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Assignment of molecular linkage groups to soybean chromosomes by primary trisomics

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Abstract Gene-linkage groups (classical linkage groups, CLGs; molecular linkage groups, MLGs) and chromosome relationship in soybean [*Glycine max* (L.) Merr., 2n = 40] is not yet established. However, primary trisomics provide an invaluable cytogenetic tool to associate genes and linkage groups to specific chromosomes. We have assigned 11 MLGs to soybean chromosomes by using primary trisomics ($2x + 1 = 41$) and SSR markers. Primary trisomics were hybridized with *Glycine soja* Sieb. and Zucc. (2n = 40) in the greenhouse, F_1 plants with 2n = 40 and 41 were identified cytologically and 41 chromosome plants were selfed. A deviation from the 1:2:1 ratio in the F_2 population suggests a marker is associated with a chromosome. Of the possible 220 combinations involving 20 MLGs and 11 primary trisomics, 151 combinations were examined. The relationships between soybean chromosomes and MLGs are: 1 = D1a+q, 3 = N, 5 = A1, 8 = A2, 9 = K, 13 = F, 14 = C1, 17 = D2, 18 = G, 19 = L and 20 = I. This study sets the stage to establish relationship between nine remaining MLGs with the other genetically unidentified nine primary trisomics. The association of CLGs with the soybean chromosomes will be discussed.

Keywords Soybean · *Glycine max* · Chromosome · Primary trisomic · SSR · Genetic map

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Introduction

Cytogenetic investigations in the soybean [*Glycine max* (L.) Merr.] have lagged behind maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and tomato (*Lycopersicon esculentum* Mill.), despite its economic importance for human consumption, livestock feed and industrial use. The main handicap is that the soybean is not considered a model crop for cytogenetic studies because it contains 2n = 40 small (1.42–2.84 μm) and symmetrical chromosomes (Sen and Vidyabhusan 1960). The associations between classical and molecular linkage groups (MLGs) and soybean chromosomes are largely unknown.

In soybean, over 250 morphological and isozyme markers are available, and 20 classical linkage groups (CLGs) consisting of 68 genetic markers have been published (Palmer and Shoemaker 1998). Several molecular linkage maps of soybean have been developed using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers (Keim et al. 1990; Shoemaker and Olson 1993; Shoemaker and Specht 1995; Mansur et al. 1996; Keim et al. 1997; Cregan et al. 1999). Twenty consensus MLGs, based on three mapping populations, contain more than 1,400 DNA markers (Cregan et al. 1999).

Singh and Hymowitz (1988) published the first soybean chromosome map based on pachytene chromosome analysis. This laid the foundation to identify primary trisomics in the soybean. A primary trisomic individual contains a normal chromosome complement plus an extra complete chromosome ($2n = 2x + 1$) and is an invaluable cytogenetic tool to locate genes and to associate classical and molecular linkage groups to the specific chromosomes (Singh 2003). Twenty possible soybean primary trisomics ($2n = 2x + 1 = 41$) have been tentatively identified and designated as Triplo 1 (longest chromosome) through Triplo 20 (smallest chromosome) (Xu et al. 2000).

The objective of this study is to report association of MLGs, developed by Cregan et al. (1999), to the specific chromosomes of the soybean by using primary trisomics.

Materials and methods

Plant material and cytological analysis

Primary trisomics in soybean were isolated, identified and produced in the genetic background of soybean cv Clark 63 by a backcrossing procedure (Xu et al. 2000). Clark 63 was used as recurrent parent because it is adapted to central Illinois (maturity group IV) and is an excellent pollinator. Each primary trisomic was crossed with *Glycine soja* PI 407287 or PI 81762 in order to provide a high degree of molecular polymorphism. F_1 plants with 2n = 40 and 41 chromosomes were identified cytologically. Briefly, 5 to 10 seeds were germinated in vermiculite in the greenhouse. Actively growing root tips from 7- to 10-day old seedlings were collected, pretreated in 0.05% (w/v) 8-hydroxyquinoline for 4 to 5 h at 16 °C, and fixed in a 3:1 (v/v) mixture of 95% ethanol and propionic acid for 24 h. The root tips were hydrolyzed in 1 M HCl for 12 min at 60 °C, washed once with double distilled water (ddH_2O), and stained in Schiff's reagent (Sigma Incorporated, St. Louis, Mo.) for 1.5 to 2 h at room temperature. The root tips were rinsed with ddH_2O and stained with Carbol fuchsin stain overnight at 0 to 4 °C in a refrigerator. After staining, root tips were washed with cold ddH_2O three to four times in order to remove phenol and stored in cold ddH_2O in a refrigerator. Root tips were squashed in 45% acetic acid and chromosomes were counted under a Nikon Optiphot-2 microscope (Nikon Incorporated, Instrument Group, Garden City, N.Y.). After cytological analysis, the F_1 hybrids with 2n = 41 were grown in the greenhouse and allowed to produce selfed seeds. F_2 seeds from each trisomic-derived population were germinated in the greenhouse and DNAs were isolated from leaves using the method of Walbot (1988).

