Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CHARACTERIZATION OF BLUE AND WHITE VARIETIES OF *CLITORIA TERNATEA* (L.) BASED ON SEED PROTEIN PROFILING COLLECTED FROM NORTH INDIA

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ABSTRACT

The medicinal plant *Clitoria ternatea* belongs to the family fabaceae and their seeds are highly nutritive. In the present study characterization of seed proteins of its popularly growing varieties has been undertaken. A comparative study has been done in term of qualitative estimation of total seed protein among the six accessions of white and blue petal varieties. Three accessions (ACCRI, ACCR2 and ACCR3) of blue petal variety and three accessions (ACCR4, ACCR5 and ACCR6) of white petal variety were taken. With the help of protein molecular markers and electrophoresis pattern, seed proteins of above two varieties showed the presence of four categories of polypeptide masses. Some bands were seen in both the varieties shows varietal differences and genetic composition. A dendrogram was made using UPGMA which revealed the interrelationship of two varieties of *Clitoria* that indicates two clustures viz. clusture A and clusture B. Clustures A suggested that there are no changes in protein profiling among ACCR1, ACCR2 and ACCR3, but different with protein profiling among clusture B (ACCR4, ACCR5 and ACCR6).

KEYWORDS: ***Clitoria ternatea;*** **Seed proteins; SDS-PAGE; UPGMA.**

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INTRODUCTION

Clitoria ternatea L. is commonly known as butterfly pea and belong to family fabaceae. This is an important medicinal plant, pasture crop and forage legume. Plants are ornamental, perennial, climber and provide many bioactive compounds. They are mainly distributed in tropical Asia, Madagascar and Philippines islands. This speciesare gradually depleting and listed as a rare species by International union for conservation of nature and natural resources (IUCNNR). Medicinally, it is useful in the treatment of hectic fever, asthma, severe bronchitis and by tribal people to cause abortion. Humans in Philippines sometimes eat the pods. The leaves and seeds are good stock food due to highly rich in proteins and vitamin. The seeds also have powerful cathartic action. Its roots are aperients, laxative, diuretic and demulcent and are given in fever, croup, chronic bronchitis, ascites, dropsy and enlargement of the abdominal viscera (Khory and Katarak 1984.). It has antioxidant, antidiabetic and hepatoprotective potentials (Manja Lata Zingre et al, 2013). This type of hepatpprotective activity of plant based drugs against D-Galn study also done(Ramji Gupta et al 2014) Seeds are also used in abdominal cramps,promote intellect. The seeds contain 25 to 38 % proteins, 5% total sugar and 10% oil along with 13 amino acids (Barro and Ribeiro, 1983). Legumes have been searched as vegetable and source of high proteins content (Valizadeh, 2001). In the present study, three accessions of each varieties of *Clitoria* have been taken for qualitative and quantitative analysis of seed proteins. Two varieties mainly differ in petal colour and other morphological markers by visual observation. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used due to its validity and simplicity for describing genetic structure of crop germplasm, but its implication has been limited mainly to cereals due to less polymorphism in most of the legumes (Ghafoor et al., 2002). Seed storage proteins have been used and, electrophoretic pattern of protein profiling obtained to resolve the taxonomic and evolutionary systems of several crop plants (Ladizinsky and Hymowitz 1979; Das and Mukarjee 1995). The aim of the present investigation is to determine seed protein variability among three accessions of two different varieties of *Clitoria* to draw their relationship.

MATERIALS AND METHODS

Collection of samples

Seeds of two wild varieties and their accessions (blue and white petals) of *Clitoria ternatea* were collected from different places viz. Chandauli, Ghazipur and Varanasi. Seed samples were dried naturally in sunlight.

Morphological study

Different morphological parameters viz. plant height, fresh weight of the plant, dry weight of the plant and stem thickness, e.t.c were studied described as in table1.

Quantitative estimation of the proteins

Seed protein extraction

About 250 mg seeds of each species and their accessions were crushed with the help of mortar-pestle using 5 ml of 0.01M Tris-HCL buffer (pH 7.0) at 4^0C. The resulting homogenates were centrifuged at 5000 rpm for 20 minutes. Pellet was taken and added with 5 ml of 10% TCA and boiled for three minute in water bath. The Samples were again centrifuged at 5000 rpm for 20 minute and remaining pellet were solubilized in 5 ml of 0.1 N NaOH. The supernatant was discarded and homogenate was used for protein estimation following Lowery et al. (1951) using Bovine serum albumin (BSA) as standard.

