Phytochemistry, 1972, Vol. 11, pp. 2507 to 2512. Pergamon Press. Printed in England.

FLAVONOID VARIATION IN THE GENUS *BRIZA*

CHRISTINE A. WILLIAMS and B. G. MURRAY
Department of Botany, The University, London Road, Reading RG1 3AQ, Berks.

(*Received* 13 *March* 1972)

Key Word Index—*Briza*; Gramineae; chemotaxonomy; flavone *C*-glycosides; tricin glycosides; ploidy level; plant geography.

Abstract—During a survey of 6 Eurasian and 10 South American *Briza* species for leaf flavonoids, 27 components were found. Twelve of these were identified: tricin 5-glucoside, tricin 7-glucoside, quercetin 3-glucoside, kaempferol 3-glucoside, vitexin, isovitexin, orientin, iso-orientin, and the 4′*O*-glucoside of all 4 glycoflavones, 3 of which are reported for the first time. The Eurasian species, with the exception of *Briza maxima*, are remarkably uniform in their flavonoid pattern, accumulating mainly vitexin and isovitexin; whereas the South American species are characterized by the presence of orientin, iso-orientin and 9 unidentified flavonoids. In *Briza media* and the South American species, ploidy level is shown to play a large part in flavonoid variation. Examination of 12 diploid and 8 autotetraploid plants of *B. media* revealed that diploids accumulate vitexin and isovitexin, whereas tetraploids accumulate orientin and iso-orientin, autotetraploidy having apparently upset regulatory genes in the formation of the flavone *C*-glycosides. Mild alkaline treatment of both isovitexin and iso-orientin was found to give 100% conversion to the corresponding 8-*C*-glucoside.

INTRODUCTION

THE GENUS *Briza* (Gramineae) contains some 30 species,[1] the majority of which are found in subtropical and temperate regions of South America and the remaining 6 species in Eurasia. The group has been split by Pilger,[2] and Chase and Niles[3] into a number of smaller genera on morphological grounds but both Parodi[4] and Rosengurtt *et al.*[1] include the Eurasian and South American species in a single genus *Briza.* Phytochemically the group has been somewhat neglected but Harborne and Hall[5] recorded the presence of vitexin, isovitexin and a tricin-like compound in leaves of *Briza media.* Some variation in cyanogenic compounds has also been reported.[6]

The present work originated from a general survey of grasses in which the three *Briza* species examined proved to be chemically rather distinctive. A difference in leaf flavonoid content between diploid and tetraploid plants of *B. media* led to a more detailed study of this species and to a survey of the genus as a whole.

RESULTS

The results of the leaf flavonoid survey are presented in Table 1. Fresh leaf material of *Briza stricta* and all the Eurasian species except *B. humilis* was used, the remaining South American species being surveyed from herbarium material. All results are based on two-dimensional paper chromatography of alcoholic leaf extracts, the major components in the

[1] B. ROSENGURTT, B. R. ARRILAGA DE MAFFEI and P. IZAGUIRRE DE ARTUCIO, *Bol. Fac. Agron. Montevideo* **105,** 3 (1968).
[2] R. PILGER, *Bot. Jl.* **76,** 300 (1954).
[3] A. CHASE and C. D. NILES, in *Index to Grass Species*, Hall, Boston, Mass. (1962).
[4] L. R. PARODI, *Rev. Fac. Agron. Vet. Bs. Aires* **3,** 113 (1920).
[5] J. B. HARBORNE and E. HALL, *Phytochem.* **3,** 421 (1964).
[6] R. HEGNAUER, *Chemotaxonomie der Pflanzen*, Vol. II, p. 174, Birkauser, Basel (1966).

