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Seed protein analysis and meiotic studies in cultivars of Indian barley

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Abstract. A comparative study was made of the meiotic behaviour and protein electrophoretic patterns of 12 different varieties of barley (*Hordeum vulgare* L)., including chiasma frequency, pollen sterility and chromosomal behaviour during different stages of meiosis. Though all the cultivars showed a uniform somatic chromosome number of $2n = 14$, they differed from each other in details of meiotic behaviour. Proteins were extracted from the seeds and subjected to polyacrylamide gel electrophoresis. Within a certain range, each variety showed a distinct pattern.

Keywords. Meiotic configurations; protein patterns; barley cultivars.

1. Introduction

In recent years electrophoretic techniques are being used for screening enzyme patterns in the studies of genetic variability of crop plants. In barley they have been employed for varietal identification (Freydenberg *et al* 1969; Fedak and Rajhathy 1971, 1972;) in the studies of polymorphism in gene frequency (Kahler and Allard 1970) and many other aspects. Barley proteins can be studied after electrophoresis and the pattern thus obtained is successfully utilized in the identification of closely related varieties.

Earlier works in this laboratory had also indicated minor structural changes in the karyotypes of different cultivated strains of *Hordeum vulgare* L. (Sharma 1956; Sharma and Mukherji 1956). An attempt has been made in the present investigation to study the genetic variations in barley cultivars through meiotic studies and protein electrophoretic patterns.

2. Materials and methods

Seeds of 11 cultivars of *Hordeum vulgare* L. viz., Ratna, Jyoti, Clipper, BG 25, K 125, RS 6, DL 3, DL'36, DL 70, RD 31 and RD 102 were obtained from the Indian Agricultural Research Institute, New Delhi, while the last one was the local market variety. The seed samples were sown and the plants grown to maturity at the experimental garden of this department.

For the study of meiotic chromosomes, flower buds were fixed in acetic acid: ethanol (1 : 1) mixture, kept at about 22°C for 48 hr, changing the fixative after 24 hr,

and finally stored in 70% ethanol. The anthers were smeared in 2% acetic carmine following the usual technique.

To extract soluble proteins, seeds were crushed in tris-glycine buffer (pH 8·3). The extract thus obtained was spun in a cold centrifuge at 10,000 rpm for 30 min. the supernatant pipetted out and used for electrophoresis. An identical procedure was followed for the extraction of water soluble proteins, using distilled water in place of buffer.

For vertical polyacrylamide gel electrophoresis, 7·5% acrylamide gel in tris-HCl-TEMED buffer (pH 8·6) was used. The reservoir buffer was tris-glycine (pH 8·3). 0·3 ml of protein extract was added to each gel tube and run at 4 mA/tube for 2 hr. The gels were stained in 0·1 gm% amido black solution in 7% acetic acid for 20 min and destained by successive changes in 14% acetic acid until the blue-black protein bands appeared against a faint background (Smith 1968).

3. Observations

Twelve cultivars of the common barley (*Hordeum vulgare* L.) have been studied with regard to meiosis and electrophoretic analysis of seed proteins.

During meiotic studies, chiasma frequency, pollen sterility and configurations at diakinesis and different divisional stages were studied in all the varieties (figures 1 to 3).

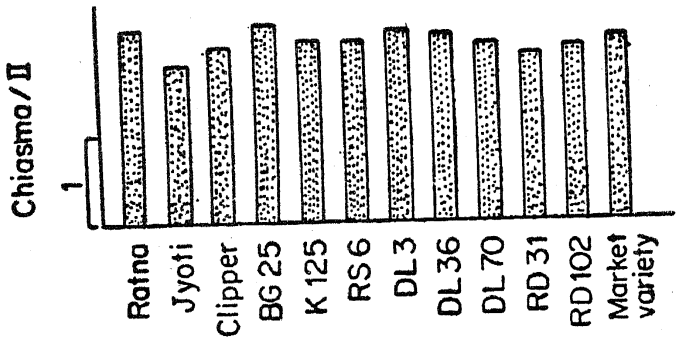

Figure 1. Histogram showing the chiasma frequencies in 12 varieties of barley

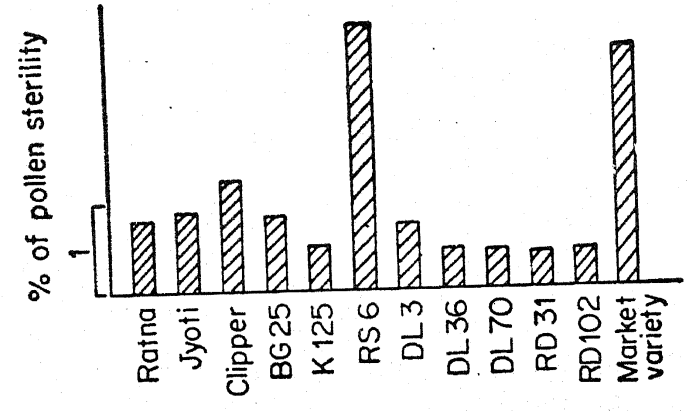

Figure 2. Histogram showing the percentages of pollen sterility in 12 varieties of barley

