Discussion
DISCUSSION

MORPHOLOGICAL ANALYSIS:

*Clitoria ternatea* L. is a native of Eastern Africa and most common in South-east Asia. Its distribution also encompasses United States of America to Uruguay, the Caribbean islands Australia, Malaesia, Micronesia and Polynesia. Twenty five populations of Ternatea were collected from diverse geographical areas of Indian states (Table-01) and have been critically analyzed for morphological characters at the collection sites, wherever possible and by raising plants under uniform conditions at the Departmental experimental garden. The observations revealed that these taxa are polymorphic and complex. The initial observation indicates that they fall into two categories, which are commonly known as single flowered and double flowered taxa. The characters studied in detail and the ranges of variation in phenotypic characters are summarized in Table-02. Habit and leaf morphology are highly variable. Even within the taxa different leaflet number are encountered such variable vegetative characters over-look discontinuities in more stable reproductive characters. It is due to
more reliance on vegetative characters Indian taxonomists were not able to delimit the natural biotic units within *C. ternatea* (Hooker, 1879; Cooke, 1908; Gamble, 1935 and Saldanha, 1984). While working on *Clitoria* of Antillarum, United States and Southeastern Asia. Fantz (1990, 1993 and 1995) emphasized on importance of leaf form, calyx, bracteoles, flower nature, colour, arrangements of stamen and pod size in delimiting the taxa within *C. ternatea*. He circumscribed two varieties of complex species *C. ternatea*, Var. Ternatea and Var. Pleniflora and six forms *C. ternatea var. ternatea f. ternatea*; *C. ternatea Var. ternatea f. albiflora*; *C. ternatea Var. ternatea f. fasciculata*; *C. ternatea Var. pleniflora f. pleniflora*; *C. ternatea Var. pleniflora f. leucopetala* and *C. ternatea Var. pleniflora f. subpolyadelpha*.

We too find in our collection variations with regard to certain subtle characters. These characters have been presented in Table-56. Characters such as Plant habitat, habit, leaf colour, leaflet morphology, nature of flower colour, medial eye spot on the banner, aestivation, stamen arrangement, pollen fertility, seed colour number of seeds per pod and pod length vary in polymorphic *C. ternatea*. On the basis of these characters five form of Fantz
(1995) can be identified in our material, except *C. ternatea* var. *pleniflora* f. *subpolyadelpha* which is characterized by actinomorphic blue flowers, stamens 10, in 2-3 fascicles of 2-6 stamens each, distributed in Sri Lanka and Thailand. We have not come across with this material.

The morphology of the studied taxa reveals that collected material exhibits certain gross morphological similarities is posing well-developed rhizomatous root system with root nodules. A woody subterranean xylopodium bears annual aerial herbaceous stems, erect or climbing, terete, strigose. Leaves odd pinnate, leaflets 5-7, terminal leaflet larger. Stipules and stippels persistent. Predominately solitary, axillary, chasmogamous, showy flowers. Occasionally 2 or 3 flowers in the same axil or a cyme with 2 or 3 flowers were also observed. Persistent 2 bracts and conspicuous bracteoles, Persistent funnel form conspicuously nerved glabrate with uncinate hairy calyx. Stamens 10, dithecous, basifixed anthers. Stipitate linear compressed many ovuled densely pubescent ovaries. Elongate geniculate near the tip, twisting, and flattened, bearded lengthwise style. Basally enclosed within calyx, subsessile beaked, splitting spirally twisting pod. Rectangular,
subreniform smooth, glabrous seeds. These morphological characters indicate the correlation within *C. ternatea*. At the same time, these 5 taxa also exhibit variations with regard to certain finer characters as presented in Table-56.

All taxa of Var. Ternatea (Single flowered) are distributed in dry to moist deciduous regions. Grow best in full sun, need moisture but do not tolerate water logging. Thrive in rich soil, but grow in ordinary well-drained soil. Slightly specific in their Rhizobium requirement (strain QA 553, Skermann *et al.*, 1988). If tips are pinched, bushy habit results, otherwise plants are vines. Leaf colour is light green compared to member of Pleniflora (Double flowered taxa), leaflets are ovate, mucronulate. Flowers are predominantly solitary, papilionaceous, colour of corolla ranges from white, whitish blue to dark blue. Banner has greenish white marking at the center or yellowish white marking. Stamens are in diadelphous condition. Pollen fertility ranges from 94.00% to 97.00%. Seed setting also varies from 6 to 9 seeds per pod. Seed colour is pod green or carnation green or shiny black or brownish black in different forms of variety of Ternatea (Table-56).
On the contrary taxa of Pleniflora (Double flowered) are very much confined to moist deciduous regions of Western Ghats. They require well-drained red acidic soil and highly specific Rhizobium strain QA 553. Grow well in full sun and do not tolerate water logging. Plants are perennial vine to low shrub. Leaf colour is dark green, leaflets are elliptic acute. Flowers mostly solitary, non-papilionaceous. Corolla colour white to dark blue. All petals are of equal size and have yellow or whitish yellow marking at the center. Stamens are 10 free. Pollen fertility (55.00%, 59.00%) is lowest compared to members of Var. Ternatea. Seed setting is also low (5-6) seeds are garnet brown or black in different forms of variety Pleniflora (Table-56).

Morphological diversity exists between 5 taxa but within taxon the plants are very uniform variations in the ecological requirements and phenotypic characters such as plant habitat, habit, leaflet shape, colour and texture symmetry of the flower, petal colour, condition of the stamens, pod size, number of seeds per pod and testa colour can be considered as one of the direct evidences of evolutionary processes at work within the populations of this species.
Tureson (1936) opinioned that morphological variants are prominent between different populations of same species, which have wider distribution. Similar variations at the intraspecific level are also met within *C. ternatea*, which has wider distribution ranging all the way from deep in continental Asia to Australia, United States of America Uruguay and the Caribbean islands. It is reasonable to assume that *C. ternatea* is ancient in origin from its distribution patterns.

The attainment of reproductive isolation between subpopulation is the key event in the formation of species. Different types of mechanisms can cause populations to be reproductively isolated, (Snustad and Simmons, 2000). In *C. ternatea* ecological isolation and mechanical isolation mechanisms like distribution patterns of forms of Ternatea, which ranges from dry to moist deciduous regions whereas forms of Pleniflora are confined moist deciduous region and these forms require well drained red acidic soil with highly specific rhizobium. Plants are vines in Ternatea and low shrub in pleniflora. Flowers papilionaceous and non-papilionaceous in the members of Ternatea and Pleniflora respectively even the colour of petals and banner eyespot also
varies in different taxa of *C. ternatea*. The arrangement of stamens also differs in these members. These isolating mechanisms will be responsible for evolution of new species.

