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RESEARCH ARTICLE

STUDIES ON ROOT ANATOMY AND STOMATA IN VARIANTS OF *CLITORIA TERNATEA* L., AN IMPORTANT MEDICINAL PLANT

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Abstract

Root anatomy for seven variants of *Clitoria ternatea* including single forms of blue, white, light blue, violet, double forms of blue, pink and white was examined. Slight anatomical variations were observed in number of epidermal cells, width of cortical region, diameter of stelar region, diameter of larger five vessels etc. The stomatal index was also calculated. From the data, 'double white' was distinct from the other variants. These variations are significant for the assessment of intraspecific variability within the species. Comprehensive anatomical studies in seven different variants of *C. ternatea* has not been carried out earlier.

Introduction:-

Clitoria ternatea L. commonly known as 'Butterfly Pea' is a member of the family Fabaceae. In Sanskrit it is known as *Aparajita* which means 'cannot be defeated' indicating its great importance to cure various ailments. It is probably a native to South America (Harinarayanan, 2005) and widely distributed in tropical and sub-tropical countries. The plant is also potential as an ornamental plant, a fodder, and a source of natural dye.

All parts of the plant are medicinally important especially the roots. The root or root bark is reported often to be an effective laxative (Fantz, 1991). It is also used as brain tonic (Terannaii & Cheeramkuzhy, 2000). It contains two important pentacyclic triterpenoids namely taraxerol and taraxerone, those promote intellectual development (Banerjee & Chakravarti, 1963; 1964). Its roots are bitter, refrigerant, intellect-promoting, anthelmintic, and are useful in dementia, hemicranias, burning sensations, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites, fever, otalgia, hepatopathy and cathartic (Nadkarni, 1976). The roots are also laxative and diuretic (Taur et al., 2009). It is recommended for the treatment of several ailments/disorders such as indigestion, constipation, eye and ear ailments, mucus disorders, sore throat, skin disorders, irritation of bladder and urethra, vaginal disorders, liver disorders, anxiety, depression, impaired learning and memory, inflammatory conditions such as rheumatoid arthritis and encephalopathy (Mukherjee, et al., 2008; Mathada et al., 2012).

Anatomy is one of the important biosystematic tools to assess the intraspecific variability in plant species. In the present study, the root of *C. ternatea* was selected for anatomical studies, considering its medicinal importance. Though several works has been reported on phytochemical, pharmacological and biochemical aspects of this highly medicinally potential plant, the information on intraspecific variability with respect to its anatomy is lacking, except a few reports on root anatomy of common blue and white varieties. This study helps to establish the inter-relationship between different variants of *C. ternatea* with respect to anatomy.

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Materials and methods:-

Seven variants in *C. ternatea* such as single forms of blue, light blue, white, violet, and double forms of blue, pink and white were subjected to the study.

Anatomical studies on the roots of seven variants and stomatal observations in leaves of seven variants in *C. ternatea* were carried out. Pieces of semi mature roots were fixed in FAA for 24 hours and hand sections were taken. The selected sections were stained with saffranin solution and mounted over slides in glycerine. Photomicrographs of the sections were taken using Leica D-2500 binocular microscope with attached DM100 digital camera. The measurements were determined using Leica applications software. For micro morphological study of stomata, mature leaves from the 5^{th} upper node were collected, chopped into 0.5cm to 1cm fragments and were boiled in alcohol for 30 minutes. Then the mixture was cleared in 5% Sodium hydroxide and incubated at 40ºC overnight. The cleared leaves were rinsed in water. Final clearing was made with 10% Sodium hypochlorite solution for 10 minutes. The lower epidermal layers were removed, rinsed in distilled water, stained with 1% saffranin and mounted on a slide in glycerol. The epidermal features were analysed and photomicrographs were taken with a compound microscope (Leica DM 100 digital camera attached to a Leica DM 2500 microscope). The quantitative measurements were recorded by the Image Anlaysing Softwrae. The cells were counted from the epidermal peels and the Stomatal Index was calculated using the standard formula (Salisbury, 1928) given below.

