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The Flavonoids in Leaves of Diploid *Triticum* Species (*Gramineae*)

By

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Abstract: Leaf flavonoids have been identified in seven species of *Triticum*, all of which have been considered at one time as putative parents of the cultivated tetraploid and hexaploid wheats. The major constituents are apigenin- and luteolin-based glycosylflavones, some of which contain various O-glycosidic attachments at the 6″-position. Four tricin glycosides are present in minor amount, as is free tricin. The flavonoid patterns link together *T. searsii, T. speltoides* and *T. squarrosa*, on the one hand, and *T. monococcum, T. boeoticum, T. thaoudar* and *T. urartu*, on the other. These results indicate that the first three taxa are more likely to be diploid ancestors to the hexaploid *T. aestivum* than the latter four species.

The origin of the cultivated hexaploid bread wheat (genome AABBDD) has been the subject of numerous investigations (FELDMAN & SEARS 1981). It is thought to contain genetic material from at least three diploid wild species, two of which are *T. monococcum* (AA) and *T. squarrosa* (DD). There is still controversy, particularly with regard to the diploid species responsible for donating the BB genome. Recent evidence from DNA-DNA hybridisation has implicated *T. searsii* (NATH & al. 1984), but other candidates include *T. urartu* and *T. speltoides*.

Biochemical data used so far have largely been derived from the macromolecules, but evidence from other sources might well help to indicate which are the most likely parental taxa. Earlier, two-dimensional TLC screening of leaf extracts has revealed the presence of at least 34 unidentified flavonoid constituents variously occurring in 26 *Triticum* species (FRÖST & HOLM 1977, HOLM & FRÖST 1979). Such flavonoid comparisons have revealed, for example, rather limited relatedness between *T. squarrosa*, the supposed donor of the D genome, and cultivated wheat. A more detailed examination of the flavonoids in leaf

tissue of wild wheat species was, therefore, undertaken in order to compare the constituents of these plants with those of the hexaploid *T. aestivum*.

Previous studies of the flavonoids of diploid and tetraploid *Triticum* species have been very limited. However, tricin, its glycosides, isovitexin and iso-orientin glycosides have been recorded in *T. monococcum* and *T. polonicum* (HARBORNE & HALL 1964). By contrast, the hexaploid *T. aestivum* has been more thoroughly investigated, and iso-orientin, lutonarin, lucenin-1, lucenin-3, vicenin-2, iso-orientin 7-rutinoside and isoswertisin (8-glucosylapigenin 7-methyl ether) 4′-glucoside have been identified (JULIAN & al. 1971). A more recent investigation of this species revealed the additional presence of vicenin-1, isoschaftoside, schaftoside and the sinapoyl ester of 6-arabinosyl-8-galactosylapigenin (WAGNER & al. 1980).

The present investigation reports the major flavonoids in leaves of seven *Triticum* species.

Materials and Methods

Plant Material. Plants were field grown from authentic seed by S. F. at Lund and dried leaves airposted to Reading. Seeds of *Triticum searsii* were kindly provided by Professor SEARS. Seeds of all other species were obtained from the *Triticum* seed collection of the USDA at Beltsville. The four samples of *T. monococcum* L. analysed came from Turkey, U.S.A., Greece and Spain, the 3 samples of *T. urartu* THUM. ex GANDIL from Lebanon, Turkey and U.S.S.R., 7 samples of *T. boeoticum* BOISS. from Iraq, Iran, U.S.S.R., Lebanon and Turkey and *T. thaoudar* REUTER ex HAUSSKN. from Turkey and Switzerland. Seven accessions of *T. speltoides* TAUSCH and *T. squarrosa* L. were also examined.

2D Chromatograms were run on microcrystalline cellulose (glass plates) in BAW and 15% HOAc. Extracts could not be directly examined by analytical HPLC because many components overlapped; however, sections of P.C. 2D chromatograms were used by cutting out and eluting the compounds present.

