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The Chromosomal Location of Factors Determining the Presence of Phenolic Compounds in Wheat (*Triticum aestivum* L.)

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Summary. Using thin-layer chromatography and nullitetrasomic and ditellosomic series of *Triticum aestivum* L. cv. 'Chinese Spring', it has been possible to relate the phenolic compounds found in adult plant leaves and 12 day-old seedling leaves with the chromosomes or chromosome arms 1 B, 2 BL, 3 BL, 5 A, 6 AL, 7 B and 7 DS.

Key words: *Triticum aestivum* – Wheat-phenolics-location

Introduction

Phenolic compounds, which are normal constituents of plants and animals, are characterized by the presence of at least one aromatic ring with a hydroxyl substituent. They are also able to form an heterogeneous group with respect to molecular structure (Harbone 1964). A number of the phenolic compounds found in plants have been structurally identified, but the physiological role of many of them is not yet well known (Alston 1965).

Thin-layer chromatography has allowed the study of phenolic compounds. The identification and classification of phenolics among species of the same genus have been reported in *Avena* (Grant and Wheter 1970), *Hordeum* (Fröst and Holm 1971; Fröst et al. 1975) and *Triticum* (Bose 1972). Phenolic compound patterns have also been seen to vary among cultivars of the same species: in *Hordeum spontaneum* (Först and Holm 1975) and *Triticum* (Först and Holm 1977). In addition, phenolic compounds have also been used as chromosome markers in wheat (May et al. 1973).

The purpose of the present investigation is the chromosomal location of leaf phenolic compounds of *Triticum aestivum* L. cv. 'Chinese Spring'.

Materials and Methods

The materials used in this study were the nulli-tetrasomic and ditellosomic series of *Triticum aestivum* L. cv. 'Chinese Spring' supplied by Proffessor E. R. Sears. The analyses were carried out with both adult plant leaves and 12 day-old seedling leaves grown at 20± 1 °C in a growth chamber with a 15-hour illumination per day.

The leaves were dried at 50 °C overnight in the dark and then crushed and extracted in methanol containing 1 per cent conc. HCl by volume (150 µl/mgr) for 15 hours in the dark at 3± 1 °C. From each extract, 20 µl was applied at the starting point of a chromatographic plate (20×20 cm) coated with cellulose powder (Merck's microcrystalline Avicel, 300 µ thick). The plates were run in one direction in the dark at 20 °C employing two different solvent systems i) n-butanol, acetic acid and water (BAW, 3:1:1) (Fröst and Holm 1973) and ii) isoamylic alcohol, acetic acid and water (AAW, 2:1:1) (Dedio et al. 1969). After drying, the plates were examined under long-wave UV-light at 360 nm and short-wave UV-light at 254 nm. Subsequently, the plates were sprayed with a conc. $AgNO_3$ solution in acetone.

Results

Using adult plant leaves and the BAW solvent system and examining the plates under 360 nm UV-light, a total of thirteen spots were identified. Under 254 nm UV-light fifteen spots were observed: a new spot (l) was found and the spot k was separated into two spots (k and k'). A total of fourteen spots were observed when the plates were sprayed with $AgNO_3$ solution (Fig. 1). Employing adult plant leaves and the AAW solvent system and observing the plates under 360 nm UV-light a total of eleven spots were identified; twelve spots were observed under 254 nm UV-light; seventeen spots were found with the $AgNO_3$ solution (Fig. 1).

When the 12 day-old seedling leaves were analyzed using BAW solvent system, eleven spots were observed under 360 nm UV-light; fourteen spots were identified under 254 nm UV-light and nineteen spots were found with the $AgNO_3$ solution (Fig. 1). However the 12 day-old seedling leaves showed eight spots under 360 nm UV-light and twelve spots under 254 nm UV-light when the AAW solvent system had been used. A total of thirteen spots were found when the plates were stained with the $AgNO_3$ solution (Fig. 1).