S.I= S/(S+E) x 100
Where,
S.I= Stomatal Index
S= Number of stomata per unit area
E=Number of epidermal cell in unit area

Results:-

Single blue:-

Cork consists of 13-14 elongated parenchyma cells. The outer region of cortex was comprised of elongated angular parenchyma and the inner region comprises angular parenchyma without intercellular spaces. The inner cortex contains abundant starch grains compared to outer cortex which were mainly in groups. They were round or oval in shape. Between outer and inner regions of cortex, only 3-4 cells contain starch grains. The width of cortex was 845.21 µm. The cortex contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-8 or single. Cortex was followed by 9-10 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were arranged along the periphery and centre of stele in scattered manner and small vessels were in between large vessels. Around 12 medullary rays were clearly visible radiating towards cortical region. Diameter of stelar region was 791.85 µm. The diameter of larger 5 vessels ranged from 59.31 µm to 68.23 µm with a mean value of 66.07µm (Table 1 & Fig 1A). The stomatal index was 12.45% (Table 2 & Fig 2A)).

Single light blue:-

Cork consists of 11elongated parenchyma cells. The outer region of cortex contains elongated parenchyma and the inner region comprises angular parenchyma without intercellular spaces. The outer and middle cortex contains more starch grains where as lesser in the inner cortex. Starch grains were round or oval in shape. The cortex also contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-11 and also as single. The width of cortex was 906.02 µm. Cortex was followed by 4-5 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were arranged at the centre and periphery of stele in a scattered manner and smaller vessels in between. Medullary rays were with elongated parenchyma. Around 4 medullary rays are clearly visible radiating towards cortical region. Diameter of stelar region was 818.43 µm. The diameter of larger 5 vessels ranged from 75.28 µm to 98.83 µm with a mean value of 88.70 µm (Table 1 & Fig 1B). The stomatal index was 20.90% (Table 2 & Fig 1B).

Single white:-

Cork consists of 9-10 elongated parenchyma cells. The outer region of cortex contains elongated parenchyma and the inner region comprises angular parenchyma without intercellular spaces. Starch grains were absent. The cortex contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-13 and also as single. The width of cortex is 911.60 µm. Cortex was followed by 5-6 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were arranged uniformly throughout the stele in a scattered manner and smaller vessels in between larger vessels. Medullary rays were with elongated parenchyma. Around 2 medullary rays were

clearly visible radiating towards cortical region. Diameter of stelar region is 1264.42 µm. The diameter of larger 5 vessels ranged from 90.03 µm to 107.95 µm with a mean value of 97.76 µm (Table 1 & Fig 1C). The stomatal index was 10.70% (Table 2 & Fig 1C).

Single violet:-
Cork consists of 8-9 elongated parenchyma cells. The outer region of cortex contains elongated parenchyma and the inner region comprises angular parenchyma without intercellular spaces. The cortex towards stele contains abundant starch grains which were less towards cork. Starch grains were round or oval in shape. The cortex also contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-14 and also single. The width of cortex was 1068.56 µm. Cortex was followed by 6-7 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were arranged throughout the stele in a scattered manner and smaller vessels were arranged in between larger vessels. Medullary rays wre with elongated parenchyma. Around 4 medullary rays were clearly visible radiating towards cortical region. Diameter of stelar region was 1260.79 µm. The diameter of larger 5 vessels ranged from 78.50 µm to 100.36 µm with a mean value of 88.19 µm (Table 1 & Fig 1D). The stomatal index was 17.71% (Table 2 & Fig 2D).

Double blue:-
Cork consists of 11-12 elongated parenchyma cells. The outer region of cortex comprises elongated angular parenchyma and the inner region comprises angular parenchyma without intercellular spaces. The outer cortex contains starch grains generally in groups. They were round or oval in shape. The inner cortical region contains a very less amount of starch grain. The width of cortex was 815.80 µm. The cortex contains irregularly scattered sclerenchymatous fibres which were in groups of 2-10 and also single. Cortex was followed by 4-5 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger and smaller vessels were arranged uniformly throughout the stelar region. Medullary rays were with elongated angular parenchyma. Around 5 medullary rays were clearly visible radiating towards cortical region. Diameter of stelar region was 1257.72 µm. The diameter of larger 5 vessels ranged from 61.23 µm to 76.82 µm with a mean value of 67.81µm (Table 1 & Fig 1E). The stomatal index was 15.10% (Table 2 & Fig 2E).

