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EVOLUTIONARY PATTERN AND CHEMICAL DIVERGENCE WITHIN *Vicia faba*

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Vicia faba is a cultivated legume showing appreciable quanta of genetic variation as expressed in large number of cultivatable varieties (cultivars). An attempt has been made to evaluate the range of chemical variation within the species selecting phenolics as the parameter. Result has shown that the inherent genetic differences manifest into divergence on record pictorises evolutionary pattern within the gene pool of *Vicia faba*.

INTRODUCTION

Vicia faba is an important legume of the tribe Vicieae of family Fabaceae. This taxon is put to cultivation on increased scale throughout the world (Harlon, 1956). Although genomic survey of the cultivars have not been done so far on a consolidated basis and only fragmentary informations are available. Furthermore, a synthetic approach appears to be imperative in order to visualize the genomic differences at the cultivar level in *Vicia faba*. That is why the tempo of evolution within *Vicia faba* was failed. In certain individual cultivars of *Vicia faba*, some cytological studies have been done which provide anchor sheet role in genomic studies.

Evolution leading to speciation works first at genic level than at the level of the biomolecules followed by organizational level, population level and other levels of higher taxonomic hierarchy. The value of chemical informations in understanding differences existing among the taxa is undepicted.

Table No. - 1

Germplasms of *Vicia faba* under investigation

Serial No	Cultivars	Source	Remarks
1	DHB - 94	Agriculture College Dholi, Pusa Samastipur	Seasonal Rabi crop.
2	DHB – 94	"	"
3	DHB – 94	"	"
4	DHB – 94	"	"
5	DHB – 94	"	"
6	DHB – 94	"	"
7	DHB – 94	"	"
8	H.V. – 1	Hisar Agriculture University	"
9	H.V. – 2	"	"
10	H.V. – 3	Agriculture College Dholi, Pusa Samastipur	In water stressed condition
11	Ranchi local	Birsa Agriculture University, Ranchi	"
12	Bhagalpur local	Agriculture College, Sabour	"

Gottleb (1980, 1982), Furguson (1980), Heywood (1984), Grant (1984) and Moore (1984) have obtained some promising results in this regard. Chemical characteristics are applied in taxonomic delimitations with profound confidence; the authenticity of results exhibit special taxonomic lusture and glare. The assessment of biomolecules on comparative basis presents a vivid picture of evolutionary work. Chemical investigations now form integral part of all serious taxonomic revision, not considering chemical data likely to be regarded as incomplete.

Chemical investigation and information so obtained become more informative which become more important in cases where other biological characters are of ambiguous nature and fail to provide a key of separation.

About 30 thousand biomoleculues have been characterized from plants (Harborne and Moore, 1984). Of these some are practically more variable chemosystematically. These chemicals fall under three broad categories, namely primary metabolites such as amino acids and other organic acids, sugars, etc; secondary metabolites which include alkaloids, polyphenols, terpenoids, oils and waxes and finally sementides such as DNA and RNA which play splendid role in cellular metabolism.

From the above groups of biomolecules phenolics have been selected to be the parameters in the present investigation.

Material and Methods:

The pure line seeds of the selected cultivars of *Vicia faba* procured from various sources were grown in the experimental garden of the Botany Department of T.N.B. College in separate plots. Fresh and healthy leaves of the plants were used as the source of material for the estimation of phenolics. Plants were used and fruits were discarded. For the extraction of phenolic compounds, the collected leaves were put in the paper bags and placed in an incubator maintained at 40° C. The drying of leaves was done for 48 hrs. and then grinded to powder and stored in dark at room temperature.

