Indian J. Genet., 55 (4): 424–433 (1995)

ANEUPLOID ANALYSIS FOR QUALITATIVE CHARACTERS IN A RECOMBINANT OF WHEAT AND RYE

RAMAVTAR SHARMA,* B. C. JOSHI AND S. M. S. TOMAR

Division of Genetics and Biotechnology Centre, Indian Agricultural Research Institute New Delhi 110012

(Received: May 11, 1992; accepted: June 10, 1995)

ABSTRACT

Monosomic analysis was used to locate genes for several qualitative traits in chromosomes of a wheat–rye recombinant, Selection-26 (Sel-26), using the monosomic series of cv. Chinese Spring. The study revealed that the anthocyanin pigmentation in leaf sheath is inherited as a single dominant gene in chromosome 1B. The character hairy rachis node is controlled by two nonlinked duplicate genes in chromosomes 5A and 7D. The symbols Hrn 1 and Hrn 2 are proposed for these two loci. The straight upper part of glume of Sel-26 is due to a single dominant gene in chromosome 6B. The gene symbol Gc (glume curvature) is proposed for this character. Three major genes governing hairy glume phenotype have been identified, two for hairiness in chromosomes 1A and 2A, and one recessive gene for glabrous glumes in 4A. Modifiers in 6B and 7B are also reported for this character. A minor recessive gene for red grain colour has been detected in chromosome 4A of Sel-26, presumably contributed by the rye parent and a modifier in 5A. Using the phenol test, the tryosinase producing gene has been mapped in chromosome 6B for glume character and in 4B for rachis.

Key words: Aneuploid analysis, qualitative characters, wheat–rye recombinant.

A complete sets of nullisomics, monosomics, trisomics and tetrasomics together with several other combinations of aneuploids are available in cv. Chinese Spring [1]. These aneuploids have been of enormous help in conducting genetic analysis in wheat. In the present paper monosomic lines (2n = 41) of cv. Chinese Spring has been used to locate genes for several qualitative characters in specific chromosomes of a wheat–rye recombinant induced by chromosome 5B manipulations [2].

*Present address: NARP Office, Rajasthan Agricultural University, Bikaner 334001.

MATERIALS AND METHODS

The materials for the study consisted of a complete set of 21 monosomic lines of wheat cv. Chinese Spring (CS) and a homoeologous recombinant of wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.), designated as Selection-26 (Sel-26).

For locating genes on specific chromosomes of Sel-26 through F_2 monosomic analysis, the following method was followed.

1. Cytological identification of monosomes in all the 21 monosomic lines of CS after which they were crossed using Sel-26 as male parent.

2. F_1 monosomic plants were identified in all the 21 lines and selfed.

3. F_2 progeny lines were raised from the F_1 monosomic plants for all the 21 chromosomes.

The parent lines and normal disomic (control cross), F_1 and F_2 families were raised. The χ^2 test was applied in F_2 to test for goodness of fit. Observations were recorded for the following six qualitative traits.

Anthocyanin pigmentation of leaf sheath. Leaf sheaths of 1st and 2nd leaf of Sel-26 develop anthocyanin pigment which becomes quite conspicuous at the time of heading.

Pubescence at rachis spikelet juncture. Chinese Spring has hairs at the rachis spikelet juncture on the node of rachis which is absent in Sel-26. Hairs were recorded after removing the spikelets on one spike from each plant.

Glume shape. It was recorded visually. CS has inward curved (concave) glumes while the glume rib of Sel-26 bends backward in the upper portion and then becomes straight.

Hairy glumes. Visual observations were recorded on hairiness of the glumes.

Red grain colour. Colour of grains was recorded visually. Three categories of red, white and amber were identified.

Phenol test. The phenol test was applied to seeds, glumes and rachis. Seeds were soaked in water by placing on wet filter paper while glumes and rachis were wrapped in wet filter paper. After 12 h of soaking they were placed on filter paper, soaked in 1% phenol solution and kept for 12–24 h at room temperature. Though most authors prefer constant temperature and a fixed time period for colour development, in the present study these limitations were not imposed so as to allow grains, glumes and rachis to express their full genetic potential and express complete phenol reaction. Chemically, the test is generally explained as involving the enzymic (tyrosinase) oxidation of phenol through diphenols to quinons and finally to dark brown melanins.

