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Some Features of the Inheritance of Avenins, the Alcohol Soluble Proteins of Oat

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Summary. The inheritance of avenin components, the prolamins (or alcohol soluble proteins) of *Avena*, is studied by means of gel electrophoresis. Avenin is composed of rather similar proteins which appear as a polymorphic group from a biochemical point of view. After a first preliminary investigation it showed a surprisingly high interspecific variability. The average number of its constituents increases with the ploidy level but it still is much lower than that of wheat gliadin.

The avenin electrophoretic patterns of 47 samples (F_4, F_5 or F_6 seeds) resulting from 3 hexaploid crosses are compared with the parental patterns. Four kinds of inheritance are observed. Roughly 50% of progeny profiles are identical to those of one of the parents. They are composed occasionally of partial sections of parental patterns. Complete additiveness occurs rather seldom. However, in one of the crosses a significant number of progeny samples show a band, one of the very slow moving constituents, which was not present in either of the parents.

The study of avenin in F_1 seeds, arising from reciprocal crosses between two homozygous parent plants, shows a significant effect of maternal gene dose in the triploid endosperm.

Because of both the variability and the relatively small number of avenin constituents, these results show that typical endosperm proteins such as oat prolamin constitute a useful tool for phylogenetic studies of the genus *Avena*.

Key words: Oat prolamins – Inheritance – Electrophoresis

Introduction

In recent years, many investigations have been carried out on the heterogeneity of storage or enzymatic proteins in relation to cereal taxonomy, phylogenetics and inheritance mechanisms. In the genus *Avena*, the inheritance of three different types of proteins have been studied. Embryo or seedling proteins, many of which are exclusively coded by nuclear DNA have been the most extensively studied. Smith (1972) and Yen and Sadanaga (1977) studied peroxidases of young oat leaves and showed they are controlled by one or two genes: one of the genes controlling one of the peroxidases is allellic to the gene controlling another band. For an oligomeric leaf enzyme such as ribulose 1-5 diphosphate carboxylase the situation is more complex: one of the subunits is coded by nuclear DNA and the other one by chloroplastic DNA (Steer 1975). The third case corresponds to endosperm proteins: Murray et al. (1970) extracted amphiploid oat seed proteins with 30% acetone, and found a nearly complete additivity in the banding paterns of the parental protein. They suggested a gene dosage effect. In this last case, prolamins, the alcohol soluble proteins of endosperm, have also been extensively studied in other cereals recently (Doekes 1973; Manghers et al. 1973; Righetti et al. 1977; Aragoncillo et al. 1978). They have been preferred to other proteins because of their presence in endosperm only and also because they are governed only by genetic factors, not by the growing conditions (Feillet and Bourdet 1967; Lee and Ronalds 1967; Auriau et al. 1976).

Moreover, prolamins are found to constitute a good biochemical material, especially in the case of wheat, with which to investigate species relationships or to identify varieties (Wrigley and Shepherd 1973, 1974; Johnson 1975; Autran and Bourdet 1975; Dhaliwal 1977).

However, until now, no research has been undertaken on the alcohol soluble proteins of oat for this purpose. In fact the heterogeneity of oat prolamin, avenin, and its biochemical properties were reported more recently than for others prolamins. In this laboratory we demonstrated that avenin, extracted from an hexaploid oat (*A. nuda* cv. 'Rhea'), reveals 8 electrophoretic constituents. Their mo-

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lecular weights determined by SDS-electrophoresis or by exclusion gel chromatography vary from 20,000 to 34,000 daltons (unpublished results). Two of those which have been isolated by chromatography have the same molecular weight (22,000), the same N-terminal amino acids (Threonin) and very similar amino acid compositions (Kim et al. 1978). These first results suggest polymorphism of avenin both in size (molecular weight) and in electric charge (starch gel electrophoresis).

In the present paper we describe the preliminary results on the polymorphism of avenin and two different features of its inheritance, by means of electrophoresis. The former deals with the additive nature of oat prolamin bands in progeny seeds after the F_2 generation; the latter corresponds to the effectiveness of the double dose of maternal genes of seed endosperm on avenin patterns.

