

INFLUENCE OF THE AFILA GENE ON GRAIN YIELD IN PEA (*PISUM SATIVUM* L.)

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Abstract

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The aim was to investigate the possibility of introducing leafless or semi-leafless features from afila cultivar "Filigreen" in early, mid-early and late pea cultivars. F₁ and F₂ generations were grown together in a single nursery during year 2006. F₁ plants with normal leaves were obtained thus proving that afila is a recessive characteristic. The total of 1490 F₂ plants segregated with 2.93 plants with normal leaves to 1.07 with afila leaves. Chi-square test confirmed monohybrid model of inheritance 3:1. Afila plants with differing maturity dates were obtained. The best recombination's, in terms of grain yield per plant, were obtained by crossing "Filigreen" x "Maja" (early cultivar), "Filigreen" x "Oskar" (mid-early cultivar) and "Filigreen" x "Zelena dolina" (late cultivar). Grain yield of F₂ afila plants was reduced by 6-18% in relation to grain yield of normal plants (depending on parents, combinations). Grain yield was reduced, in average, by 10% for afila plants compared to normal (leafy) plants in the F₂ for all possible crosses.

Key words: pea, afila, grain yield, monohybrid inheritance

Introduction

Pea is native (according to Vavilov) to the three geographic regions, Abyssinia, Kavkasia and the Mediterranean. It originates from the extinct variety *Pisum formosum*. Wild peas *Pisum elatius* can still be found in the Mediterranean mountains.

In early times, prior to beans, peas formed an important part of the diet for the people of Europe and Asia. Mature seeds were made into a soup and other delicious dishes. Nowadays, primarily immature grain is used.

Pea plant has a small number of chromosomes (2 x n = 14), a large enclosed-structured flower that can easily be castrated and crossed. It is self-pollinated and as such suitable for genetic and other biological investigations.

Scientists involved in breeding and selection of peas with the aim of developing new, better cultivars are inevitably encountered with the issue of lodging. The problem is present in all cultivars with classical leaf structure irrespective of stem length and maturing date. Lodging is most prominent during rainy seasons and seed crops were found to be more susceptible compared with market crops.

According to the literature data, the "af af" gene feature was first described by Soloveva (1958) as spontaneous mutation from the cv. Svoboda. Later on Goldenberg (1965) described afila as spontaneous mutation from the cv. Cuareton.

Blixt (1972) located the gene („leaf“) introducing the synonym „af“ in the first chromosome. It was Snoad

who introduced this gene for the first time in commercial cultivars in England (1983, according to Cousin et al., 1986). He studied two recessive pairs of genes which reduced the leaf surface: *st st*-stipules – peas without stipules and *af af* pea plants without leaflets on leaves but with stipules (Figure 1).

In literature this type of pea plants are known as „leafless“ or more often „afla“.

The occurrence of “leafless” afla pea presented a real challenge to breeders. It was no less so at the Centre for Vegetable Crops in Smederevska Palanka, Serbia. Once obtained, the cultivar Filigreen, «*af af*» genotype, was crossed with cultivars of different maturing dates with the aim of developing both leaflessness and lodging resistance in new genotypes.

The aim of the study was to monitor the features of parents and hybrids of ordinary and leafless garden pea known as afla. The investigation included the assessment of the «*af af*» gene incorporation from the English cv. Filigreen of the leafless short pea variety resistant to lodging, into a number of domestic and foreign varieties of normal leaf structure and different maturing dates and yield potential.

The objective of the study was to find answers to some essential issues with regard to pea breeding and selection. The question remains whether it is possible to successfully recombine the homozygous leafless gene with already achieved desired traits within early-, mid- and late-maturing genotypes without losing ferti-



Fig. 1. Normal pea leaf with stipules and two pairs of leaflets (*af af*) with stipules but without stipules which were transformed to tendrils

ity, quality, disease resistance and resistance to environmental stresses, in order to segregate out superior pure lines of the afla leaf type during the process of selection. These cultivars are expected to inherit quality from their parents with normal leaf structure but at the same time acquire lodging resistance from the afla pea Filigreen.