RESULTS AND DISCUSSION

ANTHOCYANIN PIGMENT ON LEAF SHEATH

Sel-26 develops a thin streak of anthocyanin pigment at the edge of leaf sheath and sometimes on the central portion of sheath blade when exposed to adequate sunlight. As indicated by Kuspira and Unrau [3], this is not a good marker as it is influenced by environment to a great extent. The F_2 populations derived from all the monosomic F_1 plants were space planted at 30 cm to get full penetrance of the gene governing this character.

Table 1. Segregation of F_2 plants derived from F_1 monosomic and disomic hybrids between monosomic Chinese Spring and Sel-26 for anthocyanin pigmentation on leaf sheath

Monosomic and parent variety	Segregation		Total	χ^2 value (3:1)	P value
	pigmented	nonpigmented			
1A	17	8	25	0.4667	0.50–0.30
1B*	30	0	30	10.00	0.05
1D	38	14	52	0.1026	0.80–0.70
2A	40	13	53	0.0063	0.95–0.90
2B	34	11	45	0.0074	0.95–0.90
2D	10	3	13	0.0256	0.90–0.80
3A	33	9	42	0.2857	0.79–0.50
3B	26	9	35	0.0095	0.95–0.90
3D	34	13	47	0.1773	0.70–0.50
4A	35	16	51	0.9412	0.50–0.30
4B	27	6	33	0.8182	0.50–0.30
4D	27	8	35	0.0857	0.80–0.70
5A	36	20	56	3.4286	0.10–0.05
5B	30	5	35	2.1428	0.20–0.10
5D	26	4	30	2.1778	0.20–0.10
6A	29	7	36	0.5926	0.50–0.30
6B	33	7	40	1.2000	0.30–0.20
6D	35	9	44	0.4848	0.50–0.30
7A	26	6	32	0.6667	0.50–0.30
7B	27	10	37	0.811	0.80–0.70
7D	42	16	58	0.2069	0.70–0.50
F_2	44	17	61	0.2677	0.70–0.50
CS	—	all	—	—	—
Sel-26	all	—	—	—	—
Total (excluding 1B)	649	211	860	0.0992	0.80–0.70

F_2 — normal disomic crosses. *Critical line.

The presence of colour on the leaf sheath in F_1 and F_2 segregation in the 3 coloured : 1 colourless ratio indicates presence of a single dominant gene for anthocyanin production (Table 1). All the 20 out of 21 monosomic F_2 populations segregated in 3:1 ratio (pigmented : nonpigmented), except for line 1B. All the plants in the line 1B were pigmented (Table 1), suggesting the presence of this dominant gene in 1B of Sel-26.

Earlier studies suggest presence of a single dominant gene for purple culm in chromosome 7B of hexaploid wheats [3, 4]. Involvement of at least 4 rye chromosomes has been reported in anthocyanin production of different plant parts: An3 in 2R [5], an1b in 2R [6], An5 in 3R [7], an1a in 1R [8], PC (purple culm) in 4R [9], and an 1a in 7R [10].

However, the gene for anthocyanin pigmentation on leaf sheath has not been located so far.

PUBESCENCE AT RACHIS SPIKELET JUNCTURE

Cv. Chinese Spring develops hairs on the rachis node at the juncture of the spikelet. This pubescence is absent in Sel-26. The normal F_1 disome along with all the F_1 monosomics were pubescent with varying degree of intensity. Observations on F_2 populations indicated segregation for this character in 15 hairy : 1 nonhairy ratio. All the F_2 lines derived from F_1 monosomic plants fit well in this ratio, except for lines 5A and 7D, which differ significantly from a 15:1 ratio. 5A showed a good fit to 3:1 ratio while 7D showed excess of nonhairy types (Table 2).