Elechrophoretic analysis

Extraction

For extraction of soluble proteins seeds of all Accessions of each variety grounded to fine powder with sampling buffer containing 150 Mm Tris(pH 6.8), 30% v/v glycerol, 15% β-mercaptoethanol, 1% w/v SDS and 0.002% w/v Bromophenol blue and incubated at room temperature for overnight next day, sample were heated as 100c in water bath for 2-3 minutes, centrifugation were done at 10000 rpm for 10 minutes, Supernatant (40 µl) was electrophoresed in the discontinuous buffer system and SDS-PAGE method was used

by the method of Laemmli (1970), 12% separating gels and 5% stacking gels, run by a protein marker medium range (Genei, Bangalure) along with molecular weight protein samples(M.WT 97.4-14.3KDa) at 100 volts. Proteins bands of different intensities and positions were obtained after staining in Coomassie brilliant blue dye R- 250 (Hames and Rickwood, 1990) and after destainig of the gels, molecular weight of protein banding pattern was analysed in GEL DOC software (UVlteck, UK).

RESULTS AND DISCUSSION

Estimated amount of total seed proteins in two varieties and it accessions shown in table.1. The amount of seed proteins varied from 36.25±0.21 to 34.52±0.21 mg/g dw of seed, among blue and white variety respectively. Whereas amount of seed protein in accessions (ACCR1, ACCR2 and ACCR3) blue varieties and have (ACCR4, ACCR5 and ACCR6) are the accessions of white varieties showed same amount of proteins. Electrophoresis patterns of seed proteins of both *Clitoria* varieties and their accessions showed variations in molecular weight of polypeptide band and band intensity, marked as quantitative protein expression. Result showed Total 15 bands were distribution among the varieties and their accessions (Lane 1-3) named ACCR1, ACCR2, and ACCR3 for blue varieties and ACCR4, ACCR5 and ACCR6 (Lane 3-6) were for accessions of white variety. Accessions blue variety (ACCR1, ACCR2 and ACCR3) showed highest aggregation of dominant polypeptide mass with high intensity bands with 54-60 KDa molecular weight protein band. Besides above 62 KDa, 58KDa, and 48 KDa molecular weight were also present. In case of accessions of white variety (ACCR4, ACCR5 and ACCR6), 62-54 KDa molecular weight proteins were common but with low intensity of bands middle polypeptide band Ranging from 29- 25 KDa were were also present in both the varieties Lowest polypeptide mass ranging from 12 to 6.5 KDa were also present in accessions of white variety (ACCR4, ACCR5 and ACCR6) expressing more intensity in comparison to accessions of blue variety (ACCR1, ACCR2, and ACCR3) belonging to the same molecular weight protein. The bands of 48 KDa molecular weight was only present in accessions of blue varieties whereas the bands of 88 KDa molecular weight polypeptide was present in accessions of blue variety but with low intensity, while they were absent in accessions of white variety. The presence of 88KDa and 50 KDa polypeptide showed differentiation and polymorphism between two varieties. The polypeptide mass of 100 KDa was present in all the varieties and their accessions, but they were less aggregated polypeptide mass present. Results suggested that bands were heterogenous between both the varities. According to the distribution pattern out of 15 bands 8 were common and 7 were not common among the varieties and their accession altogether 10 bands were visible in blue varieties and their accessions and 13 bands were in white varieties and their accessions. The result showed distribution of unequall number polypeptide band among the varieties and their accessions and differentiated the protein expression. SDS-PAGE technique is useful tool for genetically divorce group of plant accessions and variety for better selection among the germplasm collections. In order to have several collections of germplasm for maintenance the good germplasm characterization of such collections are necessary. The each accessions of blue and white varieties collected from different locations showed heterogenous clustering in differentiating into two groups in the dendrogram suggesting easily exchangeable germplasm by breeders on the other hand accession of white variety collected from different locations show homogenous clustering followed by blue variety with indicating that population were interrelated. The dendrogram analysis based on Nei and lei□s and genetic distance represented by UPGMA. Protein profiling of six accessions accommodated in two clustures, clusture, clusters A and clusture B. Clusture A represented group of three accessions of blue variety (ACCR1, ACCR2, and ACCR3) indicated no changes in genetic constitution. Similarly it was followed in clusture B showing no genetic changes among accessions of white variety (ACCR4 ACCR5 and ACCR6. The result showed that among varieties and their accessions ACCR1, ACCR2, and ACCR3 and ACCR4 ACCR5 and ACCR6 are not related to each other but the major difference was in

