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Evolutionary Pattern and Chromosomal Divergence in Order to Visualize the Mitotic Metaphase within *Vicia faba*

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ABSTRACT

Twelve cultivars of Vicia faba were analysed in order to visualize the chromosomal divergence in mitotic metaphase during the course of present investigation. Results have shown that some remarkable variations exist at the intraspecific level due to genetic divergence within the gene pool of Vicia faba. Chromosomal studies provide foundation upon which systematic conclusions are based and without exploitation of its full potential a meaningful hypothesis of evolution con not possible be created.

Key words: *Chromosomal divergence, Mitotic metaphase, Evolutionary pattern, Vicia faba*

INTRODUCTION

Vicia faba, a green lequme of the family Fabaceae has tremendous potential to be used as untraditional pulse crop. It has large number of cultivars grown on a wide scale world over. Evaluation of genetic divergence within the gene pool at the intraspecific level is not always an easy task. Several parameters are used to scale level of intraspecific variations and many such parameters do not provide sufficient clue[1,2,3]. Cytology provides anchor sheet role in genomic studies. Earlier, some very lucid advancement has been made in this field, which has very prominent mark in our understanding in chromosomal evolution[4,5,6,7,8,9,10]. This has been substantiated through the "Evolution of Genetic System" by Darlington[11], "New Systematic" by Huxley[12], "Genetics and Origin of Species" by Dobzhansky[13] and "Variations and Evolution in Plants" by Stebbins[14]. The karyotype concept offered significant push to cytology and the informations procured serve as a very efficient and reliable tool in analyzing phylogenetic distances and origin of species[11,15,16,17].

In the present investigation, some cultivars of *Vicia faba* have been selected for the study of evolutionary pattern and chromosomal divergence during mitotic metaphase.

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MATERIAL AND METHODS

In order to assess chromosomal divergence within the *Vicia faba*, it was pertinent to crave for mitotic chromosome. For this, dry pure-line seeds of selected cultivas were tried for mitotic metaphase screening. The somatic chromosomal study has been carried out in the root tips of the germinated seeds.

Seeds of the selected cultivars were treated with 0.1% mercuric chloride solution for 2 to 3 minutes, washed thoroughly in distilled water and finally allowed to germinate in an incubator at Whatman fitter paper moistened with distilled water. In order to accelerate cell division frequency, 30 minutes of light treatment was given under a five hundred-watt electric bulb before primary root-excision was done. Excised root-tips were thoroughly washed in distilled water and were pretreated in para dichloro benzene (PDB) concentrated solution for 90 minutes at 4^0C.

Then the root-tips were again washed in distilled water and fixed in freshly prepared 1:3(V/V) acetic acid-ethanol. A little pinch of ferric chloride was added to it. Root-tips were then stained in 2% acetocarime and squashed in 45% acetic acid. Camera lucida drawing was prepared from the appropriate metaphase stage and finally micro-photographs were taken using to eyepieces (10*x* or 15*x*) and oil emersion objective (100*x*). The preparations were made permanent by passing the temporary slides through acetic acid butanol series. Chromosomal measurements were done from the temporary preparations using ocular and stage micrometer.

Depending upon the length of chromosome, position of centromere, length of short and long arms during mitotic metaphase, following chromosomal parameters were taken into account.

Relative length

This is the ratio in percentage to the length of the individual chromosomes to that of the largest chromosomes. This is calculated as:

$$\frac{\text{Length of chromosome}}{\text{Length of largest chromosome}} \text{x}100$$

F percentage

This represents the percentage of the short arm length and the entire chromosomal length. Hence, F percentage is calculated as:

$$\text{F\%} = \frac{\text{Short arm length of chromosome}}{\text{Total length of the chromosome}} \text{x}100$$

TF Percentage

This is another parameter which represents ratio of the percentage of the sum total of short arm length to that of the sum total of the chromosome length. Hence, it is calculated as:

$$\text{TF\%} = \frac{\text{Total sum of the short arm length}}{\text{Sum of the chromosome length}} \text{x}100$$

For total chromatin length, the whole chromosomal compliment of the mitotic metaphase has been measured in μ and added together.

Using the above chromosomal informations, karyotypic formulae of the selected taxa have been prepared.