HPLC Systems used were (1) Partisil 5 CC5 C8 column (25 cm × 4 mm i.d) with (10 cm × 4 mm) CoPell ODS guard column; solvents were A − 5% HOAc (aq.) and B − MeOH:H_2O:HOAc; 90:5:5, initial composition 20% B in A, increasing by 2% B per minute. Flow rate 1.7 ml min^{-1}, detection at 365 or 330 nm. (2) Conditions as in 1), but initial composition 30% B in A. (3) Phenyl column replacing the C8 column, but with solvents as in (1) (HARBORNE & BOARDLEY 1984).

Isolation and Identification of Compound No. 7. It was first isolated from *T. searsii* by P. C. in BAW and 15% HOAc on 3 MM paper. Crystals readily formed on standing in 30% MeOH and these were collected. It had λ max MeOH 274, 335 nm; + $AlCl_3$ 388 nm; + NaOAc 283 nm; + NaOAc and H_3BO_3 354, 412 nm; + NaOH 405 nm. (See Table 1 for Rf and colour reactions.) Spectral data indicated an apigenin-based compound; on acid treatment there was no change, and Rf data indicated a 6,8-di-C-glycoside of apigenin. FAB-MS in glycerol gave a molecular ion $[M + 1]^+$ of 565, identical to that of schaftoside (6-arabinosyl-8-

glucosylapigenin). This structure was confirmed by co-chromatography in a variety of TLC systems with an authentic marker.

Isolation and Identification of 7′. This was first isolated from *T. monococcum* by P. C. in BAW and 15% HOAc (3 MM paper). It had λ max MeOH 259, 269, 350 m; + $AlCl_3$ 400 nm; + NaOAc 270 m; + NaOAc and H_3BO_3 373 nm; + NaOH 404 nm. (See Table 1 for colour and Rf data.) Its bright yellow colour on fuming with NH_3 was distinctive and easily picked out on 2 D chromatograms. There was no change on acid treatment, and demethylation and de-C-glycosylation with phenol and HI giving luteolin as the product, indicated that 7′ was a lucenin type. Colour and absence of an acetate shift indicated 7-methylation, 7′ having identical colour reactions to a sample of iso-orientin 7-methyl ether.

Isolation and Identification of 6/1. This was first isolated from *T. urartu* by column chromatography on Whatman CF 11 cellulose powder, elution was with H_2O. Further purification was carried out by sem-prep. HPLC on a C_8 column (25 cm × 8 mm) and elution with solvents A and B as above, composition 35% B in A throughout the run. Finally, the glycoside was run on 3 MM paper in CAW and the solvent allowed to drip off the end. Despite this, small amounts of related glycosides present in the plant were still indicated by sugar analysis. Thus, it gave mainly rhamnose, but small amounts of glucose and galactose were also detected. Spectral data were similar to iso-orientin, except only a small acetate shift was observed; however, glycosylation at the 7-position was ruled out by colour reactions. On hydrolysis (2 N HCl, 100 °C) iso-orientin and rhamnose were produced in less than 2 min, no intermediates being observed. RF data (see Table 1) indicated that one sugar was present (high in 15% HOAc) and comparison with a sample of iso-orientin 6″-O-arabinoside (Rf values-BAW 0.39; H_2O 0.14; 0.15% HOAc 0.48) confirmed that this was almost certainly iso-orientin 6″-O-rhamnoside.

Identification of 6/3. This was isolated from *T. searsi* by P. C. Rfs and colour reactions were the same as 6/1, but 6/3 ran slightly lower in BAW, and the glycosides could also be separated by HPLC (see Table 1). Hydrolysis gave iso-orientin and glucose. This compound is provisionally identified as iso-orientin 6″-O-diglucoside by analogy with 6/1. 6/2 was closely related in structure to 6/1 and 6/3 and from similar analyses to those mentioned above was provisionally identified as second iso-orientin 6″-diglycoside.

Other compounds present were identified by standard procedures and comparison with authentic markers where available. Many glycosides were present in trace amounts only and could not be fully identified.

