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Reserve Proteins in the Developing Seeds of × *Haynaldoticum sardoum.*

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Abstract. In this study the pattern of storage protein deposition in the developing seeds of × *Haynaldoticum sardoum* was examined. The accumulation of the various protein classes did not differ greatly between the two lines tested (Culmo Pieno and Culmo Vuoto). Nevertheless, some differences in the deposition pattern were revealed by polyacrylamide gel electrophoresis. In particular, the mature seeds of the two lines presented different electrophoretograms for the prolamin and glutelin proteins. These patterns reflect the differences between the two lines, which could play a role in their ability to adapt to environmental changes.

Storage proteins are localized in the endosperm of cereals and represent about 11–13 % of the reserves of this tissue (Lásztity 1984). Osborne (1895) classified seed proteins in four groups on the basis of their different solubilities: albumins (water soluble), globulins (soluble in dilute salt solutions), prolamins (soluble in aqueous alcohols) and glutelins (dilute acid- or alkali- soluble). Albumins and globulins are complex mixtures of hydrolytic or metabolic enzymes and structural proteins; they are generally present in low amounts in cereal seeds. On the contrary the prolamins together with the glutelins represent the most abundant storage proteins (over 80 %) of many cereal seeds (Larkins 1981).

In a previous paper (Capocchi *et al.* in press) we studied the proteolytic activity present in the developing seeds of × *Haynaldoticum sardoum*, an opportunistic plant native to Sardinia, Sicily and southern Italy, where it exhibits the behaviour of a typical weed (Meletti 1955). × *H. sardoum* is believed to derive from *Triticum durum* and *Haynaldia villosa* and since there is growing interest in the utilization of *Haynaldia villosa* for the genetic improvement of *Triticum* (Scarascia Mugnozza *et*

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al. 1981, Blanco *et al.* 1987), the present study was undertaken to obtain a more thorough characterization of the storage proteins of this species during its development.

MATERIALS AND METHODS

Plant Material

Caryopses of two lines of × *Haynaldoticum sardoum* Meletti et Onnis (a solid stem line, Culmo Pieno; and a hollow stem line, Culmo Vuoto; Meletti and Onnis 1975), were taken from plants cultivated under field conditions near Pisa (Italy). They were collected during ripening, according to the procedure outlined by Peterson (1965 and seeds in five stages of maturation were obtained (milky, mealy, doughy, waxy, fully ripe) (see for details Capocchi *et al.* in press).

Extraction Procedures

For each maturation stage, endosperms were isolated from twenty-five caryopses and homogenized with a Polytron homogenizer in the presence of cold acetone (–20 °C). Each homogenate was extracted for 15 min by stirring at room temperature, and then centrifuged in a bench centrifuge at the maximum speed for 10 min. This procedure was repeated three times, after which the precipitates were left at room temperature to allow the acetone to evaporate completely.

The acetonic powders were extracted according to the procedure of Rahman *et al.* (1982),as modified by Grilli *et al.* (1989). Each extraction was performed three times for 1 h with 20 volumes of solvent at room temperature. The extracts were centrifuged and the collected and pooled supernatants of each class of protein were dialyzed against water overnight and lyophilized.

The protein content was determined by the method of Bensadoun and Weinstein (1976), utilizing bovine serum albumin as standard.

Gel Electrophoresis

Gel electrophoresis was performed according to the procedure of Payne and Corfield (1979) utilizing 17 % polyacrylamide gel slabs. The samples were then resuspended in a 0.0625 M tris-HCl buffer (pH 6.8) containing 2 % sodium dodecyl sulphate (SDS) and 5 % 2-mercaptoethanol and boiled for 3 min at 100 °C. The amount of each protein applied onto the gels varied from 80 to 100 μg. A low molecular weight calibration kit (14,4–94 kDa, Pharmacia) was utilized for the molecular mass estimations. The electrophoretic runs were performed in a 0.025 M tris and 0.192 M glycine buffer (pH 8.3) containing 1 % SDS at 8 mA per slab for 19 h.