SSR marker analysis

The isolation and map positions of soybean SSR markers have been described by Cregan et al. (1999). The primer sequences of these markers can be found at http://soybase.agron.iastate.edu. PCR reactions were undertaken in 10-μl volumes containing 30 to 45 ng of template DNA, 1.5 pmol of each primer, 0.2 mM of dNTPs (Pharmacia Biotech Inc., Piscataway, N.J.), 1.5 mM of $MgCl_2$, 1× PCR buffer and 0.5 units of *Taq* polymerase (Gibco BRL Life Technologies Inc., Gaithersberg, Md.). Temperature cycling was performed in an MJ Research PTC 100 Thermal Controller using the program of 'touchdown' PCR (Don et al. 1991) with a slight modification. The amplification profile was set to run at 94 °C for 3 min followed by six cycles of denaturing at 94 °C for 30 s, annealing from 55 to 50 °C with 1 °C decreased by one cycle for 30 s and extending at 72 °C for 1 min. The final cycle (94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min) was repeated 35 times.

Amplification productions were detected by 6% denaturing polyacrylamide gel electrophoresis and silver staining. Gels were made with 6% (w/v) acrylamide bis-acrylamide (19:1), 7 M ultrapure urea, 40% (v/v) formamide, 1× TBE buffer, 1% TEMED and 10% ammonium persulfate. Following polymerization of the gel, wells were loaded with 5 μl of each amplification product mixed with 2.5 μl of loading buffer [98% formamide, 10 mM EDTA (pH 8.0), 0.075% Bromophenol Blue, 0.075% Xylene Cyanol FF]. Gels were run at 120 V constant power for 2 h. The staining protocol was according to the instruction provided by BIORAD (Hercules, Calif.).

Data analysis and statistics

Assignment of linkage groups to chromosomes was based on F_2 segregation analysis using SSR markers and primary trisomics. SSR markers from the 20 MLGs (Cregan et al. 1999) were initially used to assay polymorphism in the female, male and F_1 plants. Once polymorphic markers were identified, a group of 20 or more F_2 plants from each trisomic-derived F_2 population was analyzed and the segregation ratio was tested for goodness-of-fit to a disomic ratio (1:2:1) and to a trisomic ratio (6:11:1). In case the ratio fitted a trisomic ratio, up to 104 F_2 samples were examined. The trisomic ratio (6:11:1) is based on the 50% female transmission rate of the extra chromosome from the primary trisomic F_1s with a duplex (AAa) genotype (Singh 2003). A trisomic ratio of 5:7.5:1 is expected when the female transmission rate of the extra chromosome is 33.3% (Singh 2003). The chi-square tests were performed using MS Excel 2000. Once one SSR marker was assigned to a chromosome, additional markers from different parts of the same linkage group were tested to confirm the assignment.

Results

Eleven MLGs of soybean, described by Cregan et al. (1999), were associated with their respective chromosomes by using primary trisomics. We tested 151 of the 220 possible combinations involving 20 linkage groups and 11 primary trisomics using SSR markers. Table 1 lists all SSR-chromosome combinations studied and the segregation data. Thirty nine SSR markers that segregated in a trisomic fashion are presented in Table 2.

Triplo 1: F_2 plants of Triplo 1 × *G. soja* were analyzed with SSR markers from 18 of the 20 MLGs. Five SSR markers (Satt184, Satt368, Satt179, Satt129 and Satt360) of MLG D1a+q segregated in a trisomic fashion (6:11:1) with Triplo 1 (Table 2). This suggests that MLG D1a+q is associated with chromosome 1.

Triplo 3: F_2 plants from Triplo 3 × *G. soja* were screened with SSR markers from 12 of the 20 MLGs. Satt584 (MLG N) showed a 34:50:9 ratio with Triplo 3. Satt009 and Sat_091 from MLG N also segregated in a trisomic fashion suggesting MLG N is associated with chromosome 3.

