MATERIALS AND METHODS
(1) Genetic diversity

Ninety two diverse genotypes of Butterfly pea (Clitoria ternatea) were collected from different agro-climatic situations of tropical, sub-tropical and warm humid regions of India (Table 4 and Fig 1). These genotypes were properly accessioned and evaluated during the year 1989 to 1992. The experimental work for the present studies was carried out on five main areas of research namely gene pool collection and evaluation, germination behaviour of polymorphic seed genotypic stability, relative growth rate and compatibility of Clitoria ternatea with different pasture grasses. All the field experiments were undertaken at Central Research Farm of National Research Centre for Agroforestry (IGFRI Campus), Jhansi. The research farm is situated at 25° - 27° North, 78° - 38° East, 271 m above the sea level and falls in semi-arid plateau hills of Bundelkhand having mean annual rainfall 890 (mm) ranging from 670-1160 (mm). The average values of field capacity, wilting point and bulk density of these soils are 12 %, 46 % and 145 %, respectively. The soil is neutral in reaction, poor in nitrogen and organic content but rich in potassium (Dohre, 1981).

The experiment was laid out in randomized block design in sandy loam soils. The seeds of all the germplasm lines were sown in first week of July, 1989 in 3 m long rows spaced at 0.5 m and 10-15 cm distance between plant to plant respectively. Each plot had three lines and replicated thrice. The experiment was maintained under rainfed conditions for three consecutive years.

Data recording

The observations on various morphological, growth and fodder yielding attributes were recorded at 50 % flowering stage on three randomly selected plants from each plot. These were cut at 10 cm above the ground level for recording the data on fodder yielding attributes.

Days to 50% flowering

On the basis of visual observations, number of days to flower initiation stage was recorded. Number of days were counted from the date of sowing to the flowering initiation in 50 % plant population of entire plot.
Height of the plant was measured in (cm) from the ground base to the apex of the main shoot.

**Branch number**

Total number of branches on main shoot were recorded from first to the last node.

**Secondary branch number**

Number of branch sprouts from all the branches were counted.

**Branch length (cm)**

Length of best developed branch was measured from the place of origin on main shoot (node) to the apex of branch.

**Number of leaves/plant**

A count of total number of leaves (complete compound leaf with all three foliages) was taken for entire plant.

**Green forage yield/plant (g)**

Total above ground green biomass in (g) of the plant (both leaf+stem) was weighed just after the cutting.

**Dry matter yield/plant (g)**

For dry matter yield entire plant samples (green biomass) was put in oven at 60-70°C for more than 40 hours and dried biomass was weighed in (g).

**Leaf-stem ratio**

Leaf-stem ratio was worked out by dividing leaf dry weight by stem dry weight.

**Estimation of crude protein content**

Estimation of crude protein content was done as per the method suggested in
AOAC (1990) For this, 0.5 g oven dried and ground sample (including both leaf and stem) was taken and 0.1 g catalyst (mixture CuSO₄ and K₂SO₄ in a ratio of 1:5) was added to it. Digestion was done with 10 ml concentrated H₂SO₄ for 2-3 hours till it becomes transparent. Volume was made up to 50 ml in volumetric flask by adding distilled water. 10 ml solution from this flask was taken and distilled in micro Kjeldahl distillation apparatus with 40% NaOH. Released ammonia was collected in beaker containing 0.2% boric acid mixed with indicator. The colour of indicator changed from red to blue and released ammonia gas which was absorbed by boric acid. Then Ammonium borate was titrated with standard solution of sulphuric acid and finally ‘N’ percentage was calculated as:

\[0.1 \text{ ml of } N_\text{100} \text{ H}_2\text{SO}_4 \times 0.0014 \text{ g N}\]
Crude protein (\%) = (\%) N x 25

**Analysis of variance**

Statistical analysis was carried out using mean values of various forage yielding attributes following standard statistical procedures with the help of computer software. The significance of variance was tested by “F” value. Coefficients of variation were calculated for morphological and fodder yielding attributes.

