

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

Arun kumar P*, Elangaimannan R

Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Tamil Nadu, India.

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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 Variyankaval 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 superba*, Yield attributing characters, colchicines, HPLC.

INTRODUCTION

Glory lily is one of the modern medicine's most important plants actually facing local extinction¹. *Gloriosa superba* derives its name *Gloriosa* from the word 'gloriosus', 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 superba* 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, Srilanka, 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, Ulatchandal (Bengali); Dudhio, Vacchonag (Gujarati); Indai, Karianag, Khadyanag (Marathi); Karadi, Kanninagadde (Kannada); Adavinabhi, Kalappagadda, Ganjeri (Telugu); Mettoni, Kithonni (Malayalam); Kalappai-Kizhangu, Kannoru (Tamil); Ognisikha, Garbhoghhatono, Panjangulia, Meheriaphulo (Oriya); Kariari, Mulim (Punjabi)³.

Common Names in World: Flame lily, Isimiselo, Vlamlelie, 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 cirrhes 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 exserted, 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, subglobose, black⁴. The fruit is oblong containing about 20 globose red colored seeds in each valve^{5,6,7}.

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

*Author for Correspondence: arunkumar.agri@gmail.com

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

S.No.	Locations	Agroclimatic zones	State
1.	Arupukotai local	Southern zone	Tamil Nadu
2.	Dharapuram local	Western zone	
3.	Mulanur local	Western zone	
4.	Vedaranyam local	Cauvery Delta zone	
5.	Chittoor local	South zone	Andhra Pradesh
6.	Nellore local	South zone	



Figure 1: Sprouted tubers from different Agroclimatic Zones.

Preparation of colchicine standards.

Sample ID	Colchicine
50 ug/ml	50
100 ug/ml	101
200 ug/ml	199
400 ug/ml	365
800 ug/ml	653
1600 ug/ml	1121

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 conservatories⁸.

Distribution

A native to tropical jungles of Africa, is now found growing naturally in many parts of Tropical Asia including India, Burma, Malaysia, Srilanka⁹. 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, Savanthwadi (Maharashtra); Uttara Kannada, Hassan, Chikmangalur, Coorg, Mysore (Karnataka); Cannanore, Palakkad, Trivandrum (Kerala); Tamil Nadu and Goa¹⁰.

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 rhizome¹¹.

In traditional medicine system, tuber is used for the treatment of bruises and sprains¹² colic, chronic ulcers, hemorrhoids, cancer, impotence¹³, nocturnal seminal emissions and leprosy. Many cultures believe the species to have various magical properties¹⁴. The plump roots of the plant have been used in the treatment of parasitic skin infections, leprosy, and internal worms¹⁵.

In Ayurveda and Yunani systems of medicine, the tuber of plant is well known due to its pungent, bitter, acrid, heating, anthelmintic, laxative, alexiteric 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^{16,17}. However, ingestion of all parts of the plants is extremely poisonous and can be fatal¹⁸.