The number of pods per pea plant depends on genotype and environmental conditions. Late-maturing genotypes compared with early-maturing can form a higher pod number per plant. Also, cultivars having tiny pods were noted to have a higher number of pods per plant compared with those with larger pods. This is also true for tall compared with dwarf pea plants. The number of pods per plant is also influenced by the genetic constitution of flowering, i.e. shape of cluster. Namely, there are genotypes which form a single flower or a pod on the cluster (*Fn Fna*), two flowers (*fn Fna* or *Fn fna*) and three or more flowers (*fn fna*). This feature was analyzed by Lamprecht (1974) who eventually offered a genetic explanation. The *pn* gene from cv. Green Riva was found to reduce the number of flowers per pea cluster in the homozygous and recessive state (Omar, 1960).

According to Gritton (1975) there is an intermediary inheritance of the number of pods per plant.

According to Đorđević et al. (1994) in the F_1 generation, the number of pods per plant in the afla pea genotypes was by 13% lower than in the F_1 generation and in relation to the parents by 1% lower than in parents with normal leaves, i.e. leaf number was about 30% higher than in afla parents. The reduced number of pods per plant in the F_2 generation in afla genotypes may be attributed to the reduction of the assimilation surface.

Besides the decreased assimilation surface another important factor are global climatic changes having a major impact on the photosynthetic apparatus (Akram et al., 2008).

The exhausted natural resources may present another major threat to agricultural production. Major strategies today focus on the development of sustainable agricultural production, i.e. sustainable exploitation of natural resources. Advanced biotechnological methods and procedures may be expected to contribute to safe food production, both qualitatively and quantitatively (Pescic and Jankovic, 2009).

The number of grains per pod may range from 1 to 13. However, the number of grains typically ranges from 7 to 8 (Blixt, 1972).

The inheritance of the number of grains per pod is a complex issue being controlled by a number of genes (Gotschalk, 1964). According to Marx and Mishanec (1962, 1967) the pointed pods have a greater potential for higher grain number per pod. Cultivars with a lower number of grains in a pod crossed with those having a higher number of grains produce a F_1 generation with lower grain number predominating. In the F_2 generation it was noted that the ratio was 3:1 in favor of the lower number of grains.

Krarup and Davis (1970) were of the opinion that there is a cumulative impact of a number of recessive genes contributing to this property, i.e. it has to do with the quantitative feature.

The number of grains per plant is the principal component of yield. It is known to be influenced by genotype and the environment. Factors contributing to either higher or lower pod number per plant were also found to impact the number of grains per plant. The influence of the number of grains per pod was found to be identical.

Authors differ in their opinion with regard to the influence of the number of grains per plant on yield. According to Fedatov (1967) the property was sixth ranked and Makasheva (1975) considered it of prime importance based on the correlation coefficient for the number of grains per plant and yield per plant ($r=0.63$).

Even Dobias and Bodian (1975) considered this feature the major factor contributing to pea grain yield, along with the number of pods per plant (Johnson, 1975).

Materials and Methods

The objective of the crossing was to obtain cultivars resistant to lodging in all maturing groups and ultimately maximize pea grain yield. It is for this reason that seeds of cv. Filigreen were used and crossed with a number of domestic and foreign carefully chosen cultivars of earliest to latest maturing dates.

Filigreen is a cultivar of British descent and was purchased thanks to the German enterprise Van Waweren (Figure 2).

Filigreen is mid-maturing cultivar. Has a short stem and is dark green in color. It grows erect to a height of

45-50 cm. The stem is short, firm, zigzag in shape with closely set internodes. Flowering begins on the 11-12th node whereas on the next 5-6 nodes clusters are formed with two white flowers. Flower stems are short and pods are tiny, slim, and straight with a dull top and filled with 7-8 small green grains.

