Mapping One of the 2 Genes Controlling Lemon Ray Flower Color in Sunflower (Helianthus annuus L.)

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In an F2 population of 120 plants derived from a cross between 2 breeding lines with yellow ray flowers, we observed 111 plants with yellow-colored and 9 plants with lemon-colored ray flowers. The segregation pattern fits a 15:1 \((\chi^2_{15:1} = 0.32, P > 0.5)\) ratio, suggesting that the lemon ray flower color is conditioned by 2 independent recessive genes that had been contributed individually by each of the parents. We sampled 111 plants from the 3 F2:3 families displaying a 3 to 1 segregating ratio for genotyping with molecular markers. One of the genes, \(Yf_1\), was mapped onto linkage group 11 of the public sunflower map. A targeted region amplified polymorphism marker \((B26P17trap13-68)\) had a genetic distance of 1.5 cM to \(Yf_1\), and one simple sequence repeat marker \((ORS733)\) and one expressed sequence tag (EST)-based marker \((HT167)\) previously mapped to linkage group 11 were linked to \(Yf_1\) with distances of 9.9 and 2.3 cM, respectively.

Sunflower \((Helianthus annuus L.)\) as a cultivated crop is grown for harvesting seeds to extract the edible oil (oilseed type) or for direct consumption as snacks (confection type). In addition, sunflower is also grown as an ornamental. The ray flower color is usually yellow for both oilseed and confection types. In ornamental sunflowers, the showy flower displays a wide spectrum of color variations, from purple, red brown, orange, yellow, pale yellow to near white. Fick (1976) suggested that the ray flower color of red, orange, yellow, and lemon (light yellow) are qualitative traits controlled by 1 or 2 genes.

The sunflower genome is thought to be of allopolyploid origin (Heiser and Smith 1955), and its highly duplicated nature has been revealed by isozyme and DNA-based marker studies. Furthermore, most of the studied isozymes of sunflower had 2 or more loci (Rieseberg and Seiler 1990). Three independent studies of restriction fragment length polymorphism (RFLP) mapping (Berry et al. 1995; Gentzbittel et al. 1995; Jan et al. 1998) revealed that about 35% of the cDNA probes showed dominant segregation and about 30% of the probes detected duplicated loci throughout the genome. Tang et al. (2002) reported that 40% of the simple sequence repeat (SSR) primers amplified multiple loci. The current report suggests that the ray flower color might be also controlled by duplicated genes or different genes with same function in sunflower.

In this brief communication, we report the genetic mapping of one of the 2 genes governing the lemon ray flowering color in an F2 population derived from a cross between 2 breeding lines with yellow ray flowers.

Materials and Methods

The cross was made in March 2006. The female was HA 89 (PI 599773), a public oilseed sunflower inbred maintainer line released by US Department of Agriculture–Agricultural Research Service, and the male was a confection sunflower line designated as North China confection line (NCCL), that was introduced from China to the Northern Crop Sciences Laboratory. The F1 plants were grown in the greenhouse. One hundred twenty F2 plants were planted in the greenhouse in the winter of 2006. The selfed seeds from 92 F2 plants were planted as F2:3 family rows in the field in Fargo, ND, in 2007. Between 25 and 40 plants per family were observed for flowering color. Leaf tissues were sampled from the parents and plants in 3 families displaying a 3:1 segregation of ray flower colors for DNA marker genotyping.

The DNA samples were prepared with the Qiagen DNeasy 96 Plant Kit (Qiagen, Valencia, CA), following the manufacturer’s instructions. Target region amplification polymorphism (TRAP) and the SSR marker techniques were employed (Tang et al. 2002; Hu and Vick 2003) to map the genes controlling ray flower color. The bulked segregant analysis strategy (Michelmore et al. 1991) was adopted to rapidly establish the relationship between the phenotype
and the polymorphic markers. Sixteen fixed and 6 arbitrary primers were used to screen for polymorphism between these bulks. In addition, both SSR (Yu et al. 2003) and EST-derived markers (Lai et al. 2005) mapped on the public sunflower linkage map were used to anchor the mapped gene onto a specific linkage group. The computer program MapMaker/EXP 3.0 (Lander et al. 1987) was used for genetic map construction, and the Kosambi’s mapping function (Kosambi 1944) was used for converting recombination frequencies to genetic distances between linked markers.

Results and Discussion

Both parental lines and their F 1 hybrid had yellow ray flowers. However, in the F 2 population, 111 plants had yellow-colored and 9 had lemon-colored ray flowers. The segregation fits a 15:1 segregation ratio \( \chi^2_{(15:1)} = 0.32, P > 0.5 \) suggesting that the lemon ray flower color was controlled by 2 recessive genes that had been contributed individually by each of the parents. A successive progeny test of 92 F 2:3 families in the field confirmed the digenic inheritance of the ray flower color in the population (Figure 1). All 9 F 2 plants with lemon ray flower color produced progeny with lemon-colored ray flowers. Among the 83 F 2:3 families descended from F 2 plants with yellow ray flower, 64 produced all yellow ray flowers. The excess of these apparent non-segregating families (only about 39 families were expected to be nonsegregating) could have been due to the small number of plants per family and the low frequency of the lemon color phenotype. Among the 19 segregating families, 8 fit the 15:1 ratio (heterozygous at both loci), 5 fit the 3:1 ratio (heterozygous at one of the 2 loci), and 6 fit both ratios when a chi-square test was performed for the number of plants with yellow and lemon ray flowers.

