

## SDS-PAGE analysis of seed protein of five floral morphotypes of *Clitoria ternatea* L.



### Botany

**KEYWORDS:** *Clitoria ternatea* L., Morphotypes, Seed protein, SDS-PAGE, Polymorphism.

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### ABSTRACT

The twiner medicinal herb *Clitoria ternatea* L. of Fabaceae has five different floral types based on the combination of colour of petal, arrangement of petal and arrangement of stamens. These morphological traits have also been considered for refinement of taxonomical treatment of this species. Seed storage protein being a stable genetic character has been analyzed here to verify the justification of considering floral morphotypes for taxonomical treatment of the species at infraspecific level. Though in consideration of the amount of protein the blue double type has been noted to produce greatest amount of it, followed by the white double type as intermediate one and both single type as the least producer, polypeptide profile in SDS-PAGE has shown least polymorphism of 3.65%. In terms of this polymorphism single type of flower bearing forms, irrespective of petal colour, have been noted to be in close proximity and quite distantly placed from the double types; again then double type of white and blue petal but having solitary stamens are close to each other and distantly placed from blue double with polyadelphous stamens. Thus, even the subtle polymorphism in polypeptide profile of seed storage protein fortifies the consideration of five floral morphometric forms of *Clitoria ternatea* L.

### Introduction:

*Clitoria ternatea* L., a twiner belonging to the family Fabaceae, bears immense ethno medicinal significance due to its variety of uses for anti-depression, anxiolytic, nootropic and anti-stress properties of its biomolecules. In addition, its acetylcholinesterase inhibition property has been identified (Kumar, Mukherjee, Pal, Houghton, & Mukherjee, 2007) and it is also effectively in use for the treatment of Alzheimer's and Parkinsonism (Ngo & Li, 2013). It acts as COX inhibitor, too, (Ur Rehman et al., 2013) by inducing apoptosis. This species exhibits different floral morphotypes, which led Fantz. (1977), to classify them in five forms under two different varieties viz. 1. *Var. ternatea f. fasciculata* Fantz. (**Blue Single flower**); 2. *Var. ternatea f. albiflora*. (Voigt) Fantz. (**White Single flower**); 3. *Var. pleniflora f. leucopetala* Fantz. (**White Double flower**); 4. *Var. pleniflora f. pleniflora* Fantz. (**Blue Double flower** with solitary stamens); 5. *Var. pleniflora f. subpolyadelpa* Fantz. (**Blue Double flower** with polyadelphous stamens). Diversity at such morphological level may entail further verification in respect of a trait having genetic basis corroborating with and endorsing the creation of groups on the basis of floral phenotypes. Seed protein is widely proved to be a trait of this stature.

Seed protein profile, revealed through the electrophoretic mobility, has been in use successfully through years for discriminating and identifying taxa of lower levels like species, variety etc., in particular and thus also for dealing with taxonomic problems and diversity (Jha & Ohri, 1996), Ahmad & Slinkard, 1992 Singh, Gurtu, & Jambunathan, 1994; ; Singh, Sivaramakrishnan, Mengesha, & Ramaiah, 1991; Hussain, Ramirez, Bushuk, & Roca, 1986; Cooke, Parnell, & Draper, 1983; Ladizinsky & Hymowitz, 1979). SDS-PAGE has successfully been used in identification and characterization of plenty of plant taxa ((Hussain, Bushuk, & Roca, 1989).

Present study is aimed at scrutinizing polymorphism, if any, at seed protein profile existing amongst different floral forms of *Clitoria ternatea* L.

### Materials and Methods:

#### Protein extraction:

Healthy and mature seeds of randomly selected five different floral morphotypes of *C. ternatea* L. were selected for protein extraction. About 1g of seeds were taken and crushed in mortar and pestle prior to extraction.

#### Defatification:

The seeds of *C. ternatea* L. varieties contain adequate amount of fats along with the storage proteins. So, to isolate the storage proteins from the fats the extract was defatted.

#### Procedure:

Dried 1g of seeds were homogenised to a fine powder with the help of mortar and pestle. About 500 mg of powdered seed was taken in a test tube. To this about 15 to 20 ml of dehydrated distilled acetone was added and the test tube was made air tight with paraffin film and aluminium foil to avoid the evaporation of acetone. After shaking uniformly the test tubes were kept for 15 to 20 hrs. All fats dissolved in acetone were filtered using Whatman filter paper. The residue containing the storage proteins was collected and dried at room temperature for further storage in freezer.

