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B-Chromosomes and E-l Isozyme Activity in Mosaic Bulbs of *Scilla autumnalis* (Liliaceae)

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Abstract. The electrophoretic patterns of esterase (E-l), alcohol dehydrogenase (ADH), and glutamate oxaloacetate transaminase (GOT) isozymes were studied in two Spanish populations of the lily *Scilla autumnalis* with B-chromosome carrying individuals. The E-l isozyme activity appears only in those individuals with B-chromosomes. None of the bulbs free of B’s show it. Five bulbs, mosaic for B-content, were identified. Electrophoretic analysis shows that these bulbs are characterised by mosaicism for E-l isozyme activity. An analysis of individual roots by both electrophoretic and cytological methods shows that tissue mosaicism for B-content correlates with tissue mosaicism for E-l isozyme activity. The electrophoretic analysis of different roots from bulbs heterozygous for the Est-1 locus indicates that the structural gene for E-l is not located on the B-chromosome itself. Rather there is a derepressor effect of Bs on E-l isozyme activity. Since ADH and GOT patterns are unaffected by the presence of B-chromosomes it is clear that they do not exhibit a generalised derepressor effect.

Introduction

Supernumerary or B-chromosomes have been found in plants of *Scilla atumnalis* (Liliaceae) from Sicily (Battaglia, 1963) Palestine (Battaglia, 1964) and Spain (Ruiz-Rejbn and Oliver, 1978). The B-containing bulbs from Torre de1 Vinagre (Jaén, Spain) show a distinctive esterase (E-l) isozyme activity (Oliver, 1977; Ruiz-Rejbn et al., 1980a). We have found the same correlation in a second Spanish population.

A detailed cytological analysis of B-containing bulbs from these two populations has allowed us to identify five bulbs, mosaic for B-chromosome content. In this paper, we report on an analysis of these mosaic individuals, and show that the correlation between the presence of Bs and E-l activity exists here too. Additionally, an electrophoretic analysis of heterozygous bulbs for the Est-1 locus shows that the structural gene for this enzyme is not located on the B-chromosome itself.

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Materials and Methods

B-containing bulbs were found in only two out of ten Spanish populations sampled. The sample sizes, the number of B-containing individuals. and the geographical localities of these two populations are shown in Table 1. In all cases mitotic analysis was carried out on meristems of bulb root tips squashed in acetic orcein.

In order to protect the isozymes from oxidation, enzyme extraction was carried out by crushing the plant material in a buffered solution of several reducer agents (Valizadeh. 1977). Horizontal starch gel electrophoresis was carried out with a LiOH/Borate(pH 8.1) electrode buffer and a Tris/Citrate(pH 8.3) gel buffer (Selander et al., 1971). This system markedly improves the resolution of separation of all three enzymes here assayed by comparison with that we had previously used (Oliver and Ruiz-Rejón, 1980). Even so the E-3 zone still appears diffuse. Different gel slices were assayed for Esterases (EST, E.C. 3. I 1.1.) (Johnson et al., 1966), Alcohol dehydrogenase (ADH, E.C. 1.1.1.1.) (Pasteur, 1973), and Glutamate Oxaloacetate Transaminase (GOT, E.C. 2.6.1. 1.)(Gottlieb, 1973).

Results and Discussion

In the B-containing populations a total of 18 out of 221 bulbs sampled showed from 1 to 9 heterochromatic B-chromosomes (Ruiz-Rejón et al., 1980a; Ruiz-Rejón et al., 1980b; and see Tables 1 and 2).

One isozyme of ADH and three of GOT were detected in S. *autumnalis,* but no relationship was found between the electrophoretic patterns observed for these enzymes and the presence of B-chromosomes. The esterase isozyme patterns, on the other hand, showed a strong correlation with the presence of B-chromosomes. Three esterase isozymes (E-l, E-2, and E-3), of which E-l only appears in the B-containing individuals, have been previously described (Posse et al., 1980; Ruiz-Rejón et al., 1980a). With the use of the present electrophoretic method, and contrary to that reported in previous papers, three electromorphs (0.87, 0.83, and 0.80) can be found in the E-l zone, either in homozygous or heterozygous combinations.

Mosaicism for B-content between and within root tips, due to mitotic instability of B-s in the meristem cells (Battaglia, 1964), was detected in 5 bulbs (Table 2). The E-l isozyme phenotypes of the 5 mosaic bulbs are also shown in Table 2. When different roots and different root parts from the bulbs with unstable B-content were subjected to electrophoresis, mosaicism for E-l isozyme activity was observed, with 60 out of 127 roots analyzed showing E-l activity. When meristem and differentiated regions were examined separately, mosaicism for E-l activity within the root was found (Table 3 and Fig. 1). Therefore, those bulbs characterised by mosaicism for B-content also show mosaicism for E-l isozyme activity.