Table 2. Segregation of F_2 plants, derived from F_1 monosomic and disomic hybrids between monosomic Chinese Spring and Sel-26 for pubescence at rachis spikelet juncture

Monosomic and parent variety	Segregation		Total	χ^2 value (15:1)	P value	χ^2 value (3:1)	P value
	hairy	nonhairy					
1A	150	11	161	0.0932	0.3–0.7		
1B	204	12	216	0.1778	0.7–0.5		
1D	135	8	143	0.1049	0.8–0.7		
2A	172	11	183	0.0178	0.9–0.8		
2B	46	4	50	0.2613	0.7–0.5		
2D	18	0	18	1.2000	0.3–0.2		
3A	53	3	56	0.0762	0.8–0.7		
3B	63	5	68	0.1412	0.8–0.7		
3D	116	5	121	1.6480	0.2–0.1		
4A	78	6	84	0.1143	0.8–0.7		
4B	173	10	183	0.1927	0.7–0.5		
4D	231	13	244	0.3541	0.7–0.5		
5A*	182	63	245	158.4133	0.05	0.6667	0.5–0.3
5B	245	17	262	0.0254	0.9–0.8		
5D	256	18	274	0.0477	0.9–0.8		
6A	133	8	141	0.0799	0.8–0.7		
6B	252	15	267	0.1820	0.7–0.5		
6D	170	10	180	0.1481	0.7–0.95		
7A	177	12	189	0.0032	0.98–0.95		
7B	235	16	251	0.0066	0.95–0.90		
7D*	116	62	178	248.1633	0.05	9.1760	0.01–0.001
F_2	231	16	247	0.0217	0.90–0.80		
CS	All						
Sel-26		All					
Total (excluding 5A & 7D)	3138	200	3338	0.3803	0.70–0.50		

F_2 — normal disomic cross. *Critical line.

The segregation of F_2 populations in a 15:1 (hairy : hairless) ratio suggests duplicate gene action with hairiness acting as a dominant trait. These two genes for hairiness are present, one each in chromosome 5A and 7D, as these lines exhibited disturbed ratio. The symbol Hrn (hairy rachis node) is proposed for this character, the two genes could be designated as Hrn 1 and Hrn 2.

GLUME SHAPE

The glume midrib of CS is round (curved inward) but the midrib of Sel-26 recurves back in the upper half of the glume and then becomes straight. The control disomic F_1 as well as all the F_1 monosomes showed straightening of the glume rib, suggesting dominance of this character. The F_2 population derived from normal F_2 seed from 20 out of 21 monosomic F_1 plants segregated in 3 straight : 1 round glume midrib, indicating monogenic inheritance of this character (Table 3). Segregation of the F_2 population derived from the F_1 monosomic plant of chromosome 6B deviated significantly from the monogenic (3:1) ratio, indicating presence of the gene governing glume shape in this chromosome of Sel-26. The appearance of 5% round ribbed glumes in the critical line could be due to these plants being nullisomes. The designation Gc (glume curvature) is proposed for the gene governing this character (Table 3).

Table 3. Segregation of F_2 plants derived from F_1 monosomic and disomic hybrids between monosomic Chinese Spring and Sel-26 for glume shape

Monosomic and parent variety	Segregation for glume shape		Total	χ^2 value (1:3)	P value
	curved (cup)	straight (boat)			
1A	35	110	145	0.0575	0.90–0.80
1B	52	167	219	0.5525	0.50–0.30
1D	48	121	169	1.0434	0.50–0.30
2A	24	84	108	0.4444	0.70–0.50
2B	10	33	43	0.0698	0.50–0.30
2D	4	15	19	0.1579	0.70–0.50
3A	13	55	68	1.2549	0.30–0.20
3B	14	47	61	0.1366	0.80–0.70
3D	22	90	112	1.7143	0.20–0.10
4A	17	68	85	1.1333	0.30–0.20
4B	56	135	191	1.9005	0.20–0.10
4D	57	183	240	0.2000	0.70–0.50
5A	67	176	243	0.8573	0.50–0.30
5B	48	168	216	0.8889	0.50–0.30
5D	44	140	184	0.1159	0.80–0.70
6A	31	93	124	0.0	No deviation
6B*	11	225	236	52.0678	0.05
6D	39	138	177	0.8305	0.50–0.30
7A	60	151	211	1.3286	0.30–0.20
7B	60	162	222	0.4865	0.50–0.30
7D	45	135	180	0.0	no deviation
F_2	61	163	224	0.5952	0.50–0.30
CS	all	—	—	—	—
Sel-26	—	all	—	—	—
Total (excluding 6B*)	807	2437	3241	0.0174	0.90–0.80

F_2 — normal disomic cross. *Critical line.