Homogenization of 0.5g defatted powder with 5 - 10ml Tris HCl buffer pH-7.5 was done. Extract was then centrifuged at 10,000 rpm for 15 min. at 4°C. The supernatant containing the storage proteins was decanted slowly. The clean supernatant was used for further protein estimation and SDS-PAGE analyses. The protein content mg ml<sup>-1</sup> of extract was then estimated by Lowry et al. method (1951).

#### Protein estimation:

The blue colour developed by the reduction of the phosphomolybdic phosphotungstic components of the Folin-Ciocalteu reagent by the aromatic amino acids tyrosine and tryptophan present in the protein and the colour developed by the biuret reaction of the protein with the alkaline cupric-tartrate was measured by the Lowry's method (Lowry, Rosenbrough, Farr, & Randall, 1951) at 660 nm.

#### SDS-PAGE:

Gel Electrophoresis is used for separation and characterisation of proteins by applying electric current. In the present investigation Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970) method was used. In this method polypeptides are separated according to their molecular weights and not by their intrinsic electrical charge.

#### Statistical analysis:

Statistical analyses were performed with the help of IBM SPSS STATISTICS version 21 and Microsoft Excel.

#### Results and Discussion:

Seeds of 5 different morphotypes of *C. ternatea* L. were selected

randomly for quantitation of total protein content and analyses of polypeptide bands through SDS-PAGE. Very minor variations in seed protein content were found between floral morphotypes. Lowest amount of protein was found to be present in WS (*C. ternatea* L. var. *ternatea* f. *albiflora*) and it was 138.98mg g<sup>-1</sup>. The highest amount of total seed protein was found in BD1 (*C. ternatea* L. var. *pleniflora* f. *subpolyadelpha*) and was measured as 142.23 mg g<sup>-1</sup>. Seed protein content of BD2 (*C. ternatea* L. var. *pleniflora* f. *pleniflora*) and WD (*C. ternatea* L. var. *pleniflora* f. *leucopetala*) were 140.98 mg g<sup>-1</sup> and 139.78 mg g<sup>-1</sup> respectively. Seeds of BS (*C. ternatea* L. var. *ternatea* f. *fasciculata*) were found to have 139.10 mg g<sup>-1</sup> of protein. The trend could clearly be found from (Figure 1).

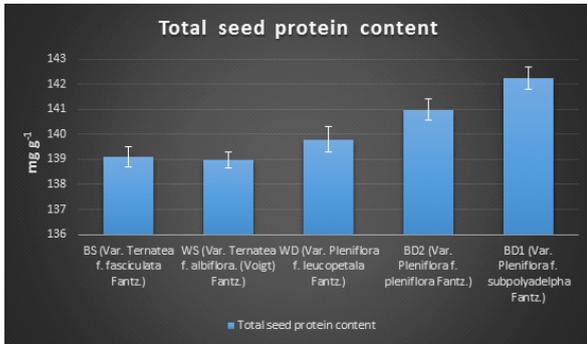


Figure 1. Total seed protein content of different morphotypes of *C. ternatea* L.

Profile of polypeptides of seed protein generated through SDS-PAGE showed altogether 192 discrete bands, out of which 9 bands were found to be polymorphic. So the polymorphism in respect of seed protein profile of the five floral morphotypes of *C. ternatea* L. was detected to be only 3.65%, which indicates the presence of very less amount of polymorphism among the floral morphotypes of *C. ternatea* L. Bands were found to be present within the range of 89 KD to 14 KD. While most of the bands are common in nature, the polymorphism is showed only by presence or absence of some bands only. Little variability was found amongst the polypeptides with higher molecular weights whereas most polymorphism was confined amongst the polypeptides with lower molecular weights (below 29 KD) (Goodrich, Cooke, & Morgan, 1985). No difference was found between the profiles of BS (*C. ternatea* var. *ternatea* f. *fasciculata*) and WS (*C. ternatea* var. *ternatea* f. *albiflora*). The highest number of polymorphic bands were found to be present in BD1 (*C. ternatea* L. var. *pleniflora* f. *subpolyadelpha*), was calculated about 9.76% (at 45.9, 44.3, 28.0 and 26.7 Kds).

Table 1. Number of bands obtained in SDA-PAGE analysis of seed protein.