Such intraspecific morphovarients are considered to be incipient species, which are important and essential at different stages of evolution, which ultimately leads to speciation. (Foseberg, 1942, Snustad and Simmons, 2000). Accordingly *C. ternatea* is in a dynamic state exhibiting intraspecific morphovarients.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Common in South East Asia, America, Australia, Africa and Asia.</td>
<td>Origin in India or Asia, Africa, America, Myanmar. Restricted distribution.</td>
<td>Only in Asia Indonesia</td>
<td>South East Asia</td>
<td></td>
</tr>
<tr>
<td>Habitat</td>
<td>Dry to moist deciduous area, well drained ordinary soil, common in drier regions of Western Ghat.</td>
<td>Dry to moist deciduous area, well drained ordinary soil, in drier regions of Western Ghat.</td>
<td>Moist deciduous region, well drained red acidic soil, un common, Restricted to Western Ghat.</td>
<td>Moist deciduous region, well drained red acidic soil, Restricted to Western Ghat.</td>
<td></td>
</tr>
<tr>
<td>Habit</td>
<td>Perennial suffrutescent vine</td>
<td>Perennial suffrutescent vine</td>
<td>Perennial suffrutescent vine</td>
<td>Perennial suffrutescent vine to low shrub</td>
<td></td>
</tr>
<tr>
<td>Rhizobium requirement</td>
<td>Moderately Specific</td>
<td>Moderately Specific</td>
<td>Slightly specific</td>
<td>Highly Specific</td>
<td></td>
</tr>
<tr>
<td>Leaf colour and leaflet shape</td>
<td>Light green, ovate mucronulate</td>
<td>Light green, ovate mucronulate</td>
<td>Light green, ovate mucronulate</td>
<td>Dark green, elliptic, acute</td>
<td></td>
</tr>
<tr>
<td>Peduncle</td>
<td>One per axil</td>
<td>1-4 per axil, fascicled</td>
<td>One per axil</td>
<td>Solitary</td>
<td></td>
</tr>
<tr>
<td>Nature of flower</td>
<td>Papilionaceous, mostly solitary</td>
<td>Papilionaceous, mostly solitary</td>
<td>Papilionaceous, mostly solitary</td>
<td>Non papilionaceous, mostly solitary</td>
<td></td>
</tr>
<tr>
<td>Colour of corolla</td>
<td>White</td>
<td>Dark blue</td>
<td>Whitsu blue</td>
<td>Dark Blue</td>
<td></td>
</tr>
<tr>
<td>Standard petal</td>
<td>Greenish white marking at the center and purplish veins</td>
<td>Yellowish white marking at the center</td>
<td>Yellowish white marking at the center</td>
<td>Whitsu yellow markings on all the petals at the center</td>
<td></td>
</tr>
<tr>
<td>Aestivation</td>
<td>Vexillary</td>
<td>Vexillary</td>
<td>Vexillary</td>
<td>Imbricate</td>
<td></td>
</tr>
<tr>
<td>Androecium</td>
<td>Stamens 10 Didelphous</td>
<td>Stamens 10 Didelphous</td>
<td>Stamens 10 Didelphous</td>
<td>Stamens 10 Filaments free</td>
<td></td>
</tr>
<tr>
<td>Pollen fertility</td>
<td>94.00%</td>
<td>96.00 %</td>
<td>97.00 %</td>
<td>59.00 %</td>
<td></td>
</tr>
<tr>
<td>Pod subsessile, L X B in cms</td>
<td>7.9 X 0.9</td>
<td>7.9 X 0.8</td>
<td>8.5 X 1.0</td>
<td>6.3 X 0.7</td>
<td></td>
</tr>
<tr>
<td>No. of seeds /Pod</td>
<td>7-8</td>
<td>6-8</td>
<td>8-9</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Seed coat colour</td>
<td>Pod green</td>
<td>Carnation green</td>
<td>Shinyblack/brownish black</td>
<td>Black</td>
<td></td>
</tr>
</tbody>
</table>

Table. 56. Comparative account Morphological characters in C. ternatea L.,
CYTOLOGICAL ANALYSIS:

The application of information on chromosome number and morphology as an adjunct to taxonomy of higher plants has been very well elucidated in various reviews (Stebbins, 1971; Jackson, 1971; Jones, 1984; Bennett, 1984; Grant, 1987 and Grielhuber and Ehrendarfer, 1988). The present day taxonomists are closely acquainted with the fact that genomes of extant higher plant species are highly variant not only with respect to chromosome number and morphology but also genome size as expressed in pg DNA. Karyomorphological data along with morphological, geographical and ecological information provides critical clues regarding phylogenetic relations between different taxa and evolutionary trends among various taxonomic units (Stebbins, 1950).

Twenty-five populations of *C. ternatea* have been subjected to cytological analysis. All possess 2n=16 and n=8 chromosomes. The earlier chromosome count of 2n=6, n=8 for *C. ternatea* by Darlington and Janaki Ammal (1945), Frahm-Leliveld (1953), Verdcourt (1970), Fedorov (1974), Sanjappa and Dasgupta (1977),
George and George (1978), Srinivastav and Raina (1980, 1982), George and Ninan (1989) and Gandhi and Patil (1993) has been confirmed. However, Bir and Kumari (1978), have reported $2n=14$ and $n=7$ for this species collected from Punjab. This is the only deviant number reported so far it needs to be confirmed. Jacob (1940); on the basis of 4 satellited chromosomes in *C. ternatea* suggested that the basic number for this species as $X=4$. In our observations as well as others (George and Ninan, 1989; Gandhi and Patil, 1993) reveals that there are only a pair of satellited chromosomes in *C. ternatea*. Hence, basic number $X=4$ cannot be accepted. Further, so for there is no report of such number in *C. ternatea*.

Perusal of available literature and chromosome analysis involving 25 accessions collected from different geographical areas within India show that diploid chromosome number as 16 and haploid chromosome number as 8. Hence, it is rational to infer that, the basic chromosome number for this species as $X=8$. On this basis *C. ternatea* is an exclusively diploid species.
a. Karyotype:

Karyotypic analysis involving 25 collections of *C. ternatea* show that the chromosomes can be distinctly classified into long, medium and short chromosomes. The ratio of long, medium and short chromosomes pairs is 4:2:2 or 4:1:3 respectively for all the forms except *C. ternatea var. ternatea f. ternatea*, which has 5:3 ratios of long and medium chromosomes. Size variations in the chromosome pairs are not gradual, indicating that karyotypes are asymmetrical. Asymmetry in karyotype is brought through unequal translocation and pericentric inversion this evidenced by occurrence of quadrivalents and bridge in the meiosis of diploid taxa of *C. ternatea* and such karyotypes are derived ones (Stebbins, 1971).

It is important to note from the karyotypic investigation that, basically most of the forms are similar in having long, medium and short chromosomes with median, nearly median and nearly submedian centromere, and a pair of medium chromosomes with interstitial satellites, which occupies fifth position in ideogram (Fig-142). This pair of chromosomes with interstitial satellites can be considered as a “marker”. On the basis of this marker
Fig. 142. Comparative ideograms of 5 taxa of *C. ternatea* L.,


142. E. *C. ternatea* Var. *ternatea f. ternatea* (Coll. No. 03).
Fig. 142. Comparative ideograms in *C. tenuata* varieties
chromosome it can be concluded that all the studied forms are closely related and originated from a common progenitor.

Karyotypic asymmetry is estimated according to the two ways system of classification proposed by Stebbins (1971). Which involves a change in the chromosomal morphology; silt of the centromere from median to sub terminal position and trend towards increasing intrakaryotypic size differences of chromosomes. Based on this classification in the present investigation, all the forms fall under 3B category, indicating that they are moderately asymmetrical suggesting that this group is in an active state of evolution (Table-5 7). In *C. ternatea Var. ternatea f. albiflora*, *C. ternatea Var. ternatea f. fasciulata* and *C. ternatea Var. pleniflora f. leucopetala* 50% of chromosomes posses arm ratio more than two showing progressive trend in the karyotypic evolution.

High degree of correlation among karyotypics of investigated taxa is evidenced from presence of three types of chromosomes, long, medium and short and fifth medium bearing interstitial satellite (Fig. 142). Hence, the inclusion of the investigated taxa under the species Ternatea seems to be justified.
Table 57. Classification of the karyotypes on the basis of Stebbins (1971) two-way system of classification of karyotype asymmetry in *C. ternatea*.

