

# COMMON VETCH (*VICIA SATIVA*) MULTI-PODDED MUTANTS FOR ENHANCED COMMERCIAL SEED PRODUCTION

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ALEKSANDAR MIKIĆ<sup>1\*</sup>, VOJISLAV MIHAILOVIĆ<sup>2</sup>, ĐURA KARAGIĆ<sup>2</sup>, BRANKO MILOŠEVIĆ<sup>2</sup>, DRAGAN MILIĆ<sup>2</sup>, SANJA VASILJEVIĆ<sup>2</sup>, SNEŽANA KATANSKI<sup>2</sup>, DALIBOR ŽIVANOV<sup>2</sup>

<sup>1</sup> Novi Sad, Serbia;<sup>2</sup> Institute of Field and Vegetable Crops, Forage Crops Department, Novi Sad, Serbia;

\* ✉ aleksandar.mikich@gmail.com

МНОГОБОБОВЫЕ МУТАНТЫ ВИКИ ПОСЕВНОЙ (*VICIA SATIVA*) ДЛЯ ПЕРЕДОВОГО КОММЕРЧЕСКОГО СЕМЕНОВОДСТВА

АЛЕКСАНДР МИКИЧ<sup>1\*</sup>, ВОЙИСЛАВ МИХАИЛОВИЧ<sup>2</sup>, ДЖЮРА КАРАГИЧ<sup>2</sup>, БРАНКО МИЛОШЕВИЧ<sup>2</sup>, ДРАГАН МИЛИЧ<sup>2</sup>, САНЬЯ ВАСИЛЬЕВИЧ<sup>2</sup>, СНЕЖАНА КАТАНСКИ<sup>2</sup>, ДАЛИБОР ЖИВАНОВ<sup>2</sup>

<sup>1</sup> Нови-Сад, Сербия;<sup>2</sup> Институт полеводства и овощеводства, отдел кормовых культур, Нови-Сад, Сербия;

\* ✉ aleksandar.mikich@gmail.com

In comparison to other annual forage legumes, such as pea (*Pisum sativum* L.), advances in breeding vetches (*Vicia* spp.) are rather modest. One of the main obstacles in increasing the cultivation area under vetches is uncertain seed production, mostly due to their indeterminate stem growth and non-uniform maturity, with the genes controlling these important traits still unattested. In contrast, in wild populations of common vetch (*V. sativa* L.) the genes have been identified, isolated in this study, and mutant plants with more than usual two pods per node tested. Crossing these mutant genotypes with wild-type ones demonstrated that the number of pods in this vetch species is controlled by two genes, orthologs to *FN* and *FNA* in pea. If both genes are recessive, a plant will have more than two flowers per each node and, depending on not yet clarified environmental factors, more than two pods per node. Developing vetch cultivars with more than two pods per node may be one of the solutions for enhancing seed production in this crop.

**Key words:** breeding, common vetch, multi-podded mutants, seed production

По сравнению с другими однолетними кормовыми бобовыми культурами, такими как горох (*Pisum sativum* L.), достижения в селекции вики (*Vicia* spp.) довольно скромные. Одно из основных препятствий на пути увеличения посевных площадей под вику – затрудненный процесс семеноводства, обусловленный главным образом неограниченным ростом стебля и неоднородным созреванием растений, так как все еще не подтверждена роль генов, контролирующих эти важные признаки. В дикорастущих популяциях вики посевной (*V. sativa* L.) эти гены, напротив, идентифицированы и выделены, а также проведен анализ растений-мутантов, образующих более двух бобов на один узел. Скрещивание этих мутантных генотипов с обычными дикорастущими показало, что число бобов у этих двух видов контролируется двумя генами, ортологичными генам *FN* и *FNA* у гороха. Если оба гена рецессивные, растение формирует более двух цветков на каждый узел и, в зависимости от еще не проясненных факторов окружающей среды, более двух бобов на узел. Выведение сортов вики с более чем двумя бобами на узел может стать одним из путей решения проблемы семеноводства этой культуры.

**Ключевые слова:** селекция, вика посевная, многобобовые мутанты, семеноводство

## Introduction

The genus vetch (*Vicia* L.) comprises more than 100 species, with bitter vetch (*V. ervilia* (L.) Willd.), faba bean (*V. faba* L.), Hungarian (*V. pannonica* Crantz), common (*V. sativa* L.) and hairy (*V. villosa* Roth) vetches as the economically most important (Mihailović et al., 2006). Most cultivated vetch species originated in the Near Eastern and Mediterranean centres of diversity (Zeven and Zhukovsky, 1975). Vetches have been present in human diets since the age of Neanderthal man, as witnessed by fossilised macroremains from modern Iraq 46,000 years old (Henry et al., 2011). Bitter vetch and faba bean are considered ones of the first domesticated plant species in the world (Tanno and Willcox, 2006). It is noteworthy that the first known extraction of ancient DNA (aDNA) in the world was done from 3,200 years old charred bitter vetch seeds (Jovanović et al., 2011).

