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Inheritance of Anthocyanin Coloration Trait in Pericarp of Sunflower Seeds

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Abstract: Article describes inheritance of pigmentation traits in seed pericarp of cultivated sunflower. Study took into account presence of anthocyanins in seed pericarp as well as throughout the plant. Segregation was analyzed based on anthocyanin coloration in pericarp and hypodermis seed coloration. Nature of inheritance was determined for anthocyanin synthesis in hypodermis as well as gene control for yellow shade coloration in white hypodermis. Two nonallelic genes with complementary type of interaction were confirmed for formation of anthocyanins in achene. Yellow shade of white hypodermis was confirmed to be inherited monogenically dominant in relation to white hypodermis. It was established that presence of anthocyanin pigmentation can mask main hypodermis pigmentation.

Keywords: sunflower, pericarp pigmentation, hypodermis, anthocyanin, inheritance

Introduction

Anthocyanin pigments are colored plant glycosides that are found in the organs of many plants (Onslow, 1916; Holton and Cornish, 1995).

In sunflower anthocyanin is accumulated in vegetative and generative tissues of plants. It can be observed in hypocotyls, stems, veins, edges of leaf blades and petioles, capitulum leaves, ray and disk flowers as well as in seed pericarp (Gavrilova and Anisimova, 2003; Miller and Fick, 1997).

Satsyiperov first isolated a gene controlling anthocyanin synthesis for the whole plant (cited from Miller and Fick, 1997). Skaloud and Kovacik (1974) described that three genes *Sa1*, *Sa2*, *Sa3* are involved in anthocyanin formation of flower style. Anthocyanin in leaf petioles is caused by *Pc1*, *Pc2*, *Pc3* genes, and genes T_1, T_2 and *Ha* regulate this pigmentation in stem, leaves and hypocotyl.

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Joshi *et al*. (1994) found that there is one Ptl_a gene with pleiotropy effect that controls anthocyanin pigmentation in leaf petioles, main leaf vein, leaf edges and top, as well as in ray flowers and stigma of disk flowers. At the same time, in each of these organs anthocyanin is controlled by separate genes *Ptlb*, *Pmd*, *Plm*, *Plt*, *Prf*, *Pst* respectively.

Gavrilova and Anisimova (2003) suggested that anthocyanin color of vegetative plant organs is caused by two genes and at least one dominant gene for stigma color, which manifests itself in the presence of an additional pair of genes in vegetative organs.

Inheritance of anthocyanins in the pericarp of sunflower seeds was studied as well. Plachek and Stebut (1915) indicated the presence of special dark red pigment in seed pericarp. Sunflower pericarp is known to consist of three layers: epidermis, hypodermis and armor layer. Hypodermis can include intracellular pigment in its structure that can be red-purple in color – called anthocyanin, its intense expression disguises pigmentation of all structural layers (Fick, 1988; Bezruchenko, 1939; Putt, 1944).

Additionally *Tf* gene was described that in dominant position determines anthocyanin color of seeds and is influenced by *T* gene (Skaloud and Kovacik, 1974). According to Mosjidis (1982) anthocyanin color of pericarp is regulated by three genes: dominant gene *C* detects color presence, *Y* gene enables non-intensive anthocyanin color, and *P* gene is responsible for intense color of achenes and corolla in disk flowers.

Demurin and Tolmachev (1986) argued that trait of anthocyanin presence in pericarp (gene T_1) is inherited monogenically only when there is absence of segragation by dominant gene T, that controls anthocyanin color of the whole plant. For the formation of anthocyanin pigmentation in pericarp simultaneous presence of dominant genes *T* and T_1 is required.

Later Tolmachev and Perestova (1997) described the results of tetrahybrid cross and also complementary interaction of genes *T-T1-* which leads to anthocyanin in pericarp hypodermis.

Anthocyanin coloration of seeds along with other morphological characteristics can be used as a marker trait for sunflower lines. Information on inheritance of this trait is fragmented and deficient and it is important to study this issue further.

Aim of this study was to research inheritance of anthocyanin coloration in F_1 and F_2 generations from the crosses of sunflower lines VIR130, AH70029Rf, SL2966, MV8, I2K439, I2K670, I2K2003-1, InK630, I2K2218, KG111, and HA298 taking into consideration pigments in hypodermal layer of pericarp with no anthocyanin.

Materials and methods

We used 11 lines from the sunflower collection (*Heliantus annuus* L.) of the Institute of Oilseed Crops of NAAS Ukraine as the basis of our research. Lines were distinguished by presence of anthocyanin pigment in plant and seed pericarp, and by hypodermis seed color (Table 1). Line VIR130 was characterized by plant anthocyanin, its absence in pericarp, and white hypodermis. Lines AH70029Rf, SL2966, MV8, I2K439 had no anthocyanin color throughout the plant and pericarp. In lines KG111 and HA298 anthocyanin was present throughout the whole plant and pericarp hypodermis. Seeds in accessions I2K670, I2K2003-1, InK630 had white pericarp hypodermis, line I2K2218 had hypodermis with a yellow shade. These lines were characterized by lack of anthocyanin in plant.

