

INHERITANCE OF DISC FLOWER COLOR IN *Helianthus petiolaris*

Gutierrez, A.^{*1}, Delucchi, C.² and Poverene, M.¹

¹ Departamento de Agronomía, Universidad Nacional del Sur and
CERZOS-CONICET, 8000 Bahía Blanca, Argentina

² INTA Estación Experimental Pergamino, ruta 32 km 4,5 B2700WAA Pergamino

Received: June 16, 2008

Accepted: June 02, 2009

SUMMARY

Disc flowers of *Helianthus petiolaris* (Asteraceae) are usually purple red due to anthocyanin pigments in corolla lobes, style and stigma. However, yellow-flowered plants are occasionally found. The inheritance of this trait was studied through crosses between different phenotypes and the results indicated that flower color is controlled by two genes with complementary action, both loci being dominant and independently inherited. This agrees with results obtained in cultivated sunflower, *H. annuus*, where one to three dominant genes have been proposed for anthocyanin expression in different organs.

Key words: anthocyanin, complementary genes, wild sunflower

INTRODUCTION

Helianthus petiolaris Nutt. (Asteraceae), known as the prairie sunflower, is an annual, diploid ($x=17$), and highly self-incompatible species (Heiser *et al.*, 1969). Native to North America, it was probably introduced in Argentina as contaminant in forage seed lots and was first described in the field by Covas (1966). At present, it has established itself in the central part of the country and is widespread on sandy soils.

H. petiolaris is well-known as source of male sterility and fertility restoring genes, which has allowed a massive production of sunflower hybrid seed (Leclercq, 1969; but see Rieseberg *et al.*, 1994). It is also a major source of genes for Verticillium wilt (*Verticillium dahliae*), downy mildew (*Plasmopara halstedii*) and rust (*Puccinia helianthii*) resistance, tolerance to sunflower moth (*Homoeosoma electellum*) and genetic improvement of cultivated sunflower (Jan and Seiler, 2007). Phenotypic genetic markers are useful for marker-assisted selection in the field and despite intense research, only a few have been identified in sunflower and its rela-

* Corresponding author: Fax 0054 291 4595127; e-mail: aguti@criba.edu.ar

tives (Gentzbittel *et al.*, 1999; Gedil *et al.*, 2001 and Carrera *et al.*, 2002). Most studies in this species refer to hybridization with *H. annuus* (Heiser, 1947; Rieseberg *et al.*, 1995; Rieseberg *et al.*, 1999; Buerkle and Rieseberg, 2001 and Poverene *et al.*, 2004) but scanty information are available about specific traits inheritance.

Studies on disc flower color inheritance have been carried out in the cultivated sunflower, *H. annuus*. The presence of anthocyanin in different organs is expressed as a single dominant trait (Leclercq, 1968). A gene with pleiotropic effects seems to be responsible for color in corolla lobes, stigma, stems and petioles (Fick, 1976) whereas two or three independently inherited genes with complementary effect have been proposed for the stigma color (Luczkiewicz, 1975 and Joshi *et al.*, 1994). Complementation is the production of the wild type phenotype when two parents contribute haploid genomes bearing different recessive mutations. The purple color in stems, petioles and bracts tips is dominant to the green color and has been assigned to two complementary genes (Deveraja and Shanker, 2005).

Among annual *Helianthus* species, disc flowers present different colors: purple in *H. agrestis*, *H. debilis* sp. *silvestris*, *H. exillis* and *H. praecox*; reddish - purple in *H. bolanderi*, *H. niveus* sp. *tephrodes* and *H. niveus* sp. *niveus*; deep purple in *H. paradoxus* and *H. argophyllus*, although this latter can also present light reddish to purple flowers (Rogers *et al.*, 1982). Disc flowers in *H. petiolaris* are usually purple red due to the presence of anthocyanins in corolla lobes, style and stigma. However, yellow flowers without anthocyanins are occasionally found in natural populations (Heiser, 1961; Rogers *et al.*, 1982) although the genetic control of this trait is still unknown.

In many other plant species, flower color depends on dominant genes for pigment synthesis showing epistatic interaction (Sreevalli *et al.*, 2002; Pahlavani *et al.*, 2004) and complementary action (Wilson and Hudson, 1978 and Imrie and Hutton, 1978). In cultivated sunflower a single dominant gene has been found (Fick, 1976 and Mosjidis, 1982) although there are variations in color shade and tint.