Results

Because of the complex mixture of flavonoids present, several methods of surveying the leaf extracts were used, including 2 D paper chromatography and HPLC on reverse phase columns; but 2 D TLC on microcrystalline cellulose proved to be far the superior procedure for resolving both major and minor components. The results of these analyses for the seven wheat species investigated are shown in Table 1 and the relative positions of the flavonoids are indicated in Fig. 1.

The individual flavonoids were then separated and purified from appropriate extracts and identified by standard procedures (see Methods).

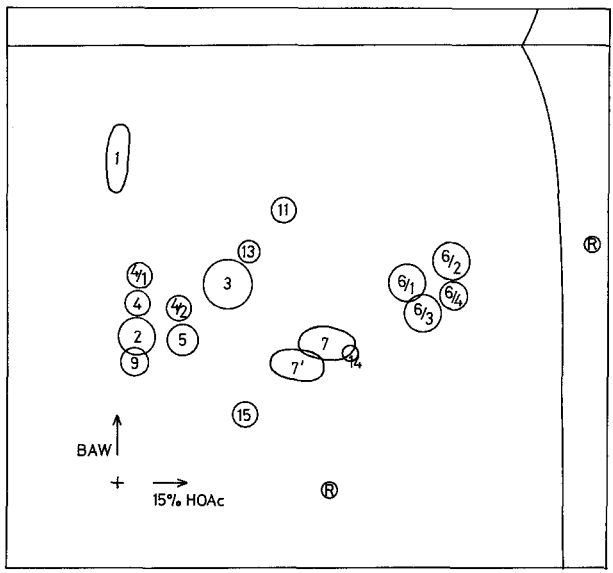

Fig. 1. 2D chromatogram of diploid wheat flavonoids on microcrystalline cellulose plates. BAW: n-BuOH-HOAc-H_2O (4:1:5, top); 15% HOAc, 15% aqueous HOAc. For structure identifications, see Table 2; 11, 13–15 are unidentified glycosylflavones; R = rutin

The results of these analyses are shown in Table 2, together with chromatographic data and colour reactions. While several of the compounds, e.g. 1–3, have been reported before in *Triticum*, others are newly described. In particular, the series of iso-orientin 6″-glycosides 6.1–6.4 (6.2 is not shown in Table 2 and 6.4 is not shown in either Table; but see Fig. 1) have not been recorded before, although they also appear to be present in *T. aestivum* (see below). As will be seen, only some of the minor components could be identified because of the difficulties of separation and analysis. A variety of trace components were also apparent on the 2D chromatoplates, but they appeared to have a fairly random distribution and were not further investigated.

As can be seen (Table 1), all seven species examined are distinguished by their flavonoid profiles, although in some cases the differences are relatively minor. Infraspecific variation might limit the value of such flavonoids as species markers, and it was not possible in this investigation to screen more than a few (at most, seven) accessions of each taxon. In the case of *T. searsii*, only one accession was available. With both *T. monococcum* and *T. urartu*, comparison of several accessions indicated minor infraspecific variation (see Table 1). This was mainly confined to

Table 1. Flavonoids of seven diploid wheat species. + + high concentration; + present; − not detected; ± present in some accessions but not in others

Species	Presence/absence of major flavonoids									
	1	2	3	4	6.1	6.2	6.3	7	7′	9
T. urartu	+	+	+ +	±	+ +	±	−	−	+ +	±
T. monococcum	+	+	+ +	−	+ +	+	−	−	+ +	±
T. thaoudar	+	+	+ +	−	+ +	−	−	−	+ +	+
T. boeoticum	+	+	+ +	+	+	+	−	−	+ +	−
T. searsii	+	+	+ +	+	−	+	+ +	+ +	−	−
T. squarrosa	+	+	+ +	+	−	+ +	+	+ +	−	+ +
T. speltoides	+	+	+ +	+	−	+ +	+ +	+ +	−	−