Materials and Methods

The following oat samples, obtained from 'Station d'Amélioration des Plantes' in Rennes and in Gembloux, were used:

a) 18 samples of 8 different species:
4 diploids *A. strigosa* and *A. brevis*.
1 tetraploid *A. abyssinica*.
13 hexaploids *A. sativa, A. sterilis, A. byzantina, A. nuda, A. fatua*.

b) 1 fixed line resulting from cross I: *A. byzantina* cv. 'Red Algerian' and *A. sativa* cr. 'Ariane'.

c) 6 lines F_4 from cross II: *A. sativa* cv. 'Astor' and *A. sterilis* ('Maroc-8').

d) 8 naked and 11 coated lines F_5 from cross III: *A. sativa* cv. 'Maris titan' and *A. nuda* '532' ('Huskless' × 'ORA').

e) 22 naked lines F_6 from cross IV: *A. sativa* '109-3' (('Flamande × Ariane') 495 × ('Bonhaw' × 'Ariane') 763-1) and *A. nuda* '542-3' ('Rhea' × 'Padarn').

f) 2 reciprocal F_1 from cross V: *A. sativa* cv. 'Sirene' and *A. nuda* cv. 'Finland'.

The extraction and starch gel electrophoresis of prolamins were performed according to the method of Kim et al. (1978). Defatted meal (1g) was extracted for 1h at room temperature with 10 ml of 45% ethanol (w/w). This has been shown to be the optimum concentration of ethanol for the extraction of avenin (Kim et al. 1978). After centrifugation, the clear supernatant was diluted with two volumes of precooled 1.5% NaCl solution and the precipitate, separated by centrifugation at 6000 × g for 30 min, was then dried under vacuum in a dessicator. Such a crude oat prolamin also contains some glutelins and salt soluble proteins but they can be distinguished from avenin in their electrophoretic behaviour. The salt soluble proteins move much faster than avenin and the glutelins do not yield any bands, as indicated in a previous paper (Kim et al. 1978). Therefore, the electrophoretic patterns shown in this paper represent exclusively oat prolamins. Gel electrophoresis was carried out on a 12% starch gel using aluminium lactate buffer-3 M urea, pH 3.2, at ca. 10-15 volts/cm. After cutting the gel (5 mm thick), staining was carried out with 0.05% nigrosin in 30% acetic acid solution. The separation of avenin compenents by starch gel elctrophoresis is known to be due principally to their electric charges.

Results

Variability of Avenin Electrophoretic Patterns

The electrophoretic pattern of Figure 1 shows the large variability of avenin in the number of bands as well as in their mobility and intensity: the oat prolamin of eighteen seed samples, corresponding to eight different *Avena* species, and to the three possible ploidy levels were submitted to electrophoresis on the same gel. The two diploid species *A. strigosa* (J and L) and *A. brevis* (P and Q) have only two or three bands of rather low mobility. The tetraploid species *A. abyssinica* (K) has four different bands, but the thirteen other samples which correspond to hexaploid oats: *A. nuda* (A, B); *A. sativa* (C, D, E, F, R); *A. byzantina* (N, O); *A. sterilis* (G, H, I) and *A. fatua* (M) show from four to eight bands, which suggests that the number of bands increases with ploidy level.

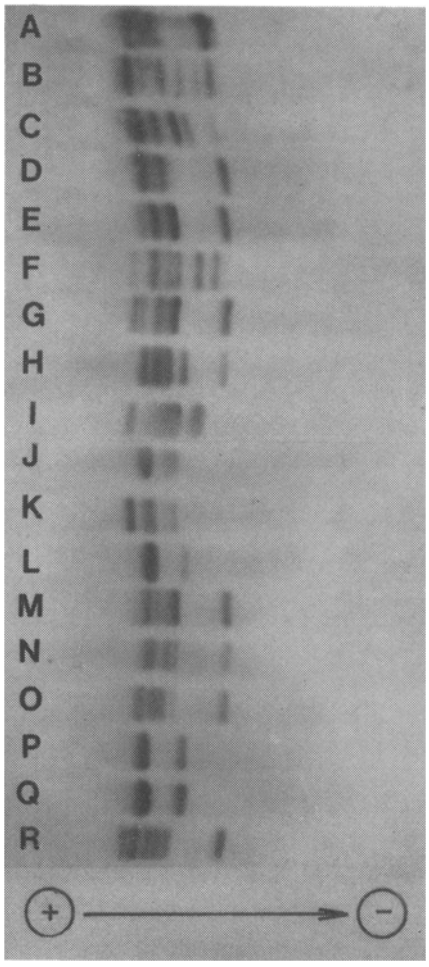

Fig. 1. Electrophoretic patterns of several oat species: A, B) *A. nuda*; C, D, E, F, R) *A. sativa*; G, H, I) *A. sterilis*; J, L) *A. strigosa*; K) *A. abyssinica*; M) *A. fatua*; N, O) *A. byzantina*; P, Q) *A. brevis*