TABLE 1. THE DISTRIBUTION OF FLAVONOID GLYCOSIDES IN LEAVES OF *Briza* SPECIES

Species	Tricin 5–G	Tricin 7–G	Vitexin	Isovitexin	Orientin	Iso-orientin	Quercetin 3–G	Kaempferol 3–G
Eurasian								
Briza australis Prokudin	+	(+)	+	+	(+)	—	+	—
B. elatior Sibth. & Sm.	+	(+)	+	+	(+)	—	+	—
B. humilis M. Bieb.	+	—	+	+	—	—	—	—
B. maxima L.	—	—	—	—	—	—	—	—
B. media L. (2*x*)	+	(+)	+	+	(+)	—	+	+
B. media L. (4*x*)	+	(+)	(+)	(+)	+	+	+	+
B. minor L.	+	(+)	+	+	(+)	(+)	—	—
South American								
Briza brizoides (Lam) O. Kuntz.	—	—	—	—	—	+	—	—
B. calotheca (Trin.) Hackel	—	—	(+)	—	—	+	—	—
B. erecta Lamarck	+	(+)	—	—	+	—	—	—
B. palaepilifera Parodi	+	(+)	(+)	—	+	+	—	—
B. paoemorpha (Presl.) Henrard	—	(+)	—	—	+	+	—	—
B. rufa (Presl.) Stuedel	—	—	—	—	—	—	—	—
B. stricta (Hook. & Arn.) Steudel	—	—	—	—	—	—	—	—
B. subaristata Lamarck	—	—	(+)	—	—	+	—	—
B. triloba Nees	—	—	—	—	—	—	—	—
B. uniolae Nees ex Steudel	+	(+)	(+)	—	+	+	—	—

Species	F1	F2	F3	F4	F5	F6	F7	F8	F9	Other components
Eurasian										
Briza australis Prokudin	—	—	—	—	—	—	—	—	—	Vitexin 4′-glucoside; Isovitexin 4′-glucoside
B. elatior Sibth. & Sm.	—	—	—	—	—	—	—	—	—	Vitexin 4′-glucoside; Isovitexin 4′-glucoside
B. humilis M. Bieb.	—	—	—	—	—	—	—	—	—	F.12
B. maxima L.	—	—	—	—	—	—	—	—	—	F.10, F.11. F.15
B. media L. (2*x*)	—	—	—	—	—	—	—	—	—	Vitexin 4′-glucoside; Isovitexin 4′-glucoside
B. media L. (4*x*)	—	—	—	—	—	—	—	—	—	Vitexin 4′-glucoside; Isovitexin 4′-glucoside; Orientin 4′-glucoside; Iso-orientin 4′-glucoside
B. minor L.	—	—	—	—	—	—	—	—	—	
South American										
Briza brizoides (Lam) O. Kuntz.	—	+	+	—	+	—	—	+	+	F.13
B. calotheca (Trin.) Hackel	+	—	—	+	—	+	+	+	+	
B. erecta Lamarck	—	—	+	—	+	+	—	—	+	
B. palaepilifera Parodi	—	+	—	—	—	—	+	—	+	
B. poaemorpha (Presl.) Henrard	—	—	+	—	+	—	+	—	—	
B. rufa (Presl.) Steudel	—	—	—	+	—	+	—	+	—	F.14
B. stricta (Hook. & Arn.) Steudel	—	—	—	—	—	—	+	+	+	
B. subaristata Lamarck	—	—	—	+	+	—	+	—	+	
B. triloba Nees	—	—	+	—	+	—	+	—	+	
B. uniolae Nees ex Steudel	+	—	—	—	—	—	+	+	—	

5-G = 5-glucoside, 7-G = 7-glucoside, 3-G = 3-glucoside. F denotes unidentified flavonoid glycoside. The R_f's of the unidentified compounds in BAW and 15% HOAc are as follows: F1 22/54; F2 49/52; F3 31/52; F4 37/12; F5 22/40; F6 31/24; F7 19/30; F8 26/29; F9 28/14; F10 17/68; F11 22/72; F12 38/73; F13 33/71; F14 21/40; F15 28/75. The colours of the unknown flavonoids in UV without and with NH_3 are as follows: F1, F3 and F5—dark to reluctant yellow; F2, F10, F12 and F14—dark to yellow; F4 and F8—dark to acid yellow; F6, F7 and F9—dark to orange-yellow; F11, F15 and F13—dark to dark.