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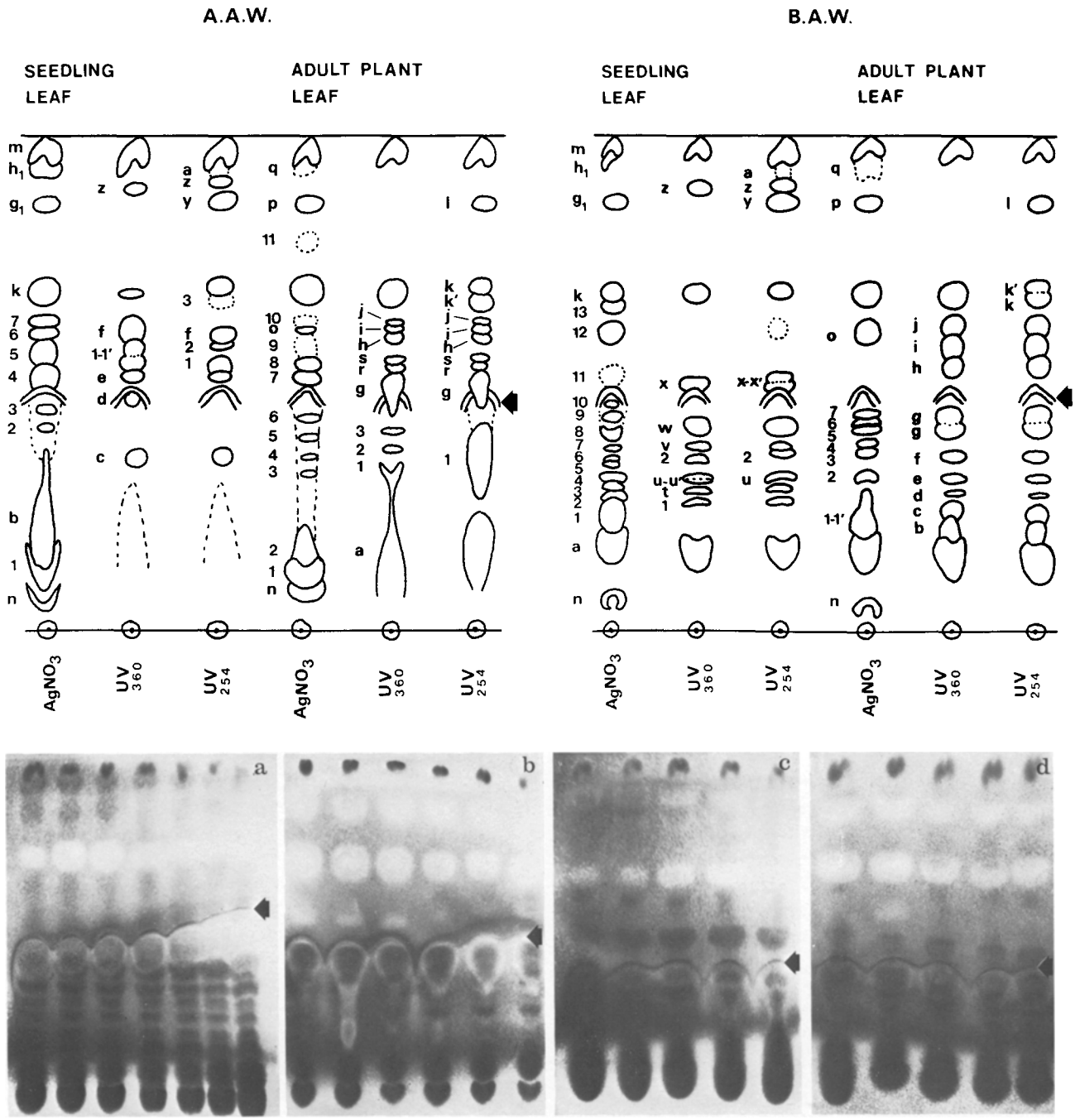

Fig. 1.a–d. Wheat phenolic compound patterns. **a** Phenolic compound patterns of the 12 day-old seedling leaves, BAW, $AgNO_3$; **b** Phenolic compound patterns of the adult plant leaves, BAW, $AgNO_3$; **c** Phenolic compound patterns of the 12 day-old seedling leaves, AAW, $AgNO_3$; **d** Phenolic compound patterns of the adult plant leaves, AAW, $AgNO_3$. The spot indicated by an arrow is produced by the extractor used

The two solvent systems used are complementary since the BAW solvent system has a great resolving power in the lower half of the plates, while the AAW solvent system has a better resolution in the upper half of the plates. This is the reason why the spots p, q and l have been observed with AAW but not with BAW (Fig. 1).

