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Karyotype analysis in lignosus bean (*Dipogon lignosus*) and lablab bean (*Lablab purpureus*)

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Abstract

The experiment was conducted in the cytogenetics laboratory of the Department of Genetics and Plant Breeding of Bangladesh Agricultural University during July 2008 to September 2009 aiming to analyze and compare the karyotypes of two genotypes of Lignosus bean (*Dipogon lignosus* L.) and Lablab bean (*Lablab purpureus* L.**)**. Root tips from germinated seeds were collected, pretreated and fixed in acetic alcohol for using in slide preparation. Chromosome measurements were done from the prints of the photomicrographs of prometaphase plates. In both the genotypes, 2n = 22 chromosomes was found. The range of length of the individual chromosome was 1.22 to 2.17µ in Lignosus bean, and 1.17 to 3.00µ in Lablab bean. In the Lignosus bean genotype, the haploid complement consisted of 11 metacentric (m) chromosomes with 3 individually identifiable ones but incase of Lablab bean the haploid complement consisted of 11 m chromosomes with 5 individually identifiable ones. All identifiable chromosomes were metacentric. This karyotype analysis of Lignosus bean and Lablab bean will be useful for further genetic studies.

Keywords: Chromosomal characteristics, Karyotype analysis, Bean, Derivation

Introduction

Lignosus bean (*Dipogon lignosus* L.) and Lablab bean (*Lablab purpureus* L.) are members of Leguminosae (Fakir *et al*., 2008). Although, Purseglove (1988) described Lignosus bean as one of the two botanical varieties of Lablab bean; but, Lignosus bean is distinctly different from Lablab bean. Lablab bean is a seasonally grown, perennial, twining herb with pods being longer and more tapering, and long axis of the seeds in the pod is parallel to the stature (Purseglove, 1988). Lignosus bean, on the other hand, is a long-lived, bushy perennial with pods being shorter and more abruptly truncated, and the long axis of the seeds in the pod is at right angle to the stature (Bari, 2000). Both Lablab bean and Lignosus bean are rich sources of protein. (Purseglove, 1988 and Rashid, 1999).

The term karyotype includes the group of characters such as the number, the form and size and other characters of the chromosomes which may be taken into account for the purpose of identifying a particular chromosome in a complement. A thorough study of the chromosome complement of any material revealing the detail chromosomal characteristics is called karyotype analysis. Detailed karyotypic information of a species is necessary for a full understanding in plant genetic studies as well as plant improvement of the material concerned (Gill and Kimber, 1974). The present study was undertaken to determine number of chromosomes of Lignosus bean (Trinidad) and Country bean (DS-106) and to analyze and compare the karyotypes of the two species in detail with special emphasis on the identification of individual chromosome.

Materials and Methods

Root tip collection and fixation

Seeds of Lignosus bean (Trinidad) and Country bean (DS-106) were germinated in the laboratory. Healthy and vigorously growing roots of 1.5-2.0cm long were collected and thoroughly washed. The roots were then pretreated in saturated aqueous solution of monobromonaphthalene (MBN) for 70 minutes with occasional stirring. MBN pretreated roots were then thoroughly washed and fixed in freshly prepared acetic alcohol. The fixed roots were left in the fixative until these were used for slide preparation. Slides were prepared within a few days of fixation of the roots. The fixation of roots and slide preparation was repeated as and when needed.

Photomicrography and measurement of chromosomes

Photomicrography of the chromosome plates was done from July 2009 to August 2009 in Post-graduate Laboratory of the Department of Genetics and Plant Breeding. Photomicrographs of the selected chromosome plates were taken with the aid of Olympus research microscope model BX40 using plan 100X objective. Fujicolour 200 ASA DX Film was used. Film processing and printings were made from commercial centres. Chromosome measurements were made from printed images and chromosome length were converted to micron (μ) based on calculated print magnification.

Karyotype analysis

The various steps followed in analysing the karyotypes were closely related to and dependent upon the results. For this reason the different procedural steps and the related discussion involving the method of analysis has been incorporated into the results section.

