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STUDY OF PHENOLIC CONSTITUENTS OF *TRITICUM* L. (POACEAE) SPECIES IN IRAN*

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Abstract – This recent study documents the phenolic constituents of the *Triticum* L. species in Iran using thin layer chromatography. Species studied are related to 55 wild and cultivated accessions of four diploid and four tetraploid species, namely *Triticum boeoticum* subsp. *boeoticum* Boiss., *T. boeoticum* subsp. *thaoudar* Reut. ex Boiss., *T. monococcum* L., *T. urartu* Tum. ex Gand., *T. turgidum* L., *T. dicoccoides* (Korn. ex Ascher. et Graebn.) Thell., *T. dicoccum* (Schrank.) Schubl. and *T. durum* Desf. collected from natural habitats and analyzed for their flavonoid compounds. The current study found that of a total of 20 flavonoid compounds, most of the derivatives were flavones, chalcones, and the others were mainly pseudobaptisin, sciadopitysin, baptigenin and fustin-3-*O*-glucoside. The basis of variation in these compounds was shown to be usable as an appropriate marker for chemotaxonomic studies.

Keywords – *Triticum*, flavonoid, Iran, Poaceae

1. INTRODUCTION

Triticum L. genus belongs to the family Poaceae and Tribe Triticeae, having 27 species throughout the world [1] and eleven species in Iran. It is vitally important economically and has always been the focus of taxonomic and biosystematic attention due to its being taxonomically intricate and showing a complex evolutionary development. In total, eleven *Triticum* species, four wild diploid species including *T. boeoticum* subsp. *boeoticum* Boiss., *T. boeoticum* subsp. *thaoudar* Reut. ex Boiss., *T. monococcum* L. and *T. urartu* Tum. ex Gand.; 2n=2x=14; x=7) and four tetraploid species counting *T. turgidum* L., *T. dicoccoides* (Korn. ex Ascher. et Graebn.) Thell., *T. dicoccum* (Schrank.) Schubl. and *T. durum* Desf. (2n=4x=28) [2, 3] grow in Iran.

Previous studies of the flavonoids in *Triticum* species have been very limited. Flavonoid research on the Poaceae detected the presence of flavones-*C*-glycosides and tricin 5-glucoside, tricin 7-glucoside, isovitexin, iso-orientin glycosides, lucenin 7-methylether [4, 5]. Moreover, the soluble phenolic compounds including flavonols were considered in *T. aestivum* [6]. Two new flavonoid-*C*-glycoside named triticuside A and triticuside B were isolated from the bran of this species [7].

Thin layer chromatographic patterns, especially of the flavonoids, could be used in the chemical taxonomy of grasses including individual genotypes and the original taxonomic relationships in the genus. The patterns were also sufficient to determine the position of the Section [8, 9]. Eleven flavonoids isolated from *Muhlenbergia* Schreb. (Poaceae) consisted of flavones, luteolin, tricin and apigenin [10]. Different phenolic profiles were apparent in *Trisetum* Pers. [11]. In addition, the most important flavonoid

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compounds present in *Hyparrhenia hirta* Stapf (Poaceae) were 7-*O*-glucoside and vitexin [4]. Vanillin and rosmarinic acid are compounds resembling those which occur in *Bromus* L., *Festuca* L. and *Dactylis* L. [9]. In addition, the flavonoid compounds in *Vetiveria nigritana* (Benth.) Stapf (Poaceae) were recognized as flavone-*C*-glycoside, tricin and luteolin [12].

Moreover, since Iran is one of the secondary centers of genetic diversity for diploid *Triticum* species, there is a need for using this genetic resource. In the present study, we report on flavonoid compounds in eight *Triticum* species, investigating the variability of these compounds among them, and explain the chemotaxonomic value of these compounds. To the best of our knowledge, this is the first report on the flavonoid contents of *Triticum* species in Iran.

2. MATERIALS AND METHODS

a) Plant material

The location of *Triticum* species and accessions (in the case of eight species and 55 accessions) collected from natural habitats of Iran is shown in Table 1. The voucher specimens were deposited in the Herbarium of Isfahan University (HIU).