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,

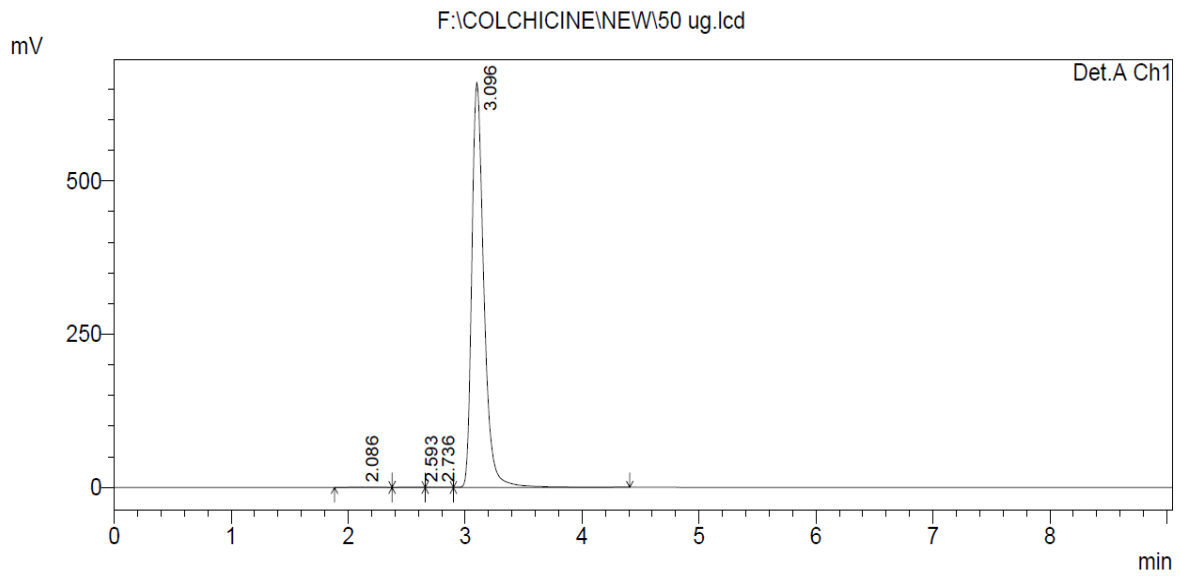


Figure 2

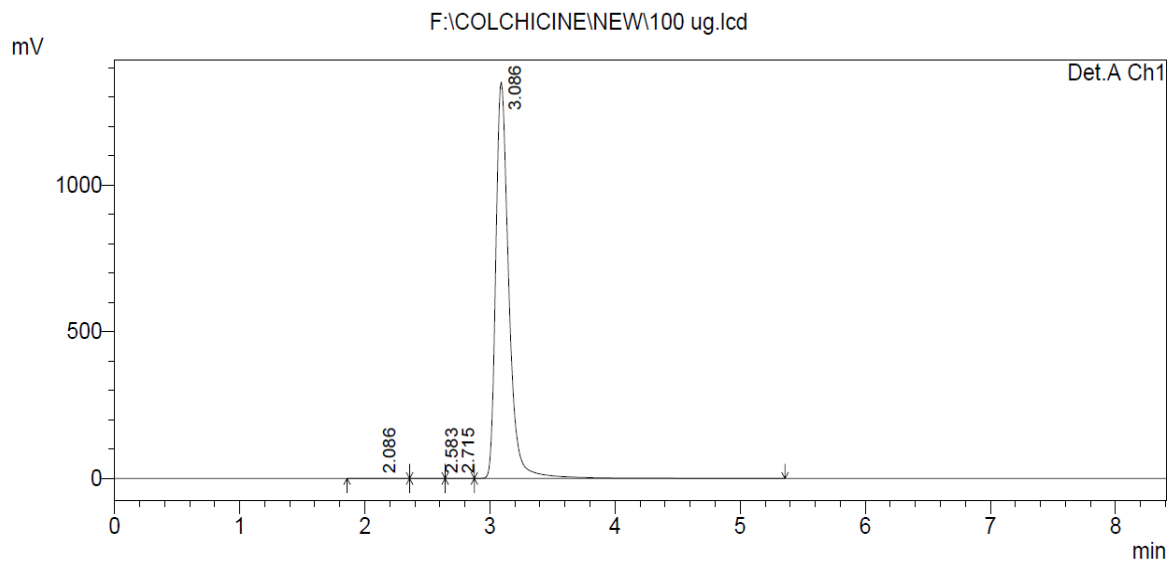


Figure 3

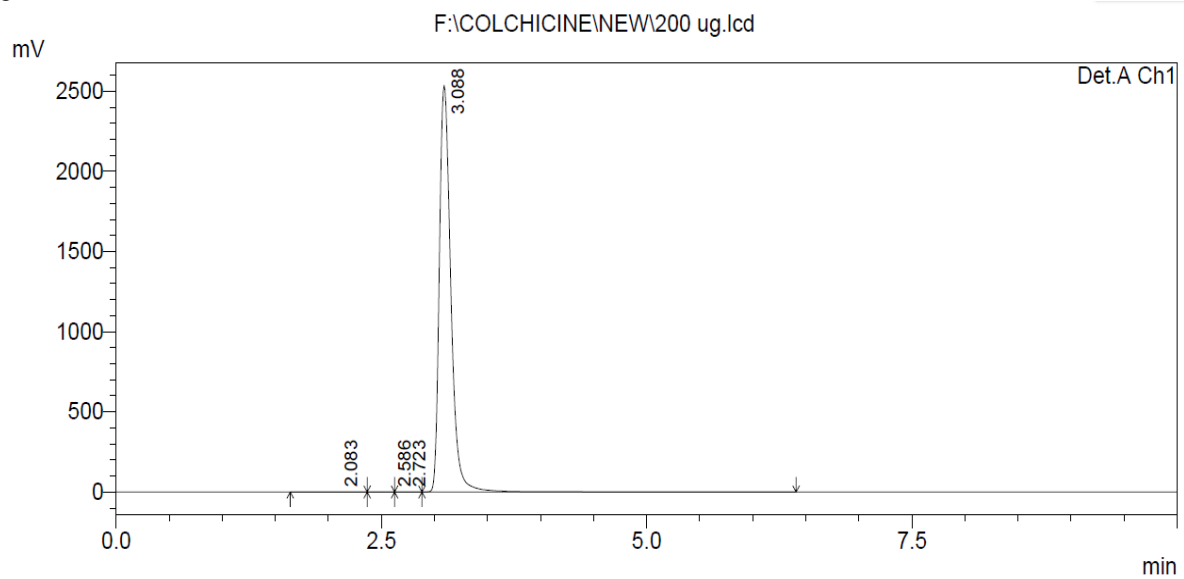


