

Do mutations modifying the leaf area (*nr3*) and the number of potential seeds (*dfc*) influence photosynthetic gas exchange characteristics in common buckwheat *Fagopyrum esculentum* Moench?

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Do mutations modifying the leaf area (*nr3*) and the number of potential seeds (*dfc*) influence photosynthetic gas exchange characteristics in common buckwheat *Fagopyrum esculentum* Moench?

Abstract: Contemporary buckwheat breeding in Russia is based mainly on a Mendelian mutation *det*. Some additional mutations are being considered for inclusion in buckwheat breeding programs. Among them are the *nr3* (narrow leaf 3) and *dfc* (determinate floret cluster). We evaluated the effects of the mutations on both the characteristics of photosynthetic gas exchange and the number of seeds per plant. The *nr3* reduces the leaf surface area by 1.4 times. The mutant plants show some compensatory increase in photosynthesis rate, which, however, is not enough to reach the level of the source ability as in the wild type since the number of seeds per plant is significantly decreased. The possibility of using this mutation in buckwheat breeding depends on the accumulation of modifiers that increase either leaf size or photosynthesis rate. The reduced number of flowers of the *dfs* mutation is compensated by an increase in flower fertility, and the number of seeds per plant does not change compared to the wild type. It explains the absence of differences between the *dfs* and wild type in terms of the photosynthesis rate. This experiment did not reveal any problems for using the *dfc* mutation in breeding. In general, the results of the work support the photosynthesis rate in buckwheat is regulated based on the source-sink ratio.

Key words: common buckwheat, photosynthesis, leaf area, source-sink ratio, breeding

Ali mutaciji, ki spreminjata listno površino (*nr3*) in število potencialnih semen (*dfc*) vplivata na značilnosti fotosintezne izmenjave plinov pri navadni ajdi (*Fagopyrum esculentum* Moench)?

Izvleček: Sodobno žlahtnjenje ajde v Rusiji temelji v glavnem na Mendlovi mutaciji *det* a so bile za vključitev v žlahtniteljske programme predlagane še dodatne mutacije. Med njimi sta mutaciji *nr3* (ozki listi 3) in *dfc* (determinantno socvetje). V raziskavi smo ovrednotili vplive obeh mutacij na značilnosti fotosintezne izmenjave plinov in na število semen na rastlino. Mutacija *nr3* zmanjša listno površino za 1,4 krat. Mutantne rastline kažejo nekatere kompenzacijske mehanizme v velikosti fotosinteze, ki pa ne zadoščajo za doseganje ravni pri divjem tipu, kar kaže značilno zmanjšanje števila semen na rastlino. Možnost uporabe te mutacije v žlahtniteljskih programih ajde je odvisna na kopičenju sprememb, ki povečujejo listno površino ali velikost fotosinteze. Zmanjšano število cvetov pri mutaciji *dfs* je kompenzirano s povečanjem plodnosti cvetov, pri čemer število semen na rastlino ni spremenjeno v primerjavi z divjim tipom. To razloži tudi odsotnost razlike v velikosti fotosinteze med *dfs* in divjim tipom. V poskusu tudi ni bilo ugotovljenih nobenih problemov v uporabi *fc* mutacije pri žlahtnjenju. Na splošno rezultati raziskave kažejo, da je velikost fotosinteze pri navadni ajdi uravnavana z razmerjem vir : ponor.

Ključne besede: navadna ajda, fotosinteza, listna površina, razmerje vir-ponor, žlahtnjenje

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1 INTRODUCTION

Common buckwheat, *Fagopyrum esculentum* Moench, is a grain and groats crop widespread throughout Eurasia (Kreft et al., 2003; Fesenko et al., 2006). Since the 1960s, buckwheat breeding in Russia has been based on mutations that are sometimes accumulated in populations not affected by scientifically based selection, i.e. approved by natural selection (Fesenko, 1983; Fesenko et al., 2006). For example, *det*-mutation causing determinate growth habit (Fesenko, 1968; Ohnishi, 1990) is a core of most contemporary Russian buckwheat varieties (Fesenko & Fesenko, 2019). The use of the *det* mutation made a local green revolution since the mass distribution of the determinate varieties doubled the average yield of buckwheat in Russia (Fesenko & Fesenko, 2019). An assessment of the groups of both determinate and indeterminate buckwheat varieties according to the intensity of photosynthesis revealed the advantage of the determinate ones at a stage of mass seed filling (Amelin et al., 2020). However, analysis of the effect of *det*-mutation *per se* using a segregated hybrid population did not reveal a significant difference with the wild type, i.e. indeterminate one. On the one hand, it clarifies the role of the *det*-mutation in control of the photosynthesis intensity is not entirely clear. But it is evident that on its background, some other complexes of genes are formed, including ones determining the photosynthesis characteristics (Amelin et al., 2020).