Abbreviations used stand as follows

Abbreviations	Stands for
SC	Secondary constriction
M	Median
Sm	Sub-median
St	Sub-terminal

RESULTS AND DISCUSSION

During the course of present investigation twelve cultivars of *Vicia faba* have been studied. Mitotic metaphase from the rool-tips have been observed. Details of different mitotic parameters have been taken into notice. Results have been depicted in Table 2 – 13. Chromosomal features of mitotic

metaphase in selected cultivars are discussed hereunder.

i. ***Vicia faba* cultivar DHB – 94**

The somatic number of this taxon is found to be 2n=12. The total Chromatin length was measured to be 145.04µ and TF percentage has been recorded to be 20.83. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 BST + 1CST

ii. ***Vicia faba* cultivar DHB – 95**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 133.74µ and TF percentage has been recorded to be 20.33. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 CST + 1CST

iii. ***Vicia faba* cultivar DHB – 96**

The somatic number of this taxon is found to be 2n = 12. The total chromatin length was recorded to be 134.72µ and TF percentage has been recorded to be 20.47. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 CST + 1CST

iv. ***Vicia faba* cultivar DHB – 97**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 134.38µ and TF percentage has been recorded to be 20.41. One pair of chromosome was sub-matacetric and had satellite chromosome. The karyotypic formulae of this cultivar is found to be

1ASM + 2 BST + 2 CST + 1CST

v. ***Vicia faba* cultivar DHB – 98**

The somatic number of this taxon is found to be 2n = 12. The total chromatin length was measured to be 132.32µ and TF percentage has been recorded to be 19, 98. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 CST + 2 CST + 1CST

vi. ***Vicia faba* cultivar DHB – 99**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 139. 16µ and TF percentage has been recorded to be 21.35. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be.

1ASM + 2 BST + 2 CST + 1CST

vii. ***Vicia faba* cultivar DHB – 100**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 141.30µ and TF percentage has been recorded to be 21.12. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 CST + 1CST

viii. ***Vicia faba* cultivar HVI**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 143.86µ and TF percentage has been recorded to be 20.82. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 BST + 1CST

ix. ***Vicia faba* cultivar – HV2**

The somatic number of this taxon is found to be 2n=12. The total chromatin length was measured to be 133.10µ and TF percentage has been recorded to be 22.11. One pair of chromosome was sub-metacentric and had satellite chromosome. The

karyotypic formulae has been found to be

1ASM + 2 BST + 2 CST + 1CST

x. ***Vicia faba* cultivar JV2**

The somatic number of this taxon is found to be 2n=14. The total chromatin length was measured to be 152.76μ and TF percentage has been recorded to be 21.50. One pair of chromosome was sub-metacentric and had satellite chromosome. The Karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 CST + 1CST + 1CM

xi. ***Vicia faba* cultivar Bhagalpur local**

The somatic number of this taxon is found to be 2n=14. The total chromatin length was measured to be 141.06μ and TF percentage has been recorded to be 20.86. One pair of chromosome was sub-metacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 CST + 2 CST + 1CST + 1DM

xii. ***Vicia faba* cultivar Ranchi local**

The somatic number of this taxon is found to be 2n=14. The total chromatin length was measured to be 146.16μ and TF percentage has been recorded to be 19.18. One pair of chromosome was sub-meatacentric and had satellite chromosome. The karyotypic formulae of this cultivar has been found to be

1ASM + 2 BST + 2 CST + 2 CST + 1DM

Table 1: Germ plasma of *Vicia faba* under investigation

Sl. No.	Cultivars	Source	Remark
1	DHB – 94	Agriculture college Dohli, Pusa, Samastipur	Seasonal Rabi Crop.
2	DHB – 95	"	"
3	DHB – 96	"	"
4	DHB – 97	"	"
5	DHB – 98	"	"
6	DHB – 99	"	"
7	DHB – 100	"	"
8	H.V. – 1	Hisar Agriculture University	"
9	H.V. – 2	"	"
10	J.V. – 2	"	"
11	Ranchi local	Birsa Agriculture University, Ranchi	In water stressed condition
12	Bhagalpur local	Agriculture College, Sabour	"

Table 2: Somatic chromosome analysis in *Vica faba* var. DHB-94 (2n=12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	10.24	7.88	2.5	20.62	1.00	38.21	SM
B	2	9.26	1.50		10.76	52.18	13.94	ST
B	2	8.96	1.42		10.38	50.33	13.68	ST
C	1	7.94	1.68		9.62	4.65	17.46	ST

Total choromatin length = 145.044μ
TF % = 20.83
Karyotypic formula = 1ASM + 2B ST + 2B ST + 1CST

Table 3: Somatic chromosome analysis in *Vicia faba* var. DHB-95 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.58	7.66	2.34	19.58	1.00	39.12	SM
B	2	8.90	1.15		10.05	0.51	11.44	ST
C	2	8.05	1.08		9.13	0.48	11.82	ST
C	1	7.45	1.46		8.93	0.45	16.57	ST

