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RESEARCH ARTICLE

PHYLOGENETIC STUDIES TO VISUALIZE MEIOTIC ABERRATIONS IN SOME CULTIVARS OF *VICIA FABA*

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ABSTRACT

Twelve cultivars of *Vicia faba* have been obtained from seed banks of different research stations for chromosomal studies during meiotic operations. Results have shown that exist some remarkable variations at the intraspecific level in order to visualize the genetic divergence. Meiotic informations provide aglimpses of gene homology indicating phylogenetic relationship between the taxa.

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INTRODUCTION

Vicia faba, an important legume of the family Fabaceae, is put to cultivation on increasing scale throughout the world (Harten, 1970). A thorough genomic survey of the cultivars have not been done so far and that only fragmentary informations are available. Evaluation of genetic divergence within the genepool at the intra-specific level is not always an easy task. Several parameters are used to scale level of intra-specific variations, and many such parameters do not provide sufficient clue (Upadhyaya and Sinha, 1992; Wen, 2008; Wang *et al.,* 2009; Ray and Georges, 2010; Tamura *et al.,* 2011). Indepth studies of food legumes are of only recent beginning (Upadhyaya, 1985; Mishra and Chourasia, 2017a, b). An excellent compendium published by Kaplan (1965) on phaseolus brans archaeology and domestication is an extraordinary attempt in this regard. Gene-evolutionary changes has undergone and put constant pressure on domestication. The other factors influencing have been the time and location of the domestication (Kumari, 1988; Roy, 1988; Upadhyaya, 1996). Evaluation of genomic differences is a prelude in assessing the narrow range of variation prevalent among the pulses. It would be very imperative to place the view in words of Harten (1970) "The study of the origin and evolution of cultivated plants is not only a work of intellectual and academic interest to the evolutionist, but a matter of enormous practical urgency in those crops that carry the burden of supporting the human population. Furthermore, a synthetic approach appears to be imperative in order to visualize the genomic differences at the cultivar level in *Vicia faba*. This would provide the grandeur and the impetus to screen the extent of down flow and the tempo of evolution within *Vicia faba;* so the detailed biosystematic perception was failed. For certain individual cultivar, some cytological studies have been done earlier by workers like Johnston *et al.* (1999), Torres *et al.*(2010), Alghamdi *et al.* (2012), Kaur *et al.* (2014) and Caracuta *et al.* (2016). In a changed cytological perception with the refinements in tool and techniques, Jackson (1984) has recommended some chromosomal informations to be useful in a biosystematic studies. These informations include basic and somatic chromosome number, relative or actual length of metaphase chromosomes arm ratios and differential staining regions if present, number of meiocytes analysed at each stage, synapsis as observed at pachytene, number and frequency of chiasma per cell and any differences among the meiotic configurations, univalent frequency, diakinesis or metaphase I, early anaphase I disjunction patterns, chromosome behaviour at anaphase I and anaphase II and pollen stainability. According to Garber (1958) chiasma frequency per chromosome yields meaningful picture indicating phylogenetic relationship between the taxa. Meiotic informations are of cryptic nature with enormous application in evolutionary studies. Stace (1984) has rightly been said that "The earlier genomic

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informations were of mere anatomical nature, number of chromosomes, their dimensions can just be utilised for taxonomic purpose like morphological traits". With the advancement in the stained technology and optics, it has now been possible to analyse the genomes in terms of nuclear DNA value. The DNA quantity per nucleus thus measured provided the genome size of the taxa analysed. The importance of nuclear DNA values in taxonomy has efficiently been suggested by some of the recent studies in various angiosperms, viz. *Lathyrus* sp. (Seal and Rees, 1982), *Arachis* sp. (Dhillon and Mikschi, 1982), *Vicia* sp. (Raina and Rees, 1988) and *Daucus* sp. (Upadhyaya, 1996). In the present investigation, phylogenetic studies have been made to visualize the meiotic aberrations in some selected cultivars of *Vicia faba*.