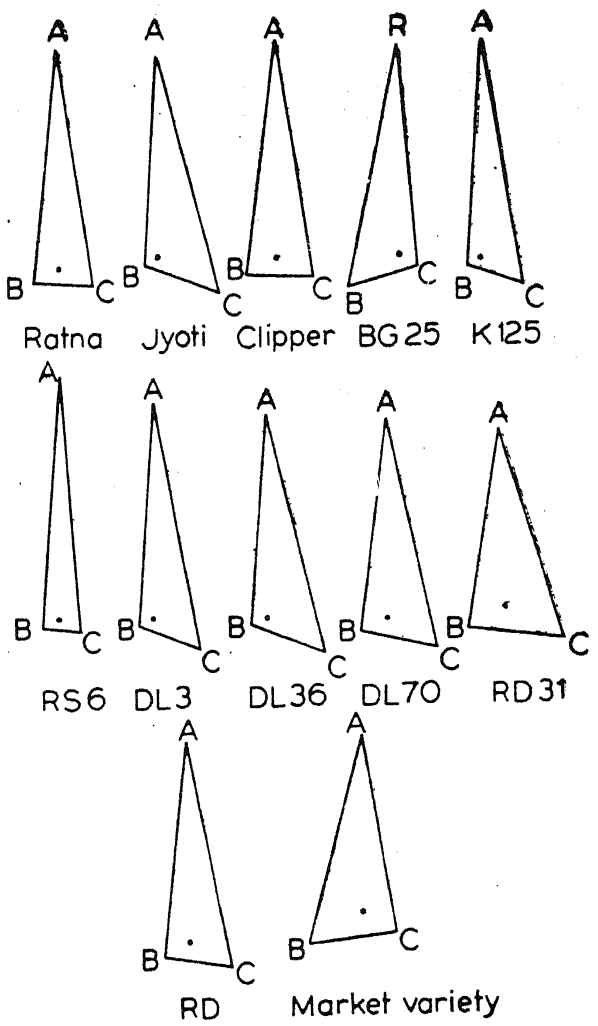

Figure 3. Polygraphs showing the configurations at diakinesis in 12 varieties of barley; R = Ring-shaped bivalents, B = Bracket-shaped or bivalent with one chiasma terminated and C = Connected rod-shaped bivalent

Chiasma frequency per bivalent was found to be nearly similar in Ratna and DL 3, Clipper and RD 102, K 125 and DL 70 and to some extent in RS 6 and market variety. The remaining varieties show chiasma frequencies distinct from the others (table 1)

The pollen sterility percentage was very low in all the cultivars, usually not exceeding 1%. Only RS 6, Clipper and the market variety showed a slightly higher percentage (table 2).

The configurations at diakinesis, in the different varieties of barley studied, showed 3 types of bivalent formation viz., ring-shaped (R), bracket-shaped or ring with one chiasma terminated (B) and connected rod-shaped bivalent (C). The R values were found to be more or less uniform in all the varieties. Both the B and C values, however, show considerable diversity (table 3).

Cells observed in the different divisional stages of meiosis viz. metaphase I, anaphase I and metaphase II, showed regular division in all the cultivars. For studying the protein band patterns, each gel was divided into 4 arbitrary zones — A, B, C, and D. As far as the number of bands in each zone is concerned, the varieties Jyoti,

Table 1. Chiasma frequency in the different varieties of barley studied

Sl. No.	Variety	Total No. of PMCs studied	Chiasma/II	Chiasma/PMC
1.	Ratna	10	2·21	15·5
2.	Jyoti	10	1·78	12·5
3.	Clipper	10	2·01	14·1
4.	BG 25	10	2·28	16·0
5.	K 125	10	2·04	14·3
6.	RS 6	10	2·07	14·5
7.	DL 3	10	2·2	15·4
8.	DL 36	10	2·13	14·9
9.	DL 70	10	2·04	14·3
10.	RD 31	10	1·88	13·2
11.	RD 102	10	2·01	14·1
12.	Market Variety	10	2·1	14·7