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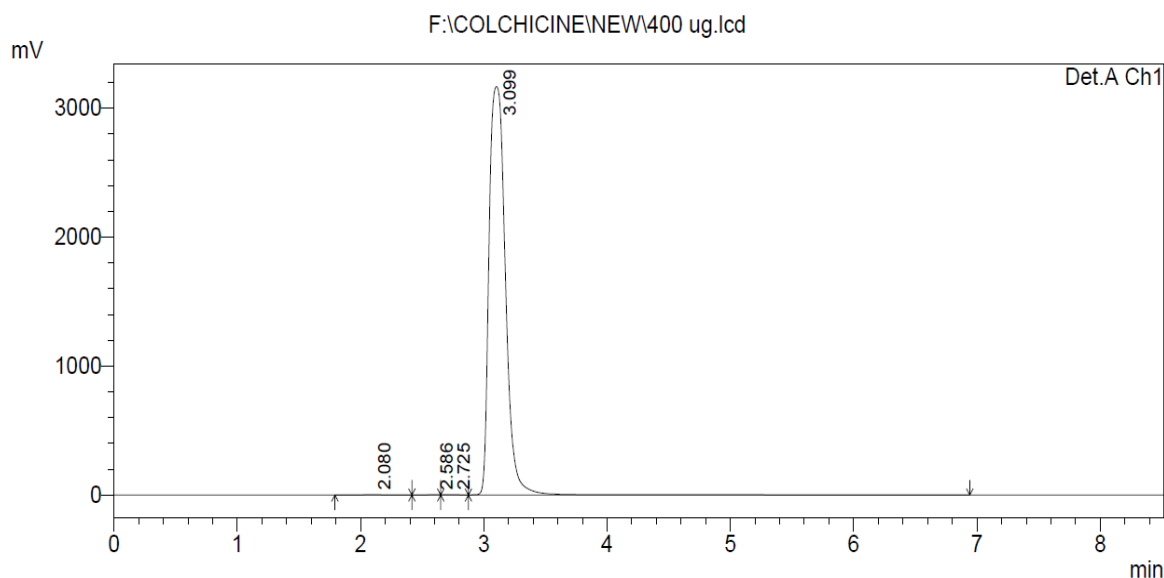


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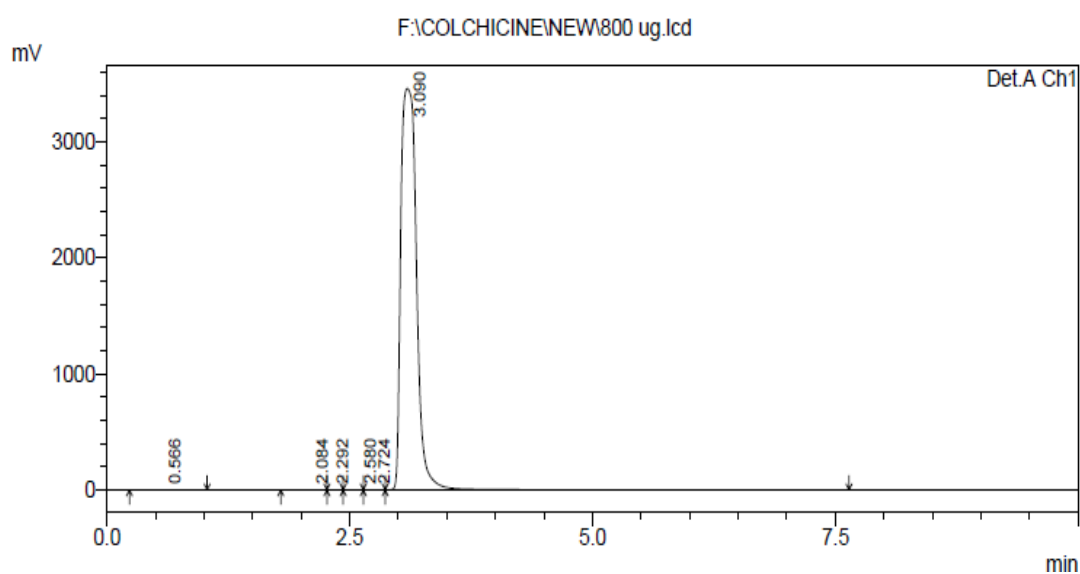