Leaves are of the «afila» type, i.e. composed of numerous tendrils which are highly developed, curling and holding to each other. This enables full resistance to lodging because plants were found to hold to each other using tendrils. This was not a common incidence in conventional cultivars.

This mother cultivar was used in a number of direct crossings and 28 cultivars and pure lines were used as father varieties for the recombination of afila properties.

Multiple crossing recombinations were employed in order to obtain numerous hybrid seeds in both direct and reciprocal hybrids.

This paper presents the results obtained in the F_1 and F_2 generations with the following 12 cultivars: Poni express, Maja, Kosmaj, Oskar, Zelena dolina and Feniks (domestic, bred at the Centre for Vegetable crops) and foreign Karina (Holland), Action (Germany), Facima (Germany), Cl-565 (Czech and Slovak), Jareh (Poland) and Pegado (Holland).

Of these the following are early maturing: Poni express, Maja, Action and Karina; mid-maturing: Kosmaj, Facima, Oskar, and late-maturing: Cl-565, Jarex, Zelena dolina, Feniks and Pegado.

The above mentioned cultivars were used as parental fathers for crossings with Filigreen and this resulted



Fig. 2. Cv. Filigreen

in seeds of the F_1 generation. Plants of the F_1 generation were produced in the next year and this eventually resulted in F_2 seeds.

Crossings were repeated. Parental seeds of the F_1 and F_2 generation were prepared and sown during the same vegetation season in spring. Optimal sowing dates were employed on 15th March. Furrows were 5-6 cm deep and seeds were sown at a 10 cm distance leaving identical vegetation space to all genotype groups. Row distance was 60 cm. Manual hoeing was employed during growth. During maturation plants were pulled out, tied into bundles and stored to be analyzed in laboratory.

Plants were counted and measured in order to obtain data with regard to:

- Number of grains per plant and
- Grain yield per plant.

The data sampled were statistically analyzed with the objective of assessing the amount of inherited features. Mean values and standard errors were computed using variation coefficient, analysis of variance, Chi-square test for proving recessive inheritance of afla and heterosis inheritance of some features with respect to the better parent.

Standard statistical methods were used. Data with regard to both afla and normal plants in the F_2 genera-

tion were used for the Chi-square test. The total number of parent plants analyzed was 575, 409 plants in the F_1 generation and 1490 in the F_2 generation (total sum 1474 plants).

Results

In the study all combinations between F_1 and afla normal sorts gave normal F_1 generation. In the F_2 generation there was an expected event afla than 3:1. To confirm this assumption the chi-square test was conducted (Table 1).

Statistical analysis shows that differences between experimental and theoretical values are not significant. Since Chi-square value is less than table value for level of significance of 0.05 and 0.01 this proves that there was no significant difference and to obtain the correct 3:1 ratio of plants with normal leaves and afla plants in the F_2 generation.

The number of grains per plant is a direct component of yield. The number of grains is far more important than grain yield given in weight units. The ultimate goal of each plant, including peas, is to ensure reproduction, i.e. progeny.

The average number of grains per plant in better yielding parents (fathers) was 36.7 and in the F_1 genera-

Table 1
Chi-square test for segregation of "leaf" and "afla" genotypes in F_2 generation

No.	Recombinations	Number of plants				Total	χ^2
		Af		af			
		Exp.	Theor.	Exp.	Theor.		
1	Filigreen x Pony expres	89	91.50	33	30.50	122	0.27
2	Filigreen x Karina	94	97.50	36	32.50	130	0.50
3	Filigreen x Action	91	96.00	37	32.00	128	1.04
4	Filigreen x Maja	90	93.00	34	31.00	124	0.39
5	Filigreen x Kosmaj	89	90.00	31	30.00	120	0.04
6	Filigreen x Oskar	93	96.75	36	32.25	129	0.58
7	Filigreen x Facima	90	91.50	32	30.50	122	0.10
8	Filigreen x Cl - 565	90	87.75	27	29.25	117	0.23
9	Filigreen x Jareh	92	90.75	29	30.25	121	0.07
10	Filigreen x Zelena dolina	93	93.75	32	31.25	125	0.02
11	Filigreen x Fenix	90	93.75	35	31.25	125	0.60
12	Filigreen x Pegado	91	95.25	36	31.75	127	0.76
	Total	1092	1117.50	398	372.50	1490	2.32