At present, some additional mutations are being considered for inclusion in buckwheat breeding programs. Two of them are *dfc* and *nr3* mutations (Figure 1). One of the significant aspects of the effects of the mutations on plants in the context of their breeding application is their influence on the characteristics of photosynthesis. The *nr3* reduces the leaves area surface, and is considered as the basis for creating varieties with reduced self-shading. At the same time, reducing leaf area changes the source potential (using the terminology of photosynthesis researchers). The *dfc* mutation drastically, by 4-5 times, reduces the number of flowers in an inflorescence (Fesenko et al., 2010). It can change the sink potential (i.e. demand for assimilates), firstly by reducing the assimilates demand for flower production, and secondly by reducing the fruiting potential. However, the latter can be leveled by increasing the fertility of flowers. So, the mutations can affect the source-sink ratio, which is the key to regulating the intensity of photosynthesis

(Paul et al., 2001; McCormick et al., 2008; Katoh et al., 2015).

An objective of this work was to evaluate both the characteristics of photosynthetic gas exchange (which reveal the source ability) and the number of seeds per plant (which shows the sinking ability) of *dfc* and *nr3* mutants vs wild type.

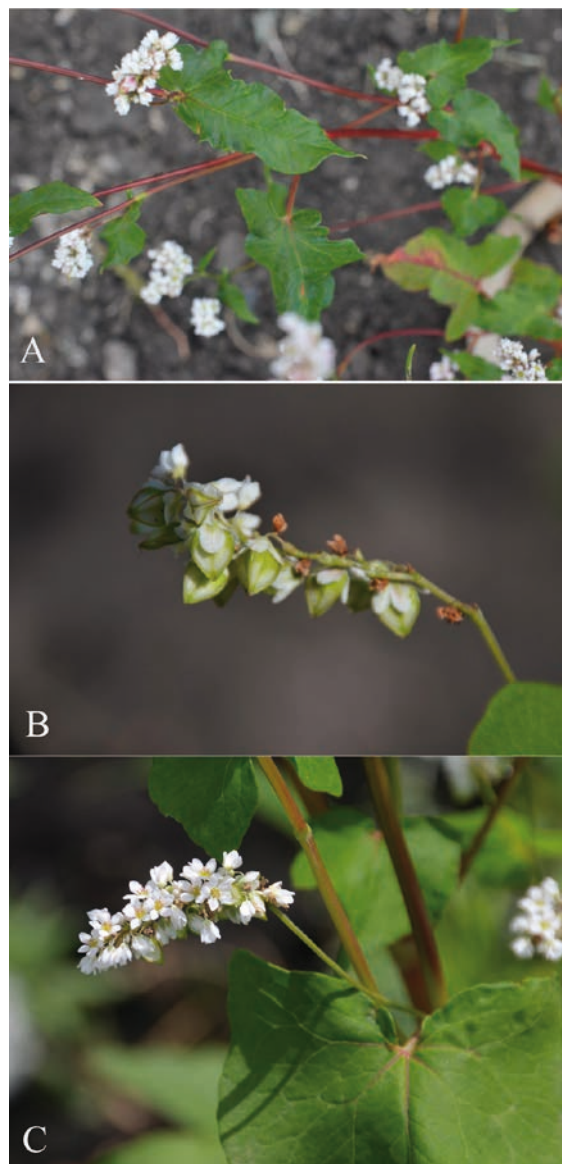


Figure 1: A) Mutant *nr3*; B) Mutant *dfc* at a stage of almost mature seeds; C) Wild type (both normal leaves and normal flower number)

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Mutants analyzed were next:

- Mutant *dfs* (determinate floret cluster) leads to a sharp, 4-5 times reduction in the number of flowers in the inflorescence. The *dfs* plants participating in crosses were determinate (genotype *det det*).

- Mutant *nr3* (narrow leaf 3) causes a change in leaf geometry due to a decrease in its width and significantly reduces the leaves' surface area of a plant. It reduces the plant's photosynthetic potential in morphological terms. The *nr3* plants participating in the work were determinate (genotype *det det*).

Both *dfs* and *nr3* mutants were isolated in the lab of buckwheat breeding, Federal Scientific Center of Legumes and Groats Crops.