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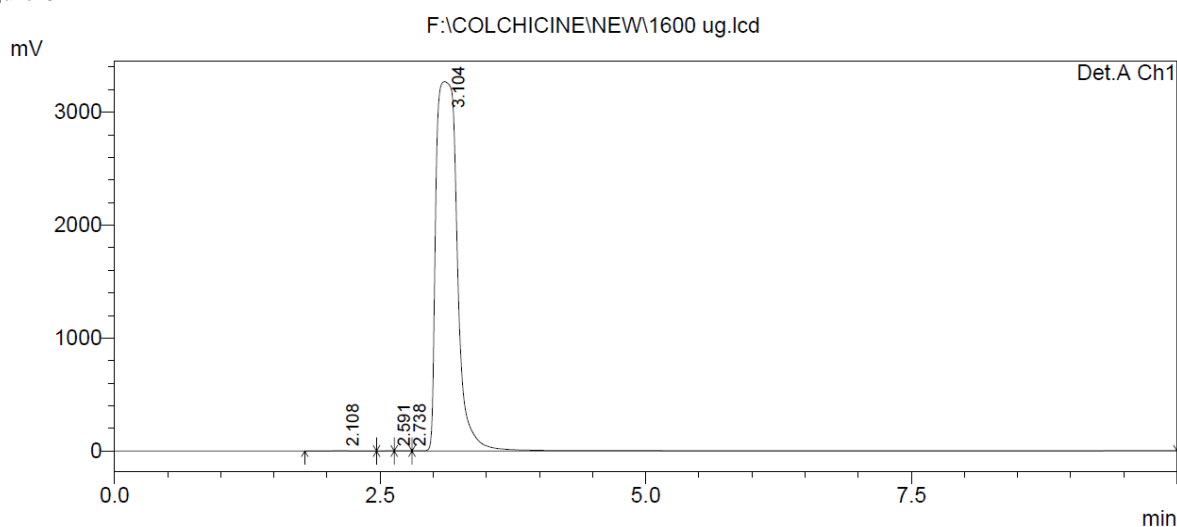


Figure 7

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

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

GENOTYPES	Plant ht(cm)	50% flowering	No. Pods plant	of per	No. of Seeds per pod	Fresh yield plant(g)	seed per yield per plant (g)	Dry seed yield per plant (g)	Length of tuber (cm)	Weight of tuber (g)
ARUPUKOTAI	137.40	103.84	24.70		58.73	162.56		50.42	16.64	64.50
CHITTOR	143.12	106.75	26.50		64.96	203.10		61.28	18.77	67.64
DHARAPURAM	116.67	101.80	20.11		41.54	131.44		40.73	16.26	59.82
MULANUR	141.62	109.86	32.95		65.65	171.40		56.91	20.72	69.10
NELLORE	126.76	105.59	23.09		55.12	135.46		42.36	20.35	68.50
VEDARANYAM	113.73	106.18	21.42		47.59	82.97		26.41	16.93	57.02
General Mean	129.88	105.67	24.79		55.60	147.82		46.35	18.28	64.43
CV	8.19	4.59	15.99		24.11	22.12		28.60	5.28	4.37
SE	6.14	2.80	2.29		7.74	18.88		7.65	0.56	1.63
SED	8.69	3.96	3.24		10.94	26.70		10.82	0.79	2.30
CD (5%)	19.36	8.82	7.21		24.39	59.49		24.12	1.76	5.13
CD (1%)	27.54	12.54	10.26		34.69	84.61		34.30	2.50	7.29

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

S.No.	Genotypes	Retention time (min)	Colchicine content (mg/g)
1.	Arupukotai local	3.090	0.760
2.	Chittoor local	3.090	0.578
3.	Dharapuram local	3.083	0.370
4.	Mulanur local	3.080	0.496
5.	Nellore local	3.075	0.384
6.	Vedaranyam local	3.073	0.144
Mean value			0.455
CV			45.944
SD			0.209
SE			0.085

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 sesamum 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 colchicine 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^{19,20}.

