CHROMOSOMAL LOCATION OF GENES CONTROLLING FLAVONOID PRODUCTION IN HEXAPLOID WHEAT

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ABSTRACT

Two-dimensional paper chromatography was performed on methanol extracts of leaves of hexaploid bread wheat, *Triticum aestivum* L. em. Thell. cultivar Chinese Spring, and of the available nullisomic-tetrasomic compensating lines, the tetrasomic lines and the ditelocentric lines. The chromatograms had 27 spots identified as flavonoids and six representing phenolic acids. Some of the areas were complex and contained more than one compound. Four flavonoids were identified as under the control of gene(s) on chromosome arms *1DS*, *4DL*, *5AS* and *6BS*. A phenolic glycoside was concluded to be controlled by a gene(s) on chromosome arm *7BL*. Gene(s) on chromosome arm *4DL* affected the amount of compounds in two other spots, and gene(s) on chromosome arm *4BS* reduced the level of all flavonoid compounds. The individual compounds in some of the complex spots may be under the control of gene(s) on homoeologous chromosomes.

IT has become accepted that the presence of secondary compounds in plants can indicate taxonomic relationships among plants (BELL 1980), and in many instances the assignment of an organism to a certain taxon has been accomplished with the use of such secondary compounds as flavonoids (HARBORNE 1975; GORNALL and BOHM 1978). Because flavonoids are almost ubiquitous in angiosperms, the analysis of these compounds has provided geneticists and systematists with objective characters on which to base taxonomic decisions.

Recently, LEVY and LEVIN (1971, 1974, 1975), ALSTON *et al.* (1965), FRANCIS and WONG (1971), BUTTERY and BUZZELL (1973) and MARTINEZ and SWAIN (1977) have used flavonoids of putative diploid parents to trace the origin of hybrids and allopolyploids in various groups of plants. The biosynthesis of a flavonoid is assumed to be the result of gene activity, which permits the association of an end product (flavonoid) with the active site in the genome coding for its production. Thus, flavonoid markers can be established for these genes and the chromosomes on which they occur. Once marker compounds are available, the origin of specific genes and chromosomes may be inferred.

MAY, VICKERY and DRISCOLL (1973) investigated the nullisomic-tetrasomic compensating lines of *Triticum aestivum* L. em. Thell. cv. Chinese Spring ($2n = 6x = 42$, genome formula *AABBDD*) with a combination of chromatographic

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TABLE 1

Phenolic acids and flavonoids of T. aestivum cv. *Chinese Spring*

Spot no. (compound)	Color on paper (color after exposure to NH_4 fumes)
Phenolic acids	
1	fy (fb)
2	y (fy/fw)
23	b (b)
34	fb (fb)
34a	fy (fy)
34b	fb (fb)
Flavonoids	
4	p (o)
5	p (y)
6	p (y)
8	p (p)
20	Brp (Br)
21A	p (yg)
21B	p (yg)
22	y (y)
24	p (y)
25 (A, B, C)	Dark Brp (Br)
26 (A, B, C)	Dark Brp (Br)
27	p (yg)
29	p (y)
30	p (y)
30a	p (o)
30b	p (o)
31	p (yg)
32	p (yg)
33	p (ygr)
35	p (y)
37	y (y)

Key: p = purple; y = yellow, g = green; gr = grey; Br = brown; b = blue; w = white; f = fluorescent; o = orange.

and electrophoretic procedures and noted that some flavonoids and phenolic glycosides could be linked to various chromosomes in this hexaploid wheat. The current investigation continues the location of genes controlling flavonoid production onto chromosomes in Chinese Spring. In addition to the nullisomic-tetrasomic compensating lines, the tetrasomic aneuploid series and some of the ditelocentric aneuploid lines have been used.

MATERIALS AND METHODS

Plant material: The *T. aestivum* L. em. Thell, cv. Chinese Spring euploid and aneuploids examined were obtained from DR. E. R. SEARS, University of Missouri, Columbia, MO 65201.

The aneuploids included 40 nullisomic-tetrasomic compensating lines (complete for all homoeologous groups except group 4, in which nullisomic (N) 4*A*-Tetrasomic (T) 4B, N4BT4A, N4DT4B and N4AT4D were the only available aneuploids); 21 tetrasomic lines (Tetrasomic for 1*A* = *T1A*, etc.); and 37 ditelocentric lines (ditelocentric for 1*A* long arm (*1AL*); 1*A* short arm (*1AS*); *2AS*; *3AL*; *3AS*; *4Aα*; *5AL*; *6AL*; *6AS*; *7AL*; *7AS*; *1BL*; *1BS*; *2BL*; *3BL*; *3BS*; *4BL*; *5BL*; *6BL*; *6BS*; *7BL*; *7BS*; *1DL*; *2DL*; *2DS*: *3DL*; *4DL*; *4DS*; *5DL*; *6DL*; *6DS*; *7DL*; *7DS*).