petal colour. Changes in petal colour could be a marker of genetic differentiation, which could be on the basis of molecular weight protein bands. The presence/absence of polypeptide band among two group of accession indicated the genotype differences (Table1) The result revealed that low intra- specific variations among the accessions, may be due to equal impact of environmental and geographical distribution which is in accordance with the findings of Ghafoor et al., (2003), as incase seed protein profiling of chickenpea. Our results also strengthened by evaluating genetic diversity of seed protein by elechtrophoresis to establish the relationship between the origin and clusturing, pattern, and even no corelation between morphological markers and seed protein profiling of the accessions collected from different locations. In our findings there was not a clear differentiation due to low level variations between blue and white petal accessions and supported a report of Ghafoor et at., (2002), in Vigna mungo and Vigna radiata which revealed low level inter-specific genetic diversity and no correlation between agronomic traits and geographical origin. It is important to note that a low level of intra-specific variations has been also reported in various legumes i.e., chickpea (Ghafoor et al., 2003)., and groundnut (Javaid et al., 2004) and black gram (Ghafoor et al., 2003) Similarly comparision between morphological and seed protein profiling have been well documented. They also showed no correlation between clusture patterns with origin as established. SDS-PAGE. However the above technique may be suitable for describing polymorphism among accessions. It is also suggested that accessions with similar banding patterns should be further characterized by 2-D gel electrophoresis for fine separation of pro in and also require a broad scale protein profiling. Protein markers in *Clitoria* may be useful discriminated between varieties and species to some extent. In additions, differentiation of protein bands could hint towards high environmental and evolutionary variability. The range of variations of protein bands in both the varieties of *Clitoria ternatea i.e.* blue and white can be useful as markers for establishing interrelationship under the seasonal and environmental impact as well as nutrient level in soil. Overall, our results concludes that on the basis of protein profiling of seed protein, there is a clear relationship among in the accessions of both varieties and only difference among protein bands could be a basis of some morphological markers like blue and white petal colour and this can be assumed as genotype relationship in the preliminary investigation.

Table 1
Characterisation of bands in different accessions of Clitoria terneatea.

Accession	MW(KDa)														
	100	94	92	90	88	75	62	58	54	48	45	40	29	25	12
ACCR1	1	0	0	0	1	1	1	1	1	0	0	1	1	1	1
ACCR2	1	0	0	0	1	1	1	1	1	0	0	1	1	1	1
ACCR3	1	0	0	0	1	1	1	1	1	0	0	1	1	1	1
ACCR4	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
ACCR5	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1
ACCR6	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1

Table- 1

Comparative account of morphological characters in three varieties of Clitoria ternatea

Parameters	*Clitoria ternatea*	
	Blue	*White*
Plant height(cm)	167± 1.00	155±1.00
Fresh weight of the plant(mg)	95.29±1.04	89.91±0.53
Dry weight of the plant(mg)	68.21±1.00	65.20±0.98
Stem thickness(cm)	0.94±0.01	1.01±0.02
Petal length(cm)	4.72±0.02	4.62±0.02
Leaf length(cm)	5.73±0.01	5.33±0.02
Leaf width(cm)	2.8±0.01	2.62±0.02
Leaf area(cm^2)	9.64±0.04	8.38±0.06
Length of pod(cm)	9.23±0.03	9.02±0.26
No of pods per plant per year	17.6±0.10	17.4±0.01
Days to flowering	47±1.00	44.67±44.67
Internodal length(cm)	8.18±0.04	8.01±0.01
Length of the rachis(mg)	5.53±0.02	5.51±0.01
Seed per pod(mg)	7.53±0.02	7.43±0.02
Length of seed(mg)	0.81±0.01	0.61±0.01
Breadth of seed (mg)	1.27±0.02	1.2±0.01
Seed weight(100 seeds)(mg)	6.12±0.05	5.01±0.01
Length of stamen(mg)	0.6±0.00	0.52±0.03
Length of style(mg)	1.27±0.03	1.2±0.01
Length of petiole(mg)	0.17±0.01	0.15±0.01