Other compounds such as lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine have been isolated from the plant²¹.

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 polyneuropathy.

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 Variyankaval, Udayarpalayam taluk of Ariyalur district, Tamil Nadu. Sprouted tubers of uniform size weighing 50-60g were selected as planting materials (fig.1). 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

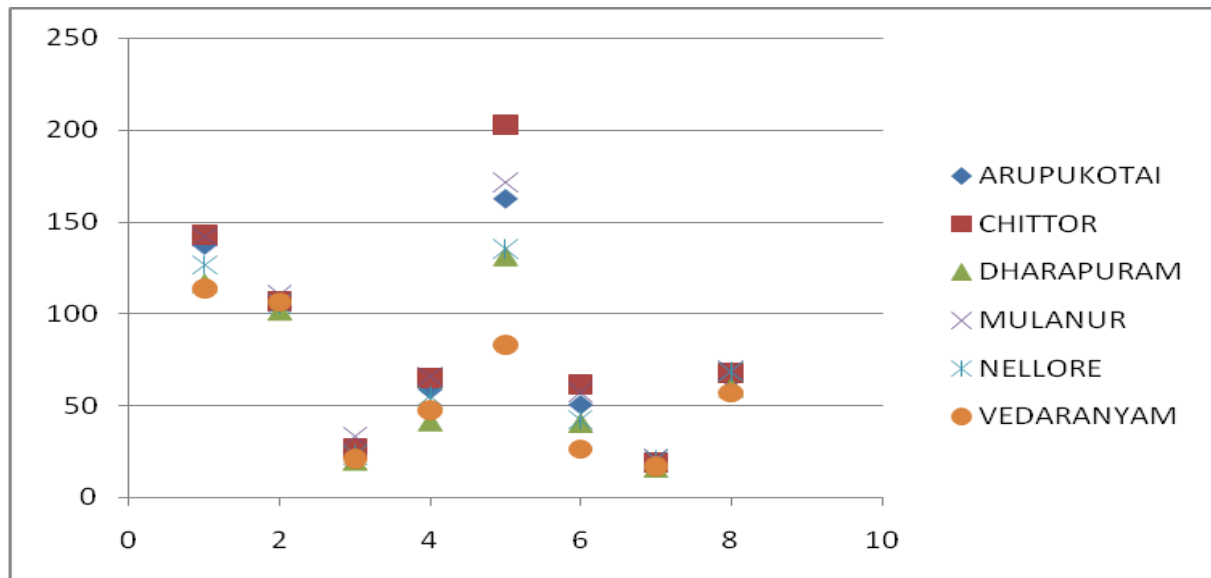


Figure 8: Performance of genotypes for various morphological traits.