Table 1. List of localities of *Triticum* species in natural habitats of Iran

Species/accession number	Locality	Height (m)
Ur40	Kurdistan-Asadabad	1977
Ur26b	Chaharmahal va Bakhtiari-Shalamzar	1980
Ur36b	Kermanshah-Kamyaran	1240
Ur72	West of Azerbayjan -Ahar	1430
Ur35	Lorestan-Islamabad	1240
Ur73	West of Azerbayjan-Zanjirboulagh, Ahar	1500
Ur74	West of Azerbayjan -Ahar	1550
Mn33	Lorestan-Islamabad	1250
Mn38	West of Azerbayjan -Zanjirboulagh, Ahar	1410
Mn38	West of Azerbayjan -Ahar	1430
Mn30	Kermanshah-gardan-e-Reno	1480
Mn31	Kermanshah-Abolvafa	1380
Mn39	Arak-Malayer	2020
Mn40	Tehran-Taleghan	1850
Bb37	Kurdistan-Janian	1770
Bb5	Lorestan-Khoramabad	1820
Bb35	Lorestan-Islamabad	1240
Bb33	Lorestan-Sepid-dasht	1800
Bb2	Isfahan-Semirom	2210
Bb1	Isfahan-Semirom	2250
Bb3	Koykilouyeh va Boyerahmad-Amirabad	1650
Bb15	Lorestan-Aligoudarz	2420
Bb9	Kermanshah-gardan-e-Reno	1370
Bt37	Kurdestan-Janian	1770
Bt29	Chaharmahalva Bakhtiari-Ardal	1820
Bt36	Kurdestan-Kamyaran	1800
Bt28	Chaharmahal va Bakhtiari-Ardal	1820
Bt31	Lorestan-Sepid-dasht	1800
Bt32	Kermanshah-Darbadam	1680
Di12428	Isfahan-Semirom	2100

Table 1. (Continued)

Di24	Kermanshah-gardan-e-Reno	1480
Species/accession number	Locality	Height (m)
Du1	Koykilouyeh va Boyerahmad-Dehbak	2210
Du4	Koykilouyeh va Boyerahmad-Dehbak	2370
Du7	Chaharmahalva Bakhtiari-Boroujen	1840
Du9	Khuzistan-Baghmalek	880
Du23	Lorestan-Khoramabad	1850
Du86	Kermanshah-Kamyaran	1350
Du94	Kermanshah-Naghiabad	1720
Du53	Koykilouyeh va Boyerahmad-Gachsaran	700
Du68	Lorestan -Sepid-dasht	1900
Du22	Khuzistan-Lordegan	1950
Du15	Khuzistan-Haftgol	550
Tu2	Koykilouyeh va Boyerahmad-Dehbak	2370
Tu84	Kermanshah-Harsin	1330
Tu128	Kermanshah-Ivan	1200
Tu194	Kurdistan-Sanandaj	1620
Tu196	Kurdistan-Divandareh	1920
Tu211	West of Azerbayjan-Khoy	2100
Tu213	West of Azerbayjan-Boukan	1900
Tu212	West of Azerbayjan-Sourin	1800
Tu11	Khuzistan-Baghmalek	950
Tu13	Khuzistan-Masjed-soleyman	450
Tu25	Lorestan -Malavi	1120
Tu37	Chaharmahal va Bakhtiari-Dashtak	2000
Do12426	West of Azerbayjan-Maragheh	1400
Do12427	Kurdistan-Janian	1770

ur: *T. urartu*, mn: *T. monococcum*, bb: *T. boeoticum* subsp. *boeoticum*, bt: *T. boeoticum* subsp. *thaoudar*, tu: *T. turgidum*, du:*T. durum*, do: *T. dicoccum*, di: *T. dicoccoides*.