Ether ethyl acetate and isoamyl alcohol extracts were obtained by mixing of leaf powder with 3 ml of NHCl, 3 ml of 95% of ethyl alcohol in a 15 ml screw top test tube and placed in 100° C water bath for four hrs. The tubes were placed at room temp, their contents were centrifuged and phenolic extracts were decanted. For the removal of chlorophyll contents, 5 ml of distilled water was added to each extract and the mixture was solvent prepared in the time mixing propionic acid and distilled water. The chromatograms were dried at room

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temperature on the chromatographic plates. $AgCl_3$ (1% in methanol) was sprayed to field florescent colors, the plates were finally subjected to UV spectral analysis, Philips TL 20 W/80. The visible phenolic spots were marked and copied on semitransparent papers and further spots total area, colours and their positions which seemed to be similar in two or more cultivators were assigned the same number. The area and intensity of colour gave rough amounts of concentration of the phenolic spots in each case. In this fashion, three chromatographs were developed to each cultivar.

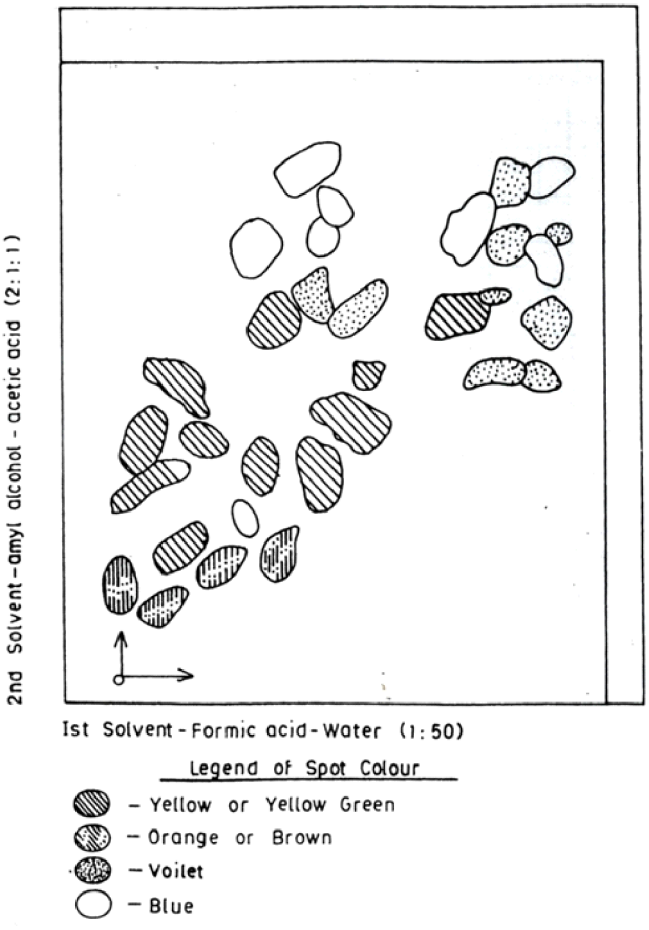

Fig. 1. Composite Chromatogram Showing Colours and Positions of Phenolic Spots in *Vicia faba* Cultivars.

In order to make a qualitative screening of phenolics between the cultigens which can be perceived to be chemotypes, the procedure of Ellison *et al.* (1962) was followed. Any two cultivars have been compared on the basis of percentage of biochemical distance or divergence. Any value above 50% in Fig. 1 exhibits chemical association and was again centrifuged. The supernatant was extracted twice with 0.5 ml of diethyl ether and also with 1 ml of ether acetate.

For the removal of anthocyanin, the extract was finally mixed with one ml of isoamyl alcohol. In order to give least touch to the extraction procedure, the extract was evaporated under a vacuum-pump in order to yield a sticky residue.

For the separation of phenolics, 2-dimensional thin layer chromatography technique was employed. The chromatographic plates have been prepared by applying a uniform coat of cellulose powder (Merck's microcrystalline avial) on the chromatographic plates, measuring 16 x 14 cm by the process developed by Nybom (1968) and further on viewed by Frost (1970). The mother residue of phenolics was diluted on the chromatographic plates with the help of micro-tip dropper and this was dried by using a hot air blower. The process was repeated many times in order to provide required concentration of the phenolics. For the sake of their separation the chromatographic plates were run into and value lower than this denotes the phylogenetic apartness between the taxa compared. Accordingly, the pair affinity value between the chemotypes, say A and B, can be obtained as follows :

$$\text{Pair Affinity(PA)} = \frac{\text{Spot common for types A + B}}{\text{Total Number of Spots in A + B}}$$

Representatives comprise a group, the interrelationship of individual cultivar with the other cultivars of the group represents the group-affinity.