(+) Present in a trace amount.

Eurasian species being identified from a detailed study of diploid and tetraploid plants of *B. media*. Unfortunately, there was insufficient material of any South American species to allow identification of the major components F1 to F9.

Plant Distribution and Flavonoid Pattern

The variation of morphological characters within the genus has a reticulate pattern, different character combinations being present in different species groups. This is reflected in the chemical variation, but here a clear separation of the species into the two geographical groups is immediately obvious.

The Eurasian species are noticeably more uniform in their flavonoid pattern. The only exception is *B. maxima*, the flavonoid pattern of which is totally different from all other Eurasian species, although it is morphologically similar to both *B. media* and *B. minor*. On the other hand, the flavonoid patterns of the South American species are extremely variable although easily distinguished from the Eurasian species on a number of points. Tricin 5-glucoside and tricin 7-glucoside are almost universal in Eurasian species, being present in 86 and 71% of the sample respectively, as compared with only 30 and 40% in the South American species. Similarly, the glycoflavones vitexin and isovitexin, predominant in Eurasian species (in 86% of the sample), are almost completely absent from the South American taxa. Conversely, orientin and iso-orientin are more common in the South American taxa, (i.e. 40 and 60% of the sample respectively). Quercetin 3-glucoside and kaempferol 3-glucoside are present only in Eurasian species (i.e. 40 and 20% of the sample, respectively), whereas the South American plants are characterised by the presence of the unidentified flavonoids F1 to F9.

Within the South American species there does not appear to be any definite pattern and the species can be arranged in a number of different groups based on a common flavonoid glycoside, none of which, however, reflects the views of those taxonomists who have split the genus into smaller groups.

It is of interest that the two morphologically very similar species *B. subaristata* and *B. triloba*, considered by Parodi[2] as two distinct species but by Rosengurtt *et al.*[1] as one. are easily separable on chemical grounds by the presence of iso-orientin in the former. Since both plants are reported to be tetraploid,[7,8] the flavonoid variation cannot be attributed to ploidy level as in *B. media*. The chemical evidence thus supports their separation into two species.

Flavonoid C-glycosides and Ploidy Level in Briza media

Briza media has a wide distribution from northern Europe to the Mediterranean and well into Russia. Plants from 16 locations as widely separated as Norway and Spain, England and Poland, were investigated for variation of flavonoid glycosides with geographic distribution. Included in this survey were 8 plants with the tetraploid chromosome number. There is little or no variation within either the diploids, tetraploids or plants with B or accessory chromosomes. There are, however, several differences in flavonoid pattern between the two chromosome races as illustrated in Table 2. In general, the diploid plants accumulate vitexin and isovitexin as major components. These compounds are replaced in the tetraploid plants by an equal quantity of orientin and iso-orientin; i.e. tetraploidy has led to a predominance of the 3′,4′-dihydroxy—rather than the 4′-hydroxyflavone *C*-glycosides. This is

[7] W. M. Bowden and H. A. Senn, *Can. J. Bot.* **40**, 1115 (1962).
[8] F. Saura, *Rev. Fac. Agron. Vet. Bs. Aires* **11**, 330 (1947).

further illustrated in that the 4′-glucosides of orientin and iso-orientin occur only in the tetraploid plants. It is also interesting to note that orientin and iso-orientin are preferentially formed in most of the South American species (Table 1) all of which are reported as being tetraploid.[7,8]

TABLE 2. LEAF FLAVONOID VARIATION WITH PLOIDY LEVEL IN *Briza media*

Flavonoid	2*x**	4*x*†
Tricin 5-glucoside	+	+
Tricin 7-glucoside	(+)	(+)
Vitexin	+++	(+)
Isovitexin	++++	(+)
Orientin	(+)	+++
Iso-orientin	—	++++
Vitexin 4′-glucoside	+	(+)
Isovitexin 4′-glucoside	+	(+)
Orientin 4′-glucoside	—	+
Iso-orientin 4′-glucoside	—	+

(+) Present in trace amount; ++++ approximately 40% of total flavonoid; +++ approximately 30% of total flavonoid; + approximately 10% of total flavonoid. Equal weights of 2*x* and 4*x* fresh leaf material yielded equal total flavone *C*-glucoside.
* 12 plants surveyed.
† 8 plants surveyed.