for $df = 1$ and $P_{0.01} = 6.635$

for $df = 12$ and $P_{0.01} = 26.217$

tion there were on average 46 grains (Table 2). This was about 25% higher yields than in better parents. The most fertile combination within the early-maturing varieties was Filigreen x Maja yielding 56.5 grains per plant. On

the other hand, the best F_1 generation was Filigreen x Oskar with an average of 90.8 grains per plant.

The analysis of variance (Table 3) shows a significant difference in the number of grains between afile in

Table 2
The average number of grains per plant

Recombinations										F_2					
	P_1			P_2			F_1			Af			af		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
1 Filigreen x Pony expres	11.3	0.29	18.41	24.3	0.98	19.45	30.2	0.24	4.40	29.1	0.27	7.04	28.4	0.30	6.09
2 Filigreen x Karina	11.3	0.29	18.41	26.3	0.40	9.69	29.3	0.17	3.00	26.1	0.18	5.40	24.2	0.16	4.05
3 Filigreen x Action	11.3	0.29	18.41	23.3	0.57	16.52	30.2	0.40	9.01	24.4	0.31	9.13	24.1	0.24	6.02
4 Filigreen x Maja	11.3	0.29	18.41	46.2	0.35	5.15	56.5	0.06	0.71	51.3	0.10	1.54	47.2	0.10	1.13
5 Filigreen x Kosmaj	11.3	0.29	18.41	32.1	0.60	12.87	40.2	0.17	2.54	37.2	0.29	5.99	31.2	0.43	7.60
6 Filigreen x Oskar	11.3	0.29	18.41	70.3	0.19	2.01	90.8	0.12	0.93	77.7	0.08	0.77	69.0	0.18	1.59
7 Filigreen x Facima	11.3	0.29	18.41	18.0	0.77	26.83	26.1	0.30	6.32	22.0	0.40	14.18	19.1	0.50	14.70
8 Filigreen x CI -565	11.3	0.29	18.41	26.4	0.40	9.81	30.4	0.42	6.68	25.2	0.21	6.51	21.4	0.34	8.22
9 Filigreen x Jareh	11.3	0.29	18.41	23.4	0.36	9.40	27.9	0.49	7.49	26.1	0.28	8.31	21.2	0.41	10.14
10 Filigreen x Zelena dolina	11.3	0.29	18.41	56.2	0.77	8.88	68.3	0.18	1.55	58.1	0.12	1.65	54.18	0.19	1.96
11 Filigreen x Fenix	11.3	0.29	18.41	50.1	0.45	5.83	63.2	0.44	3.91	50.0	0.20	3.03	40.0	0.28	4.10
12 Filigreen x Pegado	11.3	0.29	18.41	44.3	1.76	27.56	58.6	0.45	4.28	41.8	0.26	4.90	36.5	0.37	6.00
Average	11.3	0.29	18.41	36.74	0.63	12.83	45.98	0.28	4.24	39.25	0.23	5.70	34.71	0.29	5.98

Legend:

- M - Average number of grains per plant
- SD - Number of grains per plant standard deviation
- CV - coefficient of variation in percentage
- Af - Dominant Inheritance (gene)
- af - Recessive Inheritance (gene)

Table 3
Analysis of variance of Af and af for average number of grains per plant in F_2 generation

Correlation	dt	SS	MS	F
SQ Total	47	14792.2	314.73	4.55
SQ Blok	3	6491.29	2164.76	31.29
SQ Komb.	11	6018.96	547.18	7.91
SQ Error	33	2281.95	69.15	

$$LSD_{t=0.05} = 11.97$$

$$LSD_{t=0.01} = 16.09$$

$$F_{0.05} = 2.11$$

$$F_{0.01} = 2.87$$

for $df=3$ and $df=33$

the best combinations and *afila* in other combinations (Filigreen x Oskar, Maja, Zelena dolina, Feniks and Pagedo). On average, *afila* were found to be about 12% less yielding than plants having normal leaf structure, although the variability recorded was from 2 to 20%.