<table>
<thead>
<tr>
<th>Ratio Largest</th>
<th>Smallest</th>
<th>Proportion of chromosomes with arm ratio &lt; 2:1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>&lt; 2:1</td>
<td></td>
<td>1A</td>
</tr>
<tr>
<td>2:1-4:1</td>
<td></td>
<td>1B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;4:1</td>
<td></td>
<td>1C</td>
</tr>
</tbody>
</table>

*CTTT* = *C. ternatea* Var. *ternatea*, *f. ternatea*,  
*CTTF* = *C. ternatea* Var. *ternatea*, *f. fasciculata*,  
*CTTA* = *C. ternatea* Var. *ternatea*, *f. albiflora*,  
*CTPP* = *C. ternatea* Var. *pleniflora*, *f. pleniflora*,  
*CTPL* = *C. ternatea* Var. *pleniflora*, *f. leucopetala*. 


On the other hand the karyotypes of the studied taxa differ from each other with respect to total chromosome length, coefficient of variation %, F% and TF% (Tables-6-10, 58). Which implies that, varietal demarcation and the recognition of 5 investigated taxa at hand as 5 different forms. It has been suggested that varietal demarcation is a result of changes in heterochromatic part as well as repetitive sequence in genome (Flavell et al., 1977, Das et al., 2000).

There is relatively low degree of correlation between chromosome length range and gradual shift and alternation of TF% value consequently suggesting the possible role of diversification due to structural alterations in all taxa (Table-58). These structural alterations in the morphology of chromosomes are due to duplication or translocation during evolution of these varieties (Das and Mallick, 1989; Das et al., 2000).

The data on mean chromosome length indicates that *C. ternatea* Var. *ternatea* f. albiflora and *C. ternatea* Var. *ternatea* f. fasciculata come under one group with mean chromosome length 3.18 μm and 3.13 μm (Table-58). Accordingly *C. ternatea* Var.
pleniflora f. leucopetala and C. ternatea Var. pleniflora f. pleniflora comes under another group with mean chromosome length 2.96 μm and 2.85 μm respectively. However, C. ternatea Var. ternatea f. ternatea stand apart with the mean length of 4.24 μm (Table-58).

Even the total chromosome length provides the indication of closeness of C. ternatea Var. ternatea f. albiflora and C. ternatea Var. ternatea f. fasciculata. Similarly C. ternatea Var. pleniflora f. leucopetala and C. ternatea Var. pleniflora f. pleniflora also come relatively closer. Where as, C. ternatea Var. ternatea f. ternatea deviates with rest having a maximum absolute chromosome length of 33.99 μm (Table-58).

Data from Table-58 provide sufficient reason to assume the primitiveness of C. ternatea Var. ternatea f. ternatea with rest in having highest value for total chromosome length, lowest coefficient of variation and lowest TF% in its karyotype. On the other hand lowest value for total chromosome length, lower coefficient of variation and highest TF% are met in C. ternatea Var. pleniflora f. leucopetala and C. ternatea Var. pleniflora f. pleniflora suggest their advancement over rest of the 3 forms of Ternatea.
Table 58. Comparative account of Karyotypes in *C. ternatea* L.

<table>
<thead>
<tr>
<th>Forms</th>
<th>C. ternatea var. ternatea f. ternatea (Coll. No.03)</th>
<th>C. ternatea var. ternatea f. fasciculata (Coll. No.02)</th>
<th>C. ternatea var. ternatea f. albiflora (Coll. No.19)</th>
<th>C. ternatea var. pleniflora f. pleniflora (Coll. No.17)</th>
<th>C. ternatea var. pleniflora f. leucopetala (Coll. No.15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Karyotypic details</td>
<td>Somatic chromosome number</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Karyotypic formula</td>
<td>nm&lt;sub&gt;0&lt;/sub&gt; + nsm&lt;sub&gt;0&lt;/sub&gt; + nsmS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>nm&lt;sub&gt;0&lt;/sub&gt; + SM&lt;sub&gt;2&lt;/sub&gt; + nsm&lt;sub&gt;0&lt;/sub&gt; +nsmS&lt;sub&gt;2&lt;/sub&gt;</td>
<td>M&lt;sub&gt;2&lt;/sub&gt; + nm&lt;sub&gt;0&lt;/sub&gt; + nmS&lt;sub&gt;2&lt;/sub&gt; + nsm&lt;sub&gt;0&lt;/sub&gt;</td>
<td>M&lt;sub&gt;2&lt;/sub&gt; +nm&lt;sub&gt;0&lt;/sub&gt; + nmS&lt;sub&gt;2&lt;/sub&gt; + nsm&lt;sub&gt;0&lt;/sub&gt;</td>
<td>nm&lt;sub&gt;0&lt;/sub&gt; + nmS&lt;sub&gt;2&lt;/sub&gt; +nsm&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>No. of Chromosomes with interstitial satellites</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total Chromosome length in μm</td>
<td>33.99</td>
<td>25.49</td>
<td>25.08</td>
<td>23.69</td>
<td>22.81</td>
</tr>
<tr>
<td>Range of Chromosome length in μm</td>
<td>2.21-6.28</td>
<td>1.64-4.53</td>
<td>1.59-5.30</td>
<td>1.64-4.42</td>
<td>1.59-4.42</td>
</tr>
<tr>
<td>Mean Chromosome length in μm</td>
<td>4.24</td>
<td>3.18</td>
<td>3.13</td>
<td>2.96</td>
<td>2.85</td>
</tr>
<tr>
<td>Coefficient of variation %</td>
<td>34.88</td>
<td>37.44</td>
<td>41.84</td>
<td>35.80</td>
<td>35.88</td>
</tr>
<tr>
<td>TF %</td>
<td>33.93</td>
<td>33.94</td>
<td>34.56</td>
<td>35.71</td>
<td>36.78</td>
</tr>
</tbody>
</table>
Clitoria ternatea is self-pollinated hence, there is a greater uniformity in the karyotypes of each form studied. Most frequently observed karyotype consists of 3 types of chromosomes long, medium and short chromosomes. However, intraspecific karyotype variations are observed and these are due to chromosomal repatterning which are playing pivotal role in evolution of species suggesting the role of structural changes. The gradual change in TF% and occurrence of quadrivalent and bridge in meiosis corroborate this view. The variability in the chromosome structure and constitution within species could be regarded as diversification of taxa in establishing a new species (Stebbins, 1950; Clausen, 1951; Heneen, 1971; Hore, 1976, 1977 a, b; Fukuda and Grant, 1980).
b. Meiosis.

Meiosis is normal, hence pollen production is high. Pollen fertility is also good in member of Var. Ternatea, however it is quite low in members of Var. Pleniflora, in spite of good chromosome pairing in these taxa. The seed set per pod is also very low in the taxa belonging to Var. Pleniflora. The pollen sterility in these taxa is attributed to cryptic structural changes (Stebbins, 1950, 1971). Flower morphology is also responsible for low production of seeds per pod in these members.

Most of the members of *C. ternatea* show 1-2 quadrivalents indicating presence of translocations. Translocations are responsible in altering the chromosome structure. Accordingly there will repatterning of chromosomes leading to diversification of species. Such observations have been made in several groups of plants (Stebbins, 1950, Jackson, 1985).
ELECTROPHORETIC ANALYSIS:

Increasing attention is being paid to the use of biochemical criteria for the characterization of varieties within species, of the biochemical methods so far known for this purpose, electrophoresis of seed storage protein has been shown to be a powerful and versatile tool (Cooke, 1983).