**Classification and cataloguing of germplasm**

For the classification and cataloguing of germplasm score index method was followed as proposed by Anderson (1957) and accordingly, each parameter was divided into three scoring groups,

1. Low
2. Medium
3. High

These groups were denoted by numerical values ‘0’, ‘1’ and ‘2’ respectively. For score index following parameters were taken viz. days to flowering, plant height (cm), branch number, secondary branch number, branch length (cm), number of leaves/plant, green fodder yield/plant (g), dry matter yield/plant (g), leaf-stem ratio and crude protein content (%). For each character score ranged between 0-2 and accordingly maximum score by any genotype could be 20. The entire germplasm was grouped and classified.

(2) **Factors influencing the germination of Clitoria seeds**

Seeds of eight promising genotypes showing polymorphic seed coat colour with dark grey dotted testa (II.C'I-213 & II.C'I 215), blush grey dotted (II.C'I-221 & II.C'I 249), brown
collected during March/April. Collected seeds were stored in separate glass bottles at room temperature for more than 180 days in view of the dormancy reported in this species (Mullick and Chatterji, 1966 and Hall, 1992). The viability of seeds was tested with 2, 3, 5 - Tri-phenyl tetrazolium chloride (235-1TC salt). The seeds were pre-soaked in water for 24 hr, then bisected and embryo containing portion of seed was put in 01% solution of 1TC for 8 hr at 30°C. After washing the seeds developed red colour stain which indicated the viability of seeds and almost all the seeds (more than 90%) were found viable. Four different set of experiments were laid out as mentioned below.

(I) Effect of sowing depths (cm)

The seeds of eight genotypes of butterfly pea were sown in earthen pots at 2 cm, 4 cm, 6 cm and 8 cm depths. Each pot was filled with normal field soil having 0.61, 0.32, 0.18 and 0.53% organic content, nitrogen, P₂O₅ and K₂O respectively with 7.4 pH. Fifteen seeds were sown in every pot. Each treatment was replicated three and uniform environmental condition were provided to all the treatments and replicates.

(II) Effect of soil types

The seeds of each genotype were sown at 4 cm sowing depth in earthen pots. These pots were filled with different soils and soil combinations viz:

1. red soil
2. black soil
3. organic soil
4. mixed soil (mixture of all soil types in equal proportion)

**Nutrient status of soils and their combinations:**

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Organic matter</th>
<th>Nitrogen</th>
<th>P₂O₅</th>
<th>K₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red soil</td>
<td>0.59</td>
<td>0.32</td>
<td>0.15</td>
<td>0.51</td>
</tr>
<tr>
<td>Black soil</td>
<td>0.78</td>
<td>0.45</td>
<td>0.20</td>
<td>0.50</td>
</tr>
<tr>
<td>Organic soil</td>
<td>6.20</td>
<td>0.60</td>
<td>0.27</td>
<td>0.82</td>
</tr>
<tr>
<td>Mixed soil</td>
<td>1.25</td>
<td>0.57</td>
<td>0.25</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Fifteen seeds of each genotype were sown in each pot in three replications. Soils were analysed as per the method reported by Jackson (1967)
(III) Effect of temperatures (°C)

Seeds of eight genotypes were put in sterilized petri dishes with single layer of ordinary filter paper. Before sowing, the seeds were thoroughly washed and soaked in distilled water for 24 hours. Further to avoid the fungal infection the seeds were properly treated with fungicides (1% thiram + 1% D). The experiment was conducted for four different temperature treatments viz 5°C, 15°C, 25°C and 35°C. Twenty-five seeds were put on each petriplate and each treatment was replicated three. The experiment was maintained in BOD incubator under controlled temperature (flexible±2°C) and other factors being uniform.

(IV) Effect of different colours of light

Different colour treatment were adjusted by wrapping tube light in BOD incubator with cellophane paper of respective colours. Similar sterilization, fungicide treatment and method of sowing in petriplates was applied as done in case of temperature treatment. Other factors (temperature and moisture etc.) uniform maintained during the experiment.