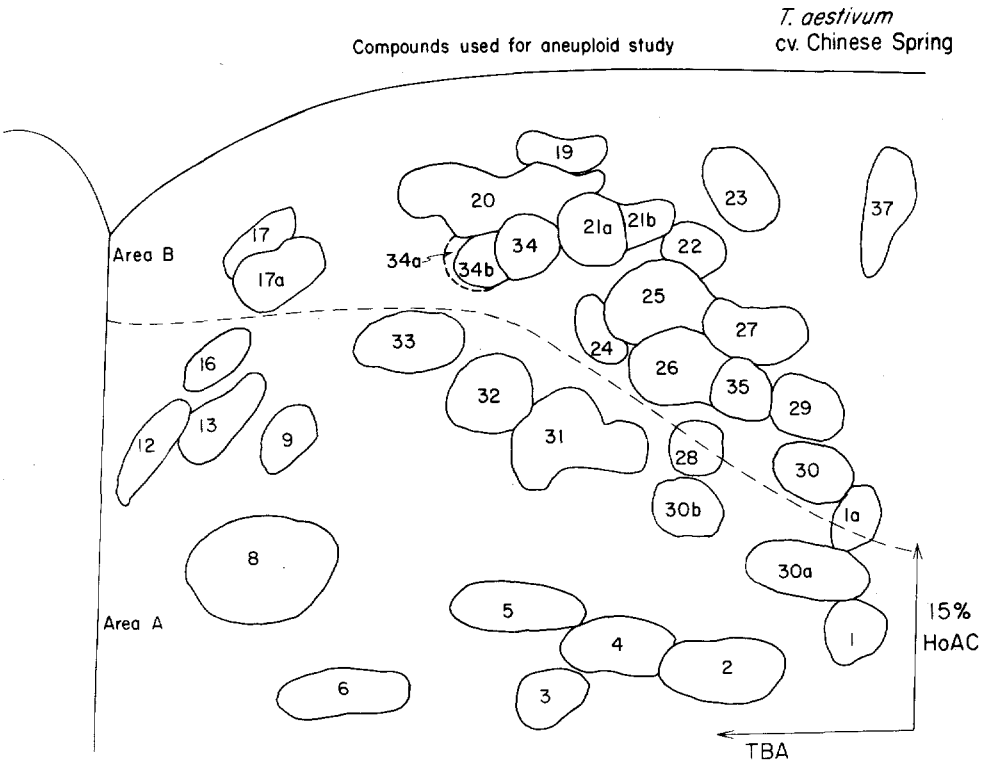

FIGURE 1.—Two-dimensional flavonoid profile of *T. aestivum* Chinese Spring euploid ($2n = 6x = 42$ *AABBDD*).

Plants were grown in 1-gallon pots in soil in the greenhouse for 8 wk, after which all the leaves were harvested. Plant material was dried at 60° in a hot air oven for 24 hr.

Extractions: Plant material was ground in a Waring blender to a medium powder. One gram of material from each of the plants was extracted in 85% methanol (MeOH), filtered, the filtrate reserved, re-extracted in 50% MeOH for an additional 24 hr and filtered. Filtrates were combined, evaporated to dryness at 47° and redissolved in 4 ml of 85% MeOH + 2 ml distilled water, for a total of 6 ml of extract. Aliquots of 500 μl were spotted onto Whatman 3 MM (47 × 56 cm) paper for two-dimensional paper chromatography in TBA (tertiary-butyl alcohol:acetic acid (HOAc):water; 3:1:1; v:v) in the first direction and 15% aqueous HOAc in the second direction.

Paper chromatograms were dried in the hood overnight between the first and second directions. Two or three replicate paper chromatograms were run.

The chromatograms were examined under UV light (366 nm), and the UV absorbing areas were circled. Color changes of the circled compounds were noted after exposure of the paper to NH_3 vapors.