S. No.	Morphotypes	Total no. of bands scored	Polymorphic c bands	Polymorphism (%)
1	BS	37	0	0
2	WS	37	0	0
3	WD	38	1	2.632
4	BD2	39	2	5.128
5	BD1	41	4	9.756
	<b>Total bands</b>	<b>192</b>	<b>7</b>	<b>3.646</b>

Table 1. 1. BS: *Var. ternatea* f. *fasciculata* Fantz. (Blue single); 2. WS: *Var. ternatea* f. *albiflora*. (Voigt) Fantz. (White single); 3. WD: *Var. pleniflora* f. *leucopetala* Fantz. (White double with solitary stamens); 4. BD2: *Var. pleniflora* f. *pleniflora* Fantz. (Blue double with solitary stamens); 5. BD1: *Var. pleniflora* f. *subpolyadelpha* Fantz. (Blue double with polyadelphous stamens).

WD (*C. ternatea* L. var. *pleniflora* f. *leucopetala*) and BD2 (*C. ternatea* L. var. *pleniflora* f. *pleniflora*) had overall 2.63% (at 45.9 KD) and 5.13% (at

45.9 and 44.3 KDs) of polymorphism respectively. Though they shared one otherwise polymorphic band common in them. So, the actual percentage of polymorphism among these latter two morphotypes was 1.29%.

The pair-wise similarity matrix for the polypeptide profile (Table 2) shows that accessions from BS i.e. variety *C. ternatea* L. var. *ternatea* f. *fasciculata* Fantz. (Blue single) and WS i.e. *C. ternatea* L. var. *ternatea* f. *albiflora*. (Voigt) Fantz. (White single) share the highest (100%) genetic similarity among themselves. The BD2 (*C. ternatea* L. var. *pleniflora* f. *pleniflora* Fantz. i.e. Blue double with solitary stamens) and WD (*C. ternatea* L. var. *pleniflora* f. *leucopetala* Fantz. i.e. White double with solitary stamens) share 97.6% of similarity. Thus, they show very little diversity among them. BS and WS have more than 95% of similarity with the WD. BD2 shows more than 92% of similarity with BS and WS. BD1 (*Var. pleniflora* f. *subpolyadelpha* Fantz. i.e. Blue double with polyadelphous stamens) has nearly 88% of similarity with BS and WS; whereas, it has more than 92% of similarity with WD and nearly 95% with Bd1.

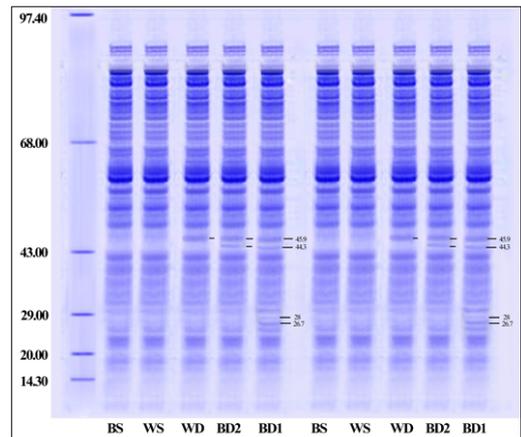


Figure 2. 1. BS: *Var. ternatea* f. *fasciculata* Fantz. (Blue single); 2. WS: *Var. ternatea* f. *albiflora*. (Voigt) Fantz. (White single); 3. WD: *Var. pleniflora* f. *leucopetala* Fantz. (White double with solitary stamens); 4. BD2: *Var. pleniflora* f. *pleniflora* Fantz. (Blue double with solitary stamens); 5. BD1: *Var. pleniflora* f. *subpolyadelpha* Fantz. (Blue double with polyadelphous stamens).

Figure 2. SDS-PAGE profile of five different floral morphotypes of *C. ternatea* L.

Table 2. Genetic similarities between different floral morphotypes of *C. ternatea* L. estimated using the Simple Matching Coefficient.