The goal of this work was to study disc flower color inheritance in *H. petiolaris*. We proposed that red color is controlled by dominant alleles responsible for anthocyanins in two independently inherited loci, while recessive alleles produce yellow flowers, without anthocyanins. Thus, several crosses between these phenotypes were made to determine how the trait is inherited.

MATERIALS AND METHODS

In this study, plants with yellow-disc flowers were collected in a wild population established in a vacant field lot near Highway 1, km 256, in La Pampa province. The seed was sown in the experiment field of the Agronomy Department in Bahía Blanca, where this species does not grow naturally. In 2004, when the seeds from open pollinated plants with yellow flowers were harvested, a plot was established in

the same field. Immature heads were covered with bags to make crosses among different flower colored plants: red \times red, yellow \times yellow, red \times yellow and yellow \times red. Consequently, ten crosses of each type were made and the following year the progenies obtained in this way were classified according to disc flower color. In order to verify self pollination, a number of heads were bagged.

In 2005, plants from the red \times yellow and yellow \times red crosses were pollinated with yellow-colored plants, as a test-cross. Each year, seedlings were raised in the greenhouse and about one month later they were transplanted to the experiment field. The immature heads were covered with polyamide bags before the ligules were visible, to make the crosses. Also, the bracts (phyllaries) and ligules were trimmed to facilitate pollination. When the stigmas appeared receptive, pollen of the chosen parent was applied with a thin brush for 3-4 consecutive days, maintaining the heads covered until seed maturity.

Chi-square and G tests were used for testing the goodness-of-fit of the observed and expected frequencies of phenotypic classes.

RESULTS AND DISCUSSION

Four viable seeds were obtained among 942 from five self-pollinated heads which reached maturity, with a mean of 187.6 aborted seeds per head and less than 1 viable seed per head. Thus, it was considered that seed from selfing would not significantly bias the expected phenotypic ratio in crosses.

In the proposed inheritance model, the red pigment of disc flowers would be controlled by dominant alleles in two loci, so the red flowers would have any of the following genotypes: $A_1A_1A_2A_2$, $A_1A_1A_2a_2$, $A_1A_1a_2a_2$, $A_1a_1A_2A_2$, $A_1a_1A_2a_2$, $A_1a_1a_2a_2$, $a_1a_1A_2A_2$, and $a_1a_1A_2a_2$ while the yellow flowers would have only one genotype, $a_1a_1a_2a_2$. According to this hypothesis, all the plants in the 2004 plot that had been obtained from open pollinated yellow plants had at least two recessive alleles, a_1 and a_2 , being the possible genotypes $A_1a_1A_2a_2$, $A_1a_1a_2a_2$, $a_1a_1A_2a_2$, and $a_1a_1a_2a_2$ in equivalent ratios. It was estimated that all the genotypes corresponding to the red flower phenotype occurred in similar frequencies, thus assuming that natural selection would not favor any particular genotype.

Red \times red crosses should involve all the possible combinations of the genotypes mentioned above, except for the last one. Consequently, considering an independent segregation of the two loci, $A_1a_1A_2a_2 \times A_1a_1A_2a_2$, $2(A_1a_1A_2a_2 \times A_1a_1a_2a_2)$, $2(A_1a_1A_2a_2 \times a_1a_1A_2a_2)$, $A_1a_1a_2a_2 \times A_1a_1a_2a_2$, $2(A_1a_1a_2a_2 \times a_1a_1A_2a_2)$, and $a_1a_1A_2a_2 \times a_1a_1A_2a_2$ would produce a 55 red : 9 yellow ratio. In the same way, red \times yellow and reciprocal crosses involving $A_1a_1A_2a_2$, $A_1a_1a_2a_2$, and $a_1a_1A_2a_2$ genotypes for red flowers would produce a 5 red : 3 yellow ratio.

The frequencies of phenotypes observed in the 2004 crosses red \times red, yellow \times yellow, and red \times yellow fit the expected ratios, whereas progeny of yellow \times red crosses showed a deviation (Table 1).

The departure from the frequency expected in the 2004 yellow \times red crosses results could be ascribed to a failure in seed set caused by environmental factors, as crosses were made in the field, not in the greenhouse. Besides, a failure in manual pollination cannot be ruled out. Although *H. petiolaris* is highly incompatible, self-pollination has been observed under mixed loads of heterospecific pollen (Desrochers and Rieseberg, 1998). However, this situation may have taken place due to the presence of *H. annuus* plants in the experiment field.