Double pink:-
Cork consists of 7-8 elongated parenchyma cells. The outer region of cortex contains elongated parenchyma and the inner region comprises angular parenchyma without intercellular spaces. The region of cortex towards stele contains abundant starch grains and no starch grains towards cork. Starch grains were round or oval in shape. The cortex also contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-11 and also as single. The width of cortex was 909.37 µm. Cortex was followed by 4-5 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were arranged uniformly throughout the stele in scattered manner and smaller vessels in between. Medullary rays were with elongated parenchyma. Diameter of stelar region was 1777.95 µm. The diameter of larger 5 vessels ranged from 76.99 µm to 108.92 µm with a mean value of 88.49 µm (Table 1 & Fig 1F). The stomatal index was 29.17 % (Table 2 & Fig 2F).

Double white:-
Cork consists of 8 elongated parenchyma cells. The outer and inner region of cortex comprises angular parenchyma without intercellular spaces. The inner and outer region of cortex contains starch grains. Starch grains were mainly seen in groups .They were round or oval in shape. The width of cortex was 617.42 µm. The cortex contains irregularly scattered sclerenchymatous fibres which were seen in groups of 2-10 and also as single. Cortex was followed by 3-4 layers of phloem parenchyma. Xylem vessels were round or oval in shape. Larger vessels were seen on the periphery and centre of stele in a scattered manner whereas smaller vessels were in between the larger vessels. Medullary rays were with elongated and oval or round shaped parenchyma. Around 8 medullary rays were clearly visible radiating towards cortical region. Diameter of stelar region was 1237.71 µm. The diameter of larger 5 vessels ranged from 60.05 µm to 68.21 µm with a mean value of 64.56 µm (Table 1 & Fig 1G). The stomatal index was 19.86% (Table 2 & Fig 2G).

Discussion:-

Variability with respect to different anatomical parameters were observed in different variants of *C. ternatea*. On comparison, the characters in 'double white' form was more distinct from others (Table 1). The quantitative leaf microscopy showed slight variation with respect to stomatal number, somatal index and palisade ratio (Table 2). The microscopical features of previously studied two varieties of *C. ternatea*, single blue and white forms clearly

showed some distinct anatomical features such as more starch grains in transverse section of root; broad patches of pericyclic fibers, more vessels with broad lumen and broad pith region in white variety. Further, the quantitative leaf microscopy of these two forms showed slight variation in stomatal number, somatal index and palisade ratio (Khatoon et al, 2015). Cortex of the root is composed of 10-12 layers of thin walled, polygonal or tangentially elongated cells, packed with mostly compound starch grains (Kiranmai, 2010, Kumar et al, 2010). All the ray cells of root are fully packed with starch grains and few contain calcium oxalate crystals (Kumar, 2010). The range in stomatal number, stomatal index and palisade ratio of both epidermis in leaves are also significant with respect to two different varieties (Khatoon et al, 2015).

Conclusion:-

Intraspecific variability in *Clitoria ternatea* was prominent in seven variants. The major variation was in width of stelar region, cortical region, number of medullary rays, distribution of starch grains, stomatal index etc. Among the variants studied, 'double white' was distinct from the rest in various parameters. Future studies of these variants may reveal other consistent differences in phytochemistry that may be useful in pharmacological applications.

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Conflict of Interest:-

The authors have no conflict of interest.