The study of seed protein profiles is a powerful method for species, varieties identification, classifying taxonomic and evolutionary problems and studying diversity (Boulter et al., 1968; Johnson, 1969; Gottleib, 1977; Ladizinsky and Hymowitz, 1979; Cooke et al., 1984; Gamal El-Din, 1984; Hussain and Salam, 1985; Hussain et al., 1986; Singh et al., 1991, 1994; Ahmad and Slinkard, 1992; Jha and Ohri, 1996) and has been successfully used with wide range of plant species.

SDS-PAGE has been used to aid the identification and characterization of varietal affinities of the varieties in *Vicia faba* (Goodrich, *et al.*, 1985), *Corchorous* species (Pathak and Chattopadhyay, 1989), *Pureria phaseolus* (Hussain, 1988), *Fagopyrum esculentum* (Rogi and Javornik, 1969) *Pisum sativum*
(Cooke, 1983) Cereal varieties (Cros and Wrigley, 1979) and many more such studies have been conducted on different species.

Recently, even in the specific cultivars and hybrids of crop plants have been identified by protein profile. This knowledge will have significant role in testing genetic purity of hybrid seeds and cultivars. On these lines work has been done in wheat Bushuk and Zilman (1978), in wheat, barley, oats and peas Cooke et al., (1984), in Vicia Goodrich et al., (1985), Agarwal et al., (1988), in Lettuce Mejia and McDaniel (1986), in Desmodium Hussain et al., (1987), in Quack grass x Blue bunch, Bennett et al., (1991), in Lolium Moller and Spoor (1993), in crop cultivars Cooke (1984), in cotton Kapse and Nerkar (1985).

In the present investigation slab gels are used, as slab gels are preferred over tube gels for cultivars identification (Stegmann, 1983) because unequivocal comparison of banding patterns is possible only in slabs. Ferguson and Grabe (1985) have confirmed this statement.

Comparing the electrophoregrame of five taxa of C. tertanea reveal some interesting features. Of the 17 bands in total 10 bands
(A, B, D, J, L, O, P, R, S, and T) are common to all 5 taxa having similar point of origin, indicating correlation between them (Figs. 136, 137 Table-49). The bands G, I and K are restricted to *C. ternatea* Var. *ternatea f. albiflora*. The band F is confined to *C. ternatea* Var. *pleniflora f. leucopetala*. The bands C and M are present in all the taxa except *C. ternatea* Var. *ternatea f. albiflora* and *C. ternatea* Var. *pleniflora f. leucopetala* respectively. Absence of H band is noticed in *C. ternatea* Var. *ternatea f. albiflora* and *C. ternatea* Var. *pleniflora f. leucopetala* (Table-49). Electrophoretically analysed data shows that *C. ternatea* Var. *ternatea f. albiflora* and *C. ternatea* Var. *ternarea f. ternatea* have maximum number of 14 bands. While *C. ternatea* Var. *pleniflora f. leucopetala* has minimum of only 12 seed protein bands and other two forms ie, *C. ternatea* Var. *ternatea f. fasciculata* and *C. ternatea* Var. *pleniflora f. pleniflora* have 13 bands each.

In all taxa little variability was detected amongst the polypeptides with higher molecular weights (above 43 kilodalton), whereas most polymorphism was confined amongst the polypeptides with lower molecular weights (below 29 kilodalton) Goodrich *et al.*, 1985; Moller and Spoor, 1993.
Electrophoretic comparison of seed protein profile of all taxa belonging to Var. Ternatea (Fig. 137) indicate that 13 bands of C. ternatea Var. ternatea f. ternatea are qualitatively similar to C. ternatea Var. ternatea f. fasciculata and C. ternatea Var. pleniflora f. pleniflora, 11 bands are similar to C. ternatea var. ternatea f. albiflora and C. ternatea Var. pleniflora f. leucopetala. In case of C. ternatea Var. ternatea f. albiflora, 11 bands are similar to C. ternatea Var. ternatea f. fasciculata and C. ternatea Var. pleniflora f. pleniflora and only 10 bands are similar to C. ternatea Var. pleniflora f. leucopetala. Where as C. ternatea Var. ternatea f. fasciculata shows 13 bands similarity with C. ternatea Var. pleniflora f. pleniflora and 11 bands similarity with C. ternatea Var. pleniflora f. leucopetala. On the basis of banding pattern similarity C. ternatea Var. ternatea f. ternatea protein profiles emphasizes closer relationships than other with C. ternatea Var. ternatea f. fasciculata and C. ternatea Var. pleniflora f. pleniflora indicating closer genetical relationships (Ladizinsky and Hymowitz, 1979).

In case of Var. Pleniflora, C. ternatea Var. pleniflora f. pleniflora 11 bands are similar to C. ternatea Var. pleniflora f. leucopetala indicating very close genetical relationships among
them. The present observations on seed protein profiles reminds us
the observations made by Shechter (1975) that, homology in
banding pattern indicate significant similarity in the structure of
the proteins and is the direct effect of identical genes among the
taxa.

**Similarity Percentage of protein profiles:**

The degree of similarity in fractionated protein is a
potential tool for estimating the relationship in different
populations. The percentage similarities suggest that there is
considerable genetic variability among the taxa although there is
not necessarily congruence with patterns of morphological
variability (Panda et al., 1986).

Percentage similarity or paired affinity index between all
possible pairs of taxa are presented in Table-60. The data indicates
that all taxa have more than 60% similarity with each other. The
data on percentage similarity of protein bands in case of *C. ternatea
Var. ternatea f. ternatea* with that of *C. ternatea Var. ternatea
f. albilora*, *C. ternatea Var. ternatea f. fasciculata*, *C. ternatea Var.
pleniflora f. pleniflora* and *C. terantea Var. pleniflora f. leucopetala*
reveal that 64.70%, 92.85%, 93.00% and 75.33% similarity respectively. On the other hand protein bands of *C. ternatea* Var. *ternatea* f. *albiflora* have 68.75%, 70.5% and 62.5% similarity with the seed protein profiles of *C. ternatea* Var. *ternairea* f. *fasciculata*, *C. ternatea* Var. *pleniflora* f. *pleniflora* and *C. ternatea* Var. *pleniflora* f. *leucopetala* respectively. Protein bands of (*C. ternatea* Var. *ternairea* f. *fasciculata* shows 98.50% and 78.57% similarity with protein profiles) of *C. ternatea* Var. *pleniflora* f. *pleniflora* and *C. ternatea* Var. *pleniflora* f. *leucopetala* respectively. Protein fractions of both the forms of Var. Pleniflora show 79.25% similarity.

On the basis of banding patterns of 3 taxa of Var. Ternatea only *C. ternatea* Var. *ternairea* f. *fasciculata* has very high degree of electrophoretic homology (98.5%) with a member of Var. Pleniflora i.e., *C. ternatea* Var. *pleniflora* f. *pleniflora*. Within three taxa of Var. Ternatea, *C. ternatea* Var. *ternairea* f. *fasciculata* shows 92.5% similarity with *C. ternatea* Var. *ternairea* f. *ternairea* on one side and 68.75% protein banding similarity with *C. ternatea* Var. *ternairea* f. *albiflora* on other side.
**Protein mobility pattern:**

Duke and Glassman (1968), while working on Drosophila, proposed that electrophoretic mobility of isozymes tended to reduce as the species become advanced. This relationship was further confirmed in rice by Siddiq *et al.*, (1972) and in Chilli by Panda *et al.*, (1986). Although the present study is not based on any structurally or functionally definite protein bands, the gross fraction suggests that the taxa differ considerably in their mobility pattern (Table-50) Among Var. Ternatea all taxa have 3 fast and 3 very fast mobility protein bands suggesting they are primitive compared to *C. ternatea Var. pleniflora f. leucopetala* which has only 2 fast and 2 very fast mobility protein fraction.