b) Sample extraction

Extraction, isolation and identification of flavonoids were based on the protocol suggested by Markham [13]. The flavonoid solution was extracted from air-dried leaves (10.5 g) from 55 accessions (Table 1) of eight *Triticum* species using crude 85% MeOH at 60°C. The extract was dissolventized using a rotary evaporator at 70°C for total solvent removal and subsequently analyzed by two-dimensional maps (2DM) on PolyamidDC-6 (15 mg, 67.5 ml H2O) thin layer chromatography (TLC; 0.25 mm). The chromatogram was developed in C2H4Cl2-MeOH-BuOH-H2O (50:25:21:4) representing an organic system, and H2O- EtOH-BuOH (70:20:10) representing an aqueous system. Spot detection with 0.1%

natural product identifiers (C14H16BNO in 1:1 MeOH and H2O) was performed under UV-254 nm. The purification of the flavonoid compounds of each accession and species was carried out using column chromatography (CC) (100 × 3 cm) with Sephadex LH-20 (Sigma-Aldrich) (Sephadex and MeOH 20% mixture) in 100 ml MeOH solution (with increasing MeOH content 20%, 30%, 40%, 50%, 60%, 80 and 100%), and extracted in fractions (the amount of packing material is 50 ml for each MeOH content 20%, 30%, 40%, 50%, 60%, 80% and 100% separately; 200 fractions). The fractions were subjected to one-dimensional maps (1DM) on polyamide TLC plates (0.25 mm). Identification of the purified compounds was performed on the basis of their UV spectra (200-500 nm), MeOH solution and shift reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/ H_3Bo_3 and MeOH [13].

3. RESULTS AND DISCUSSION

Flavonoid compounds in MeOH fractions from leaves of *Triticum* species were investigated. The individual flavonoids were separated and purified from appropriate extracts and identified by standard procedures. Based on the findings of the present study, the chromatogram spots based on color reactions (Table 2) among diploid and tetraploid *Triticum* species consisted of 4'-hydroxychalcone 5-*O*-glycoside (blue spots after treatment), 5-hydroxyflavonol (dark-yellow spots after treatment), 8-hydroxyflavones, isoflavone, 3-*O*-flavonol, dihydroflavonol and 2'-6'-hydroxychalcone (without color change in spots), 4'-hydroxychalcone (yellow spots after treatment), and 2,4-dihydroxychalcone (orange and blue spots after treatment).

From the findings on the final fraction in each accession and the UV absorption spectra via shift reagents, the variation in flavonoid patterns in diploid and tetraploid groups is present as 4-hydroxylation (*T. urartu, T. monococcum, T. dicoccum*), 5-hydroxylation (*T. boeoticum* subsp. *boeoticum, T. dicoccoides*), 7-hydroxylation (*T. boeoticum* subsp. *boeoticum*, *T. boeoticum* subsp. *thaoudar*), 2'-hydroxylation (*T. urartu, T. boeoticum* subsp. *thaoudar*), B-ring*ortho*-dihydroxylation (*T. urartu, T. boeoticum* subsp. *boeoticum, T. monococcum, T. dicoccum*), A-ring*ortho*-dihydroxylation (*T. boeoticum* subsp. *boeoticum, T. dicoccoides*), 6-oxygenation (*T. dicoccum*), 8-oxygenation (*T. urartu, T. boeoticum* subsp. *boeoticum, T. monococcum, T. turgidum*, *T. durum*), and 3'-oxygenation (*T. boeoticum* subsp. *thaoudar*). By contrast, an earlier *T. monococcum* study detected 7-methylation [5].

Table 2. Chromatogram spots of *Triticum* species

Species/spots	Pale yellow	Dark yellow	Pale blue	Orange	Violet	Brown
1	+	+	+	+	-	-
2	+	-	-	+	-	-
3	+	+	+	+	-	-
4	+	+	-	-	+	-
5	+	-	+	+	-	-
6	+	+	+	-	+	-
7	+	+	+	-	+	-
8	+	+	+	-	+	+

1: *T.boeoticum* subsp. *boeoticum*, 2: *T.boeoticum* subsp. *thaoudar,* 3: *T. monococcum*, 4: *T. urartu*, 5: *T. dicoccoides*, 6:*T. durum*, 7:*T. turgidum*, 8:*T. dicoccum*