Results and Discussion

Procedure related observations

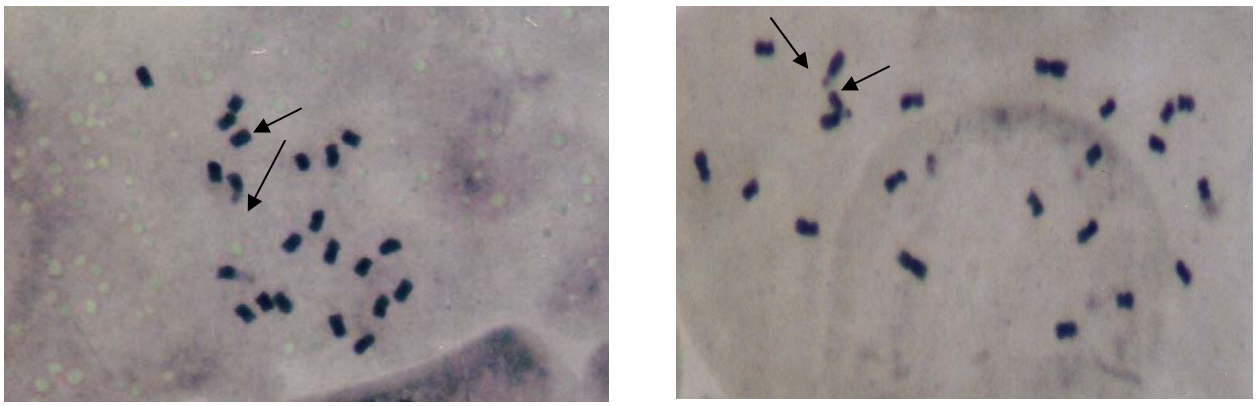
The maximum number of dividing cells for the two species, Lignosus bean and Lablab bean occurred when the roots were about 1.5cm long after germination in petridish. The duration of pretreatment with monobromonaphthalene (MBN) was important in obtaining plates with an appropriate degree of chromosome contraction. MBN pretreatment for 70 minutes was found to be optimum in obtaining a considerable number of cells in prometaphase stage with moderate degree of chromosome contraction. The fixative acetic alcohol was found suitable for the present materials. Prolonged storing of the fixed roots was avoided which caused the roots to be brittle. Hydrolysis of the roots in 10% HCl for 10-12 minutes at 60ºC was found adequate to soften the roots. Mordanting of the hydrolysed roots for 4 minutes in 2% Iron alum solution and staining for 4 mins in 0.5% haematoxyline produced satisfactory degree of staining of the chromosomes.

General observations on the chromosome complements

The somatic chromosome number of the two bean genotype Lignosus bean (Trinidad) and Lablab bean (DS-106) was 2n=22 in each case. No deviation in chromosome number was detected in case of either Lignosus bean or Lablab bean. One pair of satellited chromosome was present in the somatic complement in each of the two genotypes. But in some cases satellited chromosomes could not be detected. In each genotype satellited chromosomes were metacentric (Fig. 1). Chromosome nomenclature based on centromeric position was done following Levan *et al*. (1964).

Karyotype analysis

The method of karyotype analysis followed was as described in Ahmad *et al*. (1983), which is based on a scatter diagram technique.



A B

Fig. 1. Representative somatic chromosome complements in root tips of beans: A) Lignosus bean, B) Lablab bean. Arrow indicates satellited chromosome. Magnification: 1800x

Conceptual basis of the scatter diagram method of karyotype analysis

The scatter diagram technique of karyotype analysis is based on two assumptions. Firstly, it is assumed that in a scatter diagram of the total lengths and arm ratios of all chromosomes in a number of cells, the points produced by the same genetically individual chromosome would tend to cluster around a mean location. This mean location would be the hypothetical position of the chromosome in question with the "standard morphology" as defined above. The scatter diagram described here will be referred henceforth as 'combined scatter diagram'. Secondly, two non homologous chromosomes would be identifiable individually on the morphological basis if in a scatter diagram of several cells, the mean location of one chromosome occurred no less than one standard deviation away from that of the other, with respect to either total length or arm ratio.

Analysis of the karyotypes of Lignosus bean and Lablab bean was done separately for each material following three steps as mentioned below:

1. A scatter diagram was produced for all chromosomes in each cell. Haploid values of the chromosomes were determined by using the diploid values for both total length and arm ratio.
2. A combined scatter diagram of the haploid complements of all cells was constructed to establish standard morphology of those chromosomes which could be identified.
3. Those chromosomes which could not be identified individually were characterized through probabilistic inferences.