Superimposing the electrophoretic patterns of the 18 samples demonstrates eleven bands of different mobilities. In a more complete study of avenins extracted from different samples of the seventeen known *Avena* species (unpublished results), we have shown that at least 17 different bands occur, which have been numbered 1 to 17 in the order of increasing mobility. The same numbering is used in the present paper (Fig. 2b).

Inheritance of Avenin Constituents In Hexaploids

The electrophoregrams of two pairs of parents (A and C for cross I, D and K for cross II) and their descendants are shown in Figures 2a and b. The most different avenin patterns in the parents are found in 'Ariane' and 'Red Algerian' (A and C) which have only one common band (8). The descendant B, which has been fixed by self-pollination for several years, shows five bands. Three of them (bands 2, 4, 6) are derived from 'Ariane', one (13) from 'Red Algerian'. The fifth (8) is common to both parents.

Such a recombined type in electrophoretic pattern is found also in the lower part of Figure 2 where we can see electrophoregrams of *A. sativa* cv. 'Astor' (D), *A. sterilis* 'Maroc-8' (K) and six samples of their progeny which are F_4 lines (E to J). Further, in addition to the two descendants (E and J) which have exactly the same avenin pattern as either 'Astor' or 'Maroc-8', one descendant (G) shows all the avenin constituents of both parents. It must be also noted that of two common bands (2 and 9), one, band 9, is absent in two descendants (H, I).

Cross III (Fig. 3) between *A. sativa* cv. 'Maris titan' (A), revealing seven bands (2, 4, 6, 8, 9, 15, 16), and *A. nuda* '532' (B), with five bands (5, 7, 9, 15, 16), gives similar results. From nineteen descendants, eleven are identical to one of the parents (I, Q, R, to A, and C, D, F, K, M, N, O, S to B), and the eight others show intermediate electrophoretic patterns. For example, of the intermediate types one descendant (E) reveals seven bands, one (2) of which is derived from 'Maris titan', two (5, 7) from '532' and the others (9, 15, 16) are common bands. However, in contrast to other crosses, both parents have the fast moving band 15 and all descendants have this fast band (15) also.

Unlike the above results, some descendants of cross IV (Fig. 4) between *A. sativa* '109-3' (with the six bands 4, 6, 8, 9, 15, 16) and *A. nuda* '542-3' (with the six bands 4, 6, 7, 9, 11, 13) do have one unexpected new band: from the twenty two naked lines, nine (C, D, E, H, I, J, K, N, T) possess the slowest moving band (2) which is not expressed in any of the parents. The others show the same results as above in their electrophoretic patterns. Ten have the same avenin components as one of the parents: G, M, O, Q, V show bands 4, 6, 8, 9, 15 and 16 which are those of parent A; F, S, U, W, X show bands 4, 6, 7, 9, 11 and 13 characteristic of parent B. Besides, two descendants have intermediate patterns (L, band 4, 6, 7, 8, 11, 13, 15, 16 and P, band 4, 6, 8, 9, 11, 13, 15, 16), and only one (R) shows all the bands of both parents. The absence of