Today, faba bean is regarded almost exclusively as a grain legume crop, thus is not colloquially counted among the vetch-crops. Most cultivated vetch species are multifunctional crops

and may be used as fresh forage, forage dry matter, forage meal, grain, straw, as well as for grazing (Mihailović et al., 2004). Today, vetches are most widely grown in Turkey, Russian Federation, Spain, Ethiopia and Australia, with 90,000 ha, 75,463 ha, 75,000 ha, 73,314 ha and 48,000 ha, respectively (FAOSTAT, 2014).

In comparison to other annual legumes, such as pea (*Pisum sativum* L.) or faba bean (*V. faba*), advances in breeding common vetch are rather modest. In most European countries, it is autumn- and spring-sown landraces, mostly intercropped with cereals, that satisfy the requests by local farmers for a relatively low-input production of high-quality source of plant protein, and thus request almost no need for advanced breeding (Mikić & Mihailović 2015). In Serbia, almost all 11 registered cultivars of common vetch were developed in the Institute of Field and Vegetable Crops (IFVCNS) in Novi Sad.

One of the major problems in commercialisation of a common vetch cultivar is a reliable seed production (Karagić et al., 2009). The aim of this preliminary research was to assess the possibility to solve this important issue by introducing multi-podded common vetch mutants in breeding programmes.

**Таблица 1.** Средняя урожайность кормового сухого вещества и семян у трех отдельных линий вики посевной в связи с числом цветков/бобов на узел в условиях Римски Шанчеви, 2011–2013 гг.**Table 1.** Average forage dry matter and seed yields in three distinct common vetch lines regarding the number of flowers/pods per node at Rimski Šančevi from 2011 to 2013

Line	Genotype	Phenotype (number of flowers/pods per node)	Forage dry matter yield (t/ha <sup>-1</sup> )	Seed yield (kg/ha <sup>-1</sup> )
NS 08/12	<i>FAFA FNAFNA</i>	1	8.5	1560
NS 07/03	<i>FAFA fnafna</i>	2	8.0	1605
VSMP 04	<i>fafa fnafna</i>	3, 4	6.2	2327
<i>LSD</i> <sub>0.05</sub>			0.8	353

### Methods

A small-plot trial was carried out at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, 19°51' N, 45°20' E and 84 m asl, in the vicinity of Novi Sad, at a slightly carbonated chernozem (syn. castanosol) soil, from 2011 to 2013. It included three common vetch lines, individually selected from wild common vetch populations in the vicinity of Novi Sad, with different genetic control of the number of flowers/pods per node, namely NS 08/12, NS 08/09 and VSMP 04, with three and/or four pods per node. In all three years, they were sown in early March, as a randomised block design and with a plot size of 5 m<sup>2</sup> and six replicates, three of which were cut in full bloom and served for determining forage dry matter yield (t/ha<sup>-1</sup>), while the remaining three were harvested in full maturity of first pods and used for measuring seed yield (kg/ha<sup>-1</sup>). The obtained results were processed by ANOVA using the software STATISTICA 10 with the *t*-test applied.

In parallel, in 2009, all three lines were included in a full diallel hybridisation scheme (Table 1) in a glasshouse and a subsequent multiplication of the hybrid progenies in the field conditions, in order to understand the mode of inheritance of the flower/node number in common vetch. Since there were no available references on common vetch genetics, it was assumed that this trait could be under a similar control as in pea, that is, by two independent genes, *FN* and *FNA*, which interaction produces four genotypes and three genotypes (Sinjushin, 2013): *FNFN FNAFNA* has one flower/pod per node, *FNFN fnafna* and *fnfn FNAFNA* have two flowers/pods per node and *fnfn fnafna* have three, four or more flowers/pods per node. In other words, it was presumed that the genetic structure of the lines NS 08/12, NS 08/09 and VSMP 04 was *FNFN FNAFNA*,

*FNFN fnafna* or *fnfn FNAFNA* and *fnfn fnafna*, respectively. These hypotheses were evaluated by applying  $\chi^2$ -test.