Based on total flavonoid content, 4'-hydroxychalcone, 2'-hydroxy 4'-methoxychalcone and flavones were detected in all of the accessions investigated. The contents were significantly high, belonging to the pseudobaptisin (81.25%), 2'-hydroxy 4'-methoxychalcone (80-98%) and flavones (70-98%). In contrast, 4'-hydroxychalcone, 2',3,4-trihydroxychalcone and 6,7-dihydroxychalcone (25-12.5%), Fustin-3-*O*-glucoside, 2',4-dihydroxychalcone and 2,2'-dihydroxychalcone (17.6-10%) (Table 3), 3',4',7-trihydroxyflavone (25-16.6%) and 3,4-dihydroxychalcone (10-8.3%), 3',4',7'-trihydroxyflavone and 2-

hydroxychalcone (30-5.8%) (Table 3), 5,6,7-trihydroxyflavone (16.6-4.1%), baptigenin, sciadopitysin and 2,2',4-trihydroxychalcone (4.1%) (Table 3) had low contents. Some flavonoid compounds have been isolated for the first time from the *Triticum* species namely baptigenin, pseudobaptisin, sciadopitysin and fustin-3-*O*-glucoside. Insufficient information is available concerning the phenolic compounds analysis of *Triticum* species. Previous phytochemical studies on the Poaceae Family [8] identified flavones-C-glycoside as being the basic flavonoid in *Vetiveria* genus, and the possibility of the C-glycoside step in the flavones biosynthesis. Also, tricin-5-*O*-glucoside, apigenin, luteolin based glycosylflavone and apigenin-C-diglycoside were found in *T. aestivum* [14], apigenin-6-C-galactoside-8-C-(2-sinapoyl) arabinoside and apigenin-6-C-(2-sinapoyl) arbinoside-8-C-galactoside were found in bran *T. aestivum* [7]. The percentages of *O*-glycosidic attachment at the 6" position and glycosylation at 7 and 7'-position have been already reported in *Triticum* spp. [5]. In our research 3-*O*-glycosyl and 7-*O*-rhamnoglycosyl (pseudobaptisin compound) were found in diploid *Triticum* species, and according to Harborne et al. [5] 7-position in *Triticum* is an apigenin-based glycosylflavone. Moreover, 7-position and 7'-position were found in pseudobaptisin, 6,7-dihydroxychalcone, 5,6,7-trihydroxyflavone, 3',4',7-trihydroxyflavone, 3',4',7'-trihydroxyflavone and sciadopyticin among diploid and tetraploid species, which is in accordance with Harborne et al. [5]. However, in this study 7'-position based hydroxyflavone were observed in *T. boeoticum* subsp. *boeoticum*, but were not observed in tetraploid species, which disagrees with the findings of Harborne et al. [5]. In other taxa belonging to Poaceae, ten flavonoid compounds in *Hyparrhenia hirta* were present mainly in the case of flavones *C*-glycoside, and the 5 and 7-*O*-glucoside positions [4].

Table 3. Percentage of flavonoid constituents in *Triticum* species

compounds	1	2	3	4	5	6	7	8
4'-hydroxychalcone	58.8	30	16.6	25	50	12.5	16.6	20
2-hydroxychalcone	5.8	30	-	-	-	-	-	-
2',4-dihydroxychalcone	17.6	10	-	-	-	-	-	-
2',3',4'-trihydroxychalcone	29.4	-	-	-	-	-	-	-
2'-hydroxy4'-methoxychalcone	88.2	65	64.5	78.1	58.3	98	98	80
2'-hydroxychalcone	-	40	-	-	4.1	-	-	-
2,2'-dihydroxychalcone	-	10	-	-	-	-	-	-
3,4-dihydroxychalcone	-	10	-	-	8.3	-	-	-
2',3,4-trihydroxychalcone	-	-	-	-	-	12.5	-	-
6,7-dihydroxychalcone	-	-	-	-	-	12.5	-	-
2,2',4-trihydroxychalcone	-	-	-	-	4.1	-	-	-
Flavone	70.5	70	77	68.7	79.1	75	98	75
3',4',7'-trihydroxyflavone	5.8	-	-	-	-	-	-	-
3',4',7-trihydroxyflavone	-	-	-	-	25	25	16.6	-
5,6,7-trihydroxyflavone	-	-	-	-	4.1	-	16.6	-
3'-hydroxy4'-methoxyflavone	-	-	-	-	-	-	33.3	-
Baptigenin	-	-	4.1	-	-	-	-	-
Sciadopitysin	-	-	4.1	-	-	-	-	-
Pseudobaptisin	-	-	20.8	81.25	-	-	33.3	-
Fustin- 3-*O*-glucoside	17.6	-	-	-	-	-	-	-