MATERIALS AND METHODS

In order to assess chromosomal divergence within the *Vicia faba*, it was pertinent to crave for meiotic chromosome. For this, pure line seeds of twelve cultivars of *Vicia faba* have been procured with the courtesy of Agriculture College, Dholi, Pusa; Hisar Agriculture University, Birsa Agriculture University, Ranchi and Agriculture College, Sabour, Bhagalpur. The seeds were germinated in separate Petri dishes lined with moistened filter papers at the room temperature 26 ± 5°C. The seedlings were transferred to the experimental plots of the Botany Department of T.N.B college, T. M. Bhagalpur University, Bhagalpur. Plants were allowed to grow for 85- 95 days after sowing and flowering started in different cultivars. Flowers of different cultivars were collected in the morning between 09 - 9.30 a.m to procure better frequency of metaphase. The collected floral buds were fixed in a modified carboxy fluid (6 parts of ethanol, 3 parts of glacial acetic acid and 1 part of chloroform). A trace of saturated aquous solution of ferric chloride was also added to the fixative. The fixation was done for 20hrs. At the time of study the buds were thoroughly washed in distilled water and squashed in 2% acetocarmine solution. The chromosomal configuration at meiotic metaphase and their subsequent behavior in the later stages were studied in terms of different meiotic parameters from the temporary slides. The slides were micro-photographed and finally they were made permanent by the tertiary butyl alcohol series technique (Mishra, 2000).

RESULTS AND DISCUSSION

Chromosomal behaviour of meiosis yields some very valuable informations in establishing species resleationship. So, this gives an idea of phylogeny and informations have been used for taxonomic categorizations. With the intention to analyse the extent of gene flow and to focus attention on the tempo and direction in the evolution within the genus *Vicia faba* of twelve cultivars have been analysed. The details of meiotic informations specially the chromosomal behaviour have been presented in Table 1. The data included some established parameters selected for the purpose. The further details are discussed here under.

1. *Vicia faba* DHB - 94

The meiotic operation in this cultivar has been found to be quite smooth and ongoing. The mean number of bivalents has been considered and it was found to be 6 and regular ones. Of these 4.96 were of ring type and 1.1 were of rod-type. The mean number of chiasma in this cultivar has been found to be 20.25 and the range has been 19-21. The no. of chiasmata per bivalent was recorded as 3.61 and terminalization co-efficient of 0.52. The pollen sterility was recorded to be 2.4.

Table 1. Meiotic analysis in 12 cultivars of *Vicia faba*

Cultivars	No of meiocytes observed	Mean No of Divalent / meiocytes			Mean chiasma / meiocytes		Terminalized Xta / meiocyte	No. of Xta per bivalent	Termina-lization coefficient
		Ring	Rod	Total	Range	Mean			
DHB - 94	50	4.96 ± 0.38	1.1 ± 0.21	6	19-21	20.25 ± 0.34	10.6 ± 0.41	3.61 ± 0.39	0.52
DHB – 95	50	4.90 ± 0.38	1.1 ± 0.22	6	19-22	20.25 ± 0.42	10.8 ± 0.40	3.74 ± 0.38	0.51
DHB – 96	50	4.80 ± 0.23	1.2 ± 0.22	6	18-22	20.25 ± 0.36	10.5 ± 0.38	3.71 ± 0.36	0.51
DHB – 97	50	4.80 ± 0.21	1.2 ± 0.22	6	18-22	20.25 ± 0.38	10.5 ± 0.36	3.66 ± 0.38	0.51
DHB – 98	50	4.70 ± 0.21	1.2 ± 0.21	6	18-22	20.25 ± 0.35	10.6 ± 0.35	3.68 ± 0.39	0.51
DHB – 99	50	4.70 ± 0.27	1.5 ± 0.31	6	18-22	20.25 ± 0.42	10.6 ± 0.34	3.43 ± 0.36	0.52
DHB – 100	50	4.70 ± 0.23	1.3 ± 0.32	6	18-22	18.25 ± 0.36	10.7 ± 0.26	3.41 ± 0.35	0.56
HV – 1	50	4.60 ± 0.25	1.4 ± 0.19	6	19-21	20.25 ± 0.21	10.8 ± 0.36	3.38 ± 0.38	0.54
HV – 2	50	4.60 ± 0.28	1.4 ± 0.18	6	14-16	15.15 ± 0.28	10.5 ± 0.38	3.38 ± 0.18	0.68
JV – 2	50	4.70 ± 0.24	2.3 ± 0.23	7	18-22	18.75 ± 0.35	10.6 ± 0.28	3.36 ± 0.36	0.56
Ranchi Local	50	4.60 ± 0.24	2.5 ± 0.16	7	14-16	15.15 ± 0.16	12.2 ± 0.18	2.31 ± 0.26	0.78
Bhag. Local	50	4.40 ± 0.26	2.6 ± 0.16	7	14-16	15.15 ± 0.18	12.6 ± 0.21	2.32 ± 0.28	0.80