Derivation of haploid values

A scatter diagram for each selected cell was prepared using total lengths and arm ratio of the 22 chromosomes of the complement. Each chromosome and its corresponding points on the diagram were numbered. Scatter diagrams produced from the measurement of chromosomes of the representative plates for Lignosus bean and Lablab bean are shown in Figs. 2 and 3, respectively.

The chromosomes were then paired by circling the corresponding points on the scatter diagram based on the proximity of the two points. However, in some cases more than two points may occur in a similar degree of nearness. In such cases the concerned cell in the relevant slide was re-examined under the microscope for staining intensity and morphological similarity to establish how each pair could be ascertained best.

Thus, the 11 pairs of points in the scatter diagram were determined which represent 11 homologous chromosome pair of the complement.

The average total length and arm ratio of each of the 11 pairs of chromosomes constituted the haploid complement of that cell. The process was repeated for each of the selected cells of the 2 genotypes. Chromosome pairs were then numbered from 1 to 11 within each cell approximately, but not strictly in the increasing order of length and arm ratio.

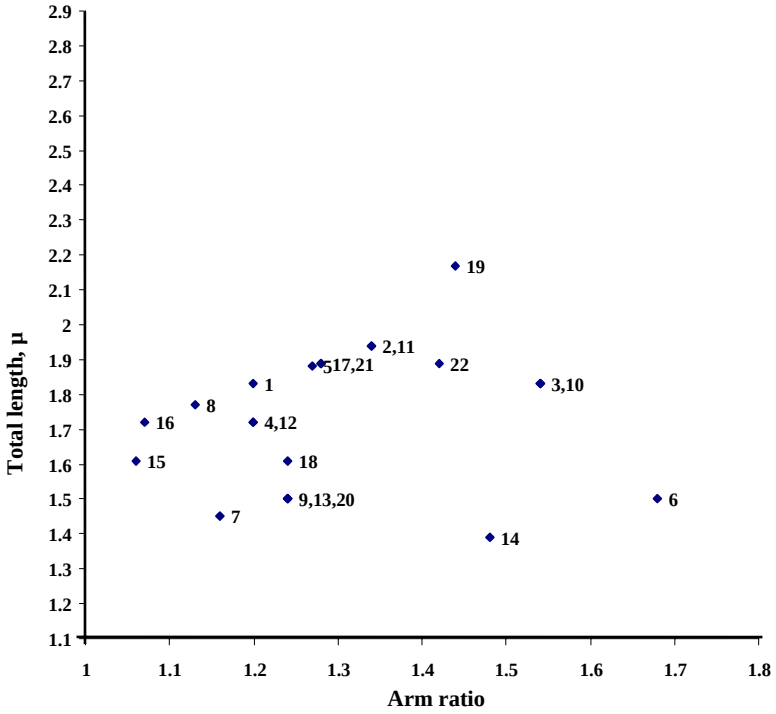


Fig. 2. Scatter diagram of the plate of Lignosus bean (Trinidad) as shown in figure 1. Each pair of points circled considered to represent a homologous pair