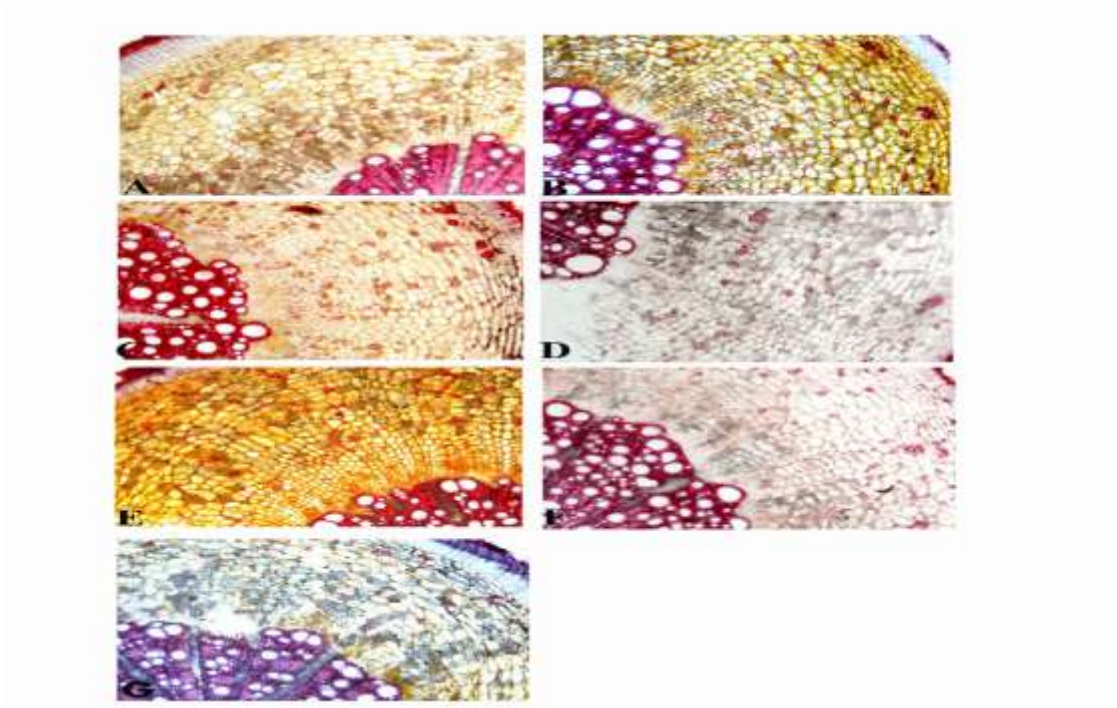

Fig. 1:- Root anatomy of seven variants of *Clitoria ternatea* A- Single blue, B- Single light blue, C- Single white, D- Single violet, E- Double blue, F-Double pink and G- Double white.

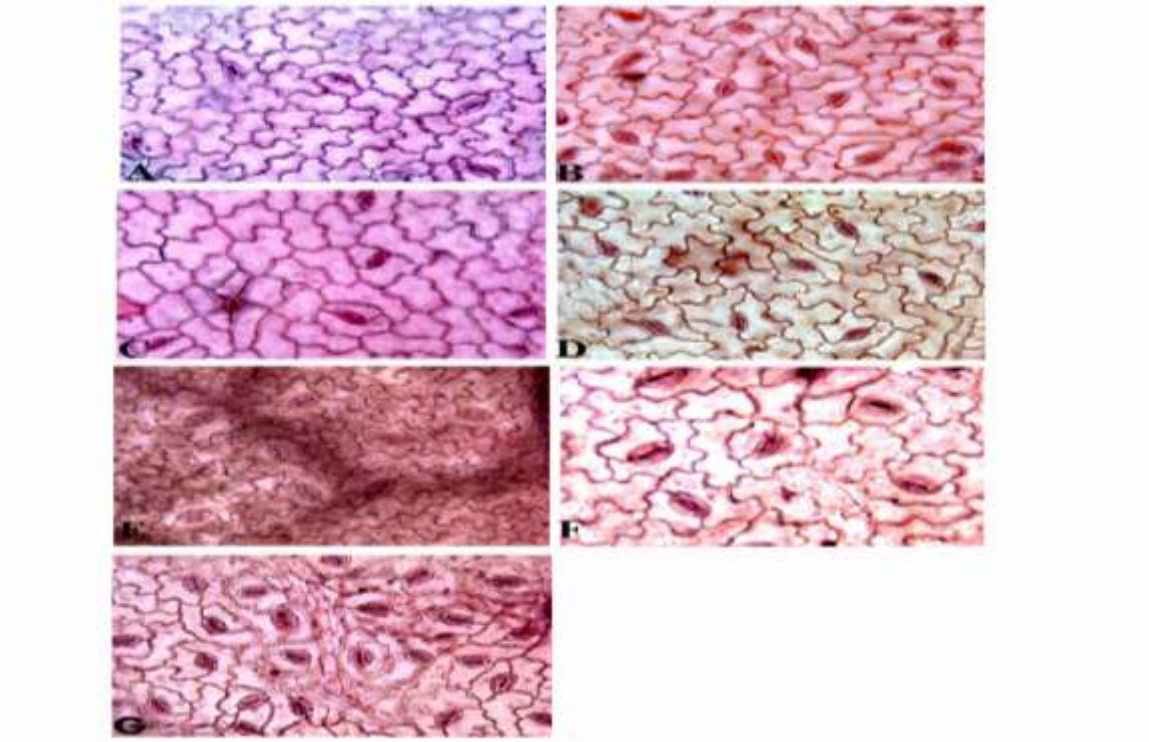

Fig. 2:-Stomatal types of seven variants of *Clitoria ternatea* A- Single blue, B- Single light blue, C- Single white, D- Single violet, E- Double blue, F-Double pink and G- Double white.