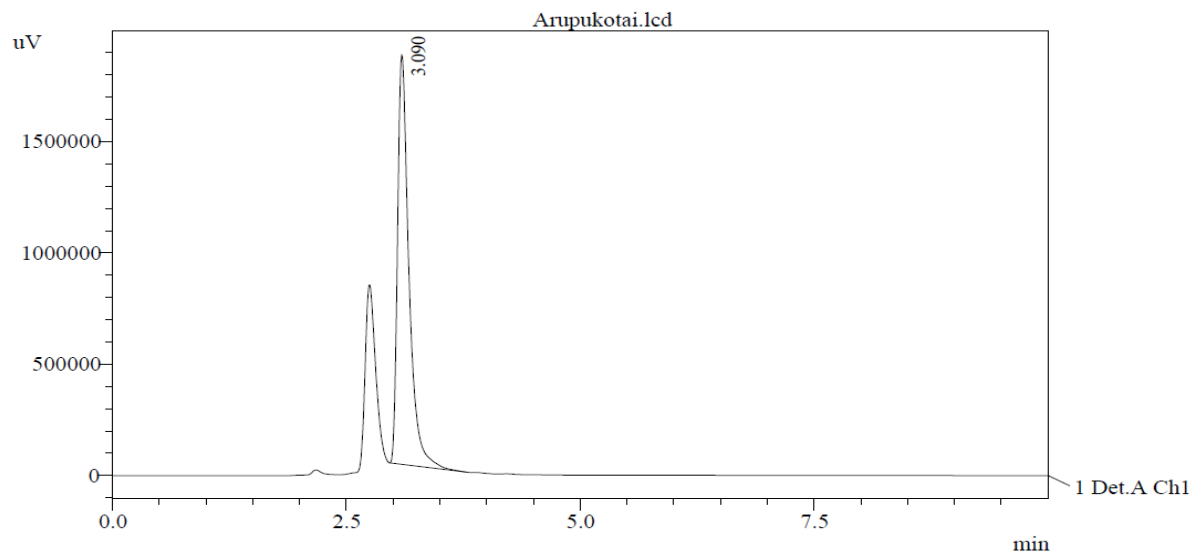


Figure 9

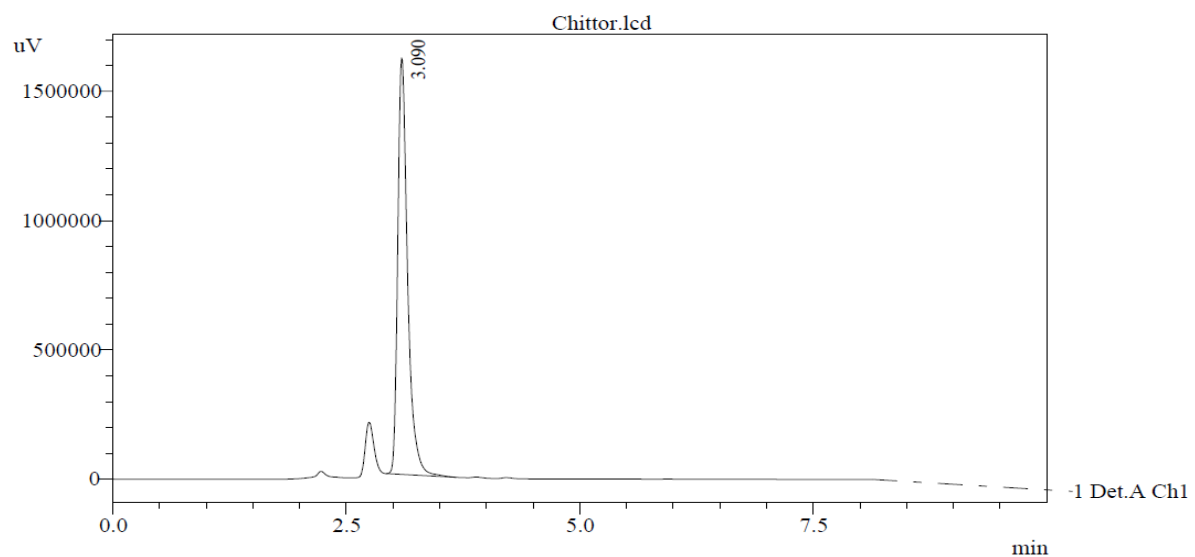


Figure 10

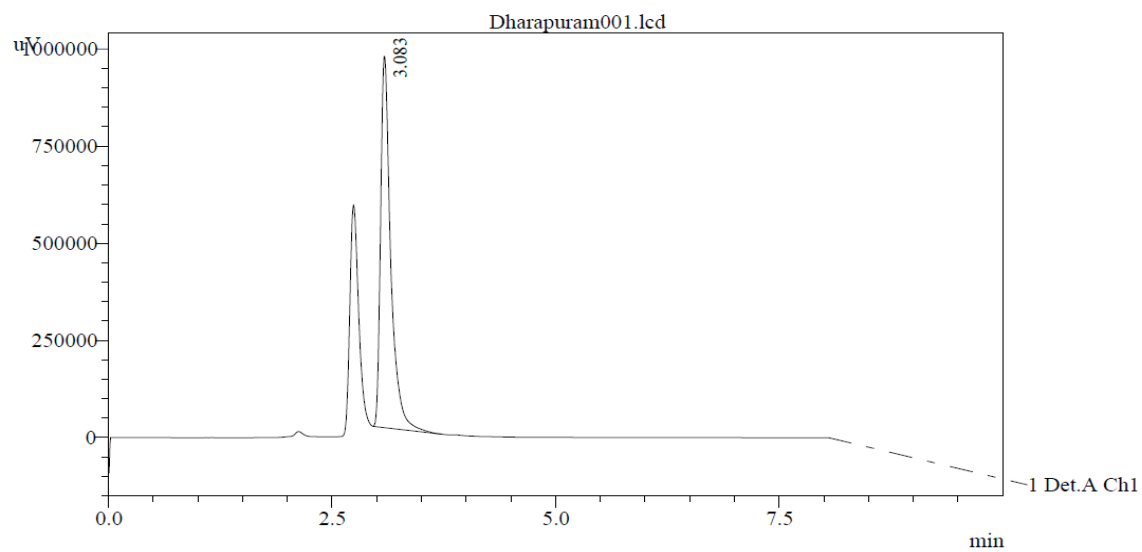


Figure 11

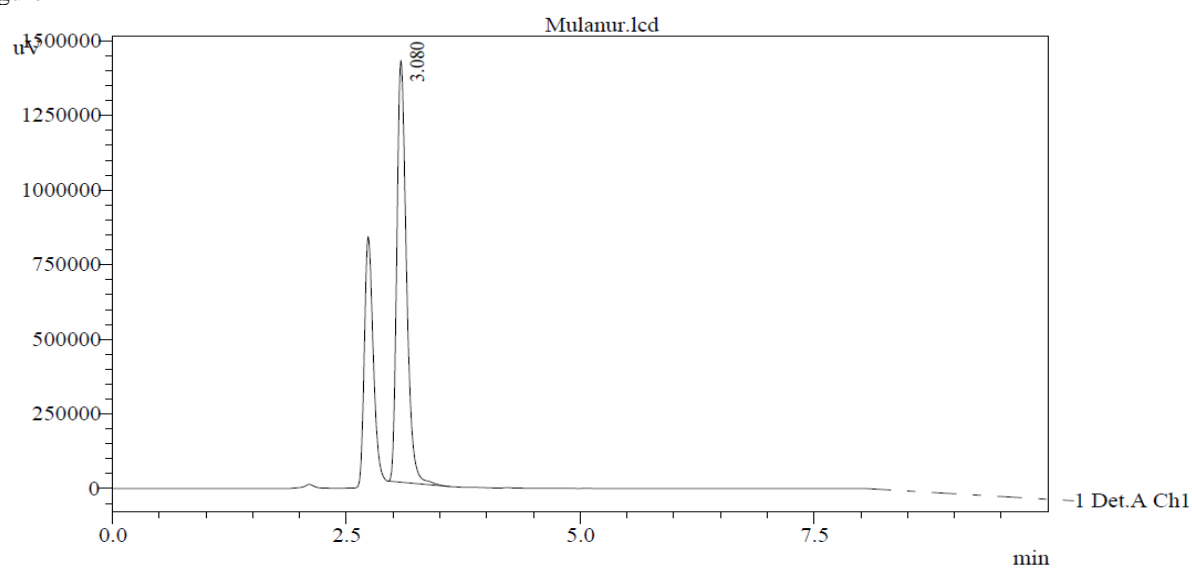


Figure 12

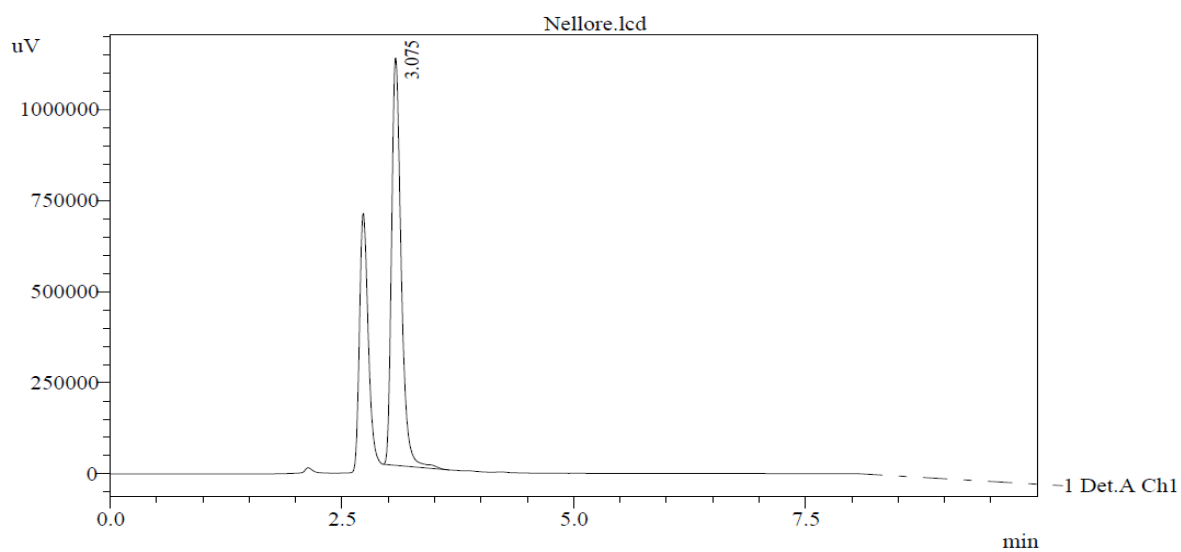


Figure 13

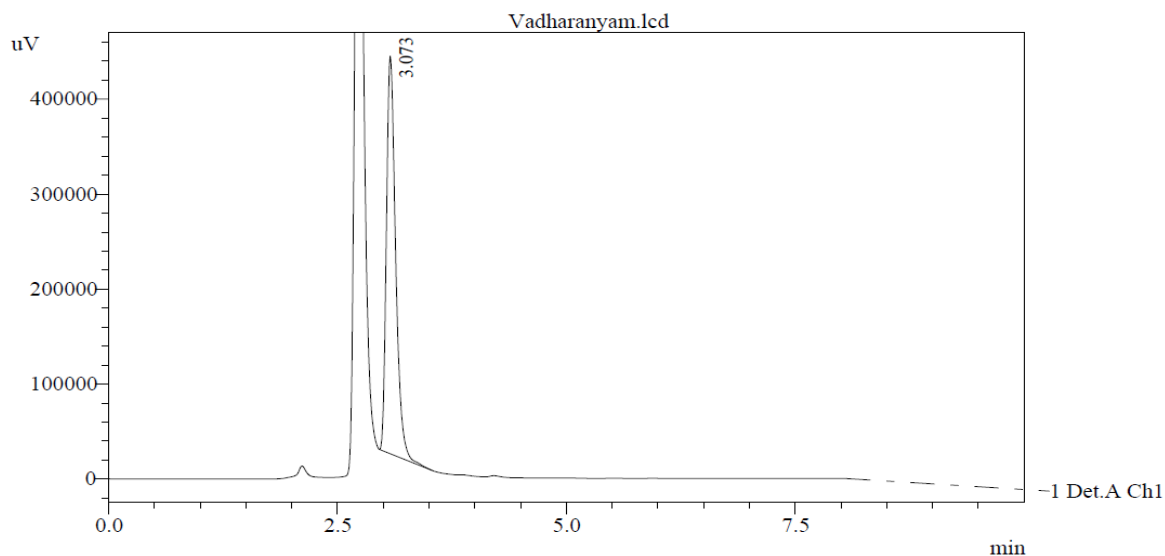


Figure 14

to 50% flowering, number of pods per plant, number of seeds per pod, fresh seed yield per plant (g), dry seed yield per plant (g), length of the tuber and weight of the tuber²².

The statistical parameters like mean, standard error and critical difference for all the characters were worked out by adopting the standard methods of the analysis²³. 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 sample²⁴. 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 scottrpw, 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 (101.80days), followed by Arupukotai local (103.86days). 