Table 2. Percentage meiotic abnormalities in 12 cultivars of *Vicia faba*

Cultivars	No. of PMC analysed	Diakinesis		Metaphase chromatin bridge	Anaphase	Laggurds	Anaphase chromatin bridge	Telophase multipolar separation	Pollen sterility
		Accessory nucleoli	Precocious separation						
DHB - 94	50	-	-	-	-	7	-	-	2.4
DHB – 95	50	1.5	1.0	1.2	2.1	-	-	-	5.2
DHB – 96	50	-	-	-	-	-	-	-	5.1
DHB – 97	50	-	-	-	-	-	-	-	6.2
DHB – 98	50	-	-	-	-	-	-	-	7.2
DHB – 99	50	-	-	2.1	1.8	-	1	-	11.8
DHB – 100	50	1.5	2.1	-	1.4	-	-	-	10.6
HV – 1	50	1.0	-	-	-	-	-	-	5.6
HV – 2	50	-	-	-	-	-	-	-	4.8
JV – 2	50	-	-	-	-	-	-	-	2.5
Ranchi Local	50	3.2	2.8	-	2.8	1.1	-	-	12.6
Bhag. Local	50	2.6	3.2	1.2	1.2	-	-	-	14.4

2.*Vicia faba* DHB - 95

This is another cultivar of DHB series where normal 6 bivalents have been found to occur at meiotic metaphase, of these 4.9 were of ring type and 1.1 were of rod-tpe. The meiosis here also was normal with pollen sterility recorded to be 5.2. The range of chiasmata in the genome has been 19-22 with mean of 20.25 like DHB 94. Terminalized chiasma per meiocyte has been found to be 10.8 and number of chiasmata per bivalent was recorded to be 3.74 with a terminalization co-efficient of 0.51.

3. *Vicia fabva* DHB - 96

This is another cultivar of DHB series where normal 6 bivalent has been found to occur at meiotic metaphase. Of these 4.8 were of ring type and 1.2 were of rod-type. The meiois here also was normal with pollen sterility found to be 5%. The range of chiasmata in the genome has been 18-22 with mean of 20.25 like DHB 94 and 95. Terminalized chiasma per meiocytes have been found to be 10.5 and no. of chiasmata per bivalent was recorded to be 3.71 with a terminalization co-efficeint of 0.5)

4.*Vicia faba* DHB - 97

This is another cultivar of DHB series where normal 6 bivalent has been found to occur at meiotic metaphase, of these 4.8 were of ring type and 1.2 pair of rod-type. The meiosis here also was normal with pollen sterility recorded to be 6.2. The range of chiasmata in the genome has been 18-22 with mean of 20.25. Terminalized chiasma per meiocyte has been found to be 10.5 and number of chiasmata per bivalent was recorded to be 3.66 with a terminalization co-efficient of 0.51.

5. *Vicia faba* DHB - 98

The meiotic operation in this cultivar has been found to be quite smooth and on going. The mean number of bivalent has been considered and it was found to be 6, of these 4.7 were of ring type and 1.2 were of rod-type. The mean number of chiasma in this cultivar has been found to be 20.25 and the range has been 18-22. The number of chiasmata per bivalent was recorded 3.68 with a termination coefficient of 0.51. The meiosis here also was normal with pollen sterility recorded to be 7.2.

6.*Vicia faba* DHB - 99

This is another cultivar of DHB-series where normal 6 bivalent has been found to occur at meiotic metaphase, of these 4.7 were of ring type and 1.5 pair of rod-type. The meiosis here also was normal with pollen sterility recorded to be 11.8. The range of chiasmata in the genome has been 18-22 with mean of 20.25. Terminalized chiasma per meiocytes have been found to be 10.6 and number of chiasmata per bivalent was recorded of be 3.43 with a terminalization co-efficient of 0.52.