HAIRY GLUMES

Th glumes of Sel-26 are hairy and those of CS are glabrous. All the

normal and monosomic F_1 plants expressed this character with varying degree. Mono-4A and mono-5B F_1s had less hairs. Variation was observed in the expression of hairiness, ranging from sparse to dense along with change in their length. Differences were also observed in the distribution of hairs. Besides fully hairy glumes, those with central portion glabrous and in some cases only glume tips hairy were also observed.

The F_2 populations derived from normal disomic F_2 seeds segregated in a 3:1 (hairy:glabrous) ratio, suggesting involvement of a single dominant gene for hairiness. Five out of 21 lines derived from the monosomic F_1s deviated significantly from 3:1 ratio: 1A, 2A and 6B lines had more hairy plants and 4A and 7A had glabrous plants in excess (Table 4). The identification of more than one critical line does not agree with monogenic inheritance. It seems that three major genes, two for hairiness (in chromosomes 1A and 2A), one recessive inhibitor (in 4A), and two modifiers (in 5B and 7B) give 3:1 ratio which could be a modified into the 54:10 ratio.

Table 4. Segregation of F_2 plants derived from F_1 monosomic and disomic hybrids between monosomic series of var. Chinese Spring and Sel- 26 for hairy glumes

Monosomic and parent variety	Segregation		Total	χ^2 value (2:1)	P value
	hairy	glab-rous			
1A*	158	29	187	8.9857	0.05
1B	169	59	228	0.0936	0.80–0.70
1D	132	36	168	1.1428	0.30–0.20
2A*	167	35	202	6.3432	0.05
2B	37	12	49	0.0068	0.95–0.90
2D	16	4	20	0.2669	0.70–0.50
3A	45	24	69	3.5271	0.10–0.05
3B	52	20	72	0.2963	0.70–0.50
3D	97	31	128	0.0417	0.90–0.80
4A	46	38	84	18.3492	0.05
4B	141	61	202	2.9109	0.10–0.05
4D	190	59	249	0.2262	0.70–0.50
5A	190	53	243	1.3182	0.30–0.20
5B	187	74	261	1.5645	0.30–0.20
5D	177	52	229	0.6419	0.50–0.30
6A	115	27	142	2.7136	0.10–0.05
6B*	229	57	286	3.9207	0.05–0.02
6D	146	38	184	1.8551	0.20–0.10
7A	130	63	193	6.0121	0.02–0.01
7B*	188	74	262	1.4707	0.30–0.20
7D	127	47	174	0.3755	0.70–0.50
F_2	187	69	256	0.5208	0.50–0.30
CS	0	all	—	—	—
Sel-26	all	0	—	—	—

F_2 — Normal disomic cross. *Critical line.

Earlier studies have assigned a single dominant gene for hairy glumes in chromosome 1A [3, 11, 12]. Digenic control of this character has also been reported [13, 14].

The hairy glume character of Sel-26 seems to have originated through interaction of genes since none of the parents involved in its pedigree have this character. Murthy and Lakhani [15] and Hayes et al. [16] also reported appearance of hairy glumes through gene interactions in material that had originated from wide crosses. The very nature of appearance of this character

through gene interaction suggests involvement of more than one gene in its expression. Interaction between genes in chromosomes 2A and 4A is suggested by the present study.

RED GRAIN COLOUR

Variety CS has red grains while the grains of Sel-26 are deep amber coloured. Transgressive segregation was observed in F_2 population, indicating presence of additional gene(s) for red grain colour (other than the one residing in chromosome 3D of CS). The F_2 population derived from 18 F_1 monosomic and normal disomic lines segregated in 1 (white + amber) : 5 (red) ratio (Table 5). Three lines derived from F_1s of monosomic 3D, 4A and 5A deviated significantly from 1:5 ratio. Plants in the 3D and 5A lines showed increase in the amber and white grain types while the 4A line had more plants with red grain.

Out of the three critical lines observed for red grain colour, the gene for this character in CS resides in 3D [1], which is presumably missing in Sel-26, which may be due to the fact that selection was exercised in breeding the wheat–rye recombinant against red grain. In the absence of the gene for red grain colour in 3D of CS (mono-3D) all the plants are expected to have amber grains, but appearance of about one-third red grains in the plants derived from mono-3D suggests presence of gene(s) for red grain colour in Sel-26 (Table 5).

