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Full Length Research Paper

Recombination frequencies between cultivated soybean (*Glycine max*) and its wild relative *Glycine soja* based on molecular marker analysis

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Close relatives of cultivated crops provide an invaluable source of genetic variation in crop improvement and exploiting such variation often forms a critical part in a breeding program. The usability of the wild soybean *Glycine soja* was investigated in this study by analyzing populations derived from two wide crosses between a common cultivar and two different *G. soja* accessions using simple sequence repeat (SSR) markers. Consistent reductions in recombination frequencies were not detected in either of these two wide crosses and the results does not seem to be confined to the particular populations or the wild genotypes used. In variance with previous reports that domestication-related traits are often controlled by one or two major loci, these recombination results strongly indicate that linkage drag should not be a major concern in transferring genes from the wild taxon into the cultigen, although backcross would still be required to minimize undesirable chromatins.

Key words: *Glycine max*, *Glycine soja*, linkage drags, recombination frequency, molecular markers.

INTRODUCTION

Creating diversity and selecting elite individuals are keys in crop improvement. To enrich genetic variation of cultivated crops, closely related taxa are routinely exploited in breeding programs. Exploiting genetic variation from related taxa has been a common practice in all major crops, such as in rice (Xiao et al., 1998; Brondani et al., 2002; Aluko et al., 2004), wheat (Huang et al., 2003), barley (Pillen et al., 2004), and tomato (Tanksley and McCouch, 1997; Monforte and Tanksley, 2000). However, difficulties at various stages may be encountered in exploiting genetic variation from related species (Zamir, 2001). Some of these can be visually obvious, such as cross incompatibility and sterility of either hybrids or their progenies. Other difficulties such as linkage drags due to reduced recombination between the chromosomes of the concerned species or genes of negative effect being tightly linked to the trait of interest, may not be visually so obvious and may need to be assessed by analyzing recombination frequencies (Liu et al., 1996).Cultivated soybean (*Glycine max*) is one of the most important crops for feed and food products and it is rich in seed protein and oil, accounting for 48% of the world market in oil crops (Zhang et al., 2004). In China, soybean is a major oilseed crop and is grown on about eight million hectares (Zhang et al., 2004). Compared with many other crops, cultivated soybean varieties are

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characterized by very narrow genetic variation (Gizlice et al., 1994; Hyten et al., 2006). To enrich genetic variation in soybean breeding, attempts have been made to introduce genes from genotypes of its annual wild relative *Glycine soja*.

Although belonging to two different taxa, cultivated soybean and *G. soja* are classified into the primary gene pool (Singh, 2007). They share a similar genome structure containing 40 chromosomes and there is no difficulty in producing fertile hybrids and progeny by crossing genotypes between these two taxa (Palmer et al., 1987; Singh et al., 1988). Genes conferring some very important traits such as protein content (Sebolt et al., 2000), SCN resistance (Wang et al., 2001; Winter et al., 2007) and yield (Concibido et al., 2003; Wang et al., 2004; Li et al., 2008) have been successfully incurporated from this wild taxon into commercial varieties.

As linkage distances are derived from recombination frequencies between loci thus can be used to assess the degree of linkage drags (Doebley and Stec, 1991, 1993; Liu et al., 1994; Mano et al., 2005). Several linkage maps have been generated for soybean, some based on populations generated between cultivated genotypes (Yamanaka et al., 2001; Zhang et al., 2004; Kassem et al., 2006) and others from populations between cultivated and wild relatives (Cregan et al., 1999; Song et al., 2004; Liu et al., 2007). However, to our knowledge, no attempt has ever been made in exploiting these linkage data for investigating the usability of *G. soja* in breeding programs. To facilitate the further exploitation of the genetic variation from this wild taxon, we assessed recombination frequencies between cultivated soybean and *G. soja* genotypes by analyzing a set of SSR markers covering each of the soybean linkage groups and reported the results obtained in this work.

MATERIALS AND METHODS

Two F_2 populations derived from two different *G. soja* accessions (ZYD2738 and ZYD2739) and a common cultivar Jidou 12 were used in this study. Both of the two wild soybean accessions were collected in China and they are typical *G. soja* genotypes showing many characteristics contrasting to those of *G. max* including rampant growth habit, twinning and indeterminate stem, pots shattering, presence of bloom and tiny seeds. Jidou 12, bred at the Institute of Cereal and Oil crops, Hebei Academy of Agricultural and Forestry Sciences, Shijiazhuang, China, is an elite variety with high protein content. Seeds of the F_2 generation were collected from a single F_1 plant for each of these two crosses in late September 2006. The F_2 populations, consisting of 85 (designated as Y8) and 96 (Y9) individuals, respectively were sown in plastic pots at the Dishang Experimental Station in Hebei, China in late June 2007.