7.*Vicia faha* DHB - 100

This cultivar is also a series of DHB where normal 6 bivalents have been recorded to occur during meiotic metaphase, of these 4.7 were of ring type and 1.3 pair of rod type. The meiosis here also was normal with pollen sterility recorded to be 10.6. The range of chiasmata in the genome has been 18-22 with mean of 18.25. Terminalized chiasma per meiocytes have been found to be 10.7 and number of chiasmata per bivalent was recorded to be 3.41 with a terminalization co-efficient of 0.56.

8. *Vicia faba* HV-1

This cultivar has shown formation of 6 normal bivalent during the meiotic metaphase. The mean number of ring bivalent per meiocyte has been found to be 4.6 and that of rod bivalent 1.4. The mean chiasma formation per meiocytes has been calculated to be 20.25 with a range of 19-21. The value of terminalized chiasmata per meiocyte has been found to be 10.8 and there has been 3.38 chiasma per meiocyte bivalent and termination co-efficient was recorded to be 0.54. Meiosis has been by and large normal bearing a appearance of accessory nucleolus in few cases. As a result of the smooth meiotic operation pollen fertility has been found to be quite high (94.40%).

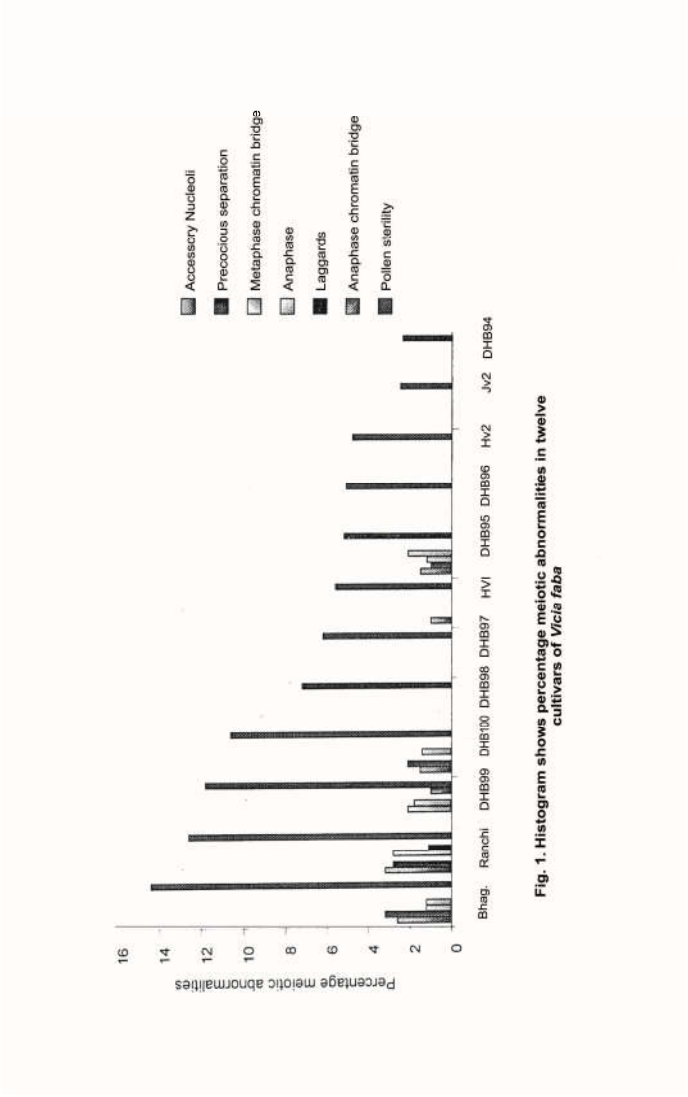

Fig. 1. Histogram shows percentage meiotic abnormalities in twelve cultivars of *Vicia faba*

9.*Vicia faba* HV - 2

In this cultivar also there has been presence of 6 normal bivalent during meiotic metaphase. The mean of ring and rod bivalents have been in the line of record of cultivar HV_1. But, here, mean of chiasma per meiocyte was quite low i.e. 15.15%. Terminalized chiasmata per meiocyte was found to be 10.5, number of chiasmata per bivalent here again was found to be 3.38 but the terminalization co-efficient here was little higher i.e. 0.68. This description is again a testimony of normal meiosis leading to very high pollen fertility of 95.2%.