The Group-Affinity values between the compared cultivars can be known by a numerical taxonomic measure. Group-Affinity of the cultivar can be obtained by adding the absolute Pair-Affinity value of the cultivars with the Pair-Affinity value of other cultivars calculated and taken together.

RESULTS AND DISCUSSION

There have been 16 phenolic spots in cultivar DHB-94, 17 in DHB-98, 14 in DHB-99, 16 in DHB-100, 14 in JV2, 11 in HV-1, 10 in HV-2, 13 in Ranchi local and 11 in Bhagalpur local of these species Some were of major types, some median type arnd others were found simply as traces. The perusal of Table No - 2 further shows that some spots are indicative of close relationships with common spots pointing to the uniqueness and chemical isolation of the cultivars.

Appraisal of the Table No - 2 furnishes yet another idea of the biochemical divergence within *Vicia faba*. Spots as such presented a picture which provides phylogenetic clue reflected in the form of spot-pattern groups. In all, four major spot-pattern groups namely A, B, C and D were noticed. Members showing a particular set of spot-pattern comprise a spot-pattern group. Spot-pattern group A includes cultivars which are chemically identical. They are members of DHB-series mostly. Spot-pattern group B includes some DHB cultivars and cultivars JV2. Third group is of spot-pattern C type which includes cultivars of HV series. The last spot-pattern group is of D which has some cultivars in common and this group is of local varieties namely Ranchi local and Bhagalpur local.

Perusal of Table No - 2 further shows that there are some spots which serve as chemical link between the spot-pattern groups. These are so-called cementing spots to bind chemically, the taxa below the level of species.

Taking advantage of data available, it has been possible to proceed for some taximetric exercises following the method of Ellison *et al*. (1962). This yields a picture of biochemical distances between the taxa for making a comparison. Such a comparison is made in two terms, one is paired affinity value (PA Value) and another is group affinity value (GA Value) from

Table No. - 2

Distribution pattern of *phenolic spots* in 12 cultivars of *Vicia faba*

Spot Sl.	DHB 94	DHB 95	DHB 96	DHB 97	DHB 98	DHB 99	DHB 100	JV-2	HV-1	HV-2	Ranchi local	Bhag. local
No	Spot pattern group - A					Spot pattern group - B			Spot pattern group - C		Spot pattern group - D	
1	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +				
2			+ + +			+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
3									+ + +	+ + +	+ + +	+ + +
4	+ + +	+ + +				+ + +	+ + +	+ + +				
5	+ + +	+ + +	+ + +			+ + +	+ + +					
6		+ + +				+ +	+ + +	+ + +				
7								+ + +	+ + +	+ + +	+ +	+
8	+ + +	+ + +	+ + +		+ + +				+ + +	+ + +		
9	+ + +	+ + +	+ + +							+ + +		+ + +
10									+ + +		+ + +	+ + +
11	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	
12		+ + +	+ + +	+ + +						+ +		
13	+ + +						+ + +	+ + +			+ + +	
14	+ + +	+	+ +				+ + +	+ + +	+ + +		+ + +	+ + +
15										+ + +	+ +	+ + +
16	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +		+ + +	
17						+ +				+ + +		
18	+ + +	++	+ +	+	+	+ + +						
19	+ + +	+ + +	+ + +	+ + +	+ + +							
20		+ + +	+ + +	+	+ +							+
21								+ + +			+ + +	+ + +
22	+ + +	+ + +		+	+ + +	+ + +						
23	+ + +	+ + +	+ + +	+ + +	+ + +							
24			+ + +			+ +	+ + +	+				
25						+ + +	+ + +	+ + +				
26						+	+ + +	+ +	+ +		+ +	+ + +
27	+ + +	+ + +										
28	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ +	+ + +	+ + +	+	+ + +
29	+ + +	++	+	+ +	+ + +							
30							+ + +		+ + +	+ +	+ + +	+