Triplo 5: Satt276, from middle of the MLG A1, segregated as 17:34:3 with Triplo 5 suggesting that this marker is located on chromosome 5. Qualitative traits *eu1* (seed urease null) and *v2* (variegated leaf) were also associated with chromosome 5 previously (Xu et al. 2000; Cregan et al. 2001). Further analysis using Satt236 confirmed that MLG A1 is associated with chromosome 5 (Table 2). All the SSR markers from the remaining 19 MLGs showed disomic ratios, indicating that only MLG A1 is located on chromosome 5.

Triplo 8: SSR markers from 11 of the 20 MLGs were used in the initial screening. Satt329 from middle of the MLG A2 showed a trisomic ratio (44:49:7) with Triplo 8. Furthermore, Satt589 and Satt177, located on the terminal, and Satt409 on the bottom region of MLG A2, were also tested with Triplo 8. All segregated in a trisomic fashion (6:11:1) indicating that these loci are located on chromosome 8 (Table 2). Thus, MLG A2 is associated with chromosome 8.

Table 1 Summary of SSR marker segregation in primary trisomic tests

Molecular linkage group[a]	Primary trisomics and type of segregation obtained[b]										
	1	3	5	8	9	13	14	17	18	19	20
A1	–	–	**T**	–	–	–	–	–	–	–	D
A2	D	D	D	**T**	–	–	D	D	–	–	D
B1	D	D	D	D	D	–	D	D	–	D	D
B2	D	–	D	D	D	–	D	D	–	D	D
C1	D	–	D	–	–	D	**T**	D	–	D	D
C2	D	D	D	D	–	–	D	D	–	D	D
D1a+q	**T**	D	D	–	–	–	D	–	–	–	D
D1b+w	D	D	D	–	–	D	D	D	D	D	D
D2	D	D	D	–	–	–	D	**T**	–	D	D
E	D	D	D	D	–	D	D	D	–	D	D
F	–	–	D	–	–	**T**	–	–	–	–	–
G	D	D	D	D	D	–	D	D	**T**	D	D
H	D	–	D	D	D	–	D	D	D	D	D
I	D	–	D	D	D	D	D	D	D	D	**T**
J	D	–	D	D	D	–	D	D	D	D	D
K	D	–	D	–	**T**	–	D	–	–	–	D
L	D	D	D	–	–	–	D	D	–	T	D
M	D	D	D	D	D	–	D	D	D	D	D
N	D	**T**	D	–	–	D	D	–	–	D	D
O	D	D	D	D	D	D	D	D	D	D	D
No. of molecular linakge groups tested	18	12	20	11	9	7	18	15	7	15	19

[a] According to Cregan et al. 1999
[b] D, disomic segregation; T, trisomic segregation; –, not tested

Triplo 9: F_2 plants of Triplo 9 × *G. soja* were analyzed with SSR markers from 9 of the 20 MLGs. Satt381 from MLG K segregates as a 35:61:8 ratio with Triplo 9. Five additional loci, Satt242, Satt046, Satt001, Satt260 and Sat_020 from MLG K, also segregated in a ratio of 6:11:1 indicating these loci are on chromosome 9 (Table 2). A classical marker gene, *fr1* (root fluorescence) from CLG 2, expressed a trisomic ratio (17:1) with Triplo 9 (Singh and Zou, unpublished results). This indicates CLG 2 is also located on chromosome 9.

Triplo 13: qualitative traits, *lx1* (seed protein lipoxygenase-1) (Xu et al. 2000; Cregan et al. 2001) and *w1* (white flower) (Sadanaga and Grindeland 1984) were located on chromosome 13. Satt114 from MLG F segregated as 22:72:2 with Triplo 13. Satt510 and Satt252 also segregated in a trisomic ratio (Table 2). This confirms the assignment of MLG F to chromosome 13 (Cregan et al. 2001).

Triplo 14: SSR markers from 18 of the 20 MLGs were analyzed against F_2 plants from Triplo14 × *G. soja*. Satt565 (MLG C1) segregated as a 29:63:7 ratio, positioning it on chromosome 14. Sat_085 and Satt180 of MLG C1 were also tested and both markers segregated in a 6:11:1 ratio, confirming MLG C1 is on chromosome 14 (Table 2).

Triplo 17: Satt226, Sct_137 and Sctt008 of MLG D2 segregated in a trisomic fashion with Triplo 17. This indicates that MLG D2 is associated with chromosome 17 (Table 2).

Triplo 18: Satt566 (MLG G) segregated in a ratio of 30:62:4 suggesting MLG G is associated with chromosome 18. Satt309 (on the top portion) and Satt472 (on the bottom portion) of MLG G also segregated in a trisomic manner with Triplo 18 (Table 2). Thus, MLG G is associated with chromosome 18.