10.*Vicia faba* JV-2

This is a cultivar with a somatic number 2n=14. As such there has been presence of 7 normal bivalent during meiotic metaphase. The number of ring bivalent is approximately double of the number of rod bivalent. Mean chiasma per meiocyte was found to be 10.6. The number of chiasmata per divalent was 3.36 and terminalization co-efficient was found to be 0.56.This cultivar particularly has shown the highest pollen fertility percentage (97.5) which is an indication of very smooth and fluent meiotic operation.

11. *Vicia faba* Ranchi Local

This cultivar is of local collection where normal 7 bivalents have been recorded to occur during meiotic metaphase. Of these bivalents, 4.6 were of ring type while 2.4 were of rod type. The range of chiasma in the genome has been found to be 14-16 with a mean value of 15.15. Terminalized chiasmata per meiocyte has been found to be 12.2 and number of chiasmata per bivalent came as 2.31 with a terminalization co-efficient of 0.78. This cultivar has shown some meiotic abnormalities as well. As per the record the percentage of accessory nucleoli, precocious separation, chromatin bridge at anaphase - 1 and laggurds were found to be 3.2, 2.8, 2.8 and 1.1, respectively. These meiotic abnormalities exhibited direct bearing on pollen fertility and here in this cultivar 12.6% of the pallen were sterile considering the genus where fertility percentage is very high. This is indeed a noticeable change.

12. *Vicia faba* Bhagalpur Local

This is the another local cultivar which is cultivated in and around the Bhagalpur city. The somatic number of this cultivar has been 2n =14. Hence, 7 normal bivalents were visible at meiotic metaphase. Of these, bivalent 4.4 were of ring type and 2.6 of rod type. The mean number of chiasmata in meiocytes has been 15.15 and terminalized chiasmata per meiocytes was recorded to be 12.6. Number of chiasma per bivalent has been 2.32 with a terminalization co-efficient value of 0.8. This local cultivar also marked by some abnormalities during meiosis. These include accessory nucleoli (2.6), precocious separation 3.2 and chromatin bridge at anaphase. Owing to these meiotic abnormality, this cultivar has shown comparatively the highest degree of pollen sterility which has been recorded to be 14.4%.

Meiotic studies become more rewarding in pulses where the range of variations accounted is very narrow. In the backdrop of this understanding, meiotic studies have been carried out is twelve cultivars of *Vicia faba* in order to evaluate the range of genetic divergence within the species. The cultivars screened during the course of present investigation has been found to possess base number either n= 6 or n = 7. In the meiocytes, therefore six and seven bivalents have been observed depending on taxon. Number of ring and rod bivalents have been observed. Number of ring and rod bivalents is one genetic parameter which gives clue about gene homology (Riley and Chapman, 1956). With this intentions, ring and rod bivalents were counted in the selected cultivars. It has been observed in all the selected cultivars that number of ring bivalents exceeded that of rod bivalents (twice or even more). High frequency of rods is indicative of genetic non-homology between the chromosomes. The number of ring and rod bivalents and other related chromosomal characteristics revealing meiotic operations have been included the Table 2. Chiasma frequency is another parameter which reveals the extent and force of gene reshuffling leading to new genetic recombinations. Less chiasma frequency is an indication of lesser gene mixing and higher chiasma frequency thus leads to high degree of gene reshuffling. On this ground in the terms of mean chiasma per meiocyte cultivars of DHB-series have high figure around mean value of 20. The local cultivars have lesser number of chiasma leading to a value of 15.15. The same trend persists when number of chiasmata per bivalent is visualized. The DHB-series here again have high value of six and plus while this figure comes to 2.31 and 2.32 for Ranchi local and Bhagalpur local, respectively. This data suggested that there has been less recombination or gene reshuffling in local cultivar in comparison to certified once developed by agriculture agencies and probably that is why these two cultivars are apparently morphologically different from the others. Result on meiotic studies further suggested that the operation is not very consistent and that certain percentage of abnormalities have been recorded in different cultivars. The percentage meiotic abnormalities have been presented in Figure 1. A perusal of tables and figure brings forth some following interesting results.