Table 1: Observed and expected progenies for red (R) and yellow (Y) disc flowers in several crosses of *Helianthus petiolaris*

Cross	Observed frequency		Expected frequency R:Y	χ^2	P	G
	Red	Yellow				
2004						
R \times R	133	22	55 : 9	0.0013	>0.95	-0.14 NS
R \times Y	70	36	5:3	0.5659	0.50-0.30	1.78 NS
Y \times R	51	53	5:3	8.0409	<0.01	7.4
Y \times Y	0	120	all yellow	0.0000	1	-
2005						
(R \times Y) \times Y	12	9	11:9	0.0389	0.90-0.70	-0.22 NS
(Y \times R) \times Y	15	8	11:9	0.9700	0.50-0.30	0.76 NS

To confirm the hypothesis, red heads of the red \times yellow and yellow \times red plots were pollinated by yellow-flowered plants. Then, in 2005, 70 progenies of each cross type were transplanted to the experiment field but most of them were unexpectedly destroyed by hares. However, progenies did fit the expected frequencies in between 21 and 23 surviving plants of each cross.

The progeny prediction was based on the expected proportions of 2004 red \times yellow and reciprocal crosses (5:3). Thus, as only red-flowered plants were test-crossed, genotypes would have been $1/5 A_1a_1A_2a_2$, $2/5 A_1a_1a_2a_2$ and $2/5 a_1a_1A_2a_2$. The expected results were 3/20 red : 1/20 yellow for the first genotype and 4/20 red : 4/20 yellow for each of the remaining two genotypes, which gave a final ratio of 11/20 red : 9/20 yellow plants. Therefore, in spite of the low number of plants that reached flowering time, the numbers observed were close to the expected ones.

There are two subspecies in *H. petiolaris*: *H. p. ssp. petiolaris*, which usually has purple anthers although they can also be red, and *H. p. ssp. fallax*, whose anthers are red but can also be purple (Rogers *et al.*, 1982). The phenotypic variation between purple and red might be due to different doses of alleles for anthocyanin synthesis, A1 and A2 being the active alleles. In this study, the crosses only involved *H. p. ssp. petiolaris*, as it is the one that seems to be present in Argentina (Poverene *et al.*, 2002). Consequently, the presence or absence of pigment was the only trait under consideration.

Table 2 summarizes different studies of anthocyanin inheritance in cultivated sunflower. Single dominant genes have been described as controlling pigmentation in vegetative and reproductive structures of cultivated sunflower yet modifier genes

were suggested in three cases. Two or three genes have also been found, with complementary and pleiotropic effects (Table 2).

Table 2: Inheritance of anthocyanin pigmentation in sunflower (*Helianthus annuus*)

Plant organ	Gene	Inheritance	Gene action	Reference
Stigma, corolla and achenes	Single dominant gene	Segregation in crosses 3:1		Leclercq 1968
Corolla, stigma and vegetative parts	Single dominant gene			Stoenescu 1974 cited by Miller and Fick 1997
Stems, petiole and leaf veins	Single dominant gene	Segregation in crosses 3:1	Modifier genes suggested	Luczkiewics 1975
Disc flowers	Single dominant gene	Segregation in crosses 3:1	Linkage or pleiotropic effects suggested	Fick 1976
Ligulated disc florets	Single dominant gene	Segregation in crosses 3:1	Modifier genes suggested	Mosjidis 1982
Stigmata	Three dominant genes	As expected for three unlinked genes	Cumulative action	Luczkiewics 1975
Stigma and style	Three dominant genes Sa_1, Sa_2, Sa_3		Complementary effect	Skaloud and Kovacik 1978
Stem, leaves and hypocotyl	Three dominant genes T_1, T_2, Ha_4		Id.	
Stigma, petiole and leaf	Single dominant gene Ptl_a and a second gene in each organ	Segregation in crosses 9:7	Pleiotropic gene	Joshi <i>et al.</i> , 1994
Stem, petiole and bract tip	Two dominant genes, Ptl_a and a second gene in each organ	Segregation in crosses 9:7	Complementary effect and pleiotropy	Deveraja and Shanker Goud 2005