Triplo 19: SSR markers from 15 MLGs were tested against F_2 plants from Triplo19 × *G. soja*. Satt462 from the middle of MLG L segregated as 38:58:3, deviating significantly from the 1:2:1 ratio. Satt182 (from the top) and Satt006 (from the bottom) of MLG L were also tested and showed trisomic segregation (Table 2). Thus, MLG L is associated with chromosome 19.

Triplo 20: after assessing SSR markers from 19 MLGs, three SSR markers (Satt440, Satt354 and Satt367) from MLG I showed a trisomic ratio with Triplo 20 (Table 2). Therefore, MLG I is associated with chromosome 20.

Discussion

Association of MLGs to soybean chromosomes

We have assigned 11 MLGs to their respective soybean chromosomes by primary trisomics. However, we were unable to associate the remaining nine MLGs to soybean chromosomes. The explanation is as follows: (1) our primary trisomic set may not be complete; Triplo 16 and Triplo 19 may be the same because both triplos showed trisomics ratio with SSR markers from MLG L (Zou et al. 2003); (2) we could not associate SSR markers with Triplo 2, 6 and 7; (3) it is possible that the reported 20 MLGs do not cover the entire soybean genome; (4) some SSR markers did not exhibit either expected (6:11:1) trisomic or (1:2:1) disomic ratios.

In soybean, most RFLP probes exhibit a multi-locus characteristic that makes the unambiguous association of the linkage group to the chromosome problematic (Cre-

Table 2 Segregation ratios for SSR markers in the F_2 generation of primary trisomics of soybean

Triplo and SSR markers	MLG	F_2 segregation[a]				Chi-square (1:2:1)		Chi-square (6:11:1)	
		T	F	S	Total	Value	Probability	Value	Probability
Triplo 1									
Satt184	D1a+q	31	58	10	99	11.828	<0.005	3.906	0.142
Satt368	D1a+q	37	52	10	99	14.980	<0.005	5.361	0.069
Satt179	D1a+q	26	36	4	66	15.212	<0.005	1.223	0.543
Satt129	D1a+q	26	59	5	90	18.511	<0.005	0.824	0.662
Satt360	D1a+q	33	57	10	100	12.540	<0.005	3.835	0.147
Triplo 3									
Satt584	N	34	50	9	93	13.968	<0.005	3.956	0.138
Sat_091	N	28	53	6	87	15.276	<0.005	0.317	0.854
Satt009[b]	N	33	51	12	96	9.563	0.005–0.10	3.647	0.161
Triplo 5									
Satt276	A1	17	34	3	54	6.368	0.01–0.05	2.766	0.251
Satt236	A1	20	27	6	53	7.415	0.01–0.05	4.376	0.112
Triplo 8									
Satt329[c]	A2	44	49	7	100	27.420	<0.005	0.021	0.528
Satt177	A2	17	26	2	45	11.089	<0.005	0.448	0.799
Satt409	A2	38	54	6	98	21.918	<0.005	1.506	0.471
Satt589	A2	38	57	5	100	23.740	<0.005	0.985	0.611
Triplo 9									
Sat_020	K	31	65	8	104	16.673	<0.005	1.275	0.529
Satt381	K	35	61	8	104	17.135	<0.005	0.961	0.619
Satt242	K	33	63	8	104	16.673	<0.005	0.940	0.625
Satt260	K	21	32	5	58	9.448	0.005–0.10	1.459	0.482
Satt001	K	21	30	3	54	12.667	<0.005	0.773	0.680
Satt046	K	21	31	4	56	10.964	<0.005	0.849	0.654
Triplo 13									
Satt510	F	22	23	2	47	17.043	<0.005	3.843	0.146
Satt252	F	15	30	4	49	7.408	0.01–0.05	0.709	0.702
Satt114[c]	F	22	72	2	96	8.239	0.01–0.05	0.116	0.137
Triplo 14									
Sat_085	C1	28	66	6	100	19.920	<0.005	1.280	0.527
Satt565	C1	29	63	7	99	17.141	<0.005	0.997	0.607
Satt180	C1	28	66	6	100	19.920	<0.005	0.034	0.983
Triplo 17									
Satt311	D2	30	66	4	100	23.760	<0.005	1.160	0.560
Sct_137	D2	30	60	7	97	16.361	<0.005	0.659	0.719
Sctt008	D2	33	57	10	100	12.540	<0.005	3.835	0.147
Satt226	D2	32	61	4	97	22.608	<0.005	0.441	0.814
Triplo 18									
Satt235	G	28	62	6	96	18.250	<0.005	0.773	0.680
Satt566	G	30	62	4	96	22.250	<0.005	0.648	0.723
Satt472	G	36	59	1	96	30.563	<0.005	4.023	0.134
Triplo 19									
Satt182[c]	L	46	50	3	99	37.364	<0.005	0.063	0.273
Satt462	L	38	58	3	99	27.667	<0.005	1.997	0.368
Satt006	L	35	56	8	99	16.434	<0.005	1.592	0.451
Triplo 20									
Satt440	I	35	56	6	97	19.660	<0.005	0.470	0.790
Satt354	I	38	52	5	95	23.779	<0.005	1.913	0.384
Satt367	I	41	47	9	97	21.206	<0.005	0.134	0.109