Data recording

Data on seed germination was recorded on alternate days from the date of first emergence to the last emergence. At final emergence, period and a total count of germinated seeds was recorded and percentage germination was worked out for all the treatments. Finally, mean values were inversely transformed and statistical analysis was done in three factorial randomized block design.

(3) Genotypic stability

A group of eight genotypes was selected from available genetic stock of butterfly pea (C. ternatea). The genotypes of diverse origin were collected from different parts of Delhi, Tamil Nadu, Rajasthan and Uttar Pradesh. The group contained sufficient genetic variability for major and minor genes. A brief picture of magnitude of qualitative and quantitative traits for each entry are given in Table 3. The material was grown in randomized block design with three replications in a plot size of 3m x 4m. Row to row distance was kept to 0.5 m accommodating eight rows of 3 m long in each plot. Plant to plant distance was adjusted at seedling stage at 15-20 cm during the kharif 1990. The trial was conducted strictly under rainfed conditions and with the naturally available soil nutrients only. It was repeated for two more consecutive years.
### Table 3  Source of origin, qualitative and quantitative characters of selected strains of Clitoria ternatea

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Qualitative characters</th>
<th>Quantitative characters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed colour</td>
<td>L/S ratio</td>
</tr>
<tr>
<td>ILCT - 213 Delhi Dark grey dotted</td>
<td>1.08</td>
<td>26</td>
</tr>
<tr>
<td>ILCT - 215 Delhi &quot;</td>
<td>1.45</td>
<td>25</td>
</tr>
<tr>
<td>ILCT - 221 Delhi Bluish grey dotted</td>
<td>1.50</td>
<td>25</td>
</tr>
<tr>
<td>ILCT - 249 Delhi &quot;</td>
<td>1.31</td>
<td>26</td>
</tr>
<tr>
<td>ILCT - 261 Tamil Nadu Brown</td>
<td>1.41</td>
<td>23</td>
</tr>
<tr>
<td>ILCT - 269 Rajasthan Brown</td>
<td>1.54</td>
<td>25</td>
</tr>
<tr>
<td>ILCT - 272 Rajasthan Black</td>
<td>1.51</td>
<td>21</td>
</tr>
<tr>
<td>ILCT - 278 Uttar Pradesh Black</td>
<td>1.55</td>
<td>25</td>
</tr>
</tbody>
</table>
Observations were recorded on five randomly selected competitive plants from four central rows in each plot. The characters studied were green fodder yield/plant (g), dry matter/plant (g), plant height (cm), branch number per plant, leaf-stem ratio. Four yield attributes viz., green fodder yield (t/ha), dry fodder yield (t/ha) and seed yield (t/ha) were also studied on plot basis. Observations for crude protein yield were recorded on sample basis drawn from each genotype and replication. The values were converted into t/ha. Statistical analysis was carried out for randomized block design (RBD) using standard procedures (Panse & Sukhatme, 1968). Analysis for gene \ environment interaction and stability was done as per model of Eberhart and Russell, 1966.

(4) Relative growth rate (RGR)

A set of eight genotypes of butterfly pea was sown in RBD with three replications. Sowing was done in the first week of July 1991. Each plot (3 x 2.5 m) had 3 m long five rows spaced at 0.5 m and 0.25 m between the rows and plants respectively.

Data recording and RGR

After 40, 50 and 60 days growth, five plants were randomly selected and harvested 10 cm above the ground level. These plants were selected from central row of the plot. Leaves and stem of plant were separated, oven dried and data on dry matter was recorded. RGR expresses the increase of dry weight in specific time period in relation to initial weight and RGR (g/g/day) can be calculated as, $RGR = \frac{\log w2 - \log w1}{t2 - t1}$ (on dry matter basis) and was computed at two growth period 40-50 days growth and 50-60 days growth. These are said to be RGR-I and RGR-II. Correlation coefficients was worked out between the RGR of leaf, stem and leaf + stem (entire plant) for respective periods of growth. Correlation coefficient was also worked out between dry matter yield/plant (g) and their respective RGR values at different harvest times (Singh and Singh, 1988).