Table 1: Characteristics of source material based on anthocyanin and hypodermis color.

Line	Plant anthocyanin	Pericarp anthocyanin	Hypodermis color
VIR130	Present	Absent	White
AH70029Rf	Absent	Absent	Brown
SL2966	Absent	Absent	Brown
MV8	Absent	Absent	Brown
I2K439	Absent	Absent	Brown
KG111	Present	Present	Brown
HA298	Present	Present	Brown
I2K670	Absent	Absent	White
I2K2003-1	Absent	Absent	White
InK630	Absent	Absent	White
I2K2218	Absent	Absent	Yellow shade

Experiments were carried out in the field. Seeds were sown in two line plots that corresponded to planting scheme 70 × 70 cm; each line had 10 nests.

Hybridization was performed using manual flower castration followed by pollination with pollen of other plants by conventional methods. For F_2 hybrid seeds plants were individually isolated before flowering and forced to self-pollinate. Presence of anthocyanin was analyzed in F_1 hybrid plants as well as pericarp color. F_2 generation from a single plant was sown in a separate plot. During flowering presence of anthocyanin was labeled when it was clearly manifested in stigmas, sepals, and bracts of disc flowers.

After ripening three achenes per plant were selected, taking into account labeled anthocyanin plants. Segregation analysis by pericarp color in F_2

generation was conducted according to Tikhomirova (1990). Separation of phenotypic groups in F_2 segregation was carried out visually and using microscope. Goodness of fit was calculated using Pearson's chi-squared test.

Results and discussion

In previous studies we have identified 13 groups by seed color (Gorohivets *et al.*, 2013; Gorohivets and Vedmedeva, 2016). Out of those three groups were characterized by presence of anthocyanin in pericarp hypodermis. However, this pigmentation masks color of pericarp structural layers. Identifying shades of anthocyanin color depends on the light, they do not have a clear segregation boundary, and without genetic analysis it is difficult to determine striped or solid pigmentation of epidermis. Therefore, after detailed studies we suggested combining these groups into one.

We have identified inheritance of anthocyanin in pericarp of some sunflower genotypes.

In VIR130 × MV8 cross paternal lines didn't have anthocyanins in achene hypodermis. However seed pericarp in F_1 plants characteristically had it. Second generation was split into two phenotypic classes: 135 plants had anthocyanin seeds, and for 92 plants anthocyanin was absent in pericarp. This ratio corresponded to model 9 to 7 ($\chi^2 = 0{,}96 < \chi^2_{0{,}05}(df = 2) = 3{,}81$) Therefore, we can assume that anthocyanins formation in pericarp is expressed by two genes.

In following cross combinations we tried to define phenotypic expression of anthocyanin for the whole plant and in hypodermis pericarp (Table 2).

Crossing line VIR130 that has white seed color and anthocyanin in plant with a line AH70029Rf (dark-brown seed, no anthocyanins in plant) F_1 generation was characterized by presence of anthocyanin in both the plant and in seed pericarp. Analyzing seed color in second generation we didn't separate anthocyanin achenes by their shades because of subjectivity and complexity in determining, attributing it all to one class. We observed segregation of seeds into three phenotypic classes with the ratio of 9 (anthocyanin plant and seeds) to 3 (anthocyanin plant but no anthocyanin in seeds) to 4 (no anthocyanin in plants or seeds). This indicates that for anthocyanin formation in achene pericarp two nonallelic genes with complementary interaction are responsible. This confirms earlier published data that for anthocyanin synthesis in pericarp hypodermis genes *T* and T_1 are responsible (Demuryn and Tolmachev, 1986)

Line VIR130 was characterized by presence of anthocyanin pigment in plant that is well manifested when determining color of stigmas, sepals, and bracts of

Table 2: Inheritance of anthocyanin coloration in plants and seed pericarp.