1. *T.boeoticum* subsp. *boeoticum*, 2: *T.boeoticum* subsp. *thaoudar,* 3: *T. monococcum*, 4: *T. urartu*, 5: *T. dicoccoides*, 6:*T. durum*, 7: *T. turgidum*, 8: *T. dicoccum*

The phenolic content such as flavonols in *Triticum aestivum* L. cultivars, reached 23-50% of the total. This evidence shows that *Triticum* cultivars differed in chemical compounds [6]. In addition, the compounds included in this research differed among the species studied and ranged from 5.8% to 98% in different species (Table 2). Flavonoid compounds including those linked at position 6 (*C*-glycosyl) and 7 (*O*-glycosyl) in *Muhlenbergia* (Poaceae, Eragrostideae), show considerable variation in flavonoid patterns

among species [10]. The present study proves that the 7-*O*-rhamnoglycosyl-position includes these variations (20.8-81.25).

Based on our findings, flavonoid content displayed in *T. monococcum* and *T. durum* exhibited the highest variability (C.V.=101.3, 95.8 respectively) (Table 4). Conversely, *T. urartu* and *T. dicoccum* showed the lowest (C.V.=41.2, 57 respectively). Among the two subspecies of *T. boeoticum*, *T. boeoticum* subsp. *boeoticum* have more variability (C.V.=85.97) than *T. boeoticum* subsp. *thaoudar* (C.V.=67.8) (Table 4). Based on previous data, *T. monococcum* and *T. urartu* indicated minor intra-specific variation [5]. Various products of secondary metabolites show extreme diversity in grass species [9], and they are often differentially distributed in taxa. Chemical differentiation might be correlated to the geographical and ecological conditions under which they grow [15, 9].

Table 4. Variability of flavonoid compounds in *Triticum* species

	1	2	3	4	5	6	7	8
Mean	36.71	34.07	31.18	63.26	32.7	39.2	44.6	58.3
S.D.	31.59	23.13	31.61	26	29.9	37.6	37.2	33.2
C.V.	85.97	67.8	101.3	41.2	79.5	95.8	83.3	57

1. *T.boeoticum* subsp. *boeoticum*, 2: *T.boeoticum* subsp. *thaoudar,* 3: *T. monococcum*, 4: *T. urartu*, 5: *T. dicoccoides*, 6:*T. durum*, 7:*T. turgidum*, 8:*T. dicoccum*

Based on previous chemotaxonomic findings, Harborne et al. [5] carried out extensive chemotaxonomic flavonoid inspections in the Poaceae Family. Phenolic profiles could be used in the chemotaxonomy of grasses as a significance marker and have been extensively used in botanical chemosystematic surveys. In the present research, among wild diploid *Triticum* species, *T. boeoticum* subsp. *thaoudar* appears to have a partially different chemical profile from *T. boeoticum* subsp. *boeoticum*, mostly because of the occurrence of 2'-hydroxychalcone, 2,2'-dihydroxychalcone, 3,4-dihydroxychalcone, while the remaining taxon has 3',4',7'-trihydroxyflavone and fustin3-*O*-glucoside. Moreover, *T. monococcum* produced baptigenin and sciadopitysin, two flavonoids that were not found in *T. urartu*. Tetraploid *Triticum* species were similar in four compounds; except for *T. dicoccum*, which is quite different from the other tetraploid species which are further correlated by the existence of ordinary flavonoids. Polyploid species showed relatively few major differences [5]. Our research showed that glucosylation and hydroxylation patterns may be considered to be specific to the Poaceae Family. Their presence could be significant in chemotaxonomy [12]. The widespread occurrence of phenolic compounds in Poaceae largely consisted of low taxonomic units [9]. To use phenolic profiles more widely as genetic markers, they would have to be not only universal, but also environmentally stable and convenient to determine [9].

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