(5) Compatibility of C. ternatea with different grasses

The study on various grass-legume intercropping system was conducted at NRCAF, Jhansi during the July 1989 to December 1992. The soil of experimental site was clay loam (approximately 46.8% sand, 39.0% clay and 14.1% silt) with 7.5 pH and soil status was 0.4% organic carbon with, available nitrogen phosphorus and Potash 161-0, 7.5 and 268 kg/ha respectively.
Grass components

1. *Chrysopogon fulvus* (Spreng) Chiov

*C. fulvus* commonly known as Dhawalu grass is a perennial grass distributed throughout the hilly regions of India, Afghanistan, Pakistan and Sri Lanka. This grass is one of the principal constituent species of *Schima-Dichanthium* grass cover in the Central and Southern plateau of the country (Dabadghao and Shankarnarayan, 1973). The grass can be grown even on rocky substratum under low to medium rainfall situations. Its herbage productivity under degraded rangeland situations is very low (0.7-1.2 t/h dry forage yield in 1-2 cuts). The quality of herbage is also poor.

2. *Heteropogon contortus* (L.) Beauv. ex Roem. & Schultt

*H. contortus* is a perennial grass and commonly known as spear or black spear grass and locally called as ‘Lampa ghas’. It is also the main component of *Schima-Dichanthium* grass cover of India (Dabadghao and Shankarnarayan, 1973). It is widely distributed throughout the grasslands of tropics and subtropics and has high adaptability to degraded poor sandy loam to clay soils with 5-7.5 pH. It is most palatable at pre-flowering stage.

3. *Cenchrus ciliaris* Linn

*C. ciliaris*, commonly known as Buffel or Anjan grass constitutes an important grass component of the *Dichanthium-Cenchrus and Lasturus* grass cover of India and grows predominantly in the arid and semi-arid areas of Gujarat, Rajasthan, Haryana, West and Central Uttar-Pradesh and northern Madhya-Pradesh. Buffel is a perennial grass, 110-145 cm erect or decumbent. It is highly adaptive to sandy and dry regions. The productivity of this grass under natural range conditions is very low (0.2 to 0.3 t/h dry matter). It resists drought condition and is considered to be the most nutritious among native grasses. It has good soil binding capacity and may check soil erosion. Grazing animals have special liking for this grass.
Subabul was introduced in domestication areas of Forest Research Institute, Dehradun as early as 1931 (Krishnaswami, 1956) and in Andmans it was grown as hedge row (Parkinson, 1977). Later, Hawai strains were introduced in Bombay state in 1948 from Philippines (Qureshi and Desai, 1981). It is adopted to degraded and denuded lands of medium rainfall zones. It yields 8-20 t/h/yr forage and about 95-96 t/h/yr fuelwood and also enriches the soil fertility. *Leucaena leucocephala* is being intensively evaluated for its varietal improvement (Gupta, 1988) and interplanted with traditional/non-traditional fodder crops and results have been quite encouraging in favour of mix cropping with cereals and grasses (Pathak, 1988, Relwani and Khandale, 1988). The green fodder and its hay can be used as a rich source of proteinous diet for animals and is recognised as ‘Protein bank’ (Hutton, 1988).

### A. Main legume species

*Chitoria ternatea* (Ct)

### B. Grass species

1. *Chrysopogon fulvus* (Cf)
2. *Heteropogon contortus* (Hc)
3. *Cenchrus ciliaris* (Ce)

### C. Tree legume species

*Leucaena leucocephala* (Sbl)