RESULTS

The two-dimensional paper chromatogram of euploid *T. aestivum* Chinese Spring ($2n = 42$) exhibited 27 spots. Twenty-one were detected as flavonoids (Table 1) and six as phenolic acids (Table 1). Some of these spots were composed of more than one flavonoid, as could be seen in an examination of the aneuploid series where the spots changed shape, size and color. When these were accounted for, the number of flavonoid compounds present increased to 37.

TABLE 2

Phenolic acid and flavonoids located to chromosomes of T. aestivum *cv. Chinese Spring*

Line (Chinese Spring)	Chromosome composition	Spot no.	Condition
Nulli-5*A* tetra-5*B*	5*B* 5*D* 5*B*	21 (A)	10.96 cm^2
Nulli-5*B* tetra-5*A*	5*A* 5*D* 5*A*	21 (A + B)	28.41 cm^2
Nulli-5*A* tetra-5*D*	5*B* 5*D* 5*D*	21 (A)	12.24 cm^2
Nulli-5*B* tetra-5*A*	5*A* 5*D* 5*D*	21 (A + B)	22.72 cm^2
Nulli-5*D* tetra-5*D*	5*A* 5*B* 5*A*	21 (A + B)	27.50 cm^2
Nulli-5*D* tetra-5*B*	5*A* 5*B* 5*B*	21 (A + B)	23.99 cm^2
Chinese Spring	5*A* 5*B* 5*D*	21 (A + B)	20.42 cm^2
Ditelocentric 5*AL*	5*AL* 5*B* 5*D*	21 (A)[a]	12.04 cm^2
Ditelocentric 5*AS*	5*AS* 5*B* 5*D*	21	N.D.[b]
Nulli-1*D* tetra-1*A*	1*A* 1*B* 1*A*	24	–
Nulli-1*A* tetra-1*D*	1*B* 1*D* 1*D*	24	+
Nulli-1*B* tetra-1*A*	1*A* 1*D* 1*A*	24	+
Nulli-1*A* tetra-1*B*	1*B* 1*D* 1*b*	24	+
Nulli-1*B* tetra-1*D*	1*A* 1*D* 1*D*	24	+
Nulli-1*D* tetra-1*B*	1*A* 1*B* 1*B*	24	–
Chinese Spring	1*A* 1*B* 1*D*	24	+
Ditelocentric 1*DL*	1*A* 1*B* 1*DL*	24[c]	–
Ditelocentric 1*DS*	1*A* 1*B* 1*DS*	24	N.D.
Nulli-4*D* tetra-4*B*	4*A* 4*B* 4*B*	25A	8.84 cm^2
Nulli-4*D* tetra-4*A*	4*A* 4*B* 4*A*	25B	6.67 cm^2
Nulli-4*D* tetra-4*A*	4*A* 4*B* 4*A*	25 (A + B)	15.51 cm^2
Nulli-4*B* tetra-4*D*	4*A* 4*D* 4*D*	25 (ABC)	23.18 cm^2
Chinese Spring	4*A* 4*B* 4*D*	25 (ABC)	17.48 cm^2
Ditelocentric 4*DL*	4*A* 4*B* 4*DL*	25 (ABC)	13.47 cm^2
Ditelocentric 4*DS*	4*A* 4*B* 4*DS*	25A[d]	11.65 cm^2
		25B	4.79 cm^2

Although all the flavonoids were noted, not all phenolic acids (fluorescent blue and yellow) were scored because their appearance was variable and independent of the presence or absence of chromosomes in the aneuploids examined. Indeed, they were sometimes present in one replicate chromatogram and absent in another.

The flavonoid profiles of Chinese Spring and its aneuploids can be divided into two general areas (Figure 1). The lower portion of the chromatogram (area A), composed of flavonoids 1, 2, 3, 4, 5, 8, 30b, 31, 32 and 33 was stable, and these flavonoids were present in all aneuploids, although the concentrations of the compounds, judged by color intensity of the spots, may vary from aneuploid to aneuploid. From this we deduced that genes on chromsomes in the *A*, *B*, and *D* genomes controlled biosynthesis of these compounds. The upper portion of the chromatogram, represented by compounds 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 34, 34a, 34b, 35 and 37, was variable, and some compounds appeared to be controlled by the chromosomes present in the aneuploid under investigation. Two compounds, 23 (blue → blue with NH_3) and 37 (yellow → yellow with NH_3) were always present within this portion of the chromatogram. In addition,