However, none of the studied cases segregated as in the *H. petiolaris* crosses, which suggested that disc flower color is controlled by two independent loci showing duplicate gene action. This would produce a 15:1 rather than 9:7 ratio in an F_2 generation. As high self-incompatibility precludes obtaining such progenies in *H. petiolaris*, different crosses were attempted in order to study gene segregation. It has been concluded that flower color inheritance in both species follows different patterns. In wild *H. annuus* plants collected in Argentina there is a variable pigment distribution as well as a variable corolla lobe color intensity. This variation consists of different tints of red, but a purple color like *H. petiolaris* was never observed. This could be explained by the segregation of a unique allele pair responsible for anthocyanin synthesis, probably interacting with modifying genes. *H. annuus* and *H. petiolaris* are diploids but they differ by a minimum of 20 breakages and fusions (Chandler *et al.*, 1986; Rieseberg *et al.*, 1995 and Burke *et al.*, 2004). Chromosome reorganizations could be the origin of the anthocyanin gene duplication in *H. petiolaris*. Gene duplications naturally arise by crossing over and segregation following chromosome rearrangements, as inversions. As linkage maps based on molecular markers are now available in *H. petiolaris* (Burke *et al.*, 2004) it would

be worthwhile to assign the herein proposed color genes to their respective linkage groups, in order to verify if those groups have undergone structural rearrangements when compared with *H. annuus* chromosomes.

ACKNOWLEDGMENTS

Thanks are due to National Research Council of Argentina (CONICET) for a fellowship to the first author. This research was supported by grant ANPCYT-PICT 08-9881.

REFERENCES

- Buerkle, C.A. and Rieseberg, L.H., 2001. Low intraspecific variation for genomic isolation between hybridizing sunflower species. *Evolution* 55: 684-691.
- Burke, J.M., Lai, Z., Salmaso, M., Nakazato, T., Tang, S., Heesacker, A., Knapp, S.J. and Rieseberg, L.H., 2004. Comparative mapping and rapid karyotypic evolution in the genus *Helianthus*. *Genetics* 167: 449-457.
- Carrera, A., Pizarro, G., Poverene, M., Feingold, S., Berry, S. and Leon, A., 2002. Variability among inbred lines and RFLP mapping of sunflower isozymes. *Genetics and Molecular Biology* 25: 65-72.
- Chandler, J., Jan, C.C. and Beard, B.H., 1986. Chromosomal differentiation among the annual *Helianthus* species. *Systematic Botany* 11: 354-371.
- Covas, G., 1966. Antófitas nuevas para la flora pampeana. *Apuntes para la Flora de La Pampa (EEA INTA Anguil)* 22: 88.
- Desrochers, A.M. and Rieseberg, L.H., 1998. Mentor effects in wild species of *Helianthus* (Asteraceae). *American Journal of Botany* 85: 770-775.
- Deveraja, T.V. and Shanker, G., 2005. Pleiotropic genes and its influence on stem, petiole and bract tip pigmentation in sunflower (*Helianthus annuus* L.). *Helia* 28(43): 107-112.
- Fick, G.N., 1976. Genetics of floral color and morphology in sunflowers. *The Journal of Heredity* 67: 227-230.
- Gedil, M.A., Wye, C., Berry, S., Segers, B., Peleman, J., Jones, R., Leon, A., Slabaugh, M.B. and Knapp, S.J., 2001. An integrated restriction fragment length polymorphism-amplified fragment length polymorphism linkage map for cultivated sunflower. *Genome* 44: 213-221.
- Gentzbittel, L., Mestries, E., Mouzeyar, S., Mazeyrat, F., Badaoui, S., Vear, F., Tourvieille de Labrouhe, D. and Nicolas, P., 1999. A composite map of expressed sequences and phenotypic traits of the sunflower (*Helianthus annuus* L.) genome. *Theoretical and Applied Genetics* 99: 218-234.
- Heiser, C.B., 1947. Hybridization between the sunflower species *Helianthus annuus* and *H. petiolaris*. *Evolution* 1: 249-262.
- Heiser, C.B., 1961. Morphological and cytological variation in *Helianthus petiolaris* with notes on related species. *Evolution* 15: 247-258.
- Heiser, C.B., Smith, D.M., Clevenger, S.B. and Martin, W.C., 1969. The North American Sunflowers (*Helianthus*). *Memoirs of the Torrey Botanical Club* 22: 1-37.
- Imrie, B.C. and Hutton, E.M., 1978. The inheritance of flower color in *Macroptilium atropurpureum*. *The Journal of Heredity* 69: 54-56.
- Jan, C.C. and Seiler, G.J., 2007. Sunflower. In: Singh R.J. (Eds). *Genetic Resources, Chromosome Engineering, and Crop Improvement. Oilseed Crops*. CRC Press, Boca Raton, pp. 103-165.
- Joshi, S.S., Basavalingappa, S. and Giriraj, K., 1994. Pleiotropy in sunflower (*Helianthus annuus* L.). *Helia* 17(20): 1-6.
- Leclercq, P., 1968. Héredité de quelques caractères qualitatifs chez le tournesol. *Annales d'Améliorement des Plantes* 18: 307-315.
- Leclercq, P., 1969. Une stérilité cytoplasmique chez le tournesol. *Annales d'Améliorement des Plantes* 19: 99-106.