Cross combination	F_1 genotype	F_2 Segregation phenotype / genotype			Segregation model	χ^2
		Anthocyanin in plant and seeds T- T_1 -	Anthocyanin in plant, no anthocyanin in seeds T-t_1t_1	No anthocyanin in plant and seeds ttT_1-, ttt$_1t_1$		
VIR130 (TTt_1t_1) × AH70029Rf (ttT_1 T_1)	Tt T_1t_1	181	58	71	9:3:4	0,80
VIR130 (TTt_1t_1) × SL2966 (tt T_1T_1)	Tt T_1t_1	39	17	18	9:3:4	0,88
SL2966 (tt T_1T_1) × VIR130 (TTt_1t_1)	Tt T_1t_1	42	13	14	9:3:4	0,87
I2K2003-1 (tt T_1T_1) × VIR130 (TTt_1t_1)	Tt T_1t_1	118	37	54	9:3:4	0,18
I2K2218 (tt T_1T_1) × VIR130 (TT t_1t_1)	Tt T_1t_1	108	50	52	9:3:4	3,74
VIR130 (TT t_1t_1) × I2K439 (tt T_1T_1)	Tt T_1t_1	70	28	39	9:3:4	1,51

Note: $\chi^2_{0,05}$(df = 2) = 5,99.

disc flowers, and a complete lack of anthocyanins in achene pericarp, due to the presence of the gene t_1, recessive alleles of which inhibit synthesis of this pigment in hypodermis. Gene *T*, which leads to the formation of anthocyanins in the plant, has dominant position. In parent form AH70029Rf anthocyanin is not phenotypically manifested throughout the plant and in pericarp hypodermis. Due to the fact that the first generation plants had anthocyanin in achene pericarp and segregation in F_2 indicated interaction between two genes, it was established that paternal line had a dominant gene T_1, which enables synthesis of anthocyanins in the seeds, but because of the lack of a dominant *T* gene it was not expressed and hypodermis had brown pigmentation.

Similar results were observed in five combinations that are presented in Table 2. It is established that the lines SL2966, I2K2003-1, I2K2218, I2K439, MV8 as well as AH70029Rf had a combination of genes *tt* T_1T_1 that determine absence of anthocyanins in achene pericarp.

However, when crossing lines VIR130 and InK630, that had no anthocyanin pigment in plant and pericarp, the first generation of plants had anthocyanin in organs but not in pericarp. In F_2 generation, this pigment was also not manifested in pericarp. Although when analysing anthocyanin in second generation plants

we observed segregation into two phenotypic classes with ratio 3 (plants with anthocyanin) to 1 (plants without anthocyanin) (χ^2 = 1,05< $\chi^2_{0,05}$(df = 1) = 3,81).

Based on the data we can assume that InK630 line had recessive alleles *tt* t_1t_1. Therefore, when it was crossed with accessions that don't have anthocyanin pericarp in phenotype, it produced hybrids in the first and second generation that didn't have this pigment as well.

However, when crossed with I2K670 line, which had white hypodermis with KG111 line (anthocyanin color in achenes) all F_1 plants, had anthocyanin pigmentation in hypodermis (Table 3).

Table 3: Inheritance of anthocyanin in seed pericarp in lines KG111 and HA298.

Cross combination	Genotype F_1	F_2 Segregation groups phenotype / genotype		Segregation model	χ^2
		Anthocyanin T-T_1-	No anthocyanin ttT_1-		
I2K670 (ttT_1T_1) × KG111 (TTT_1T_1)	Tt T_1T_1	107	42	3:1	0,81
I2K2218 (ttT_1T_1) × HA298 (TTT_1T_1)	Tt T_1T_1	145	55	3:1	0,67

Note: $\chi^2_{0,05}$(df = 1) = 3,81.

In F_2 generation we observed segregation into two classes with a ratio of 3:1, indicating that nature of anthocyanin inheritance is monogenic. At the same time, given bigenic control of anthocyanin color, this cross ratio is due to the presence of a dominant gene T_1 in both parental forms and differences in alleles of gene responsible for synthesis of anthocyanins in plants. Thus, maternal line has non-anthocyanin color in hypodermis caused by recessive alleles of the gene *t* and dominant gene T_1, which does not appear in phenotype without the presence of a dominant allele of gene *T*. Line KG111 has anthocyanin color in plant and achene pericarp (a combination of genes TTT_1T_1). This assumption is confirmed by the fact that second generation plants that have not manifested anthocyanin correspondingly had no seeds with anthocyanin hypodermis. Their genotype was ttT_1T_1, while anthocyanin plants were *T*- T_1-. Similar results were obtained in the second cross combination I2K2218 × HA298. HA298 line as well as KG111 were characterized by anthocyanin in plant and pericarp, and had genotype TTT_1T_1.

We studied accessions distinguished not only by the trait of anthocyanin presence in plants, but also pigmentation of pericarp hypodermis (Table 4).

Table 4: Inheritance of hypodermis color given anthocyanin formation in complementary gene interaction.

Cross combination/ Hypodermis color in parent lines	Phenotype F_1	F_2 segregation by hypodermis pigmentation				Segregation model	χ^2
		Anthocyanin	No anthocyanin				
			Yellow shade of white hypodermis	white	brown		
VIR130 (white) × AH70029Rf (brown)	Anthocyanin	181	–	87	42	36:21:7	4,31
VIR130 (white) × SL2966 (brown)	Anthocyanin	39	–	21	14	36:21:7	4,92
SL2966 (brown) × VIR130(white)	Anthocyanin	42	–	17	10	36:21:7	2,46
VIR130 (white) × MV8 (brown)	Anthocyanin	135	–	70	22	36:21:7	1,01
I2K2218 (yellow shade) × VIR130 (white)	Anthocyanin	108	69	33	–	36:21:7	5,25

Note: $\chi^2_{0,05}$ (df = 2) = 5,99.