With regard to grain yield per plant, the *afila* type cv. Filigreen yielded only 1.8 g of grains per plant. Other cultivars of normal leaf structure were far more yielding: 3.7 g cv. Facime to 15.2 g cv. Oskar. The most yielding parents were Maja, Oskar and Zelena dolina (Table 4).

On average grain yield was 8.57 g per plant in fathers. In the F_1 generation the yield achieved amounted to 10.1 g which was by 18% higher than in better parents. With regard to grain yield the best hybrids were

Filigreen x Maja (16.2 g), Filigreen x Oskar (17.3 g) and Filigreen x Zelena dolina (16.1 g).

In the study major attention was focused on yields in the F_2 generation. On average 8.22 g of grains per plant were obtained with the F_2 generation which was by 19% lower than the F_1 average or 4% less than the average of better parents – fathers. Plants with normal leaf structure were found to be more yielding – 9.11 g/plant. This was 10% less than in the F_1 generation and 6% higher than in the best parents (Table 4).

Based on the analysis of variance for *afila* and plants with a normal leaf structure in the F_2 generation compared with the parents, the yields for *afila* were mostly insignificant (Table 5).

Table 4
Grain yield per plant

Recombinations										F_2					
	P_1			P_2			F_1			Af			af		
	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV	M	SD	CV
1 Filigreen x Pony expres	1.8	0.06	25.0	6.1	0.11	12.46	7.1	0.30	23.38	6.7	0.25	28.81	6.2	0.21	19.19
2 Filigreen x Karina	1.8	0.06	25.0	6.3	0.12	12.38	6.8	0.19	14.41	6.4	0.16	20.47	6.0	0.16	16.33
3 Filigreen x Action	1.8	0.06	25.0	5.2	0.23	29.62	6.1	0.12	13.11	5.7	0.37	45.96	5.2	0.29	34.04
4 Filigreen x Maja	1.8	0.06	25.0	12.6	0.11	5.71	16.2	0.10	3.70	14.1	0.11	6.31	12.9	0.17	7.60
5 Filigreen x Kosmaj	1.8	0.06	25.0	8.2	0.17	13.90	9.8	0.24	14.48	9.1	0.13	10.76	8.1	0.21	14.69
6 Filigreen x Oskar	1.8	0.06	25.0	15.2	0.08	3.76	17.3	0.14	5.66	16.4	0.10	4.88	15.4	0.20	7.66
7 Filigreen x Facima	1.8	0.06	25.0	3.7	0.09	15.41	5.1	0.32	35.09	4.4	0.32	57.04	3.9	0.40	58.45
8 Filigreen x Cl - 565	1.8	0.06	25.0	5.7	0.12	13.51	6.7	0.32	23.13	6.1	0.26	33.11	5.4	0.41	39.81
9 Filigreen x Jareh	1.8	0.06	25.0	4.6	0.17	22.61	5.1	0.20	20.78	4.8	0.14	23.75	4.3	0.24	30.23
10 Filigreen x Zelena dolina	1.8	0.06	25.0	13.5	0.10	4.96	16.1	0.17	6.09	14.5	0.12	6.69	12.9	0.17	7.36
11 Filigreen x Fenix	1.8	0.06	25.0	11.6	0.13	7.16	13.4	0.37	15.52	11.4	0.15	10.00	0.4	0.22	12.66
12 Filigreen x Pagedo	1.8	0.06	25.0	10.1	0.36	24.36	11.6	0.35	16.64	9.8	0.19	15.51	9.0	0.26	17.22
Average	1.8	0.06	25.0	8.57	0.15	13.82	10.11	0.24	16.00	9.12	0.19	21.94	8.23	0.25	22.10