Fick (1976) observed a 9 yellow to 3 orange to 4 lemon segregation ratio in 2 crosses between yellow and lemon parental lines, from which he concluded that 2 genes controlled the ray flower color, and the recessive gene for lemon was assumed to be epistatic to the other gene. He also reported a 3 orange to 1 lemon segregating ratio in another cross. The difference between orange and yellow ray flower is subtle. If these 2 classes could be combined, the segregation in Fick’s 3 crosses would suggest monogenic inheritance. There was no orange ray flower observed in our study, and the segregation ratio was different. The presence of modifier genes and/or significant genotype × environment effects may account for Fick’s discrimination of an orange color class.

We propose the gene symbol \( Yf \) for the yellow ray flower and postulate that the genes that led to yellow ray flowers are duplicated in the sunflower genome and each parental line carries a different functional gene. Thus, the genotypes are \( Yf1Yf2yf2yf2 \), \( yf1yf1Yf2Yf2 \), and \( Yf1yf1Yf2yf2 \) for the female parent, male parent, and the F 1 hybrid, respectively. All these genotypes will produce yellow ray flowers. The lemon ray flower individuals possessed the double recessive genotype \( yf1yf1yf2yf2 \) and produced non-segregating F 2:3 families with lemon ray flowers.

Leaf tissues of individual plants in the F 2:3 families displaying 3:1 segregation ratios were sampled to map the yellow flower genes because we believed that the parental F 2 plants of these families must have either \( Yf1yf1yf2yf2 \) or \( yf1yf1Yf2yf2 \) genotypes. DNA from 8 plants with yellow ray flower color and 8 plants with lemon ray flower color were bulked separately for each of the 3 F 2:3 families that had population sizes sufficient for assembly of respective bulks. Sixteen fixed and 6 labeled TRAP primers were used to analyze polymorphism among the bulks. The TRAP primer combinations that produced polymorphic products in each of the 3 sets of bulks were then tested.
with the DNA from 4 individual plants with either yellow or lemon ray flower color in each F2.3 family.

Two TRAP markers, B26P17Trap13-68 (fixed primer sequence: 5'-GGTTCCGTACATCGTGA-3' and arbitrary primer sequence: 5'-GGCGGTGTATAATATC-3') and L15C24Trap13-1040 (fixed primer sequence: 5'-GAGAATGCCTCATTGCTGGA-3' and arbitrary primer sequence: 5'-GGCGGTGTATAATATC-3') were found to be associated with the ray flower color in each of the 3 families. These 2 markers segregated in the expected 3:1 ratio ($\chi^2_{(3:1)} = 0.47$, $P > 0.49$ for B26P17Trap13-68 and $\chi^2_{(3:1)} = 0.11$, $P > 0.74$ for L15C24Trap13-1040). However, the chi-square values for independent assortment between these 2 markers and the ray flower color phenotype are extremely high, 174.84 and 26.05, respectively, indicating a strong linkage between the markers and the phenotype. These 2 TRAP markers have been placed on linkage group 11 in another map constructed from our laboratory. Therefore, we deduced that the ray flower color segregation in these 3 families resulted from the 2 alleles at the same locus. Thus, we treated the 3 families as a segregating population for genetic mapping. In total, these 3 families comprised 111 plants and segregated into 79 plants with yellow and 32 plants with lemon ray colored flowers ($\chi^2_{(3:1)} = 0.87$, $P > 0.3$). We assumed that the parental F2 plants of these families have the Yf1Yf1yf2yf2 genotype and used them to map the yf2 locus.

In order to confirm the map position of yf2, we screened 10 additional SSR markers and 1 EST-derived marker from linkage group 11. One SSR marker (ORS733) and one EST-derived marker (HT167, forward primer sequence: 5'-GGTGTTCCATATGCGATTGACG-3' and reverse primer sequence: 5'-TGGCACATTGCTTTACAAA-3'), together with the 2 TRAP markers, were mapped to the Yf1 locus-harboring region (Figure 2). The total genetic distance covered by those markers was 18.0 cm, and the TRAP marker B26P17Trap13-68 was closely linked to Yf2 with a distance of only 1.5 cm. Because all the plants with lemon ray flower shared the same genotype as the parent HA 89 for all 4 markers near the Yf1 locus, we deduced that HA 89 possesses the Yf1Yf1Yf2Yf2 genotype and NCCL has the yf1yf1yf2yf2 genotype. Genetic mapping of the Yf2 locus from NCCL can be achieved by using a backcross population from a cross between HA 89 and an advanced lemon ray flower line.

The yellow color of the sunflower ray flower results from the presence of carotenoids in the chromoplasts. Carotenoids are universally distributed in the plant kingdom. They dissipate excess light energy absorbed by the antenna pigments (Frank and Cogdell 1996), harvest light for photosynthesis (Yamamoto and Bassi 1996), and serve as precursors for biosynthesis of abscisic acid (Walton and Li 1995). Carotenoids are also indispensable in human and animal diets because they are the precursors for vitamin A (Fraser and Bramley 2004), they function as antioxidants to protect living cells (Krinsky 1989), and they may possess anticarcinogenic properties (Mayne 1996). Genes coding almost every enzyme required for biosynthesis of carotenoids have been identified (Cunningham and Gantt 1998). The plant materials reported here will facilitate the understanding of genetic factors controlling carotenoid biosynthesis in sunflower with an ultimate goal of enhancing the nutritional value of sunflower products for human and animal health.

We did not observe any detrimental effects of the lemon ray flower color on plant growth and development. Thus, this trait could be introduced into sunflower breeding lines as a visual marker for seed production management. Currently, commercial sunflower is nearly 100% hybrids produced with the cytoplasmic male sterility system. In rice (Oryza sativa L.), several indicator traits have been incorporated into male-sterile lines to facilitate hybrid seed production management (Cao et al. 1999). The lemon ray flower color could serve as an indicator trait for seed purity as well as for cultivar identification in sunflower hybrid seed production management.

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