Isovitexin 4′-glucoside has been reported by Litvinenko *et al.*[9] in *Vaccaria segetalis* (Caryophyllaceae) and by Krivenchuk and Litvinenko[10] in *Gypsophila* (Caryophyllaceae) but the other three 4′-glucosides are reported here for the first time (Table 3).

TABLE 3. CHROMATOGRAPHIC DATA FOR FLAVONE *C*-GLUCOSIDE 4′-*O*-GLUCOSIDES R_f (×100)

Flavone glycoside	BAW	BEW	PhOH	H_2O	15% HOAc
Vitexin 4′-glucoside*	22	12	66	24	53
Isovitexin 4′-glucoside*	33	24	81	39	67
Orientin 4′-glucoside†	16	10	32	11	40
Iso-orientin 4′-glucoside†	26	23	57	28	61

* Isolated from *Briza media* (2*x*).
† Isolated from *Briza media* (4*x*).

Mild alkaline treatment of both isovitexin and iso-orientin, carried out as a routine test for acylation during their identification, gave 100% conversion to the corresponding 8-*C*-monoglucoside. This could prove useful in the preparation of other 8-*C*-monoglycosides from the 6-*C*-isomers and in the 'simplification' of two-dimensional paper chromatographic patterns in plant extracts rich in *C*-glycosides, e.g. Gramineae and Palmae, by removing the

[9] V. I. LITVINENKO, K. AMANMURADOR and N. K. ABUBAKIROV, *Khim. Prir. Soedin.* **3**, 159 (1967).
[10] P. E. KRIVENCHUK and V. I. LITVINENKO, *Farm Zh. (Kiev)* **23**, 62 (1968).

6-isomer. Similar alkaline treatment of 6-hydroxy-luteolin and -apigenin failed to produce either corresponding 8-hydroxyflavone.

DISCUSSION

Apart from the clear correlation between geographic distribution and flavonoid content, the most interesting discovery of this survey is the effect of polyploidy on the flavonoids in *B. media* and the possibility of a similar effect in the tetraploid South American species. The addition of genomes seems frequently to result in disturbances of gene-controlled physiological processes. In *Lycopersicon esculentum*, Rick[11] reports that polyploidy has a marked effect on the level of ascorbic acid in the fruit. Recently Gale and Flavell[12] found that the substitution of chromosomes 7A and 7B from a variety of wheat with high anthocyanin content into one with a low anthocyanin content caused the latter to synthesise large amounts of anthocyanin. In *Briza* we suggest that autotetraploidy has upset the action of similar regulatory genes resulting in the accumulation of orientin and iso-orientin in the tetraploid plants in place of the vitexin and isovitexin of the diploid plant. Attempts to produce tetraploids by colchicine treatment of diploids have so far failed. However, further work to produce both artificial tetraploids and triploids is in progress, which may lead to a clearer understanding of the effect of ploidy on flavonoid synthesis.

EXPERIMENTAL

Plant sources. Leaf samples of all the Eurasian plants, except *Briza humilis*, and *B. stricta*, were taken from plants grown from seed at the University of Reading. Seed was of spontaneous origin sent by various Botanic Gardens and Collectors and voucher specimens of the plants are deposited in the Reading University Herbarium (RNG). Leaf material of the South American species was obtained from herbarium material at the Royal Botanic Gardens, Kew. They are listed under Collector's name and number as follows: *B. brizoides* Krapovickas 600, *B. calotheca* Dombrowski 1947, *B. erecta* Pedersen 7089, *B. humilis* Alston and Sandwith 1679, *B. palaepilifera* Hawkes *et al.* 3292, *B. poaemorpha* Gallinal *et al.* 1945, *B. rufa* Burkart 7603 *B. subaristata* Pedersen 7171, *B. triloba* Hatschbach & Guimaraes 19871 and *B. uniolae* Fac. Agron. Montevideo 5579.

