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A New Source of Yellow Coloration of the Sunflower Plant Top and Its Importance in Breeding

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Abstract: Inbred line Mh174B with the yellow coloration of the sunflower plant top was produced by hybridization of annual wild species with cultivated sunflower. The aim research was to evaluate genetic control of yellow coloration of the sunflower plant top in combination with other morphological traits (branchiness, pollen fertility restoration and ray flower coloration). A total of four F_2 hybrid combinations of were created: Cx1010A × Mh174B, Cx1012A × Mh174B, Cx2111A × Mh174B, Mx845A × Mh174B. Yellow coloration of the sunflower plant top was shown to follow a monogenic mode of inheritance and, presumably, be controlled by the gene *y*. This trait is inherited independently from such traits as branchiness, pollen fertility restoration and ray flower coloration. The mainstreams of use of yellow coloration of the sunflower plant top in sunflower breeding and seed production are discussed.

Keywords: *Helianthus annuus* L., yellow coloration of sunflower plant, inheritance

Introduction

For the last decade great successes have been achieved in cultivated sunflower (*Helianthus annuus* L.) genetics through molecular technologies: from generating genetic maps based on different DNA-markers, creating organ-specific gene library to the sunflower genome sequences (Badouin *et al*., 2017; Fernández *et al*., 2003; Fusari *et al*., 2011; Gentzibittel *et al*., 1995; Lai *et al*., 2005; Tang *et al*., 2002). Large-scale investigations are also conducted in mapping genes encoding morphological and biochemical traits (Dimitrijević and Horn, 2018).

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Among different types of marker systems used to solve a genetic/breeding task, in sunflower the advantage unquestionably remains with DNA-markers. However, at certain stages of breeding work under field conditions removal of off-type plants should be carried out within short periods of time. In this case morphological marker traits with a simple mode of inheritance, which can be easily identified at a given stage of the plant development in the field, become irreplaceable. They include morphological traits of leaves, stems, inflorescence etc. Genetics of the above sunflower traits is sufficiently fully studied and presented in reports (Gavrilova and Anisimova, 2003; Popov and Kirichenko, 2010). The trait of yellow coloration of the sunflower plant top first described by Hockett and Knowles (1970), who demonstrated that this trait is controlled by one recessive gene *y*, also belongs to easily detected traits.

The aim of our research was to evaluate genetic control of yellow coloration of the sunflower plant top in combination with other marker morphological traits.

Materials and methods

As a result of a research program on sunflower distant hybridization performed at the Plant Production Institute nd. a V.Ya. Yuryev of NAAS hybrid combinations between different annual wild species and cultivated sunflower were created (Popov *et al*., 2005; Yushkina *et al*., 2009). In total, 5 annual species: (*H. annuus* L. (PI 468439), *H. argophyllus* T. & G. (PI 494573), *H. debilis* Nutt. (PI 468670), *H. praecox* Eng. & Gray (PI 435847), *H. neglecthus* Heiser (PI 435765), which had been received from the North Central Regional Plant Introduction Station (USA), were involved in crosses with cultivated sunflower. These hybrids were subjected to several backcrosses with following self-pollination. As a result various source material was generated for sunflower breeding, of which plants with the trait of yellow coloration of the top were successfully selected. Source forms of plants with this trait were produced from an interspecies hybrid resulted from the initial crossing between the annual wild species *H. annuus* and inbred breeding line X1006B.

The selected plants with yellow coloration of the top were subjected to multiple self-pollination to create uniform inbred lines by major agronomic features. As a result a series of lines with this trait was created, of which line Mh174B was selected. Inbred line Mh174B was characterized by high autofertility, which allowed obtaining a sufficient amount of seeds, and stable expression of morphobiological and agronomic features. All the above factors are necessary conditions for genetic analysis.

To obtain F_1 hybrid combinations maternal sunflower inbred lines Cx1010A, Cx1012A and Cx2111A and Mx-854A (genotype $cyt^S rf_1 rf_1$) and paternal line Mh174B ($cyt^N Rf_1 Rf_1$) generated in the Plant Production Institute nd. a V.Ya. Yuryev of NAAS were used.

Inbred lines Cx1010A, Cx1012A and Cx2111A had the following morphological traits: green coloration of plants, monocalathidium, yellow coloration of marginal flowers, sterile disk flowers, and line Mx845A differed from the other lines only by lemon coloration of marginal flowers. Parental line Mh174B had the following morphological traits: yellow coloration of the plant top, yellow coloration of ray flowers, lateral development, fertile disk flowers (Figure 1). The lines were crossed on the sterile basis. Prior to anthesis of disk flowers calathids of maternal and paternal lines were isolated with parchment isolators. Pollination was carried out 3–4 times during anthesis of disk flowers.