Table 2. Pollen sterility in the different varieties of barley studied

Sl. No.	Variety	Total No. of pollen grains observed	No. of sterile pollen	% of pollen sterility
1.	Ratna	244	2	0·819
2.	Jyoti	222	2	0·9
3.	Clipper	241	4	1·24
4.	BG 25	239	2	0·836
5.	K 125	208	1	0·48
6.	RS 6	266	8	3·01
7.	DL 3	266	2	0·75
8.	DL 36	216	1	0·463
9.	DL 70	218	1	0·458
10.	RD 31	245	1	0·408
11.	RD 102	238	1	0·42
12.	Market variety	223	6	2·7

DL 36, RD 31 and market variety showed considerable similarity. Only in zone D of RD 31 the number was different from the other three (figure 4, table 4). In zone A, 4 bands are most frequent (Jyoti, BG 25, RS 6, DL 3, DL 36, DL 70, RD 31 and market variety) with only two instances of 3 bands (K 125 and RD 102) and one each of 2 (Ratna) and 5 bands (Clipper). In zone B again, 4 bands are most common (Ratna, Clipper, K 125, RS 6 and DL 3), followed by 3 bands (Jyoti, DL 36, RD 31 and market variety) and 2 bands (BG 25, DL 70 and RD 102). In zone C, 3 bands occur in maximum frequency (Ratna, Jyoti, DL 3, DL 36, RD 31 and market variety),

Table 3. Configurations at diakinesis in the different varieties of barley studied

Sl. No.	Variety	No. of PMCs observed	Configuration/PMC		
			R*	B*	C*
1.	Ratna	10	5·2	0·8	1·0
2.	Jyoti	10	4·8	0·4	1·8
3.	Clipper	10	5·1	0·9	1·0
4.	BG 25	10	5·0	1·5	0·5
5.	K 125	10	5·2	0·4	1·2
6.	RS 6	10	5·9	0·5	0·6
7.	DL 3	10	5·2	0·4	1·4
8.	DL 36	10	4·9	0·4	1·7
9.	DL 70	10	4·8	0·7	1·5
10.	RD 31	10	4·3	1·0	1·7
11.	RD 102	10	4·9	0·8	1·3
12.	Market variety	10	4·4	1·6	1·0

*R—Ring-shaped bivalent
B—Bracket-shaped bivalent (ring with one chiasma terminated)
C—Connected rod-shaped bivalent.

while there are 4 cases of 2 bands (Clipper, BG 25, RS 6 and DL 70) and one each of 4 bands (RD 102) and 1 band (K 125). Zone D, however, shows maximum frequency of 2 bands (Ratna, Clipper, BG 25, DL 70, RD 31 and RD 102) but also a high number with one band (Jyoti, RS 6, DL 3, DL 36 and market variety). There is only one instance of 3 bands (K 125). The relative width of bands in each zone, as well as their relative intensities, is given in table 4.

4. Discussion

Chromatography and electrophoresis are proving to be rather effective tools in the analysis of genetic variants in plant systems. In the varieties which do not differ sufficiently from each other in their chromosomal characters, a study of their seed protein patterns may be able to distinguish between them.

The earlier works on the identification of seed proteins mainly involved starch-gel electrophoresis followed by the identification of individual bands on zymogram (Doekes 1968, 1969, 1973; Ellis 1971; Gehrke *et al* 1964). Johnson *et al* (1967) utilised vertical polyacrylamide gel electrophoresis in analysing the relationships of polyploid wheats. The same method has also been used in the study of species relationships and intergeneric crosses in wheat (Johnson 1969), in tracing the origin of B genome (Johnson 1972a) and relationships of hexaploid wheats (Johnson 1972b).

In barley (*Hordeum vulgare* L) numerous cultivars are present, all of which show, in general, $2n = 14$ chromosomes. This work was undertaken on 11 known cultivars