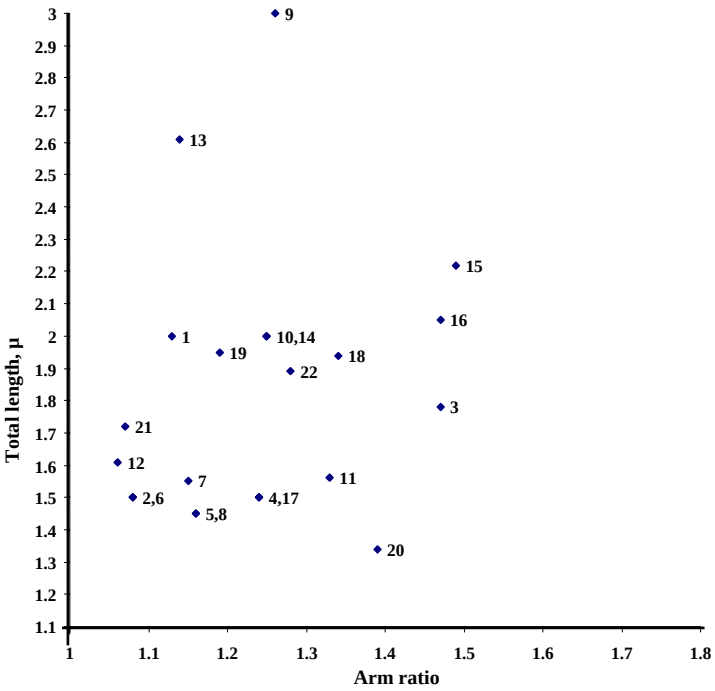


Fig. 3. Scatter diagram of the plate of Lablab bean (DS-106) as shown in figure 1. Each pair of points circled considered to represent a homologous pair

Features of the data sets of the somatic complements

The average haploid total length was 19.34±0.20µ for Lignosus bean and 19.60±0.49µ for Lablab bean. The coefficient of variation (cv) of haploid total was 2.53% for Lignosus bean and 6.13% for Lablab bean. This indicated that, on an average, degrees of contraction of the chromosomes from the different cells of the same material were similar, and this reflects the highly selected nature of those cells (Table 1).

Table 1. Haploid total lengths of chromosomes in the two bean genotypes Lignosus bean (Trinidad) and Lablab bean (DS-106)

Lignosus bean (Trinidad)				Lablab bean (DS-106)			
Root tip cell	Haploid total length (x) (µ)	$\overline{X}$ ±SE (µ)	cv (%)	Root tip cell	Haploid total length (x) (µ)	$\overline{X}$ ±SE (µ)	cv (%)
A	19.50			**A**	18.60		
B	19.07			**B**	19.76		
C	18.60	19.34±0.20	2.53	**C**	21.89	19.60±0.49	6.13
D	19.46			**D**	20.09		
E	20.21			**E**	18.99		
F	19.19			**F**	18.27		

Chromosome identification in Lignosus bean (Trinidad) and Lablab bean (DS-106)

Individually identifiable: Corresponding chromosomes in the six haploid complements were ascertained through grouping technique applied to the combined scatter diagram of the six haploid elements of each genotype. The data used for plotting were the original haploid values for arm ratio and the standardized haploid length values. Standardization was done to minimize any types of anomalies in chromosome length due to differential contraction in different cells. The haploid length of each chromosome was standardized using the following formula:

$$X'ij = Xij.\frac{\sum Xi./6}{Xi.} \quad ; i = 1.....6, \quad j = 1.....11$$

Where X´ij, and Xij, are the standardized and unstandardized length of j^{th} chromosome of i^{th} cell and Xi. is the haploid total length of the i^{th} cell. Following transformation, each complement had equal haploid total length.

The combined scatter diagram of the 66 chromosomes from the six cells of each genotype is shown in Fig. 4. Each point in the scatter diagram represented a specific chromosome in a particular haploid complement. Symbols in the scatter diagram refer to specific chromosomes in the haploid complement concerned that is letters A … F refers to the six cells studied and numbers 1 …11 represent the individual chromosomes of the concerned haploid complement assigned previously. If the morphology of all chromosome pairs would be distinct and reproducible across the cells, the six points representing the haploid homologues of the six cells should cluster and 11 such clusters would be recognizable. Consequently chromosomes of similar morphology will occur in superimposed or overlapping clusters, and will not be able to be distinguishable individually.

In each genotype, clear grouping was not possible for most of the area of the scatter diagram (Fig. 4). Three groups of six points of Lignosus bean and five groups of Lablab bean, one from each cell, could be identified clearly, each representing one specific chromosome. It was not possible to delineate groups within the remainder of the scatter diagram because the points occurred in close proximity, presumably indicating superimposition or substantial overlap of the remaining conceptual groups. Members of a group of six points as shown in Fig. 4 identified the corresponding chromosome pair of the six cells included for analysis. For each of these three and five specific chromosomes the mean, standard error of the mean and coefficient of variation with respect to both total length and arm ratio were calculated from the original diploid values.