*C. ternatea Var. pleniflora f. pleniflora* also indicates 3 fast and 3 very fast mobility protein bands suggesting that this taxa also possess certain traits of the ancestral form to some extent, since this taxa has originated due to dominant mutation most probably from *C. ternatea Var. ternatea f. fasciculata*.

According to Ladizinsky (1975), Ladizinsky and Hymowitz (1979), Seed protein profile is a specific trait. It has also been
confirmed that a cultivated and its wild progenitor share same protein profile pattern as observed in wheat (Johnson et al., 1967), barley (Mc Daniel 1970), cotton (Johnson and Thein, 1970, *Arachis* (Singh et al., 1991). In *C. ternatea*, of the 5 electrophoretically analysed taxa protein bands of only *C. ternatea* Var. *ternatea f. fasciculata*, *C. ternatea* Var. *ternatea f. ternatea* and *C. ternatea* Var. *pleniflora f. pleniflora* exhibit qualitatively similar 13 bands. Among other taxa variation is noticed with respect to 10 total numbers of protein profiles and differences in RM values.

Thus, it can be conceived from the present study that, the taxa of *C. ternatea* can be broadly grouped in to 2, namely Var. Ternatea and Pleniflora. Members of Var. Ternatea can be further subdivided on the basis of electrophoretic analysis. Study also reveals that among 3 taxa of Var. Ternatea, *C. ternatea* Var. *ternatea f. fasciculata* show more closer similarity with member of Var Peniflora ie., *C. ternatea* Var. *pleniflora f. pleniflora* on one hand and on the other hand it shows closer similarity with *C. ternatea* Var. *ternatea f. ternatea*.

Observation on electrophoretic banding in hybrids demonstrated that number of bands did not exceed parental bands.

In crosses with *C. ternatea* Var. *ternatea* f. *fasciculata* as a maternal parent polypeptide band profiles of the parents and hybrids revealed that A, C, H, T, L, M, N and O bands are common (Table-51). In hybridization experiments, *C. ternatea* Var. *ternatea* f. *ternatea* and *C. ternatea* Var. *pleniflora* f. *leucopetala* as maternal parents protein bands A, F, M are common in parents

A majority of the hybrids showed more similarity towards the maternal parent with respect to banding pattern than paternal parent (Harsh *et al.*, 1997). Few hybrids showed some bands, which were characteristic of the paternal parent (Table-50, 51 and 52). This is an interesting character, which will enable the hybrids to distinguish it from their respective maternal parent. For practical purpose it is more important to distinguish easily the hybrids seed from the seedled seeds of the maternal parents (Kapse and Nerkar, 1985).

The additional bands in hybrids could be attributed to their low frequency, which were not visible in the parental samples (Moller and Spoor, 1993).
NUCLEAR DNA CONTENT:

Once DNA was recognized as the prime genetic material in most organisms and mechanism by which it encodes genetic information was first understood, it quickly became a major focus in biological research. From the beginning, one significant interest concerned the size of the nuclear genome in different organisms. It soon established that the evolutionary divergence and speciation is accompanied by wide range of variations in nuclear DNA amount in plants as well as animals (Bachmann et al., 1972; Sparrow et al., 1972; Rees and Jones, 1972; Price, 1976; Bennett and Leitch, 1995, 1997). Indeed it differs over 2500 fold ranging less than 0.05 pg in Cardamine amara to 127.4 pg in Fritillaria assyriaca (Bennett 1985).

It is generally accepted that changes DNA content involves loss or increase of repetitive base sequences. (Cavallini and Natali, 1991). This is evident in the species with high DNA content have larger proportion of repeated DNA sequences than those species with low DNA amount (Rees et al., 1976). The nuclear genome might influence rearrangements from translocation, deletion, unequal crossing over and other divergence mechanisms. This cycle
is proposed to generate differences in DNA content among species
(Flavell et al., 1981, Flavell, 1982, 1986; Cavallini and Natali,
1991). It is well known that proliferation or deletion of nuclear DNA
sequences does occur in case of divergence and evolution of species
(Bennett and Smith, 1976; Price, 1988), but nuclear DNA content
variations may also be found among and within populations or
cultivars of a single species (Bennett, 1985; Cavallini and Natali,
1991; Bennett and Leitch, 1997; Greilhuber, 1998).

The 2C DNA value in twenty-five populations involving five
taxa of C. ternetaea collected from different ecological area is
presented in Table-54. All the varieties show somatic chromosome
number as 2n = 16 and are diploid. 2C DNA values in different taxa
of C. ternetaea ranges from 9.220 pg in C. ternetaea Var. ternetaea
f. ternetaea to 6.456 pg in C. ternetaea Var. pleniflora f. leucopetala
(Table-54). There exist 17.759 % variation between taxa of
C. ternetaea. Interaspecific variations in DNA were considered to be
rare (Bennett et al., 1982, Ohri and Khoshoo, 1986) is now
widespread in angiosperms species (Bennett, 1985; Rayburn et al.,
1985; Price, 1988; Cavallini and Natali, 1991; Caeccarelli et al.,
1992; Ohri et al., 1994). The range of variations may be very high (5.1 to 294.4 %, Cavallini and Natali, 1991).

*C. ternatea* taxa from India exhibit great range of diversity in morphological, phenological characteristics and DNA content as well as adaptation to diverse ecological conditions. Price (1988) is of opinion that variations in nuclear DNA content less than 20% are subject to selection. We too think that similar patterns of DNA content difference among the studied taxa of *C. ternatea* are having selection/adaptive values.

The mean DNA content of 5 taxa in *C. ternatea* indicates the reduction in genome size (Table-59). *C. ternatea* Var. *ternatea* f. *ternatea* is morphologically, phenotypically, geographically and karyotypically primitive taxa and has highest mean DNA content of 9.128 pg where as in advanced taxa *C. ternatea* Var. *pleniflora* f. *leucopetala* indicates lowest mean DNA content (6.596 pg). The genome size in other taxa fall between these two values (Table-59). Such evolutionary reduction in DNA content has been well documented in the angiosperms (Price and Bachmann, 1975; Rees and Hazarika, 1967; Jones and Brown, 1976). Lower DNA values were observed in Var. *Pleniflora* (C. *ternatea* Var. *pleniflora*
Table 59. Mean 2C DNA percentage variations in different taxa of *C. ternatea*.

<table>
<thead>
<tr>
<th>Taxa and mean 2C DNA content in pg</th>
<th>CTTT 9.128</th>
<th>CTTF 8.260</th>
<th>CTTA 7.505</th>
<th>CTPP 6.977</th>
<th>CTPL 6.596</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTT 9.128</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTTF 8.260</td>
<td>4.991%</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTTA 7.505</td>
<td>3.476%</td>
<td>4.789%</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTPP 6.977</td>
<td>13.356%</td>
<td>8.420%</td>
<td>3.645%</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>CTPL 6.596</td>
<td>16.102%</td>
<td>11.200%</td>
<td>6.446%</td>
<td>2.807%</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 60. Percentage similarity for protein profile of different taxa in *C. ternatea*.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>CTTT 100</th>
<th>CTTF 92.85</th>
<th>CTTA 64.70</th>
<th>CTPP 93.00</th>
<th>CTPL 73.33</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTTT</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTTF</td>
<td>100</td>
<td>68.75</td>
<td>98.50</td>
<td>78.57</td>
<td></td>
</tr>
<tr>
<td>CTTA</td>
<td>100</td>
<td>70.50</td>
<td>62.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTPP</td>
<td>100</td>
<td>79.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTPL</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTTT = *C. ternatea* Var. ternatea f. ternatea, CTTF = *C. ternatea* Var. ternatea f. fasciculata
CTTA = *C. ternatea* Var. ternatea f. albiflora, CTPP = *C. ternatea* Var. pleniflora f. pleniflora.
CTPL = *C. ternatea* Var. pleniflora f. leucopetala.
f. pleniflora and C. ternatea Var. pleniflora f. leucopetala), which are confined to moist deciduous region, with well-drained red acidic soil of Western Ghat and rhizobium requirement is highly specific. Probably, this may be genomic strategy to dispense with less important DNA associated with heterochromatic segment for ecological adaptation (Srivastava and Lavania, 1991; Singh et al., 1996). However, Raina and Bisht (1988) are of opinion that both evolutionary increase and decrease in nuclear DNA content is not uncommon.