Major: + + + Medium: + + Trace: +

which after deducing PA value, a cultivar is compared with other. Table-3 depicts are paired-affinity value of the selected cultivars. The perusal of Table No - 3 further shows that the member of DH3 series is very intimately related showing a high pair-affinity value which goes as high as 19.90. The cultivars of DHB-series do exhibit high pair-affinity value more than 80% in majority of cases. Other cultivars like HV1 and HV2 were closely related showing very high pair value which they are least related to the cultivars of other series. Local cultivars like Ranchi local and Bhagalpur local were quite near showing a PA value 85.71%. This is indeed an indication of their chemical nearness in terms of phenolics.

Table No. - 3

Paired Affinity (PA) value of twelve *Vicia faba* cultivars in terms of phenolics

DHB-94	DHB-95	DHB-96	DHB-97	DHB-98	DHB-99	DHB-100	HV-1	HV-2	JV-2	Ranchi Local	Bhag. Local
	90.9	9.32	78.57	82.75	62.5	60	51.61	35.71	30.76	38.7	20.68
		93.75	82.75	85.66	54.54	51.61	43.75	34.48	29.62	11.25	20
			81.48	86.71	58.06	48.27	46.66	37.03	24	33.33	21.42
				96	57.14	46.15	30.03	25	27.27	29.62	24
					55.17	44.44	37.03	32	26.08	28.57	23.07
						80	77.41	42.85	41.66	55.17	51.85
							89.65	51.85	48	60	50
								51.85	48	60	50
									90.9	81.48	80
										80	78.26
											85.7

Another approach was taken into group affinity value of the selected cultivars. The results have been depicted in Table No - 4. The highest group affinity value has been found to be in cultivar HV2. The lowest group affinity value was 804.64 for Bhagalpur local varieties. It is, however, pertinent to mention that all the cultivars have shown high group affinity which is on the line of expectation because taxa below the level of species have been compared.

Table No. - 4

Spot pattern groups and group affinity value of *Vicia faba* cultivars in terms of phenolics

Sl. No.	Cultivars	Spot Pattern group	Group affinity value
1	DHB 94	A	742.5
2	DHB 95	A	719.01
3	DHB 96	A	720.03
4	DHB 97	A	736.35
5	DHB 98	A	729.09
6	DHB 99	B	697.48
7	DHB 100	B	683.83
8	HV 1	B	678.01
9	HV 2	C	663.15
10	JV 2	C	624.55
11	Ranchi local	C	612.04
12	Bhagalpur local	C	604.99

CONCLUSION

As it has been stated earlier, phenolic compounds are more valuable for phylogenetic considerations of all biomolecules since it has been very positively influenced by evolutionary operations (Ferguson, 1980). For this purpose, twelve *Vicia faba* cultivars have been analysed in terms of phenolics in order to visualize chemical divergence within the group. Spot-pattern analysis has shown that the cultivars fall under four broad chemical categories. These are spot-pattern group "A", spot-pattern "B", spot-pattern "C" and spot-pattern "D". The distribution pattern indicates an appreciable range of chemical divergence within the group. On this account it has been found that the cultivars of DHB-series have higher group

affinity value with figure of seven hundred and plus. The local cultivars, however, have shown very low group affinity value which is around six hundred. This is testimony to the inherent chemical evolution and range of diversification within the group. Through another class of taximetric exercise paired-affinity value (PA) of the cultivars can be known. The cultivars of DHB-series once again have shown great chemical affinity which is sometimes above 90%. This series has shown a very low paired affinity value with local varieties that have come as different chemovars.

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