TABLE 2—*Continued*

Line (Chinese Spring)	Chromosome composition	Spot no.	Condition
Nulli-*6B* tetra-*6D*	*6A 6D 6D*	26 (A	11.55 cm^2
		B)	19.26 cm^2
Nulli-*6D* tetra-*6B*	*6A 6B 6B*	26 (A/BC)	33.16 cm^2
Nulli-*6B* tetra-*6A*	*6A 6D 6A*	26 (A[e]	8.32 cm^2
		B)	16.30 cm^2
Nulli-*6A* tetra-*6B*	*6B 6D 6B*	26 (A/BC)	33.83 cm^2
Nulli-*6A* tetra-*6D*	*6B 6D 6D*	26 (A/BC)	28.66 cm^2
Nulli-*6D* tetra-*6A*	*6A 6B 6A*	26	36.02 cm^2
Chinese Spring	*6A 6B 6D*	26	23.65 cm^2
Ditelocentric *6BL*	*6A 6BL 6D*	26A[e]	8.18 cm^2
		26B	10.78 cm^2
Nulli-*7A* tetra-*7B*	*7B 7D 7B*	34	+[g]
Nulli-*7B* tetra-*7A*	*7A 7D 7A*	34	−
Nulli-*7D* tetra-*7B*	*7A 7B 7B*	34	+
Nulli-*7B* tetra-*7D*	*7A 7D 7D*	34	−
Nulli-*7A* tetra-*7D*	*7B 7D 7D*	34	+
Nulli-*7D* tetra-*7A*	*7A 7B 7A*	34	+
Ditelocentric *7BL*	*7A 7BL 7D*	34	+
Ditelocentric *7BS*	*7A 7BS 7D*	34[f]	−

[a] The area of that portion of the spot remaining when *5AS* is not present. The missing portion of the spot is associated with *5AS*.

[b] N.D. = no data; not available for study.

[c] Because the spot was missing when *1DS* was missing, spot 24 may be associated with *1DS*, although this telocentric was not available for study.

[d] Although the areas occupied by the compounds present in the ditelocentrics are similar, the appearance of two spots when arm *4DL* is absent indicates that 25C is associated with this arm of the chromosome.

[e] Because this spot appears as two flavonoids when *6B* is nullisomic, the assumption is three compounds are present. There are two compounds evident when *6BS* is missing, therefore one compound (26C) may be associated with this chromosome. Additionally, the other two unassigned flavonoids may be associated with chromosomes *6D* and *6A*, although because the *6BS* flavonoid is masking the effect of these chromosomes, actual assignments cannot be made.

[f] Compound 34 is a marker for chromosome *7B* (long arm).

[g] + = spot present; − = spot absent.

there were four flavonoid compounds (27, 29, 30 and 35) whose presence or absence could not be correlated with specific chromosomes.

An examination of the chromatograms of the nullisomic-tetrasomic compensating lines indicated five flavonoid spots could be associated with specific chromosomes; when the chromosome was present, the flavonoid was present; when the chromosome was absent, the flavonoid was also absent (Table 2).

Compound 24, a luteolin C-glycoside derivative, was absent when chromosome *1D* was nullisomic. When *1D* was tetrasomic, the quantity of this flavonoid was increased, as evidenced by greater intensity of spot color under UV light. In the chromatograms of the ditelocentric line *1DL*, compound 24 was absent; *i.e.*, when the short arm of chromosome *1D* was absent, compound 24 was absent. This allowed us to locate the gene(s) controlling compound 24 to chromosome *1DS*.

Spot 34 was a phenolic glycoside that has been associated with the long arm of chromosome *7B*; *i.e.*, the compound was present in plants that contain the

7BL portion of the chromosome and was absent when this was missing, as in ditelocentric *7BS*.

Some flavonoid spots appeared to be composed of more than a single compound. These spots included 20, 21, 25 and 26. That these spots were indeed representative of at least two compounds each could readily be demonstrated by their behavior in the aneuploid series. For example, the mean area occupied by spot 21 (representing two apigenin C-glycoside derivatives) measured on chromatograms of euploid Chinese Spring plants was 20.42 cm^2. In those instances where chromosome *5A* was nullisomic (N5AT5B and N5AT5D), this area was markedly reduced to 10.96 and 12.24 cm^2, respectively. On those chromatograms for plants that were tetrasomic for chromosome *5A* (N5BT5A and N5DT5A), this area of spot 21 was increased over the area of spot 21 in aneuploids N5DT5B and N5BT5D where either *5B* or *5D* were tetrasomic in the presence of *5A* (28.41 and 27.50 *vs.* 23.99 and 22.72 cm^2, respectively). When the chromatograms for ditelocentric *5AL* were examined, the area of spot 21 was much reduced, and the absence of the short arm of chromosome *5A* was associated with the absence of the portion of spot 21 (21B) which was similar to the nullisomic *5A* condition. This allowed us to locate the gene(s) controlling compound 21B on chromosome *5AS*. When chromosomes *5D* and *5B* were tetrasomic in the N5BT5D and N5DT5B combinations, the area of spot 21 was somewhat larger than that in euploid Chinese Spring. This may be interpreted to indicate that chromosomes *5B* and *5D* also carried gene(s) that contribute to the remainder of complex spot 21.