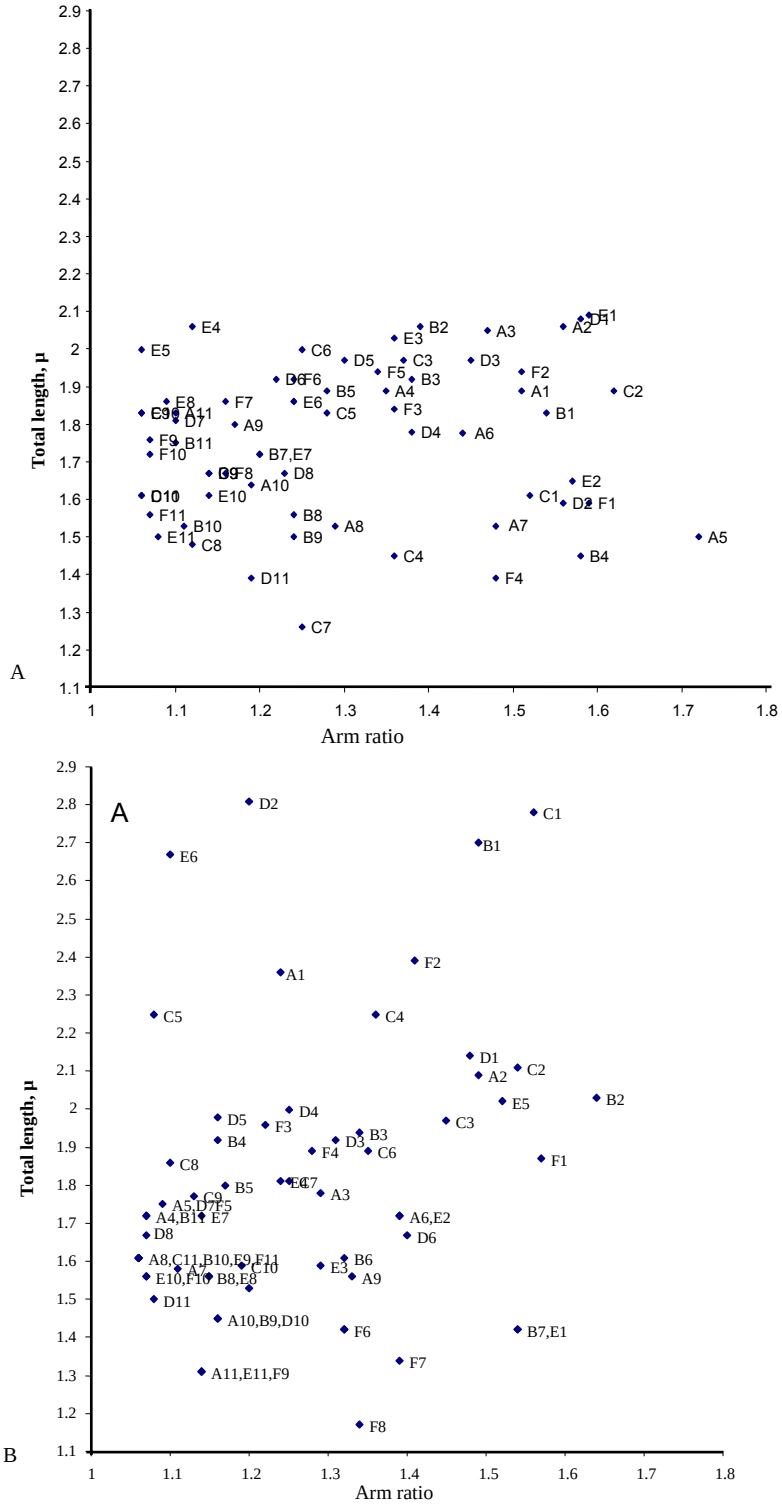


Fig. 4. Scatter diagram of the 11 haploid chromosome values, total lengths and arm ratios from each of six cells of A) Lignosus bean and B) Lablab bean; points A1-A11, plate A; points B1-B11, plate B; ……; points F1-F11, plate F. Group boundaries drawn around those chromosomes which were recognizably homologous from each of the six cells. Intersecting lines within the groups represent the group mean for total length and arm ratio ± one standard deviation for either variable for that group. Epithets m_1, m_2, m_3, within the groups are the specific name of the chromosomes concerned (Table 2).