Total choromatin length = 133.74μ
TF % = 20.33
Karyotypic formula = 1ASM + 2B ST + 2C ST + 1C ST

Table 4: Somatic chromosome analysis in *Vicia faba* var. DHB-96 (2n=12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.62	7.65	2.42	19.69	1.00		SM
B	2	8.92	1.22		10.14	0.51	12.44	ST
C	2	8.12	1.08		9.20	0.46	11.82	ST
C	1	7.45	1.54		8.93	0.45	17.57	ST

Total choromatin length = 134.20μ
TF % = 20.47
Karyotypic formula = 1A SM + 2B ST + 2C ST + 1C ST

Table 5: Somatic chromosome analysis in *Vicia faba* var. DHB-97 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.10	7.62	2.35	19.57	1.00	38.93	SM
B	2	3.92	1.20		10.12	0.51	11.85	ST
C	2	8.10	1.10		9.20	0.47	11.95	ST
C	1	7.48	1.50		8.98	0.46	16.70	ST

Total choromatin length = 134.38μ
TF % = 20.41
Karyotypic formula = 1A SM + 2BST + 2CST + 1CST

Table 6: Somatic chromosome analysis in *Vicia faba* var. DHB-98 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.52	7.60	2.30	19.42	1.00	39.13	SM
C	2	8.86	1.08		9.94	0.52	10.86	ST
C	2	8.00	1.02		9.02	0.46	11.30	ST
C	1	7.40	1.42		8.82	0.45	16.09	ST

Total choromatin length = 132.32μ
TF % = 19.98
Karyotypic formula = 1A SM + 2C ST + 2C ST + 1C ST

Table 7: Somatic chromosome analysis in *Vicia faba* var. DHB-99 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.72	7.75	2.48	19.95	1.00	38.84	SM
B	2	8.98	1.42		10.40	0.52	13.65	ST
C	2	8.35	1.26		9.61	0.48	13.11	ST
C	1	7.86	1.76		9.60	0.48	18.21	ST

Total choromatin length = 139.16μ
TF % = 21.35
Karyotypic formula = 1A SM + 2B (ST) + 2C ST + 1C ST

Table 8: Somatic chromosome analysis in *Vicia faba* var. DHB-100 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.72	7.75	2.48	19.95	1.00	38.84	SM
B	2	8.62	1.40		10.09	50.57	13.87	ST
C	2	8.42	1.36		9.78	49.02	13.90	ST
C	1	7.64	1.66		9.03	46.61	17.84	ST

Total choromatin length = 141.30μ
TF % = 21.12
Karyotypic formula = 1A SM + 2B ST + 2C ST + 1C ST

Table 9: Somatic chromosome analysis in *Vicia faba* var. HV 1 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.98	6.59	2.46	19.05	1.00	34.59	SM
B	2	9.26	1.98		11.24	59.00	17.61	ST
B	2	8.84	1.48		10.64	55.85	13.90	ST
C	1	7.48	1.64		9.12	47.87	17.98	ST

Total choromatin length = 143.86μ
TF % = 20.02
Karyotypic formula = 1A SM + 2B ST + 2B ST + 1C ST

Table 10: Somatic chromosome analysis in *Vicia faba* var. HV 2 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.46	6.61	2.64	18.31	1.00	33.91	SM
B	2	8.48	1.75		10.23	55.87	17.10	ST
B	2	7.92	1.66		9.58	52.32	17.32	ST
C	1	6.88	1.74		8.62	47.07	20.18	ST

Total choromatin length = 133.01μ
TF % = 22.11
Karyotypic formula = IA SM + 2B ST + 2C ST + 1C ST

Table11: Somatic chromosome analysis in *Vicia faba* var. JV 2 (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.66	6.84	2.26	18.76	1.00	36.46	SM
B	2	8.48	1.68		10.16	54.15	16.53	ST
C	2	8.22	1.72		9.94	52.98	17.30	ST
C	1	7.64	1.46		9.10	48.50	16.04	ST
C	1	6.66	1.42		8.30	44.24	17.10	M

Total choromatin length = 152.76μ
TF % = 21.50
Karyotypic formula = 1A SM + 2B ST + 2C ST + 1C ST + 1CM

Table 12: Somatic chromosome analysis in *Vicia faba* var. Ranchi local (2n = 12)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μ)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	9.69	6.20	2.35	18.34	1.00	33.8	SM
B	2	8.75	1.28		10.03	54.68	12.76	ST
C	2	8.26	1.22		9.48	51.69	12.86	ST
C	1	6.66	1.56		8.22	44.82	18.97	ST
D	1	6.24	1.26		7.05	40.89	16.8	M