Legend:

- M - Average grain yield per plant in grams
- SD - grain yield per plant standard deviation in grams
- CV - coefficient of variation in percentage
- Af - Dominant Inheritance (gene)
- af - Recessive Inheritance (gene)

Table 5
Analysis of variance of Af and af for grain yields per plant in F₂ generation

Correlation	dt	SS	MS	F
SQ Total	47	936.83	19.93	5.03
SQ Blok	3	380.65	126.88	32.04
SQ Komb.	11	425.45	38.68	9.77
SQ Error	33	139.73	3.96	

$$LSD_{t-0.05} = 2.87$$

$$LSD_{t-0.01} = 3.86$$

$$F_{0.05} = 2.90$$

$$F_{0.01} = 4.46$$

for df=3 and df=33

Discussion

The results of our investigations were found to be in accordance with those of other authors. When crossed with cultivars having a normal leaf structure, genotypes of normal leaves were found to predominate in the F₁ generation. A population with substantially greater number of plants having normal leaves and lower number of afila was found only in the F₂ generation following gene segregation of heterozygous af af F₁ generation. The ratio of normal leaf structure plants and afila pea plants was found 3:1, i.e. 75% of plants recorded were normal leaf structure plants and 25% were afila pea plants. The hypothesis on the inheritance of the monogenic recessive gene controlling leaflessness was proved using Chi-square test.

Having this genetic feature controlling leaflessness, breeders consider the afila trait fixation in as an easy job because in the generations to follow there will be no segregation in the chosen afila plants for the afila trait. The numbers of 398 single afila plants form the 12 F₂ populations derived form 12 F₁ crosses were sampled for further investigation.

With regard to the inherited number of grains per pod, in the F₁ generation pods were fuller compared with better parents pointing to heterosis. However, it was eventually concluded that the genetic capacity with regard to the number of grains per pod was better used. When hybrids, cultivars overloaded with genetic inbreeding were found to lack the ability of using their own potential for the number of grains per pod.

A 14% lower grain number per pod in the afila F₁ in relation to the F₁ generation was registered. The best combinations with regard to this feature were noted with cv. Maja, Oskar and Zelena dolina.

Average grain yield per plant of the F₁ hybrids Fili-green x different fathers having normal leaf structure was by about 18% higher in relation to better parents. On the other hand, a 4% lower grain yield per pod was noted in the F₂ in relation to the same parents. Plants with normal leaf structure yielded 6% higher grain number per plant in relation to the average for fathers.

Of the total 12 hybrids of the F₁ and F₂ generations, three hybrids had favorable combination potentials with regard to the performance of yield components and yield. Among the various maturing groups the following cultivars were found to meet agronomic requirements: cv. Maja (early maturing), cv. Oskar (mid-maturing) and Zelena dolina (late-maturing genotype).

Conclusions

Based on the statistical analysis of the obtained data processed afila parents and normal pea leaves and their F₁ and F₂ hybrid generation, we can conclude the following:

A 14% lower grain number per pod in the afila F₁ in relation to the F₁ generation was registered. The best combinations with regard to this feature were noted with cv. Maja, Oskar and Zelena dolina.

Average grain yield per plant of the F₁ hybrids Fili-green x different fathers having normal leaf structure was by about 18% higher in relation to better parents. On the other hand, a 4% lower grain yield per pod was noted in the F₂ in relation to the same parents. Plants with normal leaf structure yielded 6% higher grain number per plant in relation to the average for fathers.

Of the total 12 hybrids of the F₁ and F₂ generations, three hybrids had favorable combination potentials with regard to the performance of yield components

and yield. Among the various maturing groups the following cultivars were found to meet agronomic requirements: cv. Maja (early maturing), cv. Oskar (mid-maturing) and Zelena dolina (late-maturing genotype).

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