i. That meiotic operations in the selected cultivar is by and large very smooth and this reflects in terms of high pollen fertility.
ii. That some cultivars such as DHB-96, DHB-97, DHB-98, HV2 and JV2 have shown very smooth and consistent meiotic operations with very high percentage of pollen fertility reaching a maximum figure of 97.5% in JV2.
iii. That chromosomal behaviour in local cultivars during meiosis has been not that consistent and smooth showing an increased percentage of accessory nuclei, precocious separation of chromosome, anaphase bridges and legards. These abnomalities have sliced down pollen fertility in comparison to other cultivars.
iv. Inspite of the fact that the selected taxa are inbreeders but some accidental out crossing might had occurred in the past which has added some tinch of hybridity in the local cultivars with a marked dilution in the normal meiotic operations.
v. That occurrence of accessory nucleoli is often associated with the presence of secondary constrictions. Further, accidental outcrossing which atleast the local taxa might had experienced in the past during their course of evolution may be instrumental in the dispersion of nucleolar organizer which was left latent and finally become active under some environmental pressure resulting into appearance of multiple nucleoli (Rees, 1984).
vi. That precocious separation as evidenced in cultivars like Bhagalpur local, Ranchi local, DHB-100 and DHB-95 might be due to some extent of non-homology of genes.
vii. That apart from some marginal chromosomal abnormalities during meiosis, the taxa analysed have been found to be fairly stable cytologically and exhibit some degree of asymmetry is their karyotypes which speaks of its evoluionary dynamism and this may lead to speciation.

The monumental work carried out by Watson and Crick (1953) on the structure and function of genetic material (DNA) has thoroughly revolutionarized our understanding on genomic composition of species. DNA of an organism provides

tremendously vast scope for variations generated by base addition, substitution and deletion. Through Feulgen cytophotometry, it has now become possible to estimate genome size in terms of DNA-C value quite great deal of variations have been observed while making camparisons on this terms and this is what is called C-value paradox. Physical parameter of genome such as DNA amounts and perhaps average base composition and amount of satellite DNA, directly influences the phenotype, nuclear and cell volume, surface volume ratio and nuclear and cell cycle duration. A large genome means large nuclei, large cell and slow cell cycle. Minimum generation period in herbaceous plants can be positively correlated with genome size or DNA amount (Bennet and smith, 1976). The fact behind the evolutionary operations leading to speciation within a genus or species can proficiently known by looking into and by making comparative analysis of the chromosomal behaviour is the meiocysed of the taxa compared. This class of study would fit into the things which provides prudent and reliable clues while evolutionary status of the taxa meiotic informations have some definite indications in understanding the way and tempo of evolution leading to speciation. These intentions at the same time would provide some data which can hardly be procured through other sources while making a comparison. Variations have been found to be the strength guiding evolution and variations that can be of morphological, chemical or genetic nature. The genetic divergence ultimately leads to biochemical divergence and this very aspect has given birth to very formidable branch of systematics called biochemical systematics or chemosystematics. Chemosystematics has established with vast mass of data published and has led to a sober appraisal of the principle, practice and achievements of this young discipline. The chemical data are used on fairly increasing scale for confirming relationships already elucidated from various ecological and geographic relationships. Plant breeders sometimes rely on quantity production and ignore qualitative values. Under this backdrop some of the workers have earlier evaluated cultivars of some common pulses such as *Cajanus* (Kumar *et al.* 2016; Singh, *et al.,*2017) *Cicer* (Tripathi *et al.*, 2012), *Pheseolus* (Singh, 2013; Singh *et al.* 2014) and *Vigna* (Upadhyaya, 1985). Conclusively, it can be concluded that *Vicia faba* has been studied by the cytological studies yielded a wealth of informations revealing genetic architecture of the speices at the cultivar levels. The cytogenetic mechanism of cultivated species has been influenced by domestication. Appreciable variations within the species have been recorded at the level of nuclear DNA. The wealth of informations thus collected, though, are of basic nature but carry enormous practical utility in future breeding programmes. Our country after a successful "Green Revolution" most eagerly and urgently awaits a "Yellow Revolution" involving legumes. This is a dream specially for a country like India where people are vegetarian due to ethical and economic reasons.

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