According to the criterion proposed earlier, all of these three and five chromosomes fall in the category of individually identifiable ones (Fig. 4). Consequent to the similarity in morphology, the remaining groups of points occurred in superimposed or overlapping conditions, and could not be distinguished individually in the combined scatter diagram. Thus remaining eight chromosomes of the Lignosus bean and six chromosomes of Lablab bean complement were not identifiable individually on morphological basis.

All the idenfiab1e chromosomes were metacentric (m), arm ratio (L/S) was less than 1.7. These idenfiab1e chromosomes were numbered in the decreasing order of mean length values. Chromosome type together with this number constituted the specific name of the chromosome concerned (Fig. 4, Table 2). Furthermore, since standardization was based on the mean of the haploid total lengths, each of the points from a cell would be displaced vertically in one direction following transformation. Thus following transformation, members of the same group of chromosomes from the different cells would approach more closely to the group mean; that is, the groups would be more compact, while their mean locations would remain unchanged.

Table 2. Mean lengths and mean arm ratios of the identified chromosomes in Lignosus bean (Trinidad) and Lablab bean (DS-106)

Chromosome name	Total length		Arm ratio	
	$\overline{X}$ ±SE **(µ)**	**cv (%)**	$\overline{X}$ ±SE **(µ)**	**cv (%)**
Lignosus bean (Trinidad)				
m_1	2.00±0.023	4.00	1.40±0.014	3.60
m_2	1.98±0.035	6.06	1.56±0.014	3.21
m_3	1.56±0.037	8.33	1.59±0.029	6.30
Lablab bean (DS-106)				
m_1	2.62±0.058	7.63	1.33±0.055	14.29
m_2	2.04±0.040	6.86	1.55±0.026	5.81
m_3	1.87±0.026	4.81	1.30±0.014	3.85
m_4	1.60±0.072	15.63	1.45±0.026	6.21
m_5	1.54±0.023	5.20	1.28±0.029	7.81

Table 3. Proportion of the complement total length occupied by the identified chromosomes in Lignosus bean (Trinidad) and Lablab bean (DS-106)

Cell	Haploid total length (µ)	Total length of the identified 3 chromosomes (µ)	Proportion of the haploid total lengths occupied by the 3 chromosomes (%)	Mean (%)
Lignosus bean (Trinidad)				
A	19.50	5.61	28.77	
B	19.07	5.34	28.00	
C	18.60	5.47	29.41	28.70
D	19.46	5.64	28.98	
E	20.21	5.77	28.55	
F	19.19	5.47	28.50	
Lablab bean (DS-106)				
A	18.60	9.51	51.13	
B	19.76	9.70	49.09	
C	21.89	10.34	47.24	49.41
D	20.09	10.07	50.12	
E	18.99	9.51	50.08	
F	18.27	8.91	48.77	

The three identified chromosomes of Lignosus bean occupied on an average, approximately 28.70 percent and the five identified chromosomes of Lablab bean occupied on an average, approximately 49.41 percent of the complement total, and this was quite consistent in different cells included for analysis (Table 3). If the distribution would have been quite dissimilar in different cells, the proportion of total length accounted for the same identified chromosomes in those cells would be expected to be variable.

Table 4. Allocation of not identified chromosomes in Lignosus bean (Trinidad) and Lablab bean (DS-106) karyotype to different morphological categories

Length (x) classes (μ)	Arm ratio (y) classes	Total no. of chromosomes in six haploid sets	Mean no. of Chromosome per haploid set	No. of identified chromosomes with names*	Proposed no. of unidentified chromosomes	Total no. of chromo-somes	Assigned chromo-somes. no.
Lignosus bean (Trinidad)							
2.10≤x<2.35 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y						
1.85≤x<2.10 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	12 13	2.00 2.17	$2(m_1,m_2)$	2	2 2	1,2 3,4
1.60≤x<1.85 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	19 6	3.17 1.00		3 1	3 1	5,6,7 8
1.35≤x<1.60 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	8 6 1	1.33 1.00 0.17	$1(m_{3)}$	2	2 1	9,10 11
x<1.35 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	1	0.17				
	Total	66	11	3	8	11	
Lablab bean (DS-106)							
2.60≤x ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	2 2	0.33 0.33	$1(m_1)$		1	1
2.35 ≤x<2.60 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	1 1	0.17 0.17				
2.10≤x<2.35 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	1 3	0.17 0.50	$1(m_2)$		1	2
1.85≤x<2.10 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	8 6	1.33 1.00	$1(m_3)$	1	1 1	3 4
1.60≤x<1.85 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	18 3	3.00 0.50	$1(m_4)$	3	3 1	5,6,7
1.35≤x<1.60 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	14 2	2.33 0.33	$1(m_5)$	1	2	9,10
x<1.35 ,, ,,	y<1.35 1.35≤ y<1.70 1.70 ≤ y	4 1	0.67 0.17		1	1	11
	Total	66	11	5	6	11	