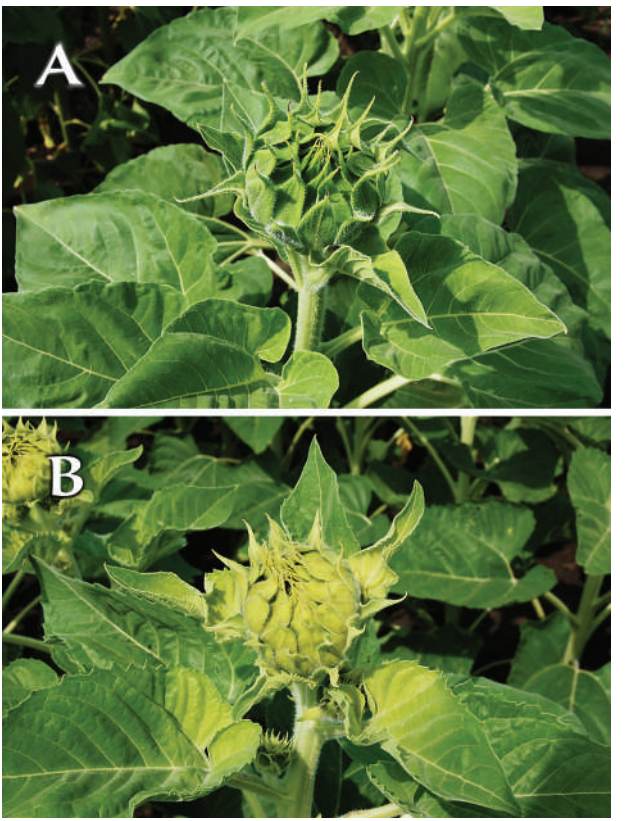

Figure 1: Green coloration (A) and yellow coloration (B) of the plant top.

Segregating F_2 populations were obtained by means of self-pollination of individual F_1 calathids under parchment isolators. A total of four F_2 hybrid combinations of were created (Cx1010A × Mh174B, Cx1012A × Mh174B, Cx2111A × Mh174B, Mx845A × Mh174B) in which 119, 133, 148 and 191 plants were analyzed, respectively. Segregated F_2 population with the following characteristics of morphological traits were involved: Cx1010A × Mh174B, Cx1012A × Mh174B, Cx2111A × Mh174B (yellow coloration of the plant top, lateral development, fertile disk flowers) and Mx845A × Mh174B (yellow coloration of the plant top, yellow coloration of ray flowers, lateral development, fertile disk flowers).

The testing of the null hypothesis of conformity of actual segregation to the theoretically expected one and gene linkage test were performed using χ^2 analysis (Atramentova and Utevskaya, 2008).

Results and discussion

Crossing of sunflower inbred lines with green (Cx1010A, Cx1012A, Cx2111A and Mx-854A) and yellow (line Mh174B) coloration of the plant top gave F_1 with all plants green, i. e. we observed uniformity of the test trait. These results suggest dominance of green coloration over yellow coloration. Analysis of the traits of ray flower coloration, stem branchiness and pollen fertility restoration in F_1 also showed uniformity, indicating homozygosis of the test traits in the sunflower source lines. In all hybrid combinations F_1 plants had the following morphological traits: yellow coloration of ray flowers, monocalathidium and disk flower fertility.

In all hybrid combinations we observed segregation of the trait of coloration of the plant top in F_2 with preponderance of the normal coloration of plants. Segregation in F_2 conformed to the theoretically expected ratio 3:1, typical for monogenic inheritance (Table 1).

Table 1: Segregation of the trait of yellow coloration of the sunflower plant top in F_2.

Crossing combination	Plant Number in F_2, pcs.		χ^2
	green	yellow	
Cx1010A × Mh174B	94	25	1.00
Cx1012A × Mh174B	101	32	0.05
Cx2111A × Mh174B	105	43	1.29
Mx845A × Mh174B	141	50	0.14

Inheritance of this plant was first described in E. Hockett and Knowles (1970). In this article the gene of yellow coloration of the sunflower plant top was denoted by the symbol *y*. It is also known that in the genetic collection of the All-Russia Research Institute of Oil Crops there is line DN47 generated from the variety Donskoy Nizkoroslyy (oral report by V.V. Tolmachyov), which is a source of yellow coloration of the sunflower plant top; there is no available information on involving this line in crosses to determine mode of inheritance of this trait. The genetic analyses of this trait conducted by Hockett and Knowles (1970) and by us were made on different material, so it is quite possible that a mutation changing plant coloration can be localized not in one but in several genes. Confirmation of this fact requires an allelism test, which implies crossing of plants having this trait with each other.