Table 1:-Microscopic characters of root of seven variants of *C. ternatea*

	Single blue	**Single light blue**	**Single white**	**Single violet**	**Double blue**	**Double pink**	**Double white**
Outer cortex	Elongated angular parenchyma without intercellular spaces	Elongated angular parenchyma without intercellular spaces	Elongated parenchyma without intercellular spaces	Elongated parenchyma without intercellular spaces	Elongated parenchyma without intercellular spaces	Elongated parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces
Inner cortex	Elongated angular parenchyma without intercellular spaces	Elongated angular parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces	Angular parenchyma without intercellular spaces
Starch grain in cortex	Inner cortex contain abundant starch grains compared to outer cortex. Between outer and inner regions of cortex only 3-4 cells contain starch	Outer and middle cortex contain starch grains and the inner cortex contain less starch grains	Absent	Cortex towards stele contains abundant starch grains and towards cork contains less starch grains	Outer cortex contains starch grains mainly in groups inner cortical region contains a very less amount of starch grain	Cortex towards stele contains abundant starch grains and towards cork contains no starch grains	Inner and outer region of cortex contains starch grains in groups

	grains and other cells are without starch grains						
Shape of starch grain	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval
Schleren chymatous Fibres in cortex	Irregularly scattered	Irregulaly scattered	Irregulary scattered	Irregularly scattered	Irregularly scattered	Irregularly scattered	Irregularly scattered
Arrangement of sclerenchymatous fibres	Groups of 2-8 and also single	Groups of 2-11 and also single	Groups of 2-13 and also single	Groups of 2-14 and also single	Groups of 2-10 and also single	Groups of 2-11 and also single	Groups of 2-10 and also single
No of phloem parenchyma	9-10	4-5	5-6	6-7	4-5	4-5	3-4
Shape of xylem vessels	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval	Round or oval
Arrangement of vessels	Larger vessels are arranged on the periphery and centre of stele in a scattered manner and small vessels are arranged between large vessels in a scattered manner	Larger vessels are arranged at the centre and periphery of stele in a scattered manner. Between larger vessels smaller vessels are arranged in a scattered manne	Larger vessels are arranged uniformly throughout the stele in a scattered manner and smaller vessels are arranged between larger vessels in a scattered manner	Larger vessels are arranged uniformly throughout the stele in a scattered manner and smaller vessels are arranged at the centre and between larger vessels in a scattered manner	Larger and smaller vessels are arranged uniformly throughout the stelar region. They are arranged in a scattered manner	Larger vessels are arranged uniformly throughout the stele in a scattered manner and smaller vessels are arranged between larger vessels in a scattered manner.	Larger vessels are seen on the periphery and centre of stele in a scattered manner whereas smaller vessels are arranged between the larger vessels in a scattered manner.
Number of medullary rays	12	4	2	4	5	3	8
No of epidermal cells	13-14	11	9-10	8-9	11-12	7-8	8
Width of cortical region (µm)	854.21	906.02	911.60	1068.56	815.80	909.37	617.42
Diameter of stelar region(µm)	791.85	818.43	1264.42	1260.79	1257.72	1777.95	1237.71
Larger 5 vessels diameter(µm)	59.31-68.23, 66.07	75.28-98.83, 88.70	90.93-107.95, 97.76	78.50-100.35, 88.19	61.23-76.82, 67.81	76.99-108.92, 88.49	60.05-68.21, 64.56
Frequency of epidermal cells on lower side	260	269	217	223	253	233	238
Frequency of stomata per unit area	37	71	26	48	45	96	59
Stomatal index (%)	12.45	20.90	10.70	17.71	15.10	29.17	19.86

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