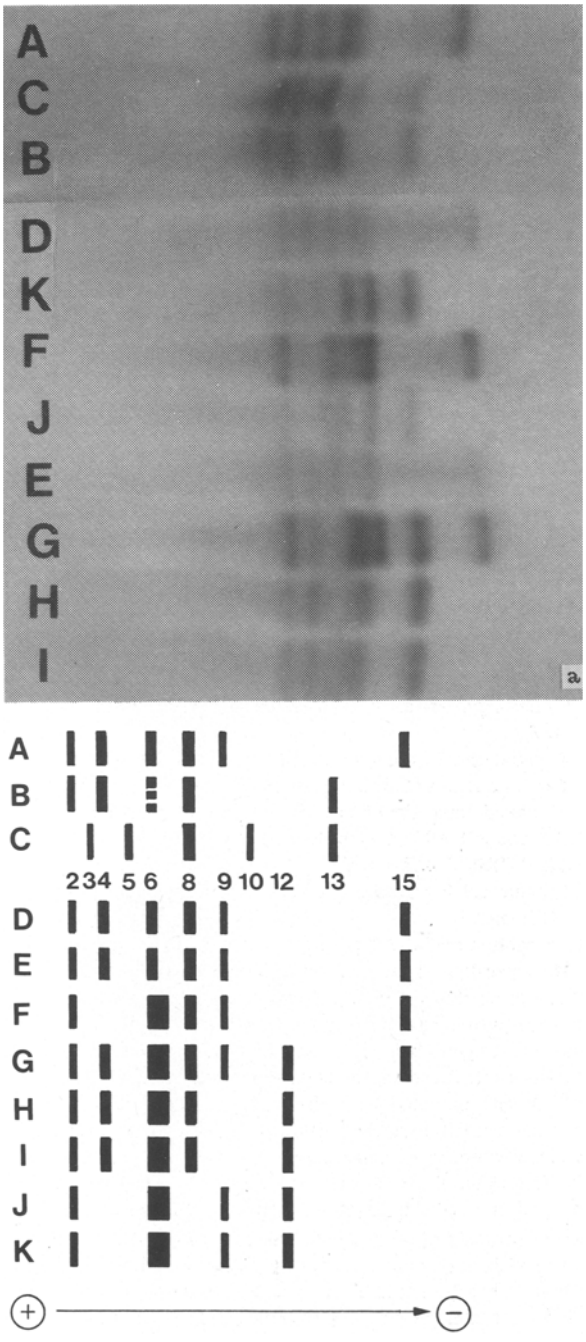

Fig. 2a-b. Electrophoregrams of cross I and II. **a** photos, **b** diagram: Cross I – A) *A. sativa* cv. 'Ariane'; B) one fixed progeny; C) *A. byzantina* cv. 'Red Algerian'. Cross II – D) *A. sativa* cv. 'Astor'; E ~ J) 6 F_4; K) *A. sterilis* ('Maroc'-8)

the common band (4) is observed in two descendants (D, J) as in cross II (H, I).

Reciprocal Crosses: Maternal Gene Dosage Effect

The two descendants of the cross V (Figs. 5a and b) represent another feature of avenin inheritance. Because of the triploid nature of the endosperm tissue, the amount of genetic information in endosperm is different from that of the embryo in heterozygous plants: endosperm cells of the F_2 generation may contain 0, 1, 2 or 3 doses of genes unique to one of the parents. Consequently, it is possible to observe the effect of dosage or interaction of genes, especially on proteins such as prolamins which exist only in the endosperm. In the first cross (B), 'Sirene' acts as a mother plant (2 doses of genes of 'Sirene' and 1 of 'Finland') and in the second (C) as a father plant (1 dose of genes of 'Sirene' and 2 of 'Finland').

The intensities of two bands of 'Sirene', the fastest and the slowest ones, and the fastest band of 'Finland' decrease according to gene dosage. The additivity of bands in F_1 descendants is also pointed out.