DNA isolation and SSR analysis

Young leaves, collected from plants (about one month old) of the two F_2 populations, were ground in liquid nitrogen, and DNA was extracted using the Tiangen DNAquick Plant System (Beijing). DNA samples were quantified and diluted to 15 ng/µl prior to PCR amplification. Based on the consensus soybean genetic linkage map published by Song et al. (2004), 151 SSR markers distributed throughout the 20 genetic linkage groups were selected to test the three parents involved in the two populations. Primer sequences of the SSR markers used were obtained from the SoyBase website of the USDA, ARS Genome Database (http://soybase.agron.iastate.edu/). Polymerase chain reactions (PCR) were carried out in a final volume of 20 µl, containing 1 × PCR Buffer, 100 µM of each dNTP, 0.4 µM of each primer, 30 ng genomic DNA, and 1 U of Taq DNA polymerase. DNA amplification was carried out over 35 cycles of 94°C for 30 s, 47°C for 30 s, 72°C for 30 s, then followed by a final 5 min extension at 72°C. PCR products were fractionated by 6% SDS-polyacrylamide gel electrophoresis and visualized by silver staining. Polymorphic SSR markers were then used to analyse the F_2 populations. Genotypes of the individuals of the F_2 populations were classified as ‘A’ (allele from Jidou 12, ‘B’ (allele from either of the wild *G. soja* accessions) or ‘H’ (heterozygous loci containing alleles from both parents).

Map construction and linkage distance comparison

Linkage analysis was performed using the Mapmaker/Exp 3.0 (Lincoln et al., 1993), with the selection of Kosambi for Map Function and 0.05 probability for linkage criterion. Segregation distortion was defined by Chi-square test. The map distances between markers from the two wide crosses were then compared with that of the consensus map which was compiled by combining data from five different populations (Song et al., 2004) using the ‘ANOVA’ in SAS (version 9.1). As the genotyping data for the consensus map was not available, 10% was arbitrarily set to indicate the existence of difference between linkage distances for a given pair of markers between those obtained from either of the two wide populations used in this study and that in the consensus map.

RESULTS

Of the 151 SSR markers analyzed in this study, 121 (80.1%) detected polymorphism between the two parents of Y8. All of the 121 polymorphic markers were used to detect the F_2 population. Of them, 114 (94.2%) showed the expected 1:2:1 segregation. The remaining seven markers (5.8%) showed distorted segregation; one (Satt407 on linkage group MLG D1a) with excessive heterozygous genotypes, two (Satt399 on MLG C1 and Satt274 on MLG D1b) with fewer than expected heterozygous genotypes, three (Satt162 and Satt623 on MLG I and Satt617 on MLG K) with excessive *G. soja* alleles and the remaining one (Satt549 on MLG N) with excessive alleles of the cultivated genotype (Figure 1). One hundred and nine (109) of the 121 polymorphic markers formed 24 linkage groups, each containing between two and eight markers. The distances between these markers varied between 0.7 and 35.7 cM. All but one of the SSR markers used in the current study mapped in the same linkage groups between the map of Y8 and the consensus map. The only exception was Satt102 which was mapped on MLG I in Y8 but on MLG K in the consensus map. It is of interest to note that the same discrepancy was also noted in a population reported by Winter et al. (2007).