- Luczkiewicz, T., 1975. Inheritance of some characters and properties in sunflower (*Helianthus annuus* L.). *Genetica Polonica* 16: 167-184.
- Miller, J.F. and Fick, G.N., 1997. The genetics of sunflower. In: Schneiter A.A. (eds) *Sunflower Technology and Production*, Madison, Wisconsin: American Society of Agronomy, Inc. pp. 441-495.
- Mosjidis, J.A., 1982. Inheritance of color in the pericarp and corolla of the disc florets in sunflower. *The Journal of Heredity* 73: 461-464.
- Pahlavani, M.H., Mirlohi, A.F. and Saeidi, G., 2004. Inheritance of flower color and spininess in safflower (*Carthamus tinctorius* L.). *The Journal of Heredity* 95: 265-267.
- Poverene, M.M., Cantamutto, M.A., Carrera, A.D., Ureta, M.S., Salaberry, M.T., Echeverria, M.M. and Rodriguez, R.H., 2002. El girasol silvestre (*Helianthus* spp.) en la Argentina: Caracterización para la liberación de cultivos transgénicos. *Revista de Investigaciones Agropecuarias RIA* 31: 97-116.
- Poverene, M., Carrera, A., Ureta, S. and Cantamutto, M., 2004. Wild *Helianthus* species and wild-sunflower hybridization in Argentina. *Helia* 27(40): 133-142.
- Rieseberg, L.H., Van Fossen, C., Arias, D. and Carter, R.L., 1994. Cytoplasmic male sterility in sunflower: Origin, inheritance, and frequency in natural populations. *The Journal of Heredity* 85: 233-238.
- Rieseberg, L.H., Van Fossen, C. and Desrochers, A.M., 1995. Hybrid speciation accompanied by genomic reorganization in wild sunflowers. *Nature* 375: 313-316.
- Rieseberg, L.H., Kim, M.J. and Seiler, G.J., 1999. Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *International Journal of Plant Science* 160: 102-108.
- Rogers, C.E., Thompson, T.E. and Seiler, G.J., 1982. *Sunflower Species of the United States*. National Sunflower Association, Fargo, ND pp. 75.
- Skaloud, V. and Kovacik, A., 1978. Survey on inheritance of sunflower characters which are conditioned by a small number of genes. In: *Proc. 8th Int. Sunflower Conf.*, Minneapolis, MN, International Sunflower Association, Paris, France. pp. 490-496.
- Sreevalli, Y., Kulkarni, R.N. and Baskaran, K., 2002. Inheritance of flower color in periwinkle: orange-red corolla and white eye. *The Journal of Heredity* 93: 55-58.
- Wilson, V.E. and Hudson, L.W., 1978. Inheritance of lentil flower color. *The Journal of Heredity* 69: 129-130.

HERENCIA DEL COLOR DE LAS FLORES DEL DISCO EN *Helianthus petiolaris*

RESUMEN

En *Helianthus petiolaris* (Asteraceae) las flores del disco son generalmente rojo púrpura debido a los pigmentos de antocianina en los lóbulos de la corola, estilo y estigma. Sin embargo, de vez en cuando se encuentran plantas con flores amarillas. La herencia de este rasgo fue estudiada a través de cruces entre diversos fenotipos y los resultados indicaron que el color de la flor es controlado por dos genes con acción complementaria, ambos loci son dominantes y de herencia independiente. Esto está de acuerdo con resultados obtenidos en girasol cultivado, *H. annuus* donde de uno a tres genes dominantes se han propuesto para la expresión de la antocianina en diversos órganos.

HÉRÉDITÉ DE LA COULEUR DES FLEURS DU DISQUE DANS *Helianthus petiolaris*

RÉSUMÉ

Dans *Helianthus petiolaris* (Asteraceae) des fleurs du disque sont en général de couleur rouge violacé à cause des pigments anthocyaniques dans les

lobes de la corolle, le style et le stigmate. Cependant, on peut trouver occasionnellement des plantes avec des fleurs jaunes. L'hérédité de ce caractère a été étudiée à travers de croisements entre différents phénotypes et les résultats ont indiqué que la couleur des fleurs est contrôlée par deux gènes avec une action complémentaire. Ces résultats sont en accord avec ceux obtenus dans le tournesol cultivé, *H. annuus* pour lequel de un à trois gènes ont été proposés pour l'expression des anthocyanes dans des organes différents.