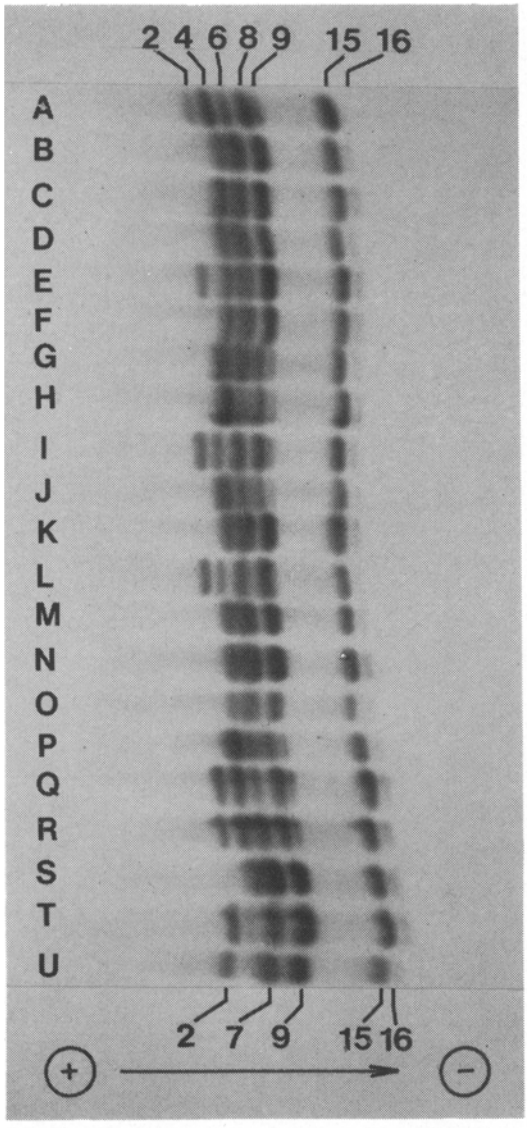

Fig. 3. Electrophoretic patterns of cross III: A) *A. sativa* cv. 'Maris titan'; B) *A. nuda* '532'; C ~ J) 8 naked F_5; K ~ U) 11 coated F_5

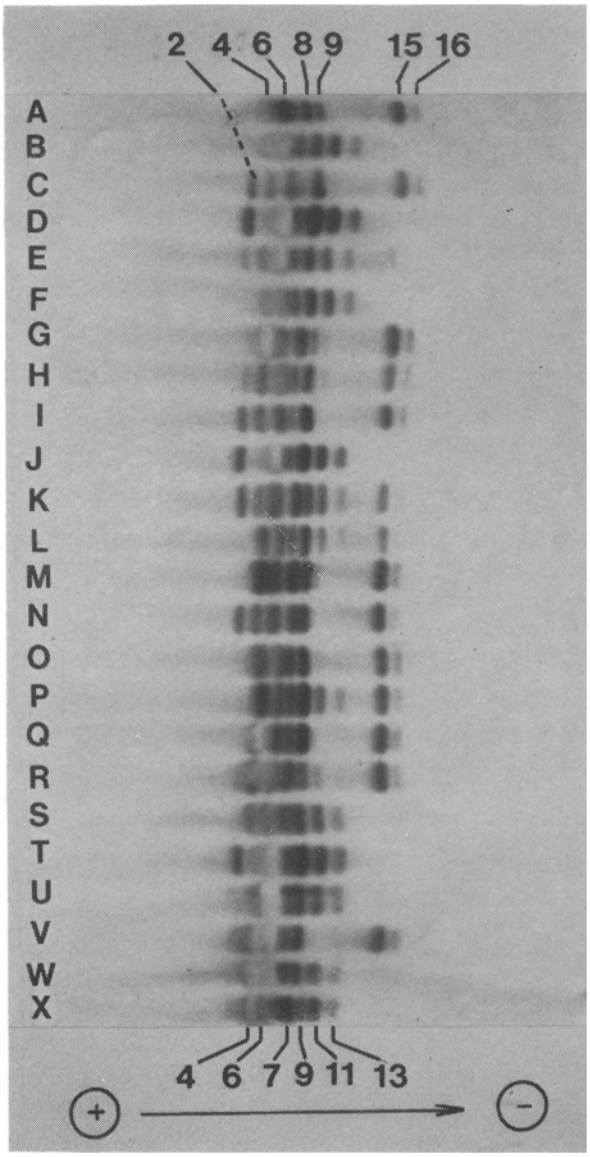

Fig. 4. Electrophoretic patterns of cross IV: A) *A. sativa* '109-3'; B) *A. nuda* '542-3'; C ~ X) 22 naked F_6