* Distribution based on mean values shown in Table 3.

Individually not identifiable: The eight chromosomes of Lignosus bean and six chromosomes of Lablab bean that were not individually identifiable were characterized morphologically as follows.

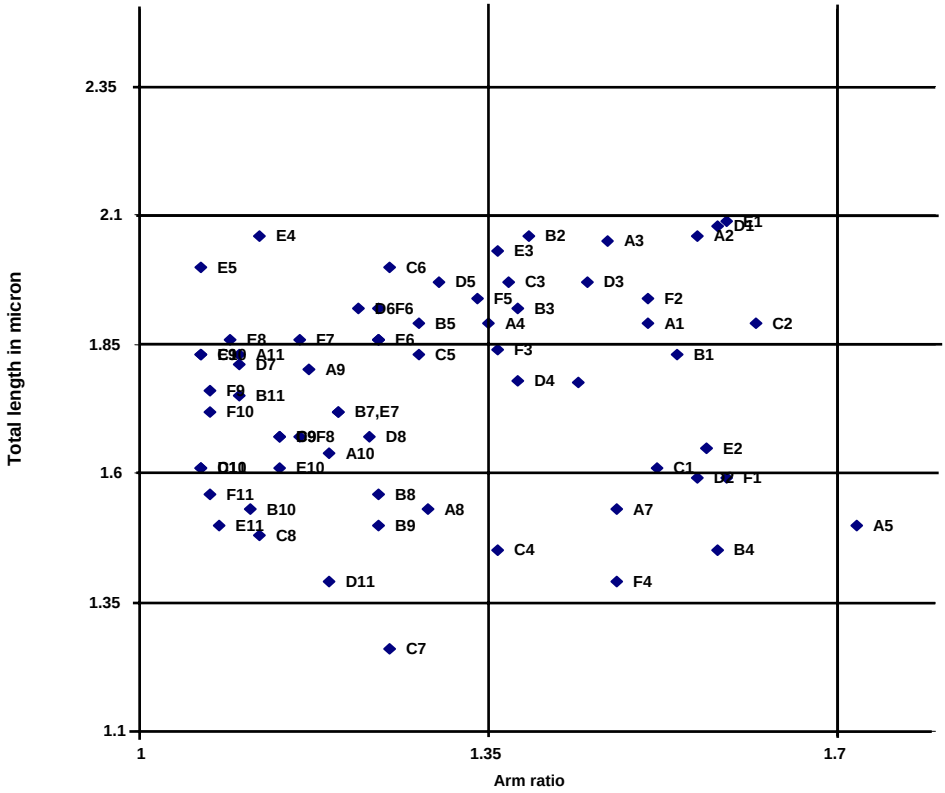
All chromosomes in the six haploid complements were classified in different morphological categories, based on total length and arm ratio within the length classes (Table 4, column 1-3). This was referred to as a second order classification. The class interval of 0.25μ for the length attribute was taken arbitrarily but of convenience, similarly the arm ratio ranges was divided in suitable classes as found convenience. This classification was superimposed on the scatter diagram of the haploid complements as a grid of length and arm ratio classes (Fig. 5). The unidentified chromosomes were distributed to the various morphological categories using probabilistic inferences on:

1. Chromosome frequency in a given class per haploid set (column 4 in Table 4);
2. Occurrence of points in the combined scatter diagram (Fig. 5); and
3. The examination of the original data on total length and arm ratio of the chromosomes

The number of unidentified chromosomes allocated to various second-order classes is shown in column 6 of Table 4. All chromosomes in the haploid complement, both identified as well as unidentified, were numbered from 1 to 11 (column 8 in Table 4) beginning with the longest one. Within each length class, chromosomes were numbered in the increasing order of arm ratio. Subsequently, identity of each chromosome will be indicated using the serial number assigned at this time (column 8 in Table 4).