### Results and Discussion

**Forage and seed yields.** There were significant differences in both forage dry matter yield and seed yield among the three tested lines of common vetch at a level of 0.05 (Table 1). The lines with one and two flowers/pods per node, NS 08/12 and NS 07/03, had significantly higher three-year values of forage dry matter yield (8.5 t/ha<sup>-1</sup> and 8.0 t/ha<sup>-1</sup>) in comparison to the line with three and more flowers/pods per node, VSMP 04 (6.2 t/ha<sup>-1</sup>). The forage dry matter yields in all three cultivars were lower than in a previously carried out study comprising more common vetch genotypes in the same environment, with an average forage dry matter yield of 8.8 t/ha<sup>-1</sup> (Mikić et al. 2014). On the other hand, VSMP 04 had significantly higher three-year values of seed yield (2327 kg/ha<sup>-1</sup>) than NS 08/12 and NS 07/03 (1560 kg/ha<sup>-1</sup> and 1605 kg/ha<sup>-1</sup>). The line VSMP also had much higher average seed yield than the average of a trial including a larger number of common vetch accessions of diverse origin and status in the same agroecological conditions, with 1744 kg/ha<sup>-1</sup> (Mikić et al. 2013).

The hybridisation among the three distinct common vetch lines was carried out according to the full diallel scheme in a glasshouse in 2011, with 30 crosses per combination, with the *F*<sub>1</sub> and *F*<sub>2</sub> plants grown in the field conditions during 2012 and 2013, respectively (Table 2).

The results of the applied  $\chi^2$ -test show a high probability that the number of flowers/pods in common vetch is controlled by two independent genes, orthologs to the genes *FN* and *FNA* in pea, a botanically very close species (Tables 3, 4 and 5).

**Таблица 2.** Результаты гибридизации трех отдельных линий вики посевной в связи с числом цветков/бобов на узел в условиях теплицы (2011) и у поколений *F*<sub>1</sub> и *F*<sub>2</sub>, выращенных в полевых условиях в Римски Шанчеви (2012–2013 гг.).**Table 2.** Results of the hybridisation of three distinct common vetch lines regarding number of flowers/pods per node in a glasshouse in 2011 and with *F*<sub>1</sub> and *F*<sub>2</sub> progenies grown in the field conditions at Rimski Šančevi in 2012 to 2013

Crosses (♀ × ♂)	Number of crosses	Number of <i>F</i> <sub>1</sub> pods	Number of <i>F</i> <sub>1</sub> seeds	Number of <i>F</i> <sub>1</sub> plants	Number of <i>F</i> <sub>2</sub> seeds	Number of <i>F</i> <sub>2</sub> plants
NS 08/12 × NS 07/03	30	26	132	117	705	663
NS 07/03 × NS 08/12	30	25	123	113	566	543
Average	30	26	128	115	635	603
NS 08/12 × VSMP 04	30	18	72	55	274	233
VSMP 04 × NS 08/12	30	16	80	62	370	303
Average	30	17	76	58	322	268
NS 07/03 × VSMP 04	30	22	88	81	486	432
VSMP 04 × NS 07/03	30	24	120	113	564	513
Average	30	23	104	97	525	473

**Таблица 3. Результаты тестирования критерия хи-квадрат ( $\chi^2$ ) у потомства линий NS 08/12 × NS 07/03, предположительно относящихся к генотипам *FNFN FNAFNA* и *FNFN fnafna***

**Table 3. The results of a  $\chi^2$ -test of the progenies between the lines NS 08/12 × NS 07/03, with assumed genotypes of *FNFN FNAFNA* and *FNFN fnafna***

Parental lines	<i>FNFN FNAFNA</i>	<i>FNFN fnafna</i>
Gametes	<i>FN FNA</i>	<i>FN fna</i>
F <sub>1</sub>	<i>FNFN FNAfna</i>	
F <sub>2</sub>	<i>FN FNA</i>	<i>FN fna</i>
<i>FN FNA</i>	<i>FNFN FNAFNA</i>	<i>FNFN FNAfna</i>
<i>FN fna</i>	<i>FNFN FNAfna</i>	<i>FNFN fnafna</i>
Phenotype	<i>FN FNA</i>	<i>FN fna</i>
e	455	148
t	452.25	150.75
d = e - t	2.75	-2.75
d <sup>2</sup>	7.5625	7.5625
d <sup>2</sup> / t	0.016721946	0.050165837
$\chi^2$	0.066887783	
P	80%	

**Note:** e – real number of the plants with a specific phenotype; t – theoretical number of the plants with a specific phenotype; P – probability according to the tables of the  $\chi^2$  distribution

The  $\chi^2$ -test in the case of the hybrid progeny of the lines NS08/12 × NS07/03 revealed that the F<sub>2</sub> generation comprised two phenotypes, identical to those of the parental lines, that is, with one and two flowers/pods per node, at a ratio of 3: 1 and with a probability of 80% (Table 3).

In the F<sub>2</sub> hybrid progeny of the lines NS08/12 × VSMP 04, there were all three possible phenotypes regarding the number of flower/pods per node, namely one, two and three or more, at a ratio of 9: 6: 1 and with a probability of 75% (Table 4).