Species	Presence/absence of minor flavonoids							
	4.1	4.2	5	11	13	14	15	16.4
T. urartu	−	−	+	−	±	+	−	±
T. monococcum	−	−	+	+	+	+	−	−
T. thaoudar	−	−	+	+	−	+	−	+
T. boeoticum	−	−	+	+	−	+	−	−
T. searsii	+	+	+	+	+	−	+	+
T. squarrosa	+	−	+	+	+	−	+	+
T. speltoides	−	−	+	+	−	−	−	+

Table 2. Rf data, colour reactions and identifications of diploid wheat flavonoids. HPLC system (1) was used (see Methods). B = brown; BL = blue; d = dull; b = bright; G = green; Y = yellow

No.	Structure	Rf value (× 100) in				HPLC t_r (min)	Colours in	
		BAW	H_2O	15% HOAc	PhOH		UV	UV + NH_3
1	Tricin	81	00	04	95	22.7	dB	Y
2	Tricin 7-glucoside	40	01	14	90	19.0	dB	bY
3	Iso-orientin	55	08	40	60	13.6	dB	Y
4	Tricin 7-diglycoside	38	02	28	87	−	dB	bYG
5	Tricin 7-monoside	37	01	17	−	−	dB	bYG
6/1	Iso-orientin 6″-rhamnoside	49	58	73	51	13.6	dB	dBY
6/3	Iso-orientin 6″ diglucoside	44	58	73	51	12.6	dB	dBY
7	Schaftoside	36	20	49	79	12.3	dB	dY
7′	Lucenin 7-methyl ether	26	16	47	−	10.6	dB	bY
9	Tricin 5-glucoside	34	01	06	91	−	bBL	YG
10	Isovitexin	68	−	47	76	−	dB	B

the tricin glycosides and especially the presence/absence of tricin 5-glucoside.

In the flavonoid patterns, there is no clear-cut separation of *T. squarrosa* and *T. speltoides* from the other *Triticum* species, but this is hardly surprising in view of the close relationship between the two species and their intercrossability.

Discussion

From the chemical results (Tables 1–2), the diploid wheat species can be divided into two groups on the basis of the occurrence of compounds 7 and 7′. There are three species – *T. searsii, T. speltoides* and *T. squarrosa* – containing 7, and four species – *T. monococcum, T. boeoticum, T. thaoudar* and *T. urartu* – containing 7′. No species contains both compounds. This difference is significant in that 7 is an apigenin-based glycosylflavone and 7′ is luteolin-based. That they occur in the same region of the chromatogram is purely coincidental. Other differences that confirm these two groupings of species are in the distribution of compounds 6.1–6.3. All three compounds are based on iso-orientin (6-glucosyl-luteolin) and they have one or more sugars specifically attached in the 6-glucosyl moiety. Their patterns are complicated and difficulties in resolution limited their characterisation.

2 D patterns of leaf flavonoids were obtained for extracts of a series of wild and cultivated tetraploid and hexaploid wheats, and comparison with those of the diploid species indicated that although more compounds were present in the polyploids, there were relatively few major differences (unpubl. results). Notably, the diploid compound 7′ could not be found in any of these samples, while 7 was normally present. Because compound 7′ occurs in relatively high concentration, especially in *T. urartu* and *T. monococcum*, its absence from these other species and cultivars is surprising. Since inheritance of leaf flavonoids is usually additive in hybrids (HARBORNE & TURNER 1984), the compound 7′ would be expected to appear in the tetraploids and hexaploids if these species were ancestral. This evidence, however, does not completely rule out the involvement of these four species in the origin of the bread wheat, since some chromosomal material may well have been lost in the process of evolution of the cultivated hexaploid. Genes controlling flavonoid synthesis in *T. aestivum* have been located at particular chromosomal sites (NEUMAN & al. 1983).

In conclusion, it appears that flavonoid data support the idea that *T. searsii, T. squarrosa* and *T. speltoides* are likely diploid ancestors of hexaploid wheats, but they do not precisely rule out the involvement of any of the others. Overall, there is more similarity in flavonoid constitu-

ents within these taxa than there are differences. It seems unlikely that further work on the flavonoids of the diploid species will reveal any greater differences than those outlined above. Work is now in progress on the flavonoids of the tetraploid plants.

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