#### Treatments

<table>
<thead>
<tr>
<th>T-1</th>
<th>Pure <em>C. ternatea</em></th>
<th>(Ct)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-2</td>
<td>Pure <em>C. fulvus</em></td>
<td>(Cf)</td>
</tr>
<tr>
<td>T-3</td>
<td>Pure <em>H. contortus</em></td>
<td>(Hc)</td>
</tr>
<tr>
<td>T-4</td>
<td>Pure <em>C. ciliaris</em></td>
<td>(Ce)</td>
</tr>
<tr>
<td>T-5</td>
<td><em>C. ternatea</em> + <em>C. fulvus</em></td>
<td>(Ct + Cf)</td>
</tr>
<tr>
<td>T-6</td>
<td><em>C. ternatea</em> + <em>H. contortus</em></td>
<td>(Ct + Hc)</td>
</tr>
<tr>
<td>T-7</td>
<td><em>C. ternatea</em> + <em>C. ciliaris</em></td>
<td>(Ct + Ce)</td>
</tr>
<tr>
<td>T-8</td>
<td><em>C. ternatea</em> + <em>L. leucocephala</em></td>
<td>(Ct + Sbl)</td>
</tr>
<tr>
<td>T-9</td>
<td><em>C. fulvus</em> + <em>L. leucocephala</em></td>
<td>(Cf + Sbl)</td>
</tr>
<tr>
<td>T-10</td>
<td><em>H. contortus</em> + <em>L. leucocephala</em></td>
<td>(Hc + Sbl)</td>
</tr>
<tr>
<td>T-11</td>
<td><em>C. ciliaris</em> + <em>L. leucocephala</em></td>
<td>(Ce + Sbl)</td>
</tr>
<tr>
<td>T-12</td>
<td><em>Chitoria</em> + <em>Chrysopogon</em> + <em>Leucaena</em></td>
<td>(Ct + Cf + Sbl)</td>
</tr>
<tr>
<td>T-13</td>
<td><em>Chitoria</em> + <em>Heteropogon</em> + <em>Leucaena</em></td>
<td>(Ct + Hc + Sbl)</td>
</tr>
<tr>
<td>T-14</td>
<td><em>Chitoria</em> + <em>Cenchrus</em> + <em>Leucaena</em></td>
<td>(Ct + Ce + Sbl)</td>
</tr>
</tbody>
</table>
The experiment was conducted during 1998—1999 by using randomized block design with three replications. The plot size was 8 x 5 m and 1 m distance between the plots. Transplanting and sowing were done as follows.

(I) Pure crop of grass species were planted at 50 cm distance between the rows and also the plants in each row. In case of *Clitoria* 12-15 plants were maintained per running meter.

(II) In case of grass legume mixtures alternate row of *Clitoria* and grass species were sown maintaining a distance of 50 cm between the rows of grasses and *Clitoria*

(III) For three species intercropping experiments each plot (8 x 5 m) was divided into two equal parts of 4 x 5 m. In half of the plot *L. lenocephala* (Sbl) were transplanted at 1 m distance between the inter row of grass species.

At the time of sowing/planting no organic or inorganic fertilizer was applied. Establishment of *Clitoria* and grasses were 80-100% whereas subabul needed some gap filling (one or two plants) in a few plots.

**Data recording**

Data on various growth parameters, fodder yielding attributes and quality aspects were recorded in all the grasses & legumes at the time of harvesting. Two cuts were taken in all the inter-cropped species, first cut in 1st week of August and 2nd cut in the last week of September. The cut in *C. ternatea* and other grass species was taken at 10 cm height from the ground while subabul plants were lopped at 1 m from the base at every cutting.

**Plant height (cm)**

Plant height in *C. ternatea* was recorded on three randomly selected plants from each plot and height was measured from the ground level of the plant to the apex of main shoot. In grass species largest tiller was measured from three randomly selected tussucks.

**Branch number**

Number of branches emerging on the main shoot (right from base to apex) were counted on three randomly selected plants in each plot in *C. ternatea* whereas in grasses number of tillers (sprouts arising from the base of the grass plant i.e. tussucks) were counted from selected tussucks from all the plots and replications.

**Green fodder yield (t/h)**

After recording the data on various growth parameters on three randomly selected
Dry matter yield (t/h)

500 g sample from each plot and plant basis samples were oven dried at 60-70°C for more than 48 hr and both the samples were weighed and dry matter per plant (g) and dry matter yield (t/h) was worked out.