VIR130 line was characterized by white hypodermis, achenes of AH70029Rf, SL2966, MV8 lines – by brown pigmentation in hypodermis, and I2K2218 line by yellow shade of hypodermis. Analyzing F_2 plants segregation with T-T_1-genotype we did not determine white, yellowish or brown pigmentation in hypodermis. Presence of anthocyanins in pericarp masks main color of hypodermis because it makes it impossible to establish pigmentation. Taking into account complementary interaction of genes responsible for synthesis of anthocyanins in hypodermis, segregation of second generation should have a ratio of 36 to 21 to 7.

Results of hybrid analysis establish that white hypodermis dominates over brown and is controlled by a single gene. We assume that in plants with T-T_1-genotype anthocyanin in hypodermis doesn't suppress but masks white or brown pigmentation. This makes it impossible to determine hypodermis pigmentation in anthocyanin seeds.

In previous studies we established that line I2K2218 has a yellow shade of white hypodermis, which is different from the white color hypodermis line InK630 (Gorohivets and Vedmedeva, 2016).

To confirm this assumption we conducted analysis of cross between I2K2218 and VIR130 lines that we assigned to one group upon initial visual assessment

(Gorohivets *et al.*, 2013). Thus, when crossing these accessions we obtained F_1 seeds with anthocyanin in hypodermis. In second generation we separated three phenotypic classes by color: seeds with anthocyanin coloration, seeds with a yellow shade and seeds with white pigmentation. Thus we confirmed that yellow shade of white hypodermis in line I2K2218 is controlled by one gene, which dominates over white color of VIR130 line.

In seeds with anthocyanin in hypodermis, it is visually very difficult to determine pigmentation of epidermis, and hypodermis color can't be assessed. Therefore, to establish color of separate structural layers of pericarp in KG111 line we analysed all phenotypic classes of seed pericarp in F_2 segregation (Table 5). We separated 3 classes: achenes with anthocyanin color (9 + 3), non-anthocyanin seeds with striped epidermis, and white hypodermis (3), non-anthocyanin seeds with striped epidermis and brown hypodermis (1). In anthocyanin seed group we didn't determine hypodermis pigmentation, so when adding anthocyanin seeds to seeds with white and brown hypodermis we had ratio 12 (9 white + 3 brown hypodermis) to 3 to 1.

Table 5: Inheritance of anthocyanin color in pericarp and brown hypodermis pigmentation in cross combination I2K670 × KG111.

Traits and figures	F_2 phenotype segregation		
	Anthocyanin seeds	No anthocyanin seeds	
		White hypodermis	Brown hypodermis
Parent phenotype	P_2	P_1	
F_1 phenotype	F_1		
Observed segregation in F_2	107	30	12
Theoretical segregation in F_2	111,75	27,9	9,3
Theoretical ratio	12	3	1

Note: $\chi^2 = 1{,}13 < \chi^2_{0,05}(df = 2) = 5{,}99$

Due to the fact that there was no segregation by epidermis pigmentation trait and two classes of non-anthocyanin seeds had striped epidermis and armor layer, we can conclude that the line KG111 had pigmented epidermis in the form of stripes, like maternal I2K670 line. However, based on the fact that maternal line had white hypodermis, and by hypodermis pigmentation trait non-anthocyanin seeds were segregated into two classes: white and brown-colored, line KG111 had brown hypodermis that was masked by anthocyanin pigment.

Anthocyanin in plants can serve as a marker trait. Its use is complicated by the fact that this pigment is also manifested in the pericarp, and this affects oil quality. Research into inheritance of this trait will allow to use anthocyanin plants with non- anthocyanin seeds in seed production.

Conclusions

1. We have confirmed that two genes T and T1 with complementary type of interaction are responsible for the synthesis of anthocyanin in hypodermis pericarp.
2. Presence of a dominant allele of T1 gene in KG111 and HA298 lines was established. Anthocyanin plant color in these lines is due to dominance of gene T.
3. It is established that trait of yellow shade in white hypodermis determined in previous studies in line I2K2218 is controlled by one gene, and it is dominant over white color in the VIR130 line.
4. Phenotypic expression of yellow shade is masked by anthocyanin in crosses. White pigmentation of hypodermis is inherited monogenically and it's dominant over brown pigmentation.
5. We established that KG111 line had striped epidermal pigmentation and brown hypodermis that was masked by anthocyanin pigment.

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