The correlation between both types of mosaicism was clearly shown when different roots from the same bulb were analyzed by both electrophoretic and cytological methods. This was achieved by sectioning the root longitudinally and then analyzing each part separately. Because of the small size of such half roots, the electrophoretic study was carried out on thin starch gels of 3 mm thickness. A total of 23 roots were analyzed in this way. The electrophoretic patterns of two roots from bulb CPa-18 are shown in Fig. 2a. One of these has both B-containing cells and E-l activity; the other has neither B's nor

Table 1. Geographical localities, sample size, and number of B-containing bulbs on two Spanish populations of *S. autumnalis*

Key	Population	Bulbs sampled	B-containing bulbs
TV	Torre del Vinagre (Jaén)	160	11
CPa	Cuesta de la Palma (Granada)	61	7
	Total	221	18

Table 2. E-l isozyme electromorphs in bulbs with an unstable number of B's from the TV and CPa populations of S. *autumnalis*

Bulb No.	No. of Bs	E-l electromorphs[a]		
		0.80	0.83	0.87
1) TV-509	o-1	–	+	–
2j CPa-l	O-2	–	+	+
3) CPa-11	o-1	–	+	+
4) CPa-18	O-2	–	+	+
5) CPa-124	o-1	–	+	–

[a] Present electromorph = + ; absent electromorph = –

Table 3. E-l isozyme activity in meristem (M) and differentiated regions (D) of roots from B-containing bulbs of S. *autumnalis*

Roots sampled	In M and D	E-l activity		No E-l activity
		Only in M	Only in D	
57	8 (14.03%)	16 (28.07%)	4 (7.02%)	29 (50.88%)

E-l activity. The same correlation was found within single roots. Thus, one root from bulb CPa-124 had an unstable number (O-l) of Bs. Here, the E-l isozyme was detected in the meristem but not in the differentiated part of the root. The latter did not have B-containing cells, since none of the differentiated nuclei examined contained a B-chromocentre of the kind reported by Ruiz-Rejón et al. (1980a). Thus, at the tissue level too, E-l activity goes along with the presence of B-chromosomes.

The obvious explanation to account for these observations is that the structural gene coding for E-l isozyme is located within the B-chromosome itself. To test this, we made use of mosaic bulbs heterozygous for the Est-1 locus. The rationale of the test was as follows. Consider bulbs CPa-1 and CPa-18 which carry unstable B-chromosome numbers ranging from 0 to 2. Since these

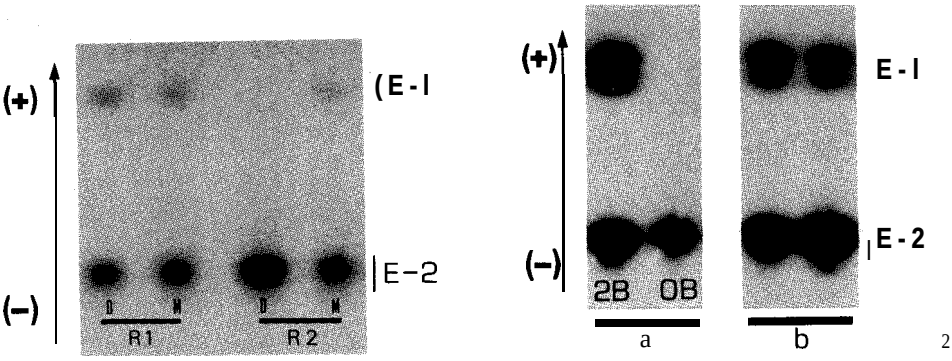

Fig. 1. E-l and E-2 isozyme patterns in different parts of two roots (RI and R2) from a same bulb of *S. autumnalis*. In R1 the E-l activity appears in both the differentiated region (*D*) and the meristem (*M*). In R2 the E-l activity only appears in M

Fig. 2. a E-l and E-2 isozymes in two roots from bulb CPa-18. One (left) has 2B's and E-l activity; another (right) has neither B's nor E-l activity. b Two banded E-l phenotype (0.87/0.83) shown by two roots of the bulb CPa-11 with 0–1B

individuals are heterozygous for this gene (Table 2) one might, according to the precise B-content of each root, expect to find roots with 0-, l-, and 2-banded phenotypes for this isozyme, if Est-1 was located on the B. A total of 42 roots from these bulbs were subjected to electrophoresis. In 22 cases no E-l bands were detected, as is expected in the case of roots which lack B's. In the remaining 20 roots two bands (0.87 and 0.83) appeared on the gels. No cases with only one of these bands were ever found. In a third bulb (CPa-11), mosaic for 0 B and 1 B, the same two banded E-l phenotype was found (Fig. 2 b). These results indicate that the structural gene Est-1 is not located on the B-chromosome itself.

Changes in isozyme electrophoretic patterns of the kind we have observed here can also reflect differential regulation of gene expression (Paigen, 1979 ; Scandalios, 1979). We therefore propose a regulatory interaction to explain the observed association between Bs and the E-l isozyme, involving a derepressor effect by the B on the expression of the Est-1 isozyme gene, located on one of the A chromosomes. Our data do not allow us to decide if this regulatory interaction takes place at the transcriptional, transcript processing, translational, or catabolic level.

A repressive effect on genetic activity has been reported for the B-chromosomes in both rye (Kirk and Jones, 1970) and maize (Ayonoadu and Rees, 1971). This appears not to be the case in *S. autumnalis* where the B's increase the number of esterase isozymes expressed in the root. Since the ADH and GOT isozyme patterns were unaffected by the presence of Bs, it is clear that the B-chromosomes of S. *autumnalis* do not produce a generalized repressive effect.

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