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New flower shapes in *Linum grandiflorum* Desf. and their inheritance

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Abstract

The inbred lines of *Linum grandiflorum* that differ in the shape of a flower, F_1 and F_2 population derived from the crosses involving the inbreds were used to study flower shape inheritance pattern. Some lines had shortened petals and in the shape of a flower resembled wild carnations. Another line was distinguished by the strongly involute edges of the petals which gave the flower a stellate shape. F_1 plants from the cross between carnation-flowered and stellate-flowered plants with wild type lines (non-shortened petals, non-stellate flower) and also among themselves had wild type of flower shape. The F_2 showed a 15 : 1 ratio of normal-petalled (wild type) and shortened-petalled plants indicating the control of the carnation shape of flower ("*cnf*" = carnation flower) by two uniquely acting genes. Stellate shape of flower ("*st*" = stellate flower) in crosses with non-stellate lines was inherited as monogenic recessive trait showing in F_2 a 3 : 1 ratio of non-stellate and stellate plants. Stellate and carnation shapes of a flower were determined by different genetic systems and inherited independently. In the case where the parents of the F_1 hybrids differed not only in the shape of the flower, but also in its color, the independent inheritance of the genes determining the carnation type of the flower and the three-locus system responsible for its coloring was established. Independent inheritance of the stellate flower gene and Si-locus, the recessive allele of which lightens the color of the petals and the spot in the center of the flower was also shown.

Key words: *L. grandiflorum*, flower shape, shortened petal, involute petal, inheritance

Introduction

Linum grandiflorum is one of the most cultivated wild species of the genus *Linum*. This unpretentious annual attracts attention primarily by the long flowering of fairly large flowers of various colors. These qualities allow them to successfully compete with many ornamental plants. Being a vivid and longtime example of floral polymorphism, in recent years *L. grandiflorum* has been used to elucidate the molecular mechanisms of heterostyly (Ushijima et al. 2012). The inheritance pattern of heterostyly is evidenced which indicates that the floral morph in this species is controlled by a single diallelic locus (Ushijima et al. 2015).

L. grandiflorum is actively involved in phylogenetic analysis of *Linum* genus. As a result of studying karyotypes of various flax species, a high similarity in chromosome C/DAPI-banding patterns, ribosomal gene localization and RAPD-spectrum between *L. grandiflorum* and *L. decumbens* was found (Muravenko et al. 2009). It was also suggested that species with chromosome no. n=8, like *L. grandiflorum*, are the progenitors of species with n=9 and n=15. Using transcriptomics to reveal a paleopolyploidy event within the *Linum* genus, *L. grandiflorum* was the closest to the cultivated flax (Sveinsson et al. 2014).

A typical flower color of *L. grandiflorum*, which occurs in nature, is crimson and bright red coloration (Joshi et al. 1961). With the efforts of breeders, the spectrum of flower colors has been significantly expanded and now there are varieties with pink, apricot, light apricot and white flower color. The presence of various colors of the flower allowed to study the genetics of this trait (Lyakh 2013). And the first steps in this direction were made more than half a century ago, when plants with apricot flowers were found (Joshi et al. 1961). At the same time, the varieties of *L. grandiflorum* are of the same type in the shape of a flower. Most of them have an open flower shape,

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although this trait is no less important than the flower coloring for giving the appearance to a ornamental plant. As a result of genetic and breeding studies with *L. grandiflorum*, we identified two previously unknown flower shapes (Лях В. А. and Сорока А.И. 2008). They are briefly described in this article, which also presents the peculiarities of their inheritance.