Table 5. F_2 segregation of plants derived from F_1 monosomic and disomic hybrids between monosomic Chinese Spring and Sel-26 for red grain colour

Monosomic and parental variety	Segregation for grain colour			Total	χ^2 value (1:5) (W+A:R)	P value
	white 1	amber 2	red 3			
1A	4	26	158	188	0.0681	0.80–0.70
1B	3	23	139	165	0.0982	0.80–0.70
1D	5	26	134	165	0.5345	0.50–0.30
2A	4	8	94	106	2.1811	0.20–0.10
2B	2	7	40	49	0.1020	0.80–0.70
2D	Not tested					
3A	2	7	56	65	0.3723	0.70–0.50
3B	2	13	64	79	0.3063	0.70–0.50
3D*	16	66	27	109	269.1541	0.05
4A*	—	3	69	72	8.1000	0.05
4B	9	15	167	191	2.3131	0.20–0.10
4D	12	10	136	158	0.8557	0.50–0.30
5A*	22	41	187	250	13.1072	0.05
5B	12	28	219	259	0.2788	0.70–0.50
5D	8	32	163	203	1.3488	0.30–0.20
6A	5	13	81	99	0.1636	0.70–0.50
6B	15	36	224	275	0.6989	0.50–0.30
6D	2	15	52	69	3.1565	0.10–0.05
7A	5	17	140	162	1.1111	0.30–0.20
7B	5	30	190	225	0.2000	0.70–0.50
7D	8	27	149	184	0.7348	0.50–0.30
F_2	9	30	156	195	1.5600	0.30–0.20
CS	—	—	Red	—	—	—
Sel-26	Dark amber	—	—	—	—	—

F_2—normal disomic cross. *Critical line.

W—white; A—amber; R—red.

The F_2 population derived from monosomic 4A contains almost all the red grained plants, indicating presence of the gene for red grain colour in this chromosome of Sel-26. It seems that either this gene is recessive or, if dominant, then it would be effective only in homozygous condition. The excess of amber and white grains in mono-5A F_1 derived population suggests the presence of a modifier in this chromosome. It appears as 3 : 13 (white + amber : red) ratio due to the genes in chromosomes 3D and 4A getting modified to 1 : 5 ratio because of the gene present in chromosome 5A.

Earlier investigators have indicated presence of three genes for red grain colour : R1 in chromosome 3DL [1], R2 in 3AL [17], and R3 in 3B [18, 19]. Though reports indicate presence of recessive gene and modifiers, the effect of environment has always been reported to produce continuous variation rather than an exact gradation which could be enhanced by the modifiers.

PHENOL TEST

Seeds of CS and Sel-26 do not show difference in their reaction to phenol. Glumes of Sel-26 gave light black colour though they had developed dark colour on the top while the glumes of CS were totally dark black. The rachis of Sel-26 developed light black colour as compared to CS which developed no colour.

The glumes of all the mono-F_1 plants were put to phenol test. Mono-6B showed slightly lighter black colour in whole glume which did not change with duration of reaction. The rachis of mono-4B had lighter colour like that of Sel-26. This suggests that chromosome 6B of Sel-26 has a gene for glume phenol reaction, or the tyrosinase producing gene in the glumes, while chromosome 4B has a gene responsible for phenol reaction in its rachis.

Zeven [20] and Wrigley and McIntosh [21] indicated that genes for phenol reaction in the grain were located in chromosomes 2A and 2D in the material studied by them. Genetic linkage of the grain phenol reaction gene with glume phenol reaction gene was suggested by Frezer and Gfeller [22]. Goud and Kamat [23] mapped the genes determining tyrosinase activity (through phenol test) in chromosomes 5B and 6B. The present study confirms the presence of one gene in chromosome 6B but the other gene has been located in chromosome 4B.

ACKNOWLEDGEMENT

The first author (RS) thanks the P. G. School, I.A.R.I., for financial assistance during the Ph.D. course.

REFERENCES

1. E. R. Sears. 1954. The aneuploids of common wheat. Missouri Agric. Exp. Sta. Res. Bull. No. 572: 59.

2. B. C. Joshi and S. M. S. Tomar. 1989. Transfer of alien genes and their effectiveness in Indian wheats. Proc. 3rd National Seminar on Genetics and Wheat Improvement (eds. A. K. Gupta, S. Nagarajan and R. S. Rana). Shimla, 1985: 205–218.

