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

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In an *F*₂ 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 ratio \( (\chi_2^{2}(15:1) = 0.32, P > 0.5) \), 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 *F*₂:₃ families displaying a 3 to 1 segregating ratio for genotyping with molecular markers. One of the genes, *Yf₁*, 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 *Yf₁*, 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 *Yf₁* 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 *F*₂ 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 *F*₁ plants were grown in the greenhouse. One hundred twenty *F*₂ plants were planted in the greenhouse in the winter of 2006. The selfed seeds from 92 *F*₂ plants were planted as *F*₂:₃ 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 trait. The bulked segregant analysis strategy involves pooling DNA samples from plants with the desired phenotype and genotyping these pools with polymorphic markers. The method allows for the rapid identification of markers linked to the trait of interest.
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 F1 hybrid had yellow ray
flowers. However, in the F2 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 con-
trolled by 2 recessive genes that had been contributed
individually by each of the parents. A successive progeny test
of 92 F2,3 families in the field confirmed the digenic inheri-
tance of the ray flower color in the population (Figure 1). All
9 F2 plants with lemon ray flower color produced progeny with
lemon-colored ray flowers. Among the 83 F2,3 families
descended from F2 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 \times
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
\( Yf_1Yf_2yf_2yf_2 \),
\( yf_1yf_1Yf_2Yf_2 \), and
\( Yf_1yf_1Yf_2yf_2 \) for the female parent, male parent,
and the F1 hybrid, respectively. All these genotypes will produce
yellow ray flowers. The lemon ray flower individuals possessed
the double recessive genotype, \( yf_1yf_1yf_2yf_2 \), and produced non-
segregating F2,3 families with lemon ray flowers.

Leaf tissues of individual plants in the F2,3 families
displaying 3:1 segregation ratios were sampled to map the
yellow flower genes because we believed that the parental
F2 plants of these families must have either \( Yf_1Yf_2yf_2yf_2 \) or
\( yf_1yf_1Yf_2yf_2 \) 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 F2,3 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

Figure 1. Segregation of the ray flower color in the field; a family exhibiting uniformity for lemon ray flower color is shown
on the right; and families segregating for yellow ray flower color and lemon ray flower color are shown on the left.
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' -GTTTTCCGTCATACTCGTTA-3' and arbitrary primer sequence: 5' -GGCGGATGATAAATTATC-3') and L15C24Trap13-1040 (fixed primer sequence: 5' -GGAA-TGTCACTTGATTTTGCT-3' and arbitrary primer sequence: 5' -GGCGGATGATAAATTATC-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 $Yf1y1yf2y2$ genotype and used them to map the $yf1$ locus.

In order to confirm the map position of $yf1$, 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' -GGGTTCCTATGTGCATTCAG-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 $Yf1$ with a distance of only 1.5 cM. Because all the plants with lemon ray flower shared the same genotypes near the $Yf2$ locus, we deduced that HA 89 possesses the $yf1yf1yf2y2$ genotype and NCCL has the $Yf1Yf1yf2y2$ 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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