Flavonoid identification. The solvents used for two-dimensional PC of direct 95% (for fresh materials) or 70% EtOH (for herbarium material) leaf extracts on Whatman No. 1 paper were: (1) BAW, (2) 15% HOAc. Individual flavonoid glycosides of 2*x* and 4*x B. media* were isolated and purified from 95% EtOH leaf extracts on Whatman No. 3 paper using standard solvents. Known glycosides were identified on the basis of R_f, UV spectral analysis, acid hydrolysis to aglycone and sugar and by direct comparison with authentic samples. All flavone *C*-glycosides were acid hydrolysed for 4 hr.

Identification of vitexin, orientin and iso-orientin 4′-O-*monoglucosides.* Isovitexin 4′-glucoside has already been reported [9,10] but as no chromatographic, spectral data or authentic sample was available these results are included for comparison with the new glycosides. Chromatographic data are presented in Table 3. Both acid and enzyme hydrolysis with β-glucosidase gave the corresponding flavone *C*-glucoside and glucose. All four glycosides appeared as dark absorbing spots in UV and remained unchanged with NH_3. Spectral data (λ_{max}) are as follows: vitexin 4′-glucoside: MeOH 273, 325, 349 (infl.); NaOAc 281, 368; alk 294, 370; H_3BO_3 275, 340. Isovitexin 4′-glucoside: MeOH 273, 323, 345 (infl.); NaOAc 281, 270; $AlCl_3$ 283, 304, 386; alk. 289, 386; H_3BO_3 275, 330. Orientin 4′-glucoside: MeOH 270, 335: NaOAc 278, 323, 355; H_3BO_3 270, 335. Iso-orientin 4′-glucoside: MeOH 272, 335; NaOAc 280, 325, 350; $AlCl_3$ 338, 280 (infl.); alk 380; H_3BO_3 273, 335. The positive $AlCl_3$ and NaOAc and shifts indicating free 5- and 7-positions together with diminution of Band II with NaOAc, dark colour in UV and enzyme hydrolysis suggest that these compounds are all 4′-monoglucosides.

Isomers of orientin and iso-orientin. Both orientin and iso-orientin were found to occur in *Briza media* (4x) as two chromatographically separable isomeric forms. R_fs: orientin: BAW 29/29, BEW 18/18, PhOH 43/43, H_2O 04/02, 15% HOAc 19/13; iso-orientin: BAW 49/48, BEW 42/43, PhOH 69/84, H_2O 07, 14/05, 11, 15% HOAc 32, 45/26, 41. UV spectra were identical and all gave 3,4-dihydroxyphenylpropionic acid on

[11] C. M. Rick, *Adv. Genet.* **8**, 267 (1956).
[12] M. D. Gale and R. B. Flavell, *Genet. Res. Camb.* **18**, 237 (1971).

reductive cleavage.[13] There was insufficient material to identify the *C*-sugar or further analyse the isomers. However, Litvinenko[14] has described four isomeric forms of both vitexin and isovitexin, in which the glucose may be α- or β-linked to the 6- or 8-position or in which it may be in either the furanose or pyranose form and Bhatia *et al.*[15] have similarly reported an optical isomer of orientin, which was similar in chemical reaction, UV and IR spectra but different in m.p. and rotation. It is probable that isomerism present in the *Briza* compounds is of this type.

Acknowledgements—The authors are grateful to Dr. J. B. Harborne for his encouragement and help in the preparation of this manuscript. We also thank Dr. Leslie Crowe for helpful comments and Dr. W. D. Clayton of the Herbarium, Royal Botanic Gardens, Kew for providing herbarium material.

[13] H. M. HURST and J. B. HARBORNE, *Phytochem.* **6**, 1111 (1967).
[14] V. I. LITVINENKO and L. I. BORODIN, *Farm. Zh Kiev* **25**, 84 (1970).
[15] V. K. BHATIA, S. R. GUPTA and T. R. SESHADRI, *Tetrahedron* **22**, 1147 (1966).