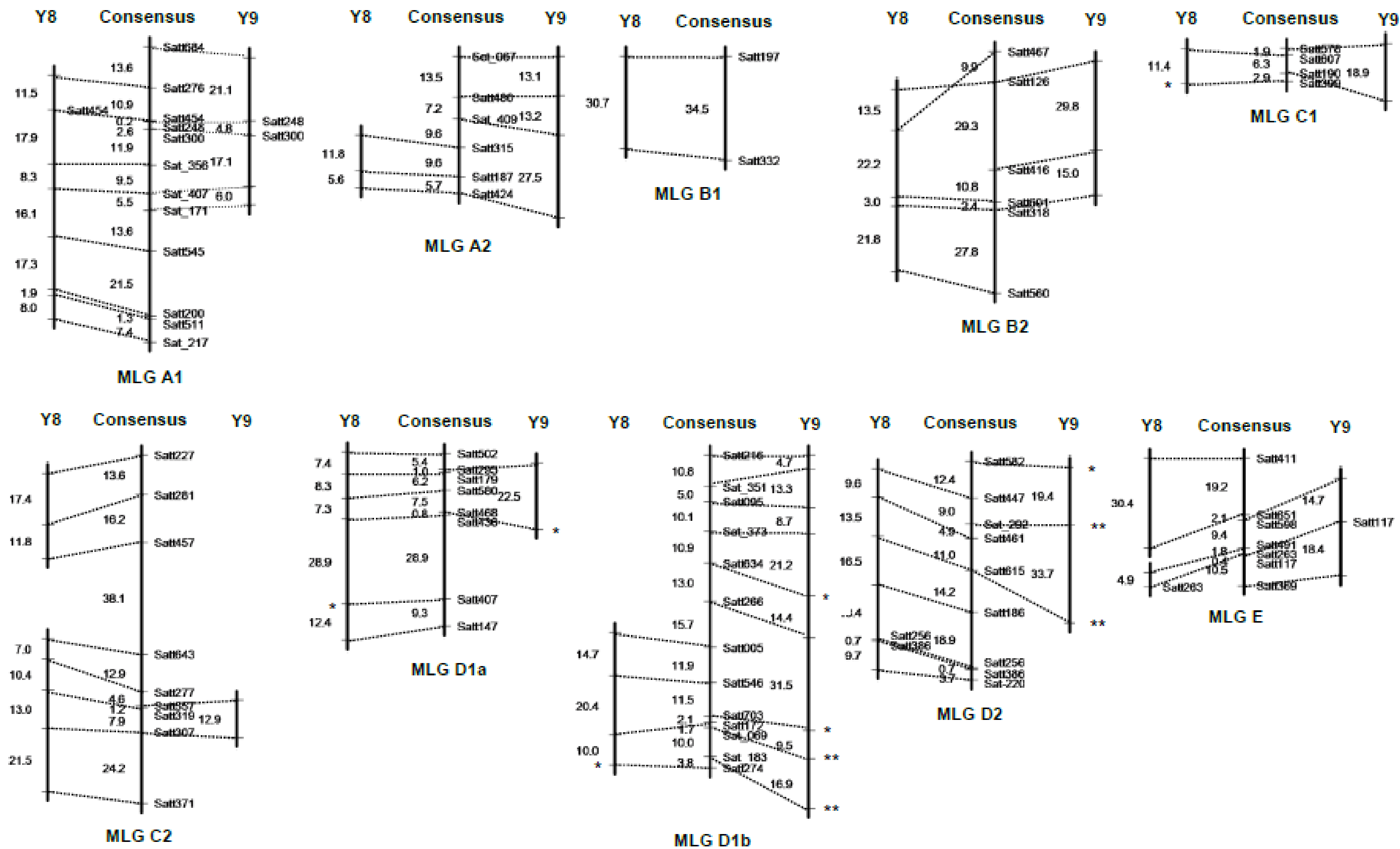

Figure 1. Linkage distances among the common intervals between the maps derived from the two wide crosses (Y8 and Y9) used this study and the consensus linkage map reported by Song et al. (2004). '*' or '**' indicates significant (p<0.05) or highly significant (p<0.01) segregation distortion of the locus concerned. Linkage groups were designated as those used in the consensus map.