Materials and methods

Material comprised of the lines of *L. grandiflorum* with a carnation-shaped, stellate-shaped and wild type (non-carnation and non-stellate) flowers, F_1 hybrids derived from the crosses between these lines, and F_2 populations derived after self-pollination of the F_1 hybrids. Carnation-like plants were obtained from the treatment of the seeds of white-flowered variety Bright Eyes with a chemical mutagen ethyl methane sulfonate (Lyakh and Lagron 2005). The mutants had a much smaller diameter of the corolla, due to the shortening the mostly uncolored part of the petal. The petals were also corrugated. This shape of a flower was called a “carnation” type because of its similaity to some of the wild carnations like *Dianthus campestris* M. Bieb. or *D. eugeniae* Kleop. Later this white-flowered mutant was involved in the hybridization and the lines are currently available with a carnation-shaped flower of crimson, copper, pink, apricot and light apricot colors. The stellate shape of the flower was described by us in one of the segregating populations in combination with apricot and light apricot flower colors. A characteristic feature of a flower of this type is a strong twisting of the lateral edges of the petals inward, which gives the corolla a stellate-like shape. In this case, the petals stay together long after opening the bud.

To study the inheritance of various flower shapes after crossing them with each other, together with their inheritance with the color of the flower, the following crosses were carried out: non-carnation (wild type), apricot (line Gr-1) × carnation, light apricot (line Gr-4) (the parent lines are contrasted in flower shape of carnation type and flower color); non-carnation, light apricot (line Gr-2) × carnation, crimson (line Gr-5) (flower shape of carnation type and its flower color); stellate, apricot (line Gr-6) × non-stellate (wild type), apricot (line Gr-1) (flower shape of stellate type); stellate, apricot (line Gr-6) × non-stellate, light apricot (line Gr-2) (flower shape of stellate type and flower color); stellate, non-carnation, apricot (line Gr-6) × non-stellate, carnation, apricot (line Gr-3) (flower shapes of carnation and stellate types); stellate, non-carnation, apricot (line Gr-6) × non-stellate, carnation, light apricot (line Gr-4) (flower shapes of carnation and stellate types, as well as flower color).

In each F_2 population the number of plants with the corresponding shape of the flower and its coloring was counted. To test if the observed frequencies of plants in F_2 populations correspond to the expected ones, a Chi-square test was applied (Griffiths et al. 2009).

Results and discussion

Three different shapes of the flower are shown in the Fig. 1. As can be seen from the figure, in contrast to

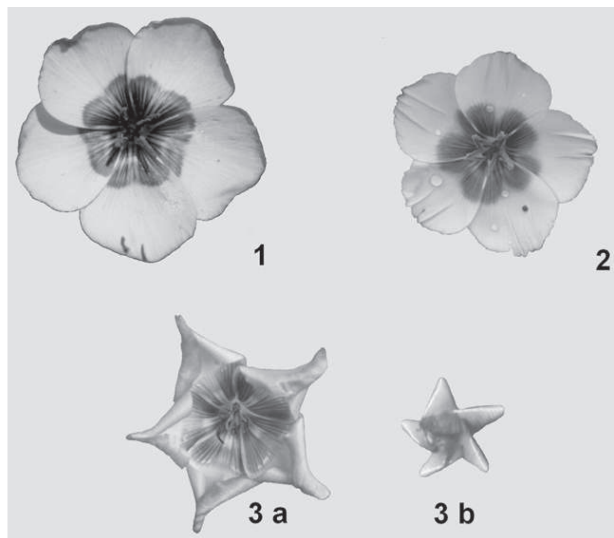

Fig. 1. Different flower shapes in *L. grandiflorum* Desf.: 1- wild type, 2 - carnation type, 3 - stellate type (a - opened flower; b - flower before opening)

the wild type (open shape of the flower), the carnation type was characterized by petals reduced in length, whereas for the stellate type of the flower twisting of the edges of the petals was unique characteristic. The buds of carnation-like plants are much shorter than buds of wild-type plants. Unblown flower of the stellate plant has a peculiar appearance due to the fact that the petals stay together for a long time. During this time the anthers have time to open.