The spots observed using the BAW or AAW solvent systems for adult plant or 12 day-old seedling leaves considered as similar are indicated by the same small letter; the spots considered as different are notated by a number. In all cases the faster migration spot (m) represents chlorophyl compounds and the spot indicated by an arrow was produced by the extractor used (Fig. 1).

Table 1. The chromosomal location of determinants of phenolic compounds of wheat

Spot	Solvent system	360 nm	254 nm	$AgNO_3$		Critical chromosome or chromosome arm	
5	BAW			×	–	7B	
d	BAW	×	×		–	1BS	
e	BAW	×	×		+– –	7B	
k	BAW			×	+– –	3BL	
k	AAW			×	+– –	3BL	
k′	BAW		×		+– –	6Al	
k′	AAW		×		+– –	6AL	
k′	AAW		×		+ +	5A	
l	BAW		×		–	6AL	
	BAW		×		+– –	3BL	
	AAW		×		+ +	5A	
o	BAW			×	+– –	3BL	Adult plant leaves
	AAW					6AL	
p	AAW			×	+ +	5A	
q	AAW			×	+ +	6AL	
s	AAW	×			–	5A	
1	BAW	×			–	6DL	
	AAW		×		+ +	1B	
2	BAW	×			+– –	1B	
2	BAW		×		+– –	1B	
7	AAW			×	–	2BL	
e_1	AAW		×		+– –	1DL	
f_1	AAW	×	×		–	2BL	
h_1	AAW			×	+ +	7DS	
k	BAW	×	×		+– –	5A	
z	BAW	×	×		–	3BL	12-day-old seedling leaves
	AAW						

– spot absence; +– – spot present with a very low intensity; + + spot present with a higher intensity

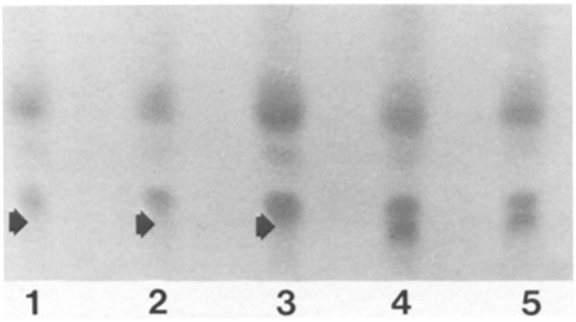

Fig. 2. Chromosomal location of the spot d. Adult plant leaves, BAW, UV-light. *1* Nulli-1B-tetra-1A, *2* Nulli-1B-tetra-1D, *3* Ditello-1BL, *4* Ditello-1BS, *5* Euploid 'Chinese Spring'. Arrows indicate spot absence

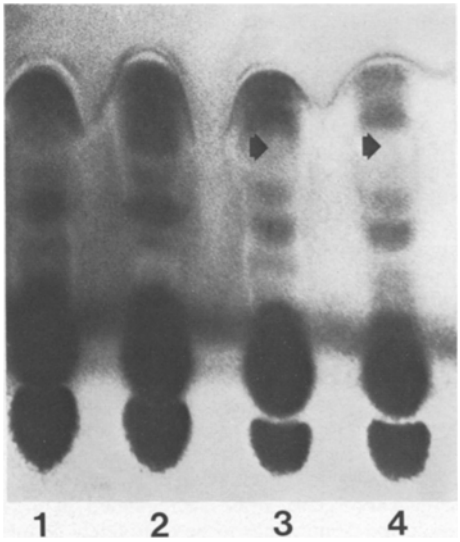

Fig. 3. Chromosomal location of the spot 5. Adult plant leaves, BAW, $AgNO_3$ *1* Ditello-7BL, *2* Ditello-7BS, *3* Nulli-7B-tetra-7A *4* Nulli-7B-tetra-7D. Arrows indicate spot absence