The gels were stained with Coomassie brillant blue according to the method of

Fullington *et al.* (1983) and destained with isopropanol-acetic acid-water (10/10/80, v/v/v).

The gels were scanned by a Vernon P. I. 6 scanner, then dried and photographed.

RESULTS

The two lines show similar deposition patterns for the main classes of endosperm reserve proteins (Fig. 1). At the beginning of the ripening period the prolamins were present in the lowest amounts, but from stage II onwards their levels increased, eventually exceeding those of the globulins and glutelins. The prolamins in the mature caryopses represented 36 % and 38 % of the total reserve proteins in Culmo Vuoto and Culmo Pieno, respectively. The albumin and globulin levels reached 34 % for Culmo Vuoto and 32 % for Culmo Pieno, whereas the glutelins totaled 29 % for both lines.

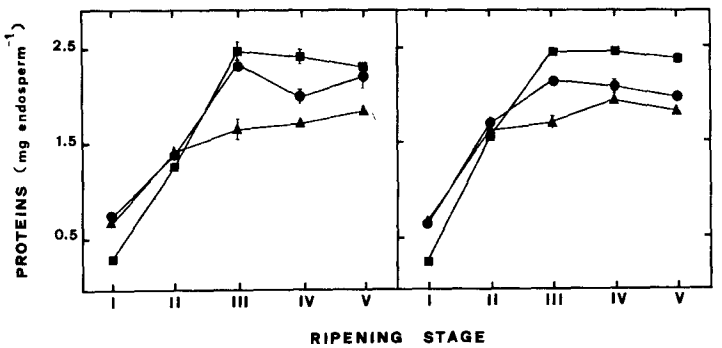

Fig. 1. Endosperm protein deposition during maturation in the two lines of ×*H. sardoum*: albumins plus globulins (•), prolamins (▪) and glutelins (▲).

The SDS-gel electrophoretograms of the reserve proteins from the ripening endosperm of Culmo Pieno and Culmo Vuoto were compared. The scans of the albumins plus globulins showed two main zones (Fig. 2): the first zone (Mr < 20 kDa) exhibited a fairly constant profile during maturation, whereas the second one (20 kDa < Mr < 67 kDa), showed continuous changes which were more pronounced for Culmo Pieno (stages IV and V) than for Culmo Vuoto. Nevertheless, no marked qualitative differences were detected for the albumin plus globulin components extracted from the fully ripe caryopses of both lines (Fig. 3).

The electrophoretic pattern of the prolamins revealed the early appearance of these proteins, (30 kDa < Mr < 43 kDa); their profile substantially maintained its characteristics during the ripening process, showing only a quantitative increase in the components. A second group of proteins, (43 kDa < Mr < 67 kDa), exhibited a complete profile from stage III onwards and finally, a third group (Mr > 94 kDa) showed profound modifications during the course of ripening (Fig. 4). Notable