Within studied each taxa of C. ternatea DNA amount varies in case of C. ternatea Var. ternatea f. ternatea it ranges from 9.020 to 9.220 pg. C. ternatea Var. ternatea f. albiflora 8.044 pg. to 8.770, C. ternatea Var. ternatea f. fasciculata 7.220 pg. to 7.98 pg, C. ternatea Var. pleniflora f. pleniflora 6.900 to 6.995 pg. and C. ternatea Var. pleniflora f. leucopetala 6.456 to 6.671 pg. These variations within the populations have adaptive significance in cultivated C. ternatea and are of potential horticultural interest. Such type of variation recorded in species by earlier workers (Furuta et al., 1975; Bennett and Smith, 1976; Price et al., 1980,
1981; Banerjee and Sharma 1985). Laurie and Bennett (1985) have shown in maize that genome size variations are due to change in heterochromatin amount.

The structural changes in chromosome leading to genetic changes as well as amount of changes in the repetitive DNA have been responsible for varietal differences. It has also been suggested that increase of DNA has a significant role in microevolution of genome. Such type of observations been recorded by Duehrssen et al., (1984) Das and Mallick (1989).

The influence of nuclear DNA content on chromosome, nuclear, cellular, tissue and organism phenotypic character has been observed among different Angiosperms (Bennett, 1973, 1985,1987; Cavalier and Smith, 1985). Several of these size, volume and duration phenomenon are decided by nuclear DNA content in two distinct ways, first by expression of its genic content and second by the biophysical effects of its mere bulk. The term nucleotype was coined to define those aspects of nuclear DNA, which affect the phenotype independent of its encoded
informational content (Bennett, 1971, 1972, 1973). In the complex multicellular higher plants nucleotypic effects are additive at successive cell cycle, so that they influence many characters, nucleotypic effects are thus very important in ecological adaptation of plants (Bennett, 1987).

Action of nuclear DNA content on many nuclear and phenotypic characters show contrasting results. Different taxa of *C. ternatea* exhibit variation in DNA content. All the taxa studied are diploid. We have studied the correlation between nucleotypic parameters and DNA content variation in *C. ternatea*. Nuclear DNA content is negatively correlated with multicellular structure like terminal leaflet size and style length (Table-55 Figs. 143, 144 and 146). Lawrence (1985) also noticed similar results. Observations on DNA content and pollen fertility, pod size shows positive correlation in *C. ternatea* (Table-55 and Figs. 147-149). In the complex multicellular structure, cell volume and structures size may vary independently especially in adult plants. It has been attributed that the effects of nuclear DNA content are then marked by the different genetic backgrounds of taxa Mowforth and Grime (1989) and (Cavallini *et al.*, 1993). Where as there is no correlation
Fig. 143. Relationship between terminal leaflet length and 2C DNA content in C. ternatea varieties

CTTT = C. ternatea Var. ternatea f. ternatea; CTTF = C. ternatea Var. ternatea f. fasciculata; CTTA = C. ternatea Var. ternatea f. alibiflora; CTPP = C. ternatea Var. pleniflora f. pleniflora and CTPL = C. ternatea Var. pleniflora f. leucopetala.

Fig. 144. Relationship between terminal leaflet breadth and 2C DNA content in C. ternatea varieties

CTTT = C. ternatea Var. ternatea f. ternatea; CTTF = C. ternatea Var. ternatea f. fasciculata; CTTA = C. ternatea Var. ternatea f. alibiflora; CTPP = C. ternatea Var. pleniflora f. pleniflora and CTPL = C. ternatea Var. pleniflora f. leucopetala.
between DNA content and rachis length and seed weight (Table-55 and Figs. 145,150). Such types of results have been observed by others (Lawrence, 1985, Ohri et al., 1986; 1994). Cavallini and Natali (1991) have rightly pointed that DNA affects the phenotype firstly by expression of its genic content, so that general behavior may be difficult to detect in different taxa.

The karyotypes of C. ternatea taxa are asymmetrical and they vary with each other with respect to chromosome types and haploid chromosome length (Table-58). Genomic chromosome length, 2C DNA per chromosome and TF % show correlation with DNA content (Table-55 and Figs. 151,152).

The present results show that 2C DNA amount differed markedly in studied taxa (Table-59). Such types of intraspecific variations in amount of DNA have been reported in many angiosperms (Raina and Rees, 1983; Greenlee et al., 1984, Bennett, 1985, Ohri and Khoshoo, 1986, Mukherjee and Sharma, 1990). In C. ternatrea Var. pleniflora f. leucopetala 2C DNA is minimum (6.596 pg) and maximum in C. ternatea Var. ternatea f. ternatea (9.128 pg). 2C DNA amount increased simultaneously with increase of genomic chromosome length (Table-55). Heterogeneity with
Fig. 145. Relationship between Rachis length and 2C DNA content in *C. ternatea* varieties

![Graph showing relationship between Rachis length and 2C DNA content in *C. ternatea* varieties.](image)

**Taxa**
- CTTT = *C. ternatea* var. ternatea
- CTTF = *C. ternatea* var. ternatea f. fasciculata
- CTTA = *C. ternatea* var. ternatea f. albiflora
- CTPP = *C. ternatea* var. pleniflora f. oleniflora
- CTPL = *C. ternatea* var. pleniflora f. leucopetala

Fig. 146. Relationship between Style length and 2C DNA content in *C. ternatea* varieties

![Graph showing relationship between Style length and 2C DNA content in *C. ternatea* varieties.](image)

**Taxa**
- CTTT = *C. ternatea* var. ternatea
- CTTF = *C. ternatea* var. ternatea f. fasciculata
- CTTA = *C. ternatea* var. ternatea f. albiflora
- CTPP = *C. ternatea* var. pleniflora f. oleniflora
- CTPL = *C. ternatea* var. pleniflora f. leucopetala
Fig. 147. Relationship between pollen fertility % and 2C DNA content in *C. ternatea* varieties.

CTTT = *C. ternatea* var. *ternatea*; CTTF = *C. ternatea* var. *fasciculata*; CTTA = *C. ternatea* var. *albiflora*; CTPP = *C. ternatea* var. *pleniflora* f. *pleniflora* and CTPL = *C. ternatea* var. *pleniflora* f. *leucopetala*.

Fig. 148. Relationship between Pod length and 2C DNA content in *C. ternatea* varieties.