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 (203.10g), followed by Mulanur local (171.40) 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 length 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 genotypes²⁵. It was suggested that the selection should be applied mainly in the lines exhibiting high mean and variability²⁶.

The crosses or families with the highest mean could be effectively utilized to identify the superior segregates²⁷. The mean performance served as a primary criterion for selecting desirable plants²⁸. Accordingly, the highest mean value for fresh seed yield (203.10g) 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 (Fig 8). Thus, these three genotypes showed superior performance for morpho-economic traits and might be serve as ideal parents for developing suitable ideotype for further crop improvement.

The colchicines content in tubers of *Gloriosa superba* was estimated for six genotypes viz. Arupukotai local, Dharapuram local, Mulanur local, Vedaranyam local, Chittoor local and Nellore local by using HPLC method (Table 3 and fig.8-14). The results revealed that, 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) that are higher than the mean value. It was identified that the Vedaranyam genotype recorded least colchicines content (0.144 mg/g) among all the six genotypes selected. This might be due to saline nature / poor quality of Vedaranyam soil type. This was in accordance that the where concentration of colchicines in *Colchicum autumnale* at limestone and serpentinite sites were much lower than other types of soils²⁹. Therefore, the genotypes collected from Arupukotai, Chittoor and Mulanur were utilized for further crop improvement.

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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