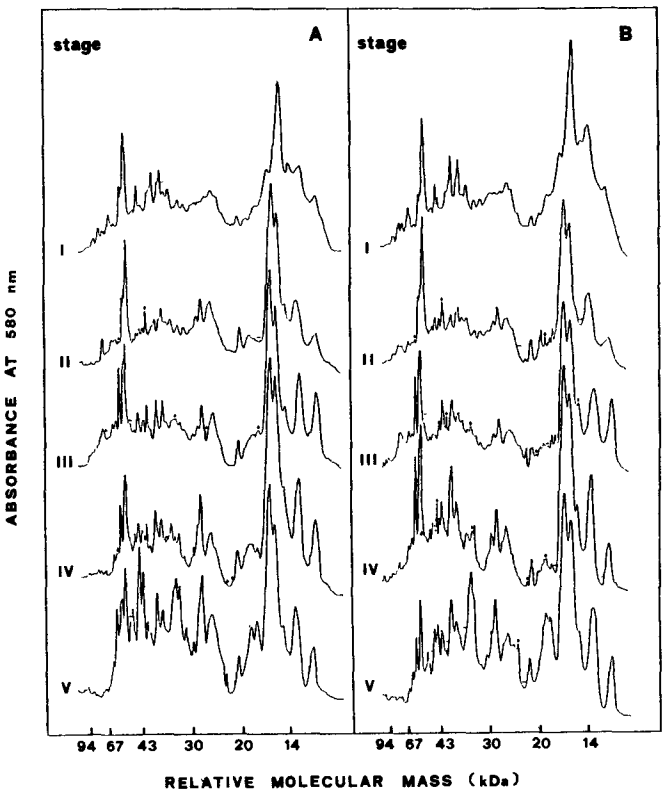

Fig. 2. Densitometric scans of the albumins plus glubulins of ×*H. sardoum* during maturation (A = Culmo Vuoto line; B = Culmo Pieno line).

qualitative differences appeared in the densitometric scans of the Culmo Pieno and Culmo Vuoto seeds and could also be seen in the mature seeds (Fig. 3).

The main components of the glutelin class appeared precociously in both lines, but their concentrations increased during seed ripening, particularly the Mr 94 kDa proteins (Fig. 5). Other proteins with an Mr lower than 67 kDa showed qualitative and quantitative changes. The mature seeds of the Culmo Pieno and Culmo Vuoto lines showed different electrophoretic patterns than the gliadin class (Fig. 3).

DISCUSSION

At twenty days from anthesis (stage I) the endosperm of the two lines of ×*H. sardoum* studied contain only low levels of each of the protein classes, but the rate of deposition increases rapidly between stage I and stage III (34 d from anthesis), where the typical concentrations of the mature caryopsis are reached. This evidence

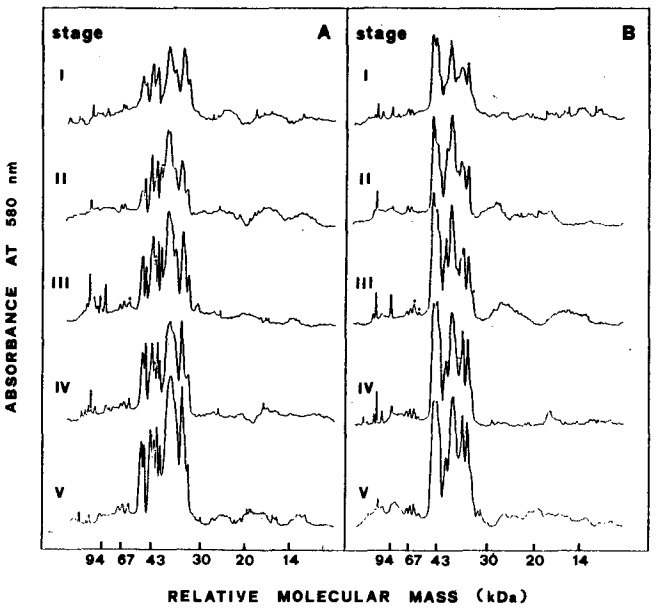

Fig. 4 Densitometric scans of the prolamins of ×*H. sardoum* during maturation (A = Culmo Vuoto line; B = Culmo Pieno line).