In non-carnation shape (wild type), apricot color × carnation shape, light apricot color cross combination in F_1 non-carnation shape of flower completely dominates over carnation shape (“*cnf*” = carnation flower). The F_2 shows a 15 : 1 ratio of wild type and carnation type plants (Table 1). This hype of

Table 1. F_2 segregation for corolla shape and color in cross of carnation-flowered and non-carnation-flowered (wild type) plants in *L. grandiflorum*

F_1 phenotype	Total F_2 plants	F_2 phenotypes										Segregation ratio tested	χ^2 (P value)
		non-carnation crimson	non-carnation copper	non-carnation apricot	non-carnation pink	non-carnation light apricot	carnation crimson	carnation copper	carnation apricot	carnation pink	carnation light apricot		
non-carnation, apricot (line Gr-1) × carnation, light apricot (line Gr-4)													
non-carnation, apricot	117	–	–	86	–	22	–	–	5	–	4	(15:1) × (3:1) = 45 : 15 : 3 : 1	3.30 (0.35)
non-carnation, light apricot (line Gr-2) × carnation, crimson (line Gr-5)													
non- carnation, crimson	100	33	10	21	20	12	1	1	1	0	1	(15:1) × (27:9:12:12:4) =405:135:180:180: 60:27:9:12:12:4	10.88 (0.28)

χ^2_{05} (d.f.3) = 7.82; χ^2_{05} (d.f.9) = 16.92

Table 2. F_2 segregation for corolla shape and color in cross of stellate-flowered and non-stellate-flowered (wild type) plants in *L. grandiflorum*

F_1 phenotype	Total F_2 plants	F_2 phenotypes				Segregation ratio tested	χ^2 (P value)
		non-stellate, apricot	non-stellate, light apricot	stellate, apricot	stellate, light apricot		
apricot (line Gr-6) × non-stellate, apricot (line Gr-1)							
non-stellate, apricot	86	63	–	23	–	3:1	0.15 (0.70)
stellate, apricot (line Gr-6) × non-stellate, light apricot (line Gr-2)							
non-stellate, apricot	102	60	25	13	4	(3:1) × (3:1) = 9:3:3:1	4.54 (0.21)

χ^2_{05} (d.f.1) = 3.84; χ^2_{05} (d.f.3) = 7.82

segregation indicated the control of the carnation shape of flower by two uniquely acting genes. In this combination of crossing, parents differ not only in the shape of the flower but also in its color. Earlier, we showed that plants of apricot and light apricot coloring differed in one pair of genes (*Si-si*), where *si* allele in the homozygous state suppresses the recessive allele of *af* gene which determines the apricot color of flower (Lyakh 2013). So, the lines having non-carnation flower of apricot color and carnation-like flower of light apricot coloring are differed by three genes. Since three genes are expected in this system, we have to obtain in F_2 a (15:1) × (3:1) = 45:15:3:1 pattern which is a modification of the three hybrid 27:9:9:3:3:3:1 Mendelian ratio due to gene interaction by the type of non-cumulative polymerism.

F_1 plants from the crossing non-canation-flowered plants of light-apricot color with carnation-flowered plants of crimson color had non-carnation flowers of crimson color. In F_2 population of 100 descendants 4 plants were of carnation type and 96 plants had non-carnation flowers, which corresponds to the 15:1 segregation model (Table 1). In turn, the 27 (crimson) : 9 (copper) : 12 (apricot) : 12 (pink) : 4 (light-apricot) ratio by the color of the flower is observed, which is explained by the epistatic effect of the recessive allele of one of the genes of the three-locus system, which determines the color of the flower in *L. grandiflorum* (Lyakh 2013). Analyzing F_2 simultaneously in the shape and color of the flower, we may presume (15:1) × (27:9:12:12:4) = 405:135: 180:180:60:27:9:12:12:4 pattern which is a modification of the five hybrid Mendelian ratio.

Stellate shape of flower ("*st*" = stellate flower) in crosses with non-stellate lines is inherited as monogenic recessive trait. In cross of apricot-petalled lines with non-stellate and stellate flowers all F_1 plants show non-stellate flowers of apricot color and F_2 segregated into 3 : 1 ratio of non-stellate and stellate plants (Table 2). A cross between the line having the apricot color of flower of stellate shape and light apricot-flowered line of non-stellate shape produces F_1 with apricot-petalled plants and non-stellate shape of flower. In this cross combination the parents are differed by two genes. And, if these genes are inherited independently, we have to obtain a typical dihybrid pattern, and the four unique phenotypes have to form a 9:3:3:1 ratio in F_2.