The results obtained using adult plant or 12 day-old seedling leaves of nulli-tetrasomic and ditellosomic series and employing BAW or AAW solvent systems, are indicated in Table 1. We have observed i) spot absence, ii) spot presence with a higher intensity and iii) spot presence with a very low intensity. Comparing the spot pattern with the absence of a chromosome or chromosome arm, it has been possible to relate several spots (phenolic compounds) with certain chromosomes or chromosome arms. Whenever these cases has been observed in the two nulli-tetrasomics but only in one ditellosomic we have just referred to the particular chromosome arm (Table 1), while we have referred it to the chromosome as a whole when the fact is observed in the two nulli-tetrasomics but not in the corresponding ditellosomics (or when the ditellosomics were not available), for example, the chromosome 5A (Table 1).

Discussion

As mentioned above, spot absence, spot presence with a higher intensity and spot presence with a very low intensity have been observed. Also we have noted that specific spots are the results of the overlapping of several phenolic compounds; because when a spot observed under UV-light is absent, another spot is observed in the same place with $AgNO_3$ solution. This is true, for example, for the spot 1 (254 nm) and the spot p ($AgNO_3$).

Several explanations can account for these three observations: i) Lack of gene or genes related with the synthesis of the enzyme or enzymes involved in the phenolic compounds metabolic pathway. This metabolic pathway could be interrupted so that the phenolic compounds following the block would not be synthesized (spot absence and spot present with a very low

intensity) while the phenolic compounds preceding the block would be accumulated (spot present with a higher intensity). Spot absence and spot presence would then be related mechanisms as they would be overlapping. According to this hypothesis it should be possible to find at the same time both spot absence and incresase or decrease of spot intensity. As seen in Table 1 when chromosome 5A is absent, the spots k', l and p (adult plant leaf) are present with a higher intensity while spot s is absent. Likewise, spot q is present with a higher intensity (adult plant leaf) when the long arm of the chromosome 6A is absent, while spot l is lacking (Table 1). Also, it is possible to find simultaneously both a decrease and an increase in spot intensity. For example, spot 1 (12 day-old seedling leaves) is present with a higher intensity when chromosome 1B is absent; at the same time spot 2 is present with a very low intensity (Table 1). Due to overlapping and that the technique used does not detect all the phenolic compounds involved in the same metabolic pathway, the differences observed between spots can not always be observed. ii) The three differences observed might be explained independently because it is known that these metabolic pathways are complex. The same chromosome or chromosome arm could be involved at different levels, having simultaneously structural and regulator genes. Therefore, the decrease of spot intensity of a given phenolic compound could be due to the existence of several metabolic pathways for the synthesis of the same phenolic compound. iii) Sometimes we have been able to observe the lack or decrease of a specific spot in the two nulli-tetrasomics while the same spot was present with normal intensity using the corresponding ditellosomics. This phenomenon could be explained if each chromosome arm were involved in a different metabolic pathway. In such a case the phenolic compound could be synthesized by two different metabolic pathways.

On the other hand, we have also observed that there are several spots which have no relation with any of the nulli-tetrasomics used. A possible explanation could be that wheat is an allohexaploid specie having three homoeologous genomes. In this case the replacement of a pair of chromosomes by either homoeologous pair would mean that there were up to 6 doses of that gene present. These genes must be regulated in the same way for the nulli-tetrasomic lines to be completely compensated (May et al. 1973).

Similar results have been obtained using a different technique by May et al. (1973). They found that some phenolic compounds were related to chromosomes 1D, 2D, 3B, 5A, 6A, and 7D. Our results agree with these. We have found a relationship between the phenolic compounds and the last four chromosomes and also with the chromosomes 1B and 2B which belong to the same homoeologous group to those indicated by May et al. (1973).

Finally, we have found that the chromosomes 7B and 6A are associated with the synthesis of phenolic compounds in adult plant leaves, while these chromosomes are not associated with 12 day-old seedling leaves phenolic compounds. We have also observed chromosomes concerned with 12 day-old seedling leaves phenolic compounds (1D, 2B, 6D, and 7D); chromosomes which are not involved with adult plant leaves phenolic compounds. This fact suggests that different genes are involved in the synthesis of phenolic compounds in different stages of development.

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