CTTT = *C. ternatea* var. *ternatea*; CTTF = *C. ternatea* var. *fasciculata*; CTTA = *C. ternatea* var. *albiflora*; CTPP = *C. ternatea* var. *pleniflora* f. *pleniflora* and CTPL = *C. ternatea* var. *pleniflora* f. *leucopetala*.
Fig. 149. Relationship between Pod breadth and 2C DNA content in *C. ternatea* varieties

CTTT = *C. ternatea* var. *ternatea f. ternatea*; CTTF = *C. ternatea* var. *ternatea f. fasciculata*; CTTA = *C. ternatea* var. *ternatea f. albiflora*; CTPP = *C. ternatea* var. *pleniflora f. pleniflora* and CTPL = *C. ternatea* var. *pleniflora f. leucopetala*.

Fig. 150. Relationship between 100 seed weight (gms) and 2C DNA content in *C. ternatea* varieties

CTTT = *C. ternatea* var. *ternatea f. ternatea*; CTTF = *C. ternatea* var. *ternatea f. fasciculata*; CTTA = *C. ternatea* var. *ternatea f. albiflora*; CTPP = *C. ternatea* var. *pleniflora f. pleniflora* and CTPL = *C. ternatea* var. *pleniflora f. leucopetala*.
Fig. 151. Relationship between Total chromosome length and 2C DNA content in C. ternatea varieties

- **CTTT**: C. ternatea Var. ternatea f. ternatea
- **CTTF**: C. ternatea Var. ternatea f. fasciculata
- **CTTA**: C. ternatea Var. ternatea f. albiflora
- **CTPP**: C. ternatea Var. pleniflora f. pleniflora
- **CTPL**: C. ternatea Var. pleniflora f. leucopetala

Taxa

- --- 2C DNA amount (pg)
- --- Total chromosome length

Fig. 152. Relationship between 2C DNA per chromosome and 2C DNA content in C. ternatea varieties

- **CTTT**: C. ternatea Var. ternatea f. ternatea
- **CTTF**: C. ternatea Var. ternatea f. fasciculata
- **CTTA**: C. ternatea Var. ternatea f. albiflora
- **CTPP**: C. ternatea Var. pleniflora f. pleniflora
- **CTPL**: C. ternatea Var. pleniflora f. leucopetala

Taxa

- --- 2C DNA amount (pg)
- --- 2C DNA per chromosome (pg)
respect to DNA amount in *C. ternatea* may be playing an important role in genome divergence and evolution.

A proportionate increase in DNA is also noticed per chromosome in the taxa of *C. ternatea* (Table-55). These changes have played an important role in varietal demarcation. Although the functions of repetitive DNA are still the subject of speculation, it has been shown that evolution of species and genera is accompanied by changes in types of DNA present (Flavell *et al.*, 1977). It has also suggested by Duehrssen *et al.*, 1984 that DNA amplification is responsible for varietal difference in *Daucus carota*. The gradual shift and alteration of TF% indicate the role of structural alterations in the genome (Table-55). These structural changes lead to genic changes as well as amount of changes in the repetitive DNA and are responsible for varietal differences. It has also been indicated that amplification or loss of DNA has a significant role in the microevolution of genomes (Das and Mallick, 1989; Chattopadhyaya and Sharma, 1990; Cavallini and Natatali, 1991; Das, *et al.*, 2000 a; Das *et al.*, 2000 b).
From above discussion it is evident that intraspecific DNA variation cannot be considered an exceptional. On the contrary, it is more and more apparent the important of the phenomenon in the adaptation of plants to different environment, divergence and evolution of species.
BREEDING/HYBRIDIZATION:

In living system sexual reproduction is a critical phase. It is not just to fulfill the need of propagation but to produce an offsprings, which will be fittest in the changing environment. To accomplish this living organisms have specialized or evolved in various ways. In plant system shape, size, colour, arrangement of petals, reproductive organs and maturity of reproductive organs etc. assist to achieve either self or cross pollination even to gain perfect self pollination flowers and reproductive organs have to be specialized. This is what we observe in taxa of Var. Ternatea. Wherever, there is change in floral architecture such as actinomorphic condition, sexual reproduction has suffered. Such situation is noticed in taxa of Var. Pleniflora.

Winkler (1920) who introduced the term genome. It refers to the haploid set of chromosomes. The term genome can be defined as the complement of all DNA sequences found in the

Classical method of establishing genonmic relationship is through assessing the chromosome pairing in F₁ hybrid (Kihara, 1930). Formation of bivalents is considered as an indication of bivalent homology between two parents of the hybrid. On the other hand non-pairing is an indication of lack of genetic relationship.
Bivalent formation reflects homology between nucleotide sequences of paired chromosomes. However, chromosome pairing may be influenced by Ph gene as in wheat (Riley and Chapman, 1958), which controls the pairing, environmental factors (Solbrig, 1968). Thus, one has to be careful while assessing genome relationship. Inspite of these limitations chromosome pairing in hybrid is considered to be best in elucidating genome homology (Kimbler et al. 1981). This technique has generated wealth of information in understanding genomic relationships in crop plants (Simmonds, 1976; Tsuchiya and Gupta, 1991; Jauhar and Joppa, 1996).

Knowledge concerning genomic relations among wild and cultivated plants is of great significance to plant breeder, systematists, evolutionists and molecular biologists. Information on genetic relationships in polymorphic, complex species *C. ternatea* is not at all there. In the present work intraspecific crosses were attempted in the various combinations (Table-11) to understand the genetic relations among different taxa of *C. ternatea*.

In crossing experiments of garden and wild taxa of *C. ternatea Var. ternatea f. albiflora* and *C. ternatea Var. ternatea fasciculata* resulted in production of fertile hybrids. This indicates
that there is unrestricted gene flow between the individuals of the same taxon, which implies that, there is no crossing barrier between garden and wild taxa. Only selection with respect to flower size has played important role in differentiating garden and wild varieties.

Crossability denotes the genetic relationship. Higher crossability indicates closer affinity between two taxa. Crossability data of taxa belonging to Var. Ternate show that, they cross readily and produce fertile F₁ hybrid. However, finer details of chromosome pairing, pollen fertility, seed production and seed germination in F₁ hybrid indicate certain interesting results.

*C. ternatea* Var. *ternatea f. albiflora* is widely distributed in India. It is found in wild and also cultivated for its large white papilionaceous flower and for medicinal purpose. *C. ternatea* Var. *ternatea f. fasciculata* originated in Myanmar or India has restricted distribution found in wild as well in cultivation. It is cultivated for its large papilionaceous flower having dark blue colour, which is lacking in most ornamentals. Crossability reveals the genetic relationship between them. Crossability data indicate that seed set per pod is 6, with hybrid seed germination of 69.95%. It appears
that crossability results are not strong enough to indicate existence of a reproductive barrier. Meiotic pairing in hybrid show that mean chromosome pairing of $0.172 + 7.224 + 0.275$ per cell. 68.9% of cells show 8 bivalents. Predominance of bivalents in hybrid, fertile F$_1$ hybrid with 81% pollen fertility suggests that genomes of C. ternatea Var. ternatea f. albiflora and C. ternatea Var. ternatea f. fasciculata are closer. Gene interaction for petal colour and selection might have played important role in differentiating this taxon from rest of the members of Var. Ternatea.