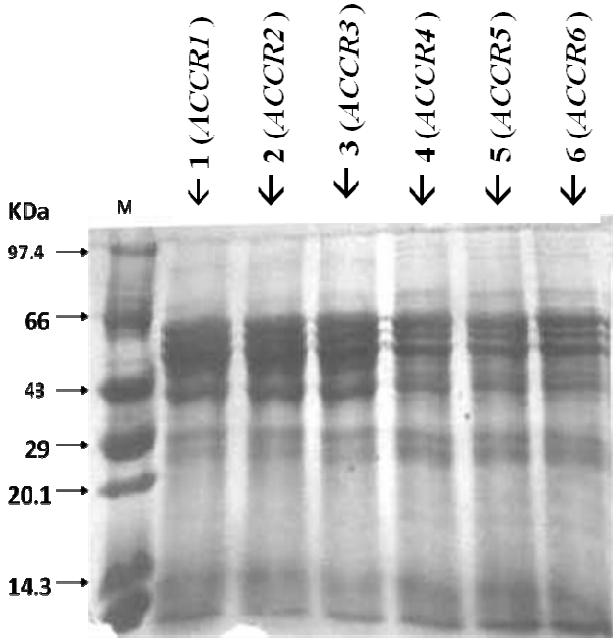

Fig 1. Accessions of blue and white variety of *C. ternatea* showing different molecular weight (KDa) in different lane(1-6). While ACCR1, ACCR2 , ACCR3 are the accessions of blue varieties of different locations, Chandauli, Ghazipur and Varanasi respectively and ACCR4 , ACCR5 and ACCR6 are that of white varieteis.

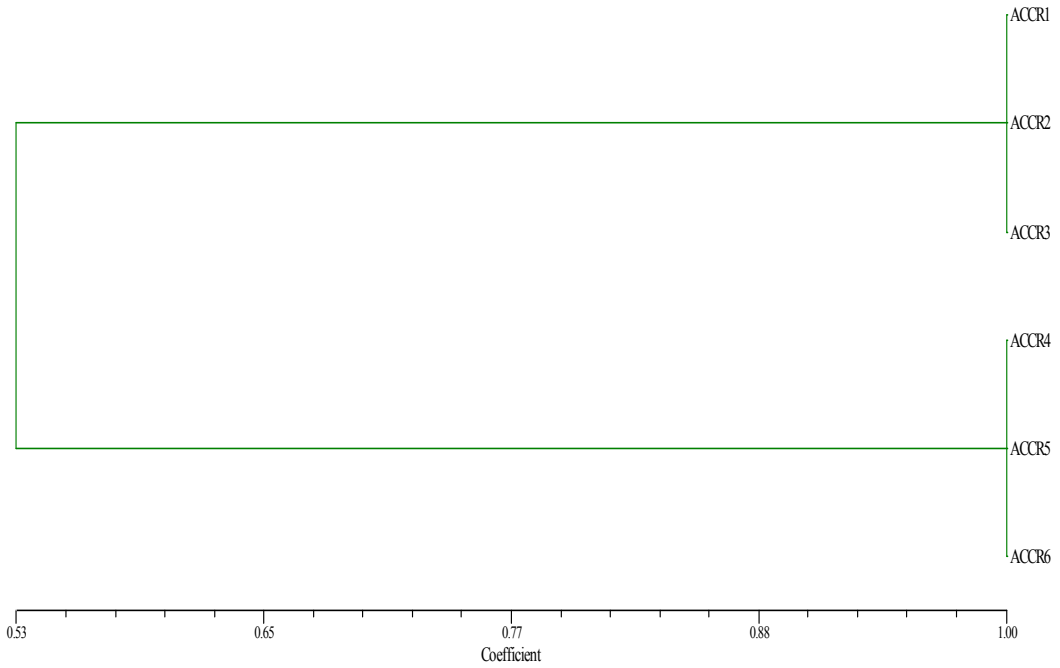

Figure 2
Dendrogram of the relationships among 6 Accessions of Clitoria ternatea based on SDS-PAGE of seed storage proteins.

CONCLUSION

In conclusions, electrophoresis (SDS-PAGE) of seed storage proteins can be economically used to assess genetic variation and identification of superior germplasm and also to differentiate varieties from their both genotypes. Moreover, seed storage protein based markers can be used for identification of genotypes i.e. white and blue petal color of the flower. In this study some bands are common in both the varieties and their accessions and indicated as specific markers of this genus which are helpful in differentiating accessions of the adapted varieties. Aggregation of polypeptide were more observed in blue petal plants suggested that these have more variable proteins than white verities, but some bands are common in both varieties, in white petal cultivars with low intensity of that indicated the dependence on the genetic expression of proteins. It may be that expression of high proteins also depends upon environment and nutrient availability in the soil. Results concludes that SDS-PAGE is not best suitable for describing polymorphism among Accessions, the information on polymorphism using RAPD in *Clitoria* genotypes is useful in the assessment of genetic diversity, genetic relationships and phylogenetic relationships. It is also suggested that accessions with similar banding patterns should be further characterized by 2-D gel electrophoresis for fine separation of protein and also require a broad scale protein profiling.

ACKNOWLEDGEMENT

Authors are thankful to University Grant Commission, New Delhi for financial assistance. The authors express a deep sense of gratitude to the Head, Department of botany, BHU, Varanasi for constant encouragement and support.

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