local (203.10 g), followed by Mulanur local (171.40 g) and Arupukotai local (162.56). The genotype...
Chittor local was recorded the highest mean value for dry seed yield per plant (61.28), followed by Mulanur local (56.91) and Arupukotai local (50.42). The highest mean value for weight of the tuber was recorded in mulanur local (20.72), followed by Nellore (20.35) and Chittor local (18.77). The genotype Mulanur local has been recorded the highest mean value for the weight of the tuber (69.10), followed by Nellore (68.50) and Chittor local (67.64) (Table 2).

Yield is governed by genetic and environmental factors and it varied with the genotypes. It was suggested that the selection should be applied mainly in the lines exhibiting high mean and variability. The crosses or families with the highest mean could be effectively utilized to identify the superior segregates. The mean performance served as a primary criterion for selecting desirable plants. Accordingly, the highest mean value for fresh seed yield (203.10 g) and dry seed yield (61.28g) was observed in Chittor local. The genotype Mulanur local has recorded the highest mean value for number of pods per plant (32.95) and number of seeds per pod (65.65) and this might be due to the potentiality of tuber mass. Arupukotai local excelled the general mean for the traits seeds per pod, fresh and dry seed yield and also for tuber characters with highest colchicine content (0.760 mg/g).

CONCLUSION
The present study revealed that the genotype Chittor local occupied the highest mean value for fresh seed yield (203.10g) and dry seed yield (61.28g) with colchicines content of 0.578 mg/g. The genotype Mulanur local contains the highest mean value for number of pods per plant (32.95) and number of seeds per pod (65.65). This might be due to the higher tuber mass that support the yield traits with lesser colchicines content (0.496 mg/g) among the three high yielding genotypes selected. Arupukotai local excelled the general mean for the traits seeds per pod, fresh and dry seed yield and also for tuber characters with highest colchicine content (0.760 mg/g).

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