Crude protein yield (t/h)

Methods of estimation of crude protein content were followed as mentioned earlier (A O A C 1999). After conversion and multiplying with factor the CP yield was worked out in t/h. In the text C-1 and C-2 denotes first and second cutting, respectively. Similarly the treatments are denoted as 1-1 to 1-14 while Y-1, Y-2 and Y-3 shows the first, second and third year, respectively.

Meteorological observations

Meteorological data on rainfall (mm), temperature (°C), humidity (%) and evaporation (mm/day) for the period of June 1989 to December 1992 is given in Figure 2-4. During the experimentation period minimum precipitation 668.8 mm was received during the establishment year 1989 while maximum rainfall 1161.2 mm was recorded in 1990 followed by 1991 and 1992. Highest number of rainy days were recorded in second year i.e. 1990 which is given below.

<table>
<thead>
<tr>
<th>Year</th>
<th>Rainfall mm</th>
<th>Number of rainy days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989*</td>
<td>668.8*</td>
<td>27</td>
</tr>
<tr>
<td>1990</td>
<td>1161.2</td>
<td>46</td>
</tr>
<tr>
<td>1991</td>
<td>957.7</td>
<td>35</td>
</tr>
<tr>
<td>1992</td>
<td>786.4</td>
<td>44</td>
</tr>
</tbody>
</table>

* Rainfall received from June - December, 1989

The onset of monsoon was well in time in 1989. Early rains were recorded in June and July, while most of the precipitation (more than 80% of the total rainfall) was received during
Rainfall (mm) and relative humidity (%) pattern during study period

Figure-2
The year 1990 experienced normal onset of monsoon during third week of June (25th standard week) and was well distributed in 40 rainy days throughout the monsoon season. The monsoon remained active up to 38th standard week and during last spell more than 340 mm rainfall was recorded within 5 rainy days (15th to 19th September). A good amount of precipitation (39.5 mm) was also recorded in second week of February. Maximum temperature (44.8°C) was recorded during the last week of May (29th May) while minimum (6.1°C) was observed in first week of January. Highest rate of evaporation (19.8 mm/day) was recorded during the last week ending on 29th May (Fig 2, 3 & 4).

During 1991, monsoon rains were received quite late in 29th standard week (third week of July) and maximum precipitation (415 mm) was recorded in July followed by September. The monsoon was most effective during 7 weeks and over all normal rains (878.2 mm) were received in 24 rainy days but for crop growth the distribution was not favorable. A good amount of rainfall (22.2 mm) was also received during rabi season. High value with respect to temperature (45.4°C) and evaporation (21.0 mm/day) was recorded during the last week of May and first week of June 1991 respectively.

Again during the year 1992, a late onset of monsoon was experienced in second week of July (28th standard week) thereafter, the monsoon was effective for next 10 weeks. During this period a total rainfall (723 mm) was well distributed in 36 rainy days. In second week of October about 38 mm rainfall was observed in three rainy days. Maximum and minimum temperature were recorded on 8th June (46.0°C) and December 29th (6.2°C), respectively. Highest rate of evaporation (18.0 mm/day) was recorded on June 8th (Fig 4).

During the course of experimentation, on an average, precipitation was mainly received during the period of June to September every year. The late onset of monsoon and decreasing trend for mean total rainfall was observed from 1989 to 1992. Relative humidity (%) and rainfall (mm) trend is being presented in Fig 2. An increasing trend in both sides of RH% was observed from establishment year to terminating year and was positively correlated with rainfall pattern. Generally, the maximum RH% was recorded in September and December-January.

An inter-relationships between temperature and rainfall was drawn in Ombrothermic diagram (Fig 3) which indicated that the temperature and rainfall patterns was positively correlated. An increasing trend in temperatures and decreasing trend in precipitation (with scanty or without rains) was experienced during the Kharif season in respect of September, 1989, 91 and August 90 while during 1992 a normal trend in both the parameters was recorded.
Ombrothermic diagram obtained during study period

Figure-3
Temperature (°C) and evaporation (mm/day) pattern during study period

Figure-4