Total choromatin length = 146.16μ
TF % = 19.18
Karyotypic formula = 1A SM + 2B ST + 2C ST + 2C ST + 1DM

Table 13: Somatic chromosome analysis in *Vicia faba* var. Bhagalpur local (2n = 14)

Type of chromosome	No. of pair	Length of component arms (in μ)			Total length of choromosome (in μu)	Relative length of chromosome	Centromeric index (F%)	Position of centromere
		Long	Short	Sat				
A	1	8.94	5.88	2.25	17.07	1.00	34.44	SM
C	2	8.64	1.26		9.90	57.99	12.72	ST
C	2	7.48	1.74		9.22	54.01	18.87	ST
C	1	6.88	1.62		8.50	49.79	19.05	ST
D	1	6.24	1.22		7.44	43.58	16.39	M

Total choromatin length = 141.06μ

TF % = 20.86

Karyotypic formula = 1A SM + 2C ST + 2C ST + 1C ST + 1DM

In karyomorphological analysis some parameters like total chromatin length, TF percentage, F% karyotypic formula have been said to enormous phylogenetic significance of the twelve cultivars selected. Another parameters have been total chromatin length, the highest length of chromatin have been found to be 152.76u in cultivar JV_2 and the smallest value (132.32μ) came in DHB – 98. It is interesting to note that the total chromatin length in Bhagalpur local is 141.06μ inspite of having 2n=14. Perusal of the tables showing karyomorphological data suggested that increase in chromosome number is not always accompanied by enhancement in total chromatin length. This is for the relevance by the observation that the cultivars DHB – 94 with 2n=12 has more chromatin length than Bhagalpur local cultivar which has 2n=14. This wide range of differences in the total

chromatin length among the cultivars might be due to various genetic reasons and factors such as deletion, duplication, translocation and inversion. The taxon with lesser chromatin may be the product of deletion that too in heterochromatic part. Such taxa can be thought to be more involved in comparison to others because they have extricated extra genetic burden.

Upadhyay[18] has stressed upon the role of heterochromatin in determining the chromosomal aberrations and role of heterochromatic segments during the course of evolution of the speices. Yet another reason behind the differences in the chromosome length of related taxa may be due to gene duplicity either through random fashion or through polyteny[14]. But the total chromatin length itself does not bear the conclusive responsibility while assessing evolutionary status of the species, since it does not prelude the possibility of the other factors operating within the genus. Therefore, it must be born in mind that this information may not be basic but of supplementary value in the service of taxanomy.

The highest TF% (22.11) was noticed in cultivar HV_2. Among the cultivars, the high T.F.% come in cultivars like JV 2 (21.50) followed by DHB-99 (21.35) and DHB-100(21.12). The lowest T.F.% was recorded in Ranchi local (19.18), DHB-97 (20.41) and Bhagalpur local (20.86), the rest values come between these two levels in different cultivars. On the basis of these parameters, some phylogenetic trends can be derived, inspite of very narrow range of deviation in the TF.

An evaluation of karyotypic formulae and T.F.% if taken together in the selected cultivars of *Vicia faba*, it appears that evolution within the species is operative in accordance with the following trends.

a) From larger to smaller chromosomes which has been resulted after elimination of the extra or excess inert part of chromatin.
b) From median to sub-median and finally to sub-terminal centromere and
c) Both a and b has laid to symmetrical to little more asymmetrical types of karyotypes.

CONCLUSION

Since, *Vicia faba* has undergone a very long period of domestication, hence, the evolutionary operations within the genus has been greatly influenced by man's interference and the taxon as assumed a particular shape. The real problem faced by geneticists and plant breeders in improvement of pulses has been a very narrow range of variations among them. It was, therefore, thought productive to visualize the range of variations present at the intra specific level in *Vicia faba*.

Chromosomal informations reveal more reliable and explicit picture of genetic diversity and these information have been utilized by the systematists with profound confidence in classifications. Chromosomal studies, therefore, provide solid foundation upon which systematic conclusions are based and that without exploiting these informations a meaningful hypothesis on evolution can not possible be derived. The genetic divergence ultimately leads to biochemical divergence and this very aspect has given birth to a very formidable branch of systematic called biochemical systematic of chemosystematics.

Quality of informations obtained through the present investigation are of course more or less of basic nature but definitely, these carry tremendous applied value in future breeding programmes on legums, which does carry great potential to provide helping hand in the improvement of pulses. This is indeed very urgent to user a "yellow revolution" in our country.

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