Locations of the chromosomes are shown in Fig. 5 by solid boxes for the individually identified ones and open boxes for the unidentified ones in both the genotypes, respectively. The serial number of each chromosome and the specific name for the identified ones are shown beside the boxes. The symbols for the identified chromosomes were positioned in the figure at the mean locations determined earlier (Fig. 5). The symbols for the remaining unidentified chromosomes of both the genotypes were positioned arbitrarily but in a convenient manner within the respective class boundaries and do not indicate any specific value for length and arm ratio on an individual chromosome basis.

A.



B.

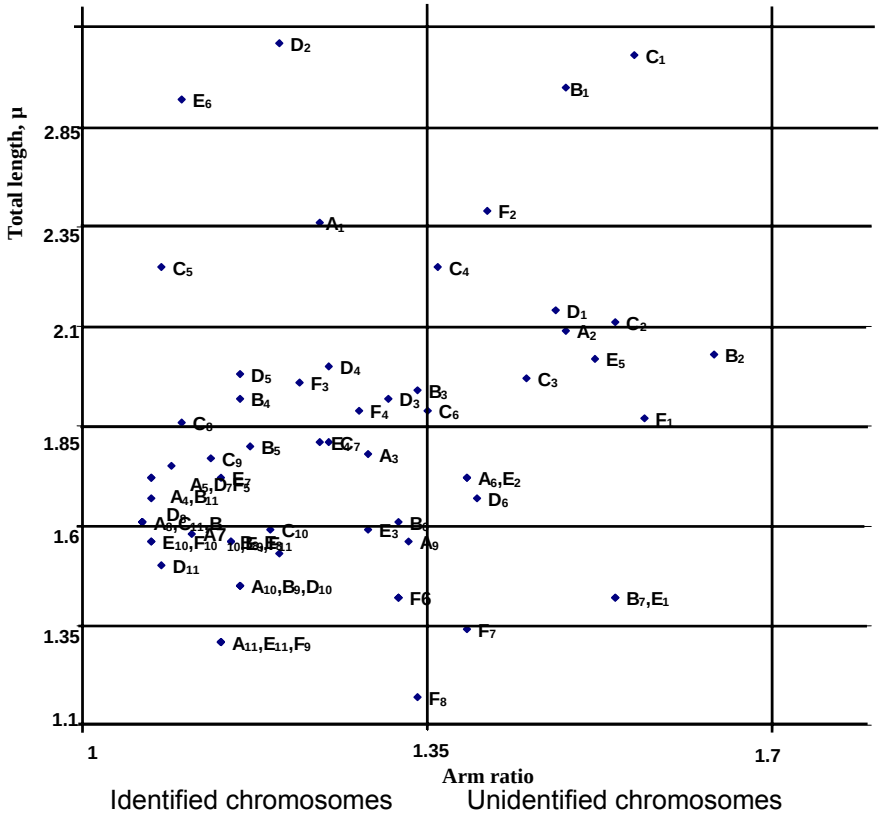


Fig. 5. Location of chromosomes of A) Lignosus bean (Trinidad) and B) Lablab bean in a two dimensional array, based on total length and arm ratio

Standard karyotype for Lignosus bean consisted of three metacentric (M1, M2 and M3) chromosomes as identified individually based on morphological measurements in total length and arm ratio. The remaining eight unidentified chromosomes were m type as characterized on probability basis. Five (M1, M2, M3, M4 and M5) individual chromosomes of the haploid set were individually identified in country bean based on morphological measurements in total length and arm ratio. All identified chromosomes were m type. The remaining six unidentified chromosomes were m type as characterized on probability basis. All chromosomes of the haploid complement were numbered serially.

Though general composition of the haploid karyotype in the studied material was same, yet some variation existed in respect of type, length and arm ratio of the individual chromosome (Table 4). Thus it is apparent that some degree of karyotypic variation was occurred in the studied materials which could be due to structural changes of some chromosomes. This detailed karyotypic information of the studied material could be helpful in their genetic studies as well as improvement programs.

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