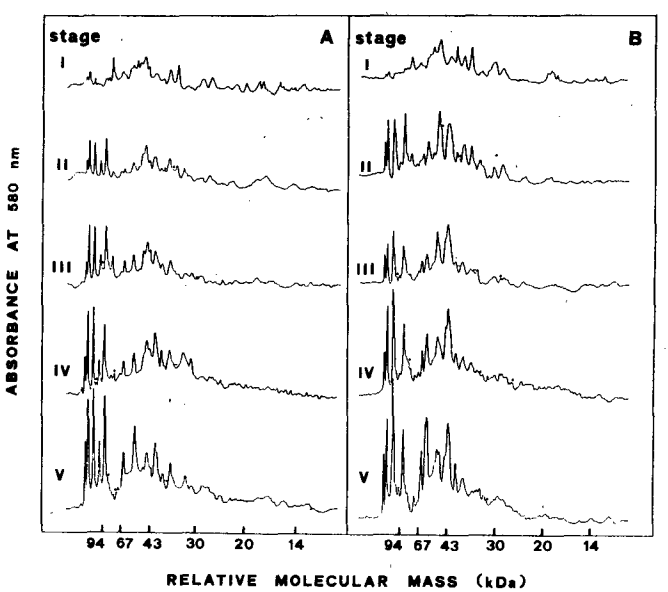

Fig. 5 Densitometric scans of the glutelins of ×*H. sardoum* during maturation (A = Culmo Vuoto line; B = Culmo Pieno line).

seems to confirm a general pattern observed in certain graminaceous seeds, according to which the synthesis of storage proteins does not start precociously ; such patterns have been observed in wheat, sorghum, rye and barley seeds (Johari *et al.* 1977, Rahman *et al.* 1982, Greene 1983, Shewry *et al.* 1983, Greene *et al.* 1985).

Nevertheless the endosperm of ×*H. sardoum* has a higher albumin plus globulin content (30 %) than wheat, malt or rye (about 20 %). This could be explained by ascribing a reserve function to a portion of the proteins in ×*H. sardoum*. In particular, the zone of proteins with Mr lower than 20 kDa is relatively stable and its subunits appear to accumulate during the course of ripening. On the contrary the remaining zones (Mr > 20 kDa) show continuous changes which could be due to variations in the enzymic and structural components resulting from the ripening process.

The main components of the prolamin class show their presence early and increase quantitatively during the course of ×*H. sardoum* ripening. The expression of the gliadin genes occurs at the very earliest stages of endosperm development in wheat (2 days after anthesis, Tercé-Laforgue *et al.* 1987), whereas their accumulation *in vivo* is delayed (Tercé-Laforgue and Pernollet 1982, Greene 1983, Greene *et al.* 1985). This could be due to a delayed triggering of the cellular machinery (Tercé-Laforgue *et al.* 1987). In ×*H. sardoum* endosperm some of the larger gliadin components (Mr 67 kDa), the ω-gliadins, seem to appear later, as has already been observed in soft and durum wheat by Tercé-Laforgue and Pernollet (1982) and Grilli *et al.* (1989).

Other components with an elevated Mr (94 kDa) seem to be high molecular mass glutelins, which can contaminate the prolamin fraction as has been reported for *in vivo* labelled gliadins of soft wheat (Tercé-Laforgue *et al.* 1987).

The pattern of glutelin deposition is very similar from the start of development until maturity in the two lines of ×*H. sardoum* seeds. This agrees with the results of Khan and Bushuk (1976) and Grilli *et al.* (1989). The mature caryopses of the two lines, however, show different electrophoretic patterns for the prolamin and glutelin classes. Wrigley *et al.* (1981) demonstrated that the electrophoretic pattern of prolamins is not influenced by environmental conditions, but rather reflects the different genetic makeup of the seeds. Because ×*H. sardoum* seeds may vary widely in their physiological and morphological characteristics (Onnis 1971, Meletti and Onnis 1975), our evidence seems to confirm the differences between the seeds of the two lines. This result agrees with some observations (data not shown) according to which the Culmo Pieno line may be a mutant present with a low frequency amids the Culmo Vuoto plants in the natural population of ×*H. sardoum*. Significantly *T. durum* plants (from which ×*H. sardoum* derives) may show the solid stem as a spontaneous or X-ray induced mutation (Bozzini and Avanzi 1962). The appearance of the Culmo Pieno line could represent an attempt at adaptation by the species ; in fact, it demonstrates the typical behaviour of a spring wheat, while Culmo Vuoto is a winter line (Onnis 1971).

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Fig. 3. at the end of the issue

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