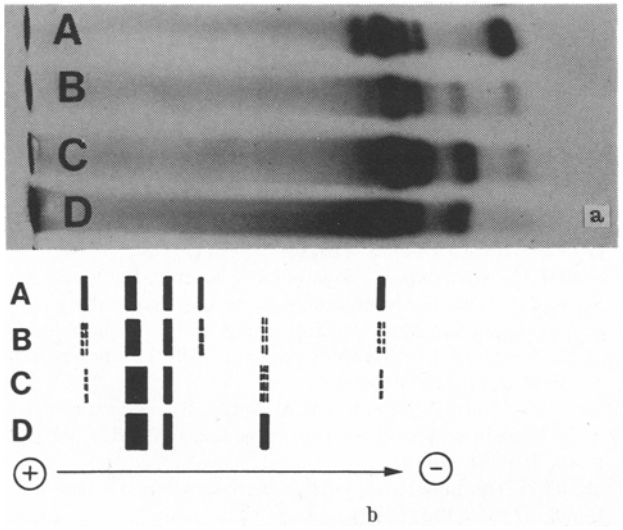

Fig. 5a and b. Electrophoregrams of cross V. a photos, b schematic diagram: A) *A. sativa* cv. 'Sirene'; B) 'Sirene' × 'Finland'; C) 'Finland' × 'Sirene'; D) *A. nuda* cv. 'Finland' (double concentration was applied in gel for C)

Discussion

A significant variability within or between species in electrophoretic patterns of oat prolamin and an increase in band number with ploidy level suggests polymorphism for polypeptide chains of the different bands. However, as in many similar experiments on other cereal proteins, we have no direct evidence that all control of avenin synthesis is transcriptional. In fact, the possibility of post-translational modifications has been already discussed by Mossé (1973) and more recently by Bray (1976). As with insulin, which arises from a much longer polypeptide precursor, it is known now that some seed proteins are submitted to *in vivo* cutting, either occasional as in concanavalin of the jack bean (Wang et al. 1971; Edelman et al. 1972) or plausibly systematical as in corn zein. This last case has been suggested by the recent exciting investigations of Burr et al. (1978). Moreover other events like deamidation or glycosylation could have occurred, but the modifications of a single polypeptide chain can not produce several bands in starch gel electrophoretic patterns at acid pH.

Such post-translational modifications of prolamin are possible but in this study we think that most of the bands of avenin electrophoretic patterns represent structural genes such as in other prolamins. The genetic regulation of electrophoretic constituents have been clearly demonstrated by many authors for cereal prolamins, and we know now the gene locations which code for wheat or barley prolamin components (Manghers et al. 1973; Righetti et al. 1977; Aragoncillo et al. 1978; Shewry et al. 1978). The results obtained with 47 samples of the progeny of cross II, III and IV can be separated into four different classes.

1. Many descendants have a band pattern identical to one of the parents (23 among 47, i.e. roughly half of them).

2. A smaller number of the descendants show a partially recombined pattern of the parents, but not for all of the components. Two cases can be observed for the inheritance of the common bands: their presence in some descendants and their absence in others.

3. A few descendants appear to have all of the bands of the parents, which correspond to complete additivity of bands.

4. For one of the crosses, several descendants have one new band which is absent in the parental patterns.

The first three cases summarized above were already described among cereals like wheat by Doekes (1973), but as far as we know, no evidence of the fourth case has been until now encountered among prolamins even though its occurrence does not seem unlikely. This last case can be considered a result of repression of the corresponding structural gene in both parents. This gene is then *derepressed* in the progeny which results in the appearance of a new band. Other kinds of gene interaction can be also suggested: a progeny band not present in either parent could arise via a modification gene in one parent thus changing a basic polypeptide subunit specified by the genome of the other parent. The absence of a common parental band in a progeny, the second case, could result from gene interaction as above but other arguments are also possible in regard to the limit of electrophoretic analysis. If we assume that the band of the same mobility in each parent represents two distinct polypeptide chains specified by different loci and that one parent carries a null allele responsible for the protein in the other parent and *vice versa*, then it could happen that the band could be missing in some advanced progeny.

The effect of gene dosage is demonstrated by a reciprocal cross test. These results are in good agreement with those found by Righetti et al. (1977) with corn seed prolamins. Nevertheless, it is too early to draw general conclusions from such a single experiment. By studing allohexaploid wheat endosperm proteins, Aragoncillo et al. (1978) recently illustrated different possible features of the gene dosage effects.

The above results suggest that the additivity of the banding patterns found by Murray et al. (1976) with oat seed protein is probably not as frequent for avenin, which is composed of endosperm specific proteins. In fact, the inheritance mechanisms of seed protein can not be explained by a simple gene absence or presence. In order to know the inheritance characteristics of avenin, further research has to be carried out to localize the genes control-

ling each component using aneuploid techniques to make near-isolines (mono, nulli, tetra and nullitetrasomic) and also to characterize its biochemical properties and synthetic mechanisms.

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