The portion of the chromatogram designated spot 25 was representative of three flavonoids. One of the compounds present (tentatively identified as an apigenin di-C-glycoside 7-methyl ether) was controlled by gene(s) on the short arm of chromosome *4D*. In ditelocentric *4DS*, where *4DL* was absent, the original very intense purple spot divided into two spots that did not overlap. In ditelocentric *4DL*, where *4DS* was absent, the spot once more resumed the appearance of a single compound. When *4D* was nullisomic (N4DT4A), the area of the two remaining compounds was 15.51 cm^2 (25A, 8.84 cm^2 + 25B, 6.66 cm^2) compared with an area of 17.48 cm^2 in euploid Chinese Spring plants. The difference in the relative areas was not great and may be explained by postulating that this third compound was superimposed on 25A and 25B, and that it may not have contributed as much to the area of the combined spot as it did to the intensity of UV absorption. Although the reciprocal nullisomic-tetrasomic line (N4AT4D) was not available for study, in the other aneuploid where *4D* was tetrasomic (N4BT4D), the total area of spot 25 was 23.18 cm^2, 5.7 cm^2 larger than the euploid plant, which indicated production of additional compound associated with tetrasomy.

Like spot 25, spot 26 was complex, being composed of three flavonoid compounds. In the absence of chromosome *6B*, this single spot divided into two adjacent spots (26A and 26B), which were easily distinguished. Moreover, the combined area of the two spots in N6BT6D was 2.35 cm^2 less than the reciprocal combination N6DT6B and, in N6BT6A, 9.21 cm^2 less than in the reciprocal combination N6AT6B. From the chromatograms for ditelocentric *6BL* we can

assign the missing flavonoid (postulated as an apigenin C-glycoside) to the short arm of chromosome *6B*. In all nullisomic-tetrasomic compensating lines for group 6 chromosomes, there appeared to be greater production of the flavonoids in spot 26 by the aneuploids than in euploid Chinese Spring (Table 2). When the area of spot 26 is measured in the chromatograms representing plants N6AT6D, and the reciprocal condition N6DT6A, the tetrasomic condition of both *6A* and *6D* increased the area of the spot over that of euploid Chinese Spring. In both of these nullisomic-tetrasomic compensating lines, chromosome *6B* was constant. After removal of spot 26C associated with *6B*, it was probable that one, or both, of the compounds that remained in spot 26 was produced as a result of gene activity on chromosomes *6A* and *6D*. If 26C were superimposed on 26A and 26B as postulated, this would prevent the appearance of two spots in those chromosome aneuploids which could permit the association of either compound with *6A* or *6D*, because *6B* would always be present to produce overlaying flavonoid.

There are two cases in which a chromosome affected more than one flavonoid. On the chromatogram of ditelocentric *4DS*, the quantities of compounds 29 and 30 were much reduced. However, a concurrent increase in any of the other compounds present was not detected. Therefore, a gene(s) on chromosome *4DL* may in part control production of compounds 29 and 30. The quantities of all flavonoids present were reduced in the chromatograms of *4BL* when chromosome arm *4BS* was missing. Since all flavonoids were reduced in quantity, this chromosome arm appeared to have a great effect on the biosynthetic pathway of flavonoids in wheat.

DISCUSSION

MAY, VICKERY and DRISCOLL (1973) associated four flavonoids with specific chromosomes of *T. aestivum* cv. Chinese Spring. These compounds were located on chromosomes *1D*, *2D*, *5A* and *7D*. Compound 18, an *O*-glycosyl-*C*-glycoflavone, was placed on chromosome *1D*. Compound 24 in this study appears to be equivalent to compound 18 of MAY, VICKERY and DRISCOLL. The chemical identity of compound 18 with our compound 24 has not been unequivocally determined because it was not identified further than *O*-glycosyl-*C*-glycoflavone by MAY, VICKERY and DRISCOLL. We assign compound 24 to the short arm of chromosome *1D*.