[a] T, Triplo; F, F_1; and S, *G. Soja*
[b] Chi-square for 5:7.5:1 (trisomic segregation ratio)
[c] Chi-square for 17:1 (trisomic segregation ratio)

gan et al. 2001). The SSR markers on the map reported by Cregan et al. (1999) were only those that produced single amplification products with each of ten homozygous soybean genotypes. In the early effort to assign linkage groups to Triplo 5 and Triplo 13, only about 15% of the SSR were polymorphic in the mapping populations derived from Triplos × experimental lines (*G. max* × *G. max*) (Cregan et al. 2001). In order to provide a high level

Table 3 Assignment of molecular and classical linkage groups to soybean chromosomes. The molecular and classical linkage groups are listed according to Palmer and Shoemaker (1998), Cregan et al. (1999) and Mahama et al. (2002)

Chromosome	MLG	CLG (classical marker)	Classical marker (not on CLG)
1	D1a+q	3 (*g, d1*)	*pd1, me*
3	N	10 (*l2, rps1, rps7, hm*)	*rpg4*
5	A1	–	*v2,eu1*
8	A2	7 (*rhg4, I, o, y13*), 9 (*pgd2, lap1, ti, ap, fr3*)	*lf1*
9	K	2 (*r, p1*), 12 (*ep, fr1*)	
13	F	6 (*df2, y11*) 8 (*ms1, w1, ms6, adh1, wm, y23, st5*), 13 (*rsv1, rpv1, rps3*)	*rpg1, lx1, b1*
14	C1	21 (*fle, dia2*)	*n*
17	D2	20 (*rxp, mdh*)	*fr2*
18	G	18 (*dt2, mpi*)	*rhg1, rps4, rps5*
19	L	5 (*pgi1, pgd1, l1, dt1, e3*)	
20	I	4 (*enp, ln, v1, p2*)	*chi*

of SSR polymorphisms, the primary trisomics were crossed with *G. soja*. Of the 260 SSR markers screened against the parent materials, 104 (40%) SSR markers were polymorphic.

Integration of MLGs and CLGs with chromosomes

The associations between 11 soybean MLGs and the cytologically identifiable chromosomes have been established. Based on this study, we also assigned 14 CLGs to the soybean chromosomes (Table 3). These associations have unified the available cytogenetic, classical and molecular mapping information. Chromosome 1 (MLG D1a+1) is the longest one among all the soybean chromosomes (Singh and Hymowitz 1988). Triplo 1 expressed unique diagnostic features, such as vigorous vegetative growth, larger branches and dark-green leaves with large pods and seeds than disomics (Xu et al. 2000). Four genes (*g, d1, pd1* and *me*), which were mapped to MLG D1a+q, now can be assigned to chromosome 1 (Table 3). Similarly, chromosome 9 (MLG K) contains a dark-staining chromomere (knob) on its long arm (Singh and Hymowitz 1988). Four genes, which belong to CLGs 2 (*r* and *p1*) and 12 (*ep* and *fr1*), were located on this chromosome (Table 3). Chromosome 13, the nucleolus organizer, carries MLG F, CLG6, 8 and 13 (Table 3). Several loci conferring resistance to diverse pathogens have been mapped on chromosome 13 (Yu et al. 1994; Ashfield et al. 1998; Demirbas et al. 2001; Penuela et al. 2002).

Construction of a universal soybean linkage map

In the last decade, the development of linkage maps in soybean has tremendously increased. However, the linkage maps of soybean remain less saturated with genetic markers compared to some other major crops, such as maize, rice, barley and tomato. The soybean classical linkage map has only 68 of the over 250 qualitative traits (pigmentation, morphological, isozyme and disease resistance genes; Palmer and Shoemaker 1998) and most of them have not been associated to any chromosomes. The slow pace in constructing the soybean genetic map is mainly due to the difficulty in counting and identifying chromosomes, making crosses and generating large numbers of hybrids. This study sets the stage to integrate MLGs and CLGs to the soybean chromosomes.

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