Table 4. Protein electrophoretic patterns in cultivars of barley studied

Sl. No.	Variety	Total No. of bands	Zone A			Zone B			Zone C			Zone D		
			No. of bands	Width	Intensity	No. of bands	Width	Intensity	No. of bands	Width	Intensity	No. of bands	Width	Intensity
1.	Ratna	11	2	1, 4	+, ++	4	4,1,1,3	+, ++, +, +	3	2,1,2	++, +, ++	2	1,1	++, +
2.	Jyoti	11	4	1,6,1,1	++, ++, ++, ++	3	1,1,1	++, +, +	3	3,1,1	+, ++	1	1	++
3.	Clipper	13	5	1,2,1,5,1	++, +, ++, +, ++	4	1,3,1,1	++, ++, +, ++	2	2,1	++, +	2	1, 2	+, ++
4.	BG 25	10	4	1,4,1,1	+, +, ++, ++	2	3,7	++, +	2	1,2	++, ++	2	1, 1	++, ++
5.	K 125	11	3	1,1,5	+, ++, +	4	2,2,1,2	+, ++, ++, +	1	1	++	3	2,2,2	+, ++, +
6.	RS 6	11	4	2,1,4,1	+, ++, ++, ++	4	2,1,1,2	+, ++, ++, ++	2	1,1	+, +	1	2	+
7.	DL 3	12	4	1,2,6,5	++, +, +, 3 ++	4	2,2,2,2	+, ++, ++, ++	3	1,1,1	++, +, +	1	1	+
8.	DL 36	11	4	1,1,1,4	++, +, +, +	3	1,1,2	++, ++, ++	3	2,2,1	++, +, +	1	2	++
9.	DL 70	10	4	1,6,1,1	++, ++, +, +	2	2,4	++, +	2	2,1	+, +	2	2,2	++, ++
10.	RD 31	12	4	1,2,4,1	++, ++, +, +	3	2,8,2	++, +, ++	3	1,1,1	++, +, +	2	2,1	++, +
11.	RD 102	11	3	1,1,4	++, +, +	2	1,2	++, ++	4	1,2,1,1	+, ++, +, +	2	1,2	+, ++
12.	Market variety	11	4	1,1,2,1	+, ++, +, ++	3	1,2,8	+, ++, +	3	1,3,2	+, ++, +	1	1	+

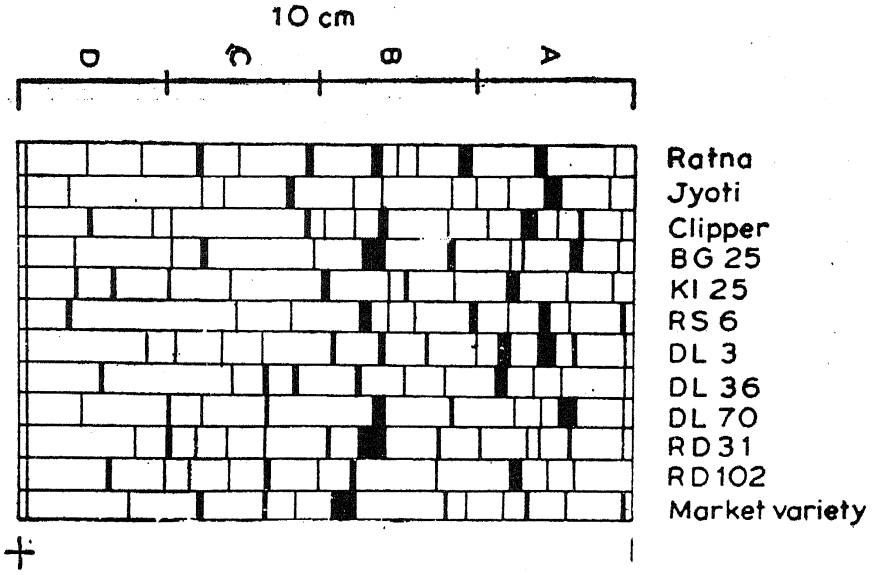

Figure 4. Electrophoretic patterns of seed proteins in 12 varieties of barley

and the common market variety, to study the genetic variations amongst the cultivars, as shown by their meiotic configurations and electrophoretic mobility of seed proteins. The chiasma frequencies differed in the different varieties (figure 1). The records for some varieties were, however, much more similar than those for the others. Similar variations were observed in the configurations at diakinesis (figure 3). The similarities observed in this case do not tally entirely with those in figure 1. Pollen sterility data (figure 2) and behaviour during meiosis gave similar observations for all the varieties, since all of them are sexually propagated with low pollen sterility and almost regular meiosis. Taken in all, the meiotic data indicate that in spite of being closely related and having a uniform somatic chromosome number of $2n = 14$, these cultivars differ from each other in details of meiotic configurations, establishing their genetic diversity.

The data presented in figure 4 clearly show that each cultivar is distinct in the electrophoretic mobility of its protein fractions. There is only a overall similarity in that the range in number of bands is between 10 and 13 (figure 4). Within this range each variety showed a clear pattern distinguishing it unequivocally from all other varieties studied. In the width of each band and intensity of its staining, the pattern for each variety was also characteristic. In the identification of cultivars of barley therefore, the banding patterns observed, following electrophoresis, may be taken as distinct criteria. This method can also be applied to other cultivars which do not otherwise show marked differences in their karyotype or other morphological characters.

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