The other three compounds located on specific chromosomes by MAY, VICKERY and DRISCOLL (1973) were not identified during this study. These compounds may be associated with the chromosomes noted, but because different chromatographic procedures for flavonoids were used in the two studies, compounds previously determined as being produced by these chromosomes could not be matched, which prevented confirmation of the published results. In our work, four additional phenolic compounds were located on chromosomes, namely spots 21B, 25C, 26C and 34.

Although not a flavonoid, compound 34 has been unequivocally associated with the long arm of chromosome *7B*. It was the only phenolic glycoside in this

study whose appearance can be predicted, although other phenolic glycosides appear in the plants investigated.

Spot 21B was associated with the short arm of chromosome 5*A*. This compound must be structurally close to the compound 21A, which shares the same area of the chromatogram; however, there are no indications that this second compound increases in concentration when 21B is absent. The compounds located on chromosome 5*A* by MAY, VICKERY and DRISCOLL were identified as 4′-O-glucosyl-isoswertisin and wyomin. These compounds have not as yet been identified in our study. MAY, VICKERY and DRISCOLL noted that when 5*A* was absent, these two compounds were reduced in quantity with a concomitant increase noted in two other compounds in the plant, which they tentatively identified as saponarin and lutonarin. In our study there was no increase in the quantity of any other compound when spot 21B was absent. Moreover, since 21B apparently disappeared completely, it served as a more effective marker than either 4′-O-glucosyl-isoswertisin or wyomin.

Spot 25 usually appeared as a very intense brownish-purple area which, after exposure to ammonia fumes, is brown when viewed under UV light. Ordinarily, flavonoids appear purple or yellow under long wavelength UV light and either remain purple or yellow or change to yellow or yellow-green when exposed to ammonia fumes, depending upon the structure of the compound (MABRY, MARKHAM and THOMAS 1970). The unusual color of spot 25 at the outset was evidence that the spot is probably composed of more than one compound. This view was supported when, in the absence of chromosome 4*D*, the spot clearly divided into two compounds. This condition persisted in the absence of the long arm of chromosome 4*D*. If spot 25 were composed of only the two compounds detected when 4*DL* was absent, one of these compounds should be expected to disappear under some other nullisomic condition such as nulli-4*A* or nulli-4*B*. Since that was not the case, there must have been a third component present, superimposed over the two remaining compounds, which masked their presence or absence. The presence of three compounds was confirmed during an examination of the area of spot 25. When only two compounds were present (when 4*D* is absent), the area of the spot was 15.51 cm^2 compared with 17.48 cm^2 in a plant with normal chromosome complement. This area should not be increased when chromosome 4*D* was tetrasomic if there was not a third compound present. However, when 4*D* was tetrasomic, the area of the spot was increased by 5.7 cm^2. The biosynthesis of the third spot present, 25C, must therefore be controlled by chromosome, 4*DL*.

Spot 26 presented a similar situation to that just described; however, the appearance of the spot did not correlate well with the area occupied by the compounds present. The suggested explanation in this case was that either or both of the other two compounds present are under the influence of the two remaining homoeologous chromosomes in group 6. It was clear that one compound in this group, 26C, was being produced by gene(s) on the short arm of chromosome 6*B* because of the division of the spot into two distinct compounds when this chromosome arm was absent. However, the areas of the compounds measured in the nullisomic-tetrasomic compensating lines of group 6 indicate

that each of the other two compounds present (26A and 26B) were increased when chromosomes *6A* and *6B* were tetrasomic. This could account for the very large differences in areas of the spot compared with that of euploid Chinese Spring plants.

Finally, when the short arm of chromosome *4B* is missing, either as a telocentric or as a nullisomic condition, there is a reduction in the total quantity of flavonoids produced in the aneuploid. There is a concomitant increase in the quantity of flavonoids produced when *4B* is tetrasomic. This suggests that there was a gene(s) present on *4BS* that influenced the early flavonoid biosynthetic pathway. Thus, although all flavonoids are being produced, there are fewer precursors available for the production of the various compounds.

In conclusion, although flavonoids are only indirect indicators of gene action, it is apparent that they can be used as marker compounds. In addition, flavonoids are easily analyzed and scored and provide convenient genetic markers that are readily available for little expense.

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