In cross of apricot-petalled lines with stellate-shaped and carnation-shaped flowers F_1 plants have wild type of flower shape – non-stellate flower and non-shortened petals (Table 3). It is assumed then, that the stellate and carnation shapes of flower are determined by different genetic systems and can designate the genotypes of the parental lines as follows: *ststCnf1Cnf1Cnf2Cnf2* (stellate, non-carnation), *StStcnf1cnf1 cnf2cnf2* (non-stellate, carnation). In population of 108 descendants, 5 plants were of carnation type and 25 plants had stellate flowers, which corresponds to the 15:1 and 3:1 segregation models, respectively. Joint inheritance of stellate and carnation shapes of flower corresponded completely to the expected 45:15:15:1 pattern. The triple recessive homozygote had its own manifestation characterized by stellate flowers and even more reduced length of the corrugated petals compared to carnation shape.

According to the results of crossbreeding stellate-shaped plant of apricot color and carnation-shaped line of light apricot color, eight phenotypes are observed in F_2 (Table 3). Since there are four genes in this cross, we have to obtain a typical four hybrid pattern in F_2. However, in F_2 the 135 (non-carnation, non-

Table 3. F_2 segregation for corolla shape and color in cross of stellate-flowered and carnation-flowered plants in *L. grandiflorum*

F_1 phenotype	Total F_2 plants	F_2 phenotypes								Segregation ratio tested	χ^2 (P value)
		non-stellate non-carnation apricot	non-stellate non-carnation light apricot	stellate non-carnation apricot	stellate non-carnation light apricot	non-stellate carnation apricot	non-stellate carnation light apricot	stellate carnation apricot	stellate carnation light apricot		
		stellate, non-carnation, apricot (line Gr-6) × non-stellate, carnation, apricot (line Gr-3)									
non-stellate, non-carnation, apricot	108	79	–	24	–	4	–	1	–	(3:1) × (15:1) = 45:15:3:1	0.86 (0.84)
		stellate, non-carnation, apricot (line Gr-6) × non-stellate, carnation, light apricot (line Gr-4)									
non-stellate, non-carnation, apricot	55	29	8	11	4	1	1	1	0	(3:1)×(15:1)×(3:1) = 135:45:45:15:9:3:3:1	2.33 (0.94)

χ^2_{05}(d.f.3) = 7.82; χ^2_{05}(d.f.7) = 14.07

stellate, apricot) : 45 (non-carnation, non-stellate, light apricot) : 45 (non-carnation, stellate, apricot) : 15 (non-carnation, stellate, light apricot) : 9 (carnation, non-stellate, apricot) : 3 (carnation, non-stellate, light-apricot) : 3 (carnation, stellate, apricot) : 1 (carnation, stellate, light-apricot) ratio is observed which is a modification of the four hybrid Mendelian ratio due to duplicate genes interaction.

As for a wide range of flower colors is concerned, it may seem that these colors are determined by different alleles of the same gene. However, this assumption is refuted when, as a result of crossing the apricot- and pink-petalled lines, wild type (crimson petals) is restored in F_1 hybrids, and phenotypes different from parents and F_1 hybrid appear in F_2. The three-locus system of non-allelic genes that interact in a certain way explains the existing variety of flower colors quite well (Lyakh 2013).

The cytological observations showed that the cells of the corolla petals of the carnation-flowered mutant were significantly shorter than the cells of the original plant. Mutant plant is also smaller in height and forms the seeds of smaller size (Лях В. А. and Сорока А.И. 2008).

As for a common mechanism of petal cell size control, it is believed that endoreduplication could be the reason for a number of plants (Kudo and Kimura 2002). This mechanism is independent of the mechanism that determines the number of flower organs affecting its architecture. Both of these mechanisms ultimately regulate floral size (Weiss et al. 2005).