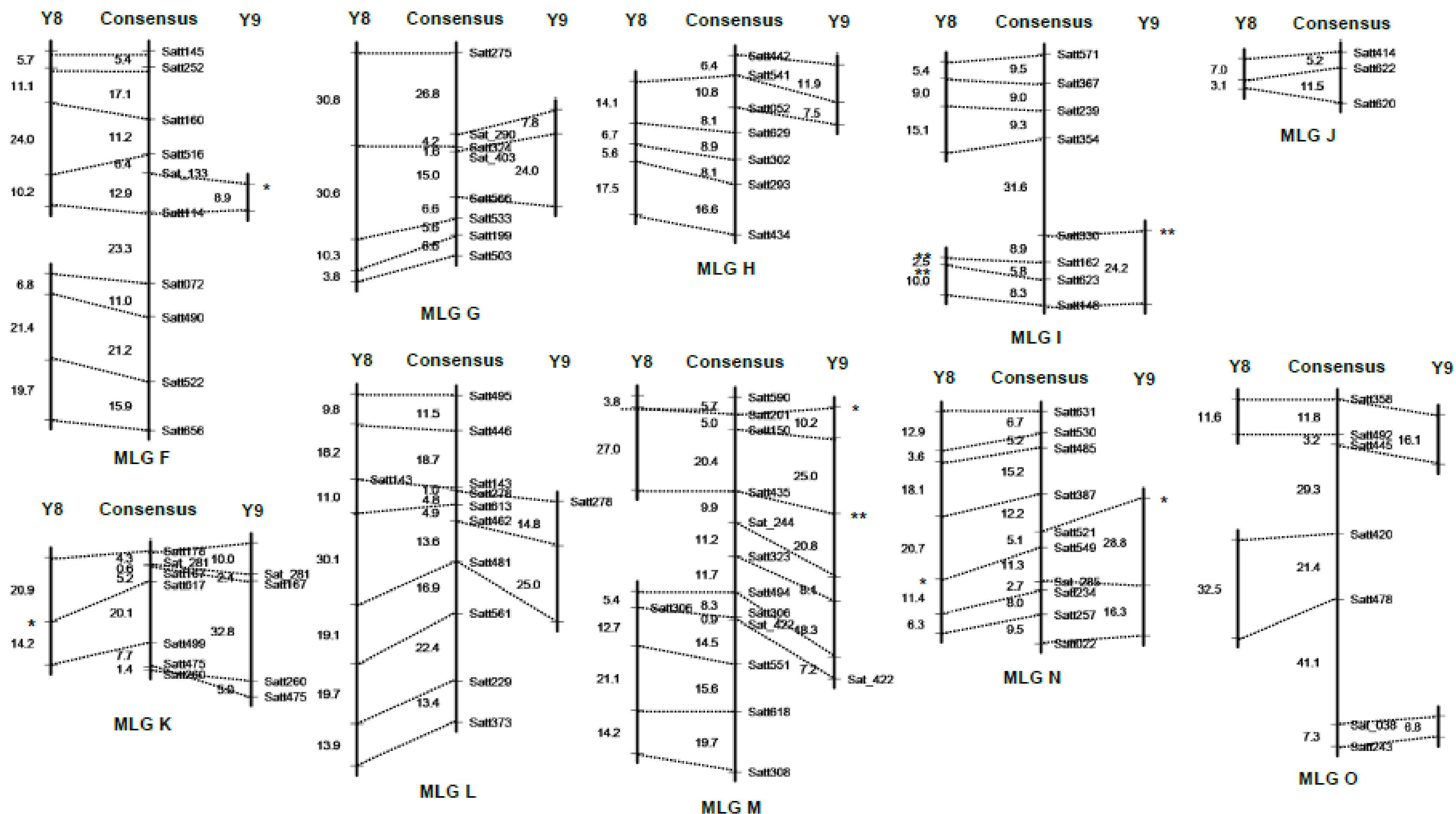

Figure 1. Contd

The 109 markers cover a total distance of 1123.8 cM which is not significantly different from the 1093.5 cM of the consensus map (p=0.66). The average distance between a pair of these markers was 13.9 cM. Linkage among the remaining 12 markers or between any of them with other markers was not detected. Eighty- two (82) shared marker intervals were found between the Y8 linkage map and the consensus map (Figure 1). Of these, 32 gave shorter distances (reduced by 10% or larger), 35 gave longer distances (increased by 10% or larger) and the differences

for the remainder 15 intervals between those in the Y8 and those in the consensus maps were less than 10%.

For Y9, 102 (67.5%) of the 151 SSR markers were detected to be polymorphism between its two parents. Seventy-two (72) of the 102 polymorphic markers were used to screen this population. Of these, 57 (79.2%) showed the expected 1:2:1 segregation ratios. Segregation ratios for the other 15 markers (20.8%) were significantly or highly significantly distorted (Figure 1). Sixty-five (65) of the 72 polymorphic markers formed 19 linkage groups, each containing between two and nine markers. The linkage distances between these markers varied between 2.4 and 33.7 cM. The 65 markers covered a total distance of 740.2 cM which was again not significantly longer than the 599.4 cM covered by the same markers in the consensus map (p=0.08). The average distance between these markers in the linkage map of Y9 was 16.1 cM. Compared with the linkage groups formed by these markers in Y9 and those in the consensus map, 46 common intervals were found (Figure 1). Of these, ten gave shorter distances in the map constructed in this study, 29 longer, and the map distances for the remaining seven intervals were similar between these two linkage maps.

DISCUSSION

Previous studies showed that linkage distance between two markers can be affected by many factors including parental genotypes as well as environments (Liu et al., 1994; Busso et al., 1995; Lashermes et al., 2001; Lenormand and Dutheil, 2005). Thus, the absence of significant differences described above may only apply to the two wild accessions used in this study. However, this does not seem to be the case. The total linkage distance obtained from a similar cross (Cregan et al., 1999) was also not any shorter than that derived from a cultivated by cultivated population (Song et al., 2004). Thus, it is not unreasonable to speculate that compared with those of between cultigens, a general reduction in recombination frequencies between cultivated soybean and *G. soja* may not exist. Transferring genes from most of the chromosomal regions of the *G. soja* genome into cultivated genotypes is thus no more difficult than transferring genes between two cultivated genotypes. The lack of recombination barriers between cultivated and the wild soybean taxon seems to be consistent with the reports that the domestication-related traits are often controlled by major loci with a few genotype-dependent minor loci (Paterson et al., 1995; Liu et al., 2007; Weeden, 2007). Nonetheless, many traits of agronomic importance such as seed size are apparently controlled by multiple genes located at different genome regions (Liu et al., 2007). Thus, to eliminate those chromatins conferring non-desirable characteristics, backcrossing would still be essential in exploiting the genetic variations from this wild taxon.

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