3. J. Kuspira and J. Unrau. 1960. Determination of gene chromosome associations and establishment of chromosome markers by aneuploid analysis in common wheat. I. F_2 analysis of glume pubescence, spike density and culm colour. Can. J. Genet., **2**: 301–310.

4. C. N. Law. 1966. The location of genetic factors affecting a quantitative character in wheat. Genetics, **53**: 487–493.

5. G. Melz. 1985. Cited in R. Schlegel, G. Melz and D. Mettin. 1986. Rye cytology, cytogenetics and genetics. Current status. Theor. Appl. Genet., **72**: 721–734.

6. W. Sturm, H. Newmann and G. Melz. 1981. Trisomenanalyse Fur Merkmal Anthocyanfarbung bei *Secale cereale* L. Arch. Zhutungsforsch., **11**: 49–53.

7. W. G. Smirnov and S. P. Sosnichina. 1984. Genetikarti. Leningrad: 1–156.

8. F. J. Zeller and C. L. Koller. 1981. Identification of a 4A/7R and a 7B/4R wheat–rye chromosome translocation. Theor. Appl. Genet., **59**: 33–37.

9. T. E. Miller. 1984. The homoeologous relationships between the chromosomes of rye and wheat. Current status. Can. J. Genet. Cytol., **26**: 578–589.

10. J. N. DeVaries and J. Sybenga. 1984. Chromosomal location of 17 monogenetically inherited morphological markers in rye (*Secale cereale* L.) using the translocation tester set. Z. Pflanzenzuchtg., **92**: 117–139.

11. K. Tsunewaki. 1960. Monosomic and conventional gene analyses in common wheat. IV. Glume hairiness and ear density. Jap. J. Genet., **36**: 55–62.

12. R. G. Anderson and R. C. McGinnis. 1960. The inheritance and chromosomal association of a gene for glume pubescence in the common wheat variety Loro. Can. J. Genet. Cytol., **2**: 331–335.

13. A. Howard and G. L. C. Howard. 1912. On the inheritance of some characters in wheat. I. Mem. Dep. Agric. India, Bot., S: 1–47.

14. A. Vacenko. 1934. Inheritance of glume pubescence and of black colour of the ear of durum wheats. Dokl. Akad. Nauk. SSSR (Compt. Rend. Acad. Sci.), **4**: 338–343.

15. G. S. Murthy and L. B. Lakhani. 1958. Genetic studies in crosses between *aestivum* varieties and the *durum* wheat Gaza. Indian J. Genet., **18**: 163–177.

16. H. K. Hayes, J. H. Parkerand and C. Kurtzwell. 1920. Genetics of rust resistance in crosses of varieties of *Triticum vulgare* with varieties of *T. durum* and *T. dicoccum*. J. Agric. Res., **19**: 523–542.

17. R. E. Allan and A. O. Vogel. 1965. Monosomic analysis of red grain colour in wheat. Crop Sci., **5**: 474–475.

18. R. J. Metzger and B. A. Silbaugh. 1970. Location of genes for seed coat colour in hexaploid wheat, *Triticum aestivum* L. Crop Sci., **10**: 495–496.

19. B. C. Joshi, D. Singh and R. N. Sawhney. 1971. Identification of the third locus governing red grain colour in *Triticum aestivum*. EWAC Newsl., **3**: 63.

20. A. C. Zeven. 1972. Identification of chromosomes carrying a locus for a gene conditioning the production of tyrosinase in wheat grains. Wheat Inf. Serv., **35**: 3–8.

21. C. W. Wrigley and and R. A. McIntosh. 1975. Genetic control of factors regulating the phenol reaction of wheat and rye grain. Wheat Inf. Serv., **40**: 6–10.

22. J. G. C. Frazer and F. Gfeller. 1936. The inheritance and use of phenol colour reaction in hard redspring wheat. Sci. Agric., **17**: 243–249.

23. J. V. Goud and R. T. Kamat. 1985. Aneuploid F_3 analysis of tyrosinase activity in bread wheat (*Triticum aestivum* L. em. Thell). Mysore J. Agric. Sci., **17**: 334–336.