References

Griffiths A. J. F., Wessler S. R., Lewontin R. C., Gelbart W. M. and Suzuki D. T. 2004. An introduction to genetic analysis. 8th ed. WH Freeman and Company, New York: 800. https://www.amazon.com/Introduction-Genetic-Analysis-Anthony-Griffiths/dp/0716749394.

Joshi A. B., Hardas M. V. and Guglani P. L. 1961. Multiple allelism for petal colour in *Linum grandiflorum* Desf. Curr. Sci., **30**: 109. http://www.currentscience. ac.in/Downloads/article_id_030_03_0109_0109_0.pdf.

Kudo N. and Kimura Y. 2002. Nuclear DNA endoreduplication during petal development in cabbage: relationship between ploidy levels and cell size. J. Exp. Bot., **53**: 1017-1023. https://academic.oup.com/jxb/article/53/371/1017/509268/Nuclear-DNA-endoreduplication-during-petal.

Lyakh V. 2013. Genetics of flower color in *Linum grandiflorum* Desf. Indian J. Genet., **73**: 335-337. http://www. indianjournals.com/ijor.aspx? target=ijor: ijgpb&volume=73&issue=3&article=017.

Lyakh V. and Lagron V. 2005. Induced mutation variability in *Linum grandiflorum* Desf. Mutation Breeding Newsletter and Reviews, **1**: 4-5. https://inis.iaea.org/search/search.aspx?orig_q=RN:36105145.

Muravenko O. V., Yurkevich O. Yu., Bolsheva N. L., Samatadze T. E., Nosova I. V., Zelenina D. A., Volkov A. A., Popov K. V. and Zelenin A. V. 2009. Comparison of genomes of eight species of cections *Linum* and *Adenolinum* from the genus *Linum* based on chromosome banding, molecular markers and RAPD analysis. Genetica, **135**: 245-255. https://link.springer.com/article/10.1007/s10709-008-9273-7.

Sveinsson S., McDill J., Wong G. K. S., Li J., Li X., Deyholos M. K. and Cronk Q. C. B. 2014. Phylogenetic pinpointing of a paleopolyploidy event within the *flax genus* (*Linum*) using transcriptomics. Ann. Bot., **113**: 753-761. https://academic.oup.com/aob/article/113/5/753/159739/Phylogenetic-pinpointing-of-a-paleopolyploidy.

Ushijima K., Ikeda K., Nakano R., Matsubara M., Tsuda Y. and Kubo Y. 2015. Genetic control of floral morph and petal pigmentation in *Linum grandiflorum* Desf., a heterostylous flax. The Hort. J., **84**: 261-268. https://www.jstage.jst.go.jp/article/hortj/84/3/84_MI-045/_html

Ushijima K., Nakano R., Bando M., Shigezane Y., Ikeda K., Namba Y, Kume S., Kitabata T., Mori H. and Kubo Y. 2012. Isolation of the floral morph-related genes in heterostylous flax (*Linum grandiflorum*): the genetic polymorphism and the transcriptional and post-transcriptional regulations of the S locus. The Plant J., **69**: 317-331. http://onlinelibrary. wiley.com/doi/10.1111/j.1365-313X.2011.04792.x/full.

Weiss J., Delgado-Benarroch L. and Egea-Cortines M. 2005. Genetic control of floral size and proportions. Int. J. Dev. Biol., **49**: 513-525. http://www.ijdb.ehu.es/web/paper/051998jw/genetic-control-of-floral-size-and-proportions.

Лях В. А. and Сорока А.И. 2008. Ботанические и цитогенетические особенности видов рода *Linum* L. и биотехнологические пути работы с ними (Botanical and cytogenetic peculiarities and biotechnological techniques for work with *Linum* L. genus species). Запорожье, ЗНУ: 182. https://scholar.google.com.ua/citations?user=JUuezpUAAAAJ&hl=ru.