Hybridization between C. ternatea Var. ternatea f. albiflora and C. ternatea Var. ternatea f. ternatea, which is cosmopolitan in distribution, resulted in production of fertile hybrids. Crossability data shows that seed set per pod is 5, hybrid seed germination is 58.33% and pollen fertility is 75%. These readings are comparatively lower than earlier cross (C. ternatea Var. ternatea f. albiflora X C. ternatea Var. ternatea f. ternatea). Even here we do not find any strong crossing barrier. Meiotic pairing in F$_1$ hybrids show regular formation of 8 bivalents in 78.33% cells and mean chromosome pairing is $7.235 + 0.299$ per cell. This pairing behaviour suggests that genomes of C. ternatea Var. ternatea f. albiflora and
C. ternatea Var. ternatea f. ternatea are fairly closer. Although fairly good chromosome pairing in hybrids only 75% of pollen are fertile and and 58.33% seeds are viable. Cryptic structural hybridity may be operating in these F1 hybrids, hence low pollen fertility (Stebbins, 1971 and Jackson 1985). Occurrence of chromosomal bridge indicates that, evolutionary divergence within taxa occurred because of paracentric inversions. (Stebbins, 1950; Grant, 1963; Singh et al., 1988).

Chromosome pairing in F1 hybrids of C. ternatea Var. ternatea f. fasciculata and C. ternatea Var. ternatea f. ternatea is of $0.275_t + 7.448_n + 0.206_r$ per cell. Nearly 75.8% cells show 8 bivalent in F1 hybrid. Predominance of bivalent formation in hybrid suggest that stronger genome homology of C. ternatea Var. ternatea f. fasciculata with C. ternatea Var. ternatea f. ternatea. Crossability data indicate that C. ternatea Var. ternatea f. fasciculata and C. ternatea Var. f. ternatea cross readily pollen fertility is 86.00% pollen fertility is 86%, seed set per pod is 7 and seed germination is 70.00% in hybrids. It appears that crossing barriers between these two taxa are not well developed. Consequently morphological, distributional pattern, ease of
crossability, fertile hybrids and chromosome pairing analysis indicate that these two taxa are closely related and karyotypic protein profile observations disclose that, *C. ternatea Var. ternatea f. fasciculata* may be evolved from *C. ternatea Var. ternatea f. ternatea* and point mutation for flower colour and selection have diverged this taxon from the rest.

Results of crossing experiments between members of Var. Ternatea and Var. Pleniflora reveal that, there is restricted gene flow between members of these two varieties. Subtle data on crossability and chromosome pairing show interesting inferences.

*C. ternatea Var. pleniflora f. leucopetala* is distributed in SouthEast Asia, and has restricted distribution in India. Its white flowers are non-papilionaceous or actinomorphic found in wild as well as in cultivation. Crossability data between *C. ternatea Var. ternatea f. albiflora* and *C. ternatea Var. pleniflora f. leucopetala* shows that seed set per pod is just 3 and hybrid seed germination is 46.45%. Pollen fertility is also minimum in this hybrid (53.00%). Meiotic pairing data in hybrid reveals that mean chromosome pairing of $0.46n + 6.268n + 0.756^r$ per cell. In about 42.00% of cells 8 bivalents are noticed. Crossability between these two taxa is also
poor. It appears that crossing barriers are strongly developed. The low crossability denotes divergent and distant relationship.

_C. ternatea Var. pleniflora f. pleniflora_ is confined to Asia and Indonesia. In India its distribution is restricted. It produces dark blue non-papilionaceous flowers. Hybridization experiments with _C. ternatea Var. ternatea f. albiflora_ shows that seed set per pod is 5, with 60.00% hybrids seed germination and pollen fertility is 55.00% in hybrid. Chromosome pairing during meiotic prophase is of $0.6\,\lambda + 7.26\pi + 0.184\nu$ per cell. 67.69% of cells show 8 bivalents. Crossability and chromosome pairing data and genomic homology are slightly better than earlier cross. From the above data it can infer that crossability barrier are well developed indicating distant relationship.

In other crosses such as _C. ternatea Var. ternatea f. fasciculata_ X _C. ternatea Var. pleniflora f. leucopetala_, _C. ternatea Var. ternatea f. fasciculata_ X _C. ternatea Var. pleniflora f. pleniflora_, _C. ternatea Var. ternatea f. ternatea_ X _C. ternatea Var. pleniflora f. leucopetala_ and _C. ternatea Var. ternatea f. ternatea_ X _C. ternatea Var. pleniflora f. pleniflora_, over all result of crossability and chromosome pairing analysis are almost similar.
However, chromosome-pairing behaviour is an indicator of genome homology. Maximum genomic homology is observed in hybrids of *C. ternatea Var. ternatea f. fasciculata X C. ternatea Var. pleniflora f. pleniflora*, and this followed by hybrids of *C. ternatea Var. ternatea f. ternatea X C. ternatea Var. pleniflora f. pleniflora*, *C. ternatea Var. ternatea f. fasciculata X C. ternatea Var. pleniflora f. leucopetala* and *C. ternatea Var. ternatea f. ternatea X C. ternatea Var. pleniflora f. leucopetala*.

Crossing experiments in *C. ternatea Var. pleniflora f. leucopetala* and *C. ternatea Var. pleniflora f. pleniflora* show that chromosome pairing is of $0.047_i + 7.427_n + 0.226^v$ per cell. 80.90% of cells show 8 bivalent crossability is, 5 seeds per pod and hybrid seed germination is 50.00%. This indicating good genomic homology between these two taxa of variety Pleniflora.

Meiotic observations in F$_1$ hybrids indicate that it is almost normal except for univalents, multivalents and laggard in few cells. Consequently pollen fertility is good in most of the F$_1$ hybrids. However, occurrence of quadrivalents during meiosis of all diploid hybrids which implies that, the genomes of these taxa are differentiated by reciprocal translocation. In a few cases F$_1$ hybrids
show low pollen fertility. This points out the role of cryptic structural hybridity in these F1 hybrids.

Over all picture of genomic relationship based on meiotic pairing in F1 hybrids C. ternatea varieties has been presented in the Fig. 153.

**Inheritance of flower colour and flower morphology.**

Although the present study is not based on any stastical analysis with respect to inheritance of flower colour and flower morphology the gross observations on these characters in F1 hybrids of C. ternatea are interesting. Inheritance of flower colour being controlled by two pairs of genes with complementary interaction (Table-15, 19 and 31 Figs. 14, 22 and 68), where as flower morphology ie., non-papilionaceous nature is controlled by monogenic dominant gene (Table-22, 25, 34,37, 40 and 43 Figs. 30, 38, 76, 84, 92 and 100). Theses observations corroborate earlier report of dominant nature of blue flower colour (Rant, 1922; Sen Krishnan, 1961;Chow, 1978). Non-papilionaceous nature of flower is due to dominant gene mutation (Sen and Krishnan1961; Saroja, 1961). Present observation indicate that flower colour and flower morphology are governed by independent genes and without
Fig. 153. Summary of proposed genomic relationships based on meiotic pairing in F1 hybrids among the varieties of Clitoria ternatea L., CTTT = C. ternatea Var. ternatea f. ternatea. CTTF = C. ternatea Var. ternatea f. fasciculata. CTTA = C. ternatea Var. ternatea f. albiflora. CTPP = C. ternatea Var. pleniflora f. pleniflora. CTPL = C. ternatea Var. pleniflora leucopetala. All carry 2n=16 chromosomes.
any pleiotropic effect. However, Thombre and Atale (1974) have reported pleiotropic of these genes.

Cytological observations indicate that structural changes and point mutations have played a pivotal role in diversifying *C. ternatea* in to five forms. Genome analysis indicates genetic homology between different forms. Morphological study shows that reproductive barriers are not well developed, however reproductive isolating mechanisms like ecological preferences, rhizobium requirements and mechanical isolation mechanisms like flower colour, morphology and stamen arrangement are noticed. The phylogenetic relationships in forms of *C. ternatea* based on present study are represented by phyletic tree (Fig. 154).

![Phylogenetic tree](CTTA_CTPL)

Fig. 154. Phylogenetic relationships of five forms of *C. ternatea*. 