Analysis of the traits of stem branchiness, pollen fertility restoration and ray flower coloration in F_2 showed preponderance of monocalathid plants over branchy ones, fertile plants over sterile ones and yellow ray flowers over lemon coloration. In F_2 population the ratio of groups with these morphological traits also conformed to the theoretically expected segregation 3:1 (Table 2). Such segregation indicates that each trait is controlled by one gene.

Genetic control of ray flower coloration, stem branchiness and pollen fertility restoration is thoroughly studied; one can get acquainted with the results in the experimental articles and reviews (Gavrilova and Anisimova, 2003; Sharypina *et al.*, 2008; Vedmedeva, 2018). Comparison of the data obtained with the theoretically expected segregation 9:3:3:1 demonstrated independent inheritance of yellow coloration of the sunflower plant top from the traits of pollen fertility restoration, branchiness and ray flower coloration (tables are not shown). The calculated χ^2 value did not exceed the theoretically expected segregation for all the combinations. Thus, the data obtained suggest a possible location the gene *y* in a linkage group other than those of the genes ray flower coloration, stem branchiness and pollen fertility restoration. In addition, it is

Table 2: Segregation of sunflower morphological traits in F_2.

Crossing combination	Ratio of classes in F_2 by traits		
	stem branchiness	pollen fertility restoration	ray flower coloration
Cx1010A × Mh174B	96:23 (χ^2 = 0.23)	89:30 (χ^2 = 0.05)	–
Cx1012A × Mh174B	92:41 (χ^2 = 0.54)	106:27 (χ^2 = 2.15)	–
Cx2111A × Mh174B	108:40 (χ^2 = 0.17)	105:43 (χ^2 = 0.09)	–
Mx845A × Mh174B	147:44 (χ^2 = 0.17)	143:48 (χ^2 = 0.09)	141:50 (χ^2 = 0.14)

known that the sunflower gene *y* is in the same linkage group with the gene *Br3* with 11.6 % recombination (Hockett and Knowles, 1970). It should be noted that in the literature there are no data on the linkage of the gene *y* with other genes of the morphological traits of sunflower (Miller and Fick, 1997). Given this information, the created hybrid combinations can be used for mapping the gene *y*, which, unlike other genes, has not been involved in such investigations (Kusterer *et al.*, 2005; Lu *et al.*, 1998; Rojas-Barros *et al.*, 2008; Yue *et al.*, 2009, 2010, 2008).

The trait of yellow coloration of the sunflower plant top is distinctive because it is identified at early ontogenetic stages, i. e. the trait is well detected long before sunflower inflorescence. Due to such expression of this trait in plants, it is possible to use it as a marker when sunflower inbred lines are grown. A peculiarity of heterosis breeding of sunflower is that cytoplasmic male sterility (CMS) is used to create commercial hybrids (Popov and Kirichenko, 2010). Use of CMS permits creating the following types of inbred lines: sterile analogue and sterility fixer, genotypes of which can be described as $cyt^{S}rf_1rf_1$ and $cyt^{N}rf_1rf_1$, respectively, inclusive of cytoplasm. These lines are absolutely identical morphologically, though their cytoplasm types are different. Specific interaction between nuclear and cytoplasmic genes leads to production either of sterile ($cyt^{S}rf_1rf_1$) or of fertile ($cyt^{N}rf_1rf_1$) pollen. Molecular mechanism of this interaction is specified in Moneger *et al.* (1994).

Seed production of maternal lines is performed in isolated plots, which are laid out by alternation of sterile analogue and sterility fixer crops, keeping up spatial isolation from other sunflower crops (Popov and Kirichenko, 2010). Fertile flowers should be removed in sterile rows during the period of anthesis. It is associated with the fact that unrogued fertile plants in sterile rows are intercrossed among themselves, reducing sterility level of a maternal line. Consequently, low sterility of the maternal line decreases hybridity level of seeds of simple sunflower hybrids, grown in isolated hybridization plots. The situation is complicated by the necessity to eliminate fertile plants in a rather large area (5–10 ha) from 6 to 8 a.m. within the sort period of anthesis. This important production stage requires considerable manpower and time, and flowering fertile plants should be removed every day. We believe that introduction of the gene *y* into a sterility fixer genotype ($cyt^{N}rf_1rf_1$) will allow removing rogues with yellow coloration of the plant top in sterile rows from emergence to anthesis, which will significantly improve seed quality at this stage of seed production. Our inbred line Mh174B is well suitable to solve this task.

Conclusions

Thus, as a result of many-year studies on interspecies hybridization of sunflower we created line Mh174B with the trait of yellow coloration of the sunflower plant top. Genetic analysis of the test trait showed that the source inbred lines differed by one gene, presumably by the gene *y*. The trait of yellow coloration of the sunflower plant top is inherited independently from lemon coloration of ray flowers, branchiness and pollen fertility restoration. This trait can be used in sunflower breeding and seed production to quickly and efficiently determine the genetic purity of inbred lines before flowering.

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