Case	BS	WS	WD	BD2	BD1
BS	1.000				
WS	1.000	1.000			
WD	.951	.951	1.000		
BD2	.927	.927	.976	1.000	
BD1	.878	.878	.927	.951	1.000

Table 2. 1. BS: *Var. ternatea* f. *fasciculata* Fantz. (Blue single); 2. WS: *Var. ternatea* f. *albiflora*. (Voigt) Fantz. (White single); 3. WD: *Var. pleniflora* f. *leucopetala* Fantz. (White double with solitary stamens); 4. BD2: *Var. pleniflora* f. *pleniflora* Fantz. (Blue double with solitary stamens); 5. BD1: *Var. pleniflora* f. *subpolyadelpha* Fantz. (Blue double with polyadelphous stamens)

From binary matrix PCA analysis was performed and the analysis included Unweighted Pair Group Method of Arithmetic Averages (Sneath & Sokal, 1973) to create a dendrogram for better understanding of relationship pattern. PCA analysis and findings of simple matching coefficient analysis suggested that cohesion pattern of stamens played the key role here to draw the infra-species relationship among different floral morphotypes of *C. ternatea* L.

Agglomeration schedule revealed that initially 2 major clusters were formed. One was comprised of BS and WS, while the second one included WD, BD2 and BD1. This suggests that primarily the arrangement patterns of petals were crucial for the formation of clusters and the finer relationship amongst them was based on cohesion patterns of stamens.

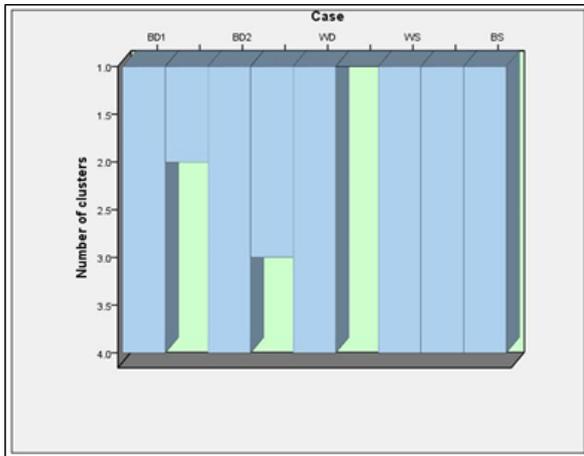


Figure 3. Agglomeration Schedule

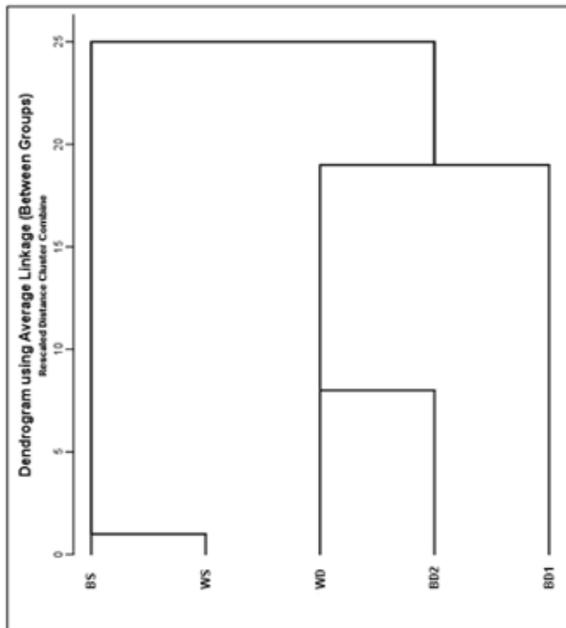


Figure 4: 1. BS: *Var. ternatea f. fasciculata* Fantz. (Blue single); 2. WS: *Var. ternatea f. albiflora*. (Voigt) Fantz. (White single); 3. WD: *Var. pleniflora f. leucopetala* Fantz. (White double with solitary stamens); 4. BD2: *Var. pleniflora f. pleniflora* Fantz. (Blue double with solitary stamens); 5. BD1: *Var. pleniflora f. subpolyadelpha* Fantz. (Blue double with polyadelphous stamens)

Figure 4. Dendrogram based on SDS-PAGE profile of seed protein of *C. ternatea L.*

**Conclusion:** Though *C. ternatea L. var. pleniflora f. subpolyadelpha* Fantz. (BD1) was kept under the form *pleniflora* along with two other members namely *C. ternatea L. var. pleniflora f. leucopetala* Fantz. (WD) and *C. ternatea L. var. pleniflora f. pleniflora* Fantz. (BD1), but it depicted relatively distant relationship with those in terms of seed protein profile. These findings as an aid to further studies may help arrive at a decision regarding the justification of the placement of the form *subpolyadelpha* under the variety *Pleniflora*.

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