Similarly to the results of the applied  $\chi^2$ -test of the lines NS08/12 × NS07/03, the F<sub>2</sub> generation of the crossings between the lines NS07/03 × VSMP 04 comprised two phenotypes, identical to the parental line ones, that is, with two and three or more flowers/pods per node, at a ratio of 3: 1 and with a prob-

ability of 85% (Table 5).

The summarised results of the applied  $\chi^2$ -test confirm that the flower/pod number per node in common vetch is inherited in a similar way to the one present in pea, that is, by means of two independent genes, here tentatively designated as *FN* and *FNA* in the same fashion like their orthologs in pea. Generally, it is regarded that the lines with more than two flowers/pods per node in pea are not promising for a wider use in production, since this trait negatively affects several other important agronomic characteristics, especially the chemical composition of grain. For this reason, developing pea cultivars with more than two flowers/pods per node, despite few opposite viewpoints (Puzio-Idźkowska, 1998), is largely abandoned.

**Таблица 4. Результаты тестирования критерия хи-квадрат ( $\chi^2$ ) у потомства линий NS 08/12 × VSMP 04, предположительно относящихся к генотипам *FNFN FNAFNA* и *fnfn fnafna***

**Table 4. The results of a  $\chi^2$ -test of the progenies between the lines NS 08/12 × VSMP 04, with assumed genotypes of *FNFN FNAFNA* and *fnfn fnafna***

Parental lines	<i>FNFN FNAFNA</i>	<i>fnfn fnafna</i>		
Gametes	<i>FN FNA</i>	<i>fn fna</i>		
F <sub>1</sub>	<i>fnfn FNAfna</i>			
F <sub>2</sub>	<i>FN FNA</i>	<i>FN fna</i>	<i>fn FNA</i>	<i>fn fna</i>
<i>FN FNA</i>	<i>FNFN FNAFNA</i>	<i>FNFN FNAfna</i>	<i>FNfn FNAFNA</i>	<i>FNfn FNAfna</i>
<i>FN fna</i>	<i>FNFN FNAfna</i>	<i>FNFN fnafna</i>	<i>FNfn FNAfna</i>	<i>FNfn fnafna</i>
<i>fn FNA</i>	<i>FNfn FNAFNA</i>	<i>FNfn FNAfna</i>	<i>fnfn FNAFNA</i>	<i>fnfn FNAfna</i>
<i>fn fna</i>	<i>FNfn FNAfna</i>	<i>FNfn fnafna</i>	<i>fnfn FNAfna</i>	<i>fnfn fnafna</i>
Phenotype	<i>FN FNA</i>	<i>FN fna</i>	<i>fn fna</i>	
e	153	99	16	
t	150.75	100.5	16.75	
d = e - t	2.25	-1.5	-0.75	
d <sup>2</sup>	5.0625	2.25	0.5625	
d <sup>2</sup> / t	0.03358209	0.02238806	0.03358209	
$\chi^2$	0.089552239			
P	75%			

**Note:** e – real number of the plants with a specific phenotype; t – theoretical number of the plants with a specific phenotype; P – probability according to the tables of the  $\chi^2$  distribution

**Таблица 5.** Результаты тестирования критерия хи-квадрат ( $\chi^2$ ) у потомства линий NS 07/03 × VSMP 04, предположительно относящихся к генотипам *FNfn fnafna* и *fnfn fnafna***Table 5.** The results of a  $\chi^2$ -test of the progenies between the lines NS 07/03 × VSMP 04, with assumed genotypes of *FNfn fnafna* and *fnfn fnafna*

Parental lines	<i>FNfn fnafna</i>	<i>fnfn fnafna</i>
Gametes	<i>FN fna</i>	<i>fn fna</i>
$F_1$	<i>FNfn fnafna</i>	
$F_2$	<i>FN fna</i>	<i>fn fna</i>
<i>FN fna</i>	<i>FNfn fnafna</i>	<i>FNfn fnafna</i>
<i>fn fna</i>	<i>FNfn fnafna</i>	<i>fnfn fnafna</i>
Phenotype	<i>FN fna</i>	<i>fn fna</i>
e	357	116
t	354.75	118.25
d = e - t	2.25	-2.25
d <sup>2</sup>	5.0625	5.0625
d <sup>2</sup> / t	0.014270613	0.042811839
$\chi^2$	0.057082452	
P	85%	

**Note:** e – real number of the plants with a specific phenotype; t – theoretical number of the plants with a specific phenotype; P – probability according to the tables of the  $\chi^2$  distribution

### Conclusion

Breeding common vetches for forage production is aimed at increasing the crop's yield, improving its chemical composition and enhancing its seed production to an economically satisfactory extent. Thus, unlike in pea, developing common vetch cultivars with more than two pods per node may increase seed

yield, especially if, as demonstrated in this research, it is possible to introduce this trait into the typical forage cultivars by simple crossing and thus merge reliable forage and seed yields.

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