To level the possible influence of some unidentified genes on the parameters of photosynthetic gas exchange F_2 hybrids 'mutant \times wild type' were used for the analyses.

As a wild type the next varieties were used:

- Dikul, a determinate common buckwheat variety bred in Federal Scientific Center of Legumes and Groats Crops. The variety was registered in 1999.

- Bogatyr, an indeterminate common buckwheat variety bred on Shatilovskaya Experimental Station (Orel region, Russia). It is the first commercial buckwheat variety in Russia which was registered in 1938.

F_2 hybrids analyzed were next:

- (1) '*dfs dfs/det det* \times Dikul'.
- (2) '*nr3 nr3/det det* \times Dikul'.
- (3) '*nr3 nr3 / det det* \times Bogatyr'.

Since Dikul is a determinate variety, the F_2 hybrids (1) and (2) manifest segregation only according to *dfs* and *nr3* alterations, respectively. F_2 hybrids with indeterminate variety Bogatyr shown expected segregation comprising four phenotypical classes (Table 1).

2.2 EXPERIMENTAL APPROACHES

The photosynthesis and transpiration intensities were evaluated on intact plants in real-time regime with a portable gas analyzer Li-COR – 6400 using the original methodology of the company Li-COR. The WUE (water use efficiency) was calculated for each plant analyzed using the formula $WUE = \text{photosynthesis rate} / \text{transpiration intensity}$.

The evaluations of photosynthesis and transpiration intensities were conducted in 2017, 2018 and 2021. All experimental plants were labeled and numbered. The

measurements within single mutant segregations (*dfs* or *nr3*) were made in order "mutant - wild type - mutant - etc" with alternation on each plant. The measurements within a segregation for the two recessives (*nr3* and *det*) were conducted in order "nr3 (non-det) - det (non-nr3) - wild type (both non-nr3 and non-det) - nr3+det - etc" with regular alternation in such order.

To measure the leaf size of F_2 hybrids 'nr3 \times Dikul' with both narrow leaves and normal leaves the largest leaf from each plant was photographed with a scale in 2021. Leaves sizes were measured on the photos using Axio Vision Software. 25 plants of both types were taken randomly.

Sowing dates were June 1 in 2017, May 23 in 2018 and May 27 in 2021. Blossom beginning dates were July 6-7 in 2017, June 26-27 in 2018 and July 1-2 in 2021. The dates of photosynthesis assessment (which are mentioned in the Table 1) fell on the period of mass filling of seeds.

The number of filled grains per plant was evaluated on August 15 in 2017, on August 14 in 2018, and on August 10 in 2021.

The significance of differences was assessed using ANOVA (Software Statistica 7).

3 RESULTS

3.1 ANALYSES OF PHOTOSYNTHETIC GAS EXCHANGE CHARACTERISTICS AND SEED PRODUCTIVITY OF INDIVIDUAL PLANTS WITHIN POPULATIONS SEGREGATED ACCORDING TO *DFC*, *NR3*, AND *NR3+DET* ALTERATIONS

3.1.1 *dfs*-mutant

The experiment revealed no differences between the mutant and non-mutant plants in photosynthesis and transpiration intensities (Table 1). Also, there were no significant differences in the number of seeds per plant between the groups of plants with normal (wt) and reduced (*dfs*) number of flowers. Apparently, it points out that at this ontogenesis stage, the demand for assimilates is mainly formed by developing seeds.

This mutation is considered the basis for creating varieties both with more simultaneous maturation and more early ripening. According to the results of the work *dfs*-mutation does not disturb the system of regulation of physiological processes associated with photosynthesis. It simplifies any application of the mutant for buckwheat breeding.

Table 1: Characteristics of mutant and non-mutant (wt) classes in segregated populations in terms of photosynthetic gas exchange and seed productivity (Mean \pm SD)

Hybrid combination (date of analysis)	Phenotype class	N	t (leaf), °C	Photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Transpiration ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Water use efficiency	Seeds per plant
F_2 (Dikul \times dfc) (July 28, 2017)	wt	42	24.7 \pm 0.62	8.32 \pm 3.85	5.84 \pm 1.73	1.40 \pm 0.56	44.87 \pm 10.31
	dfc	42		8.69 \pm 3.63	6.12 \pm 1.92	1.40 \pm 0.49	45.80 \pm 6.84
One-way ANOVA							
F_2 (Dikul \times nr3) (July 26, 2021)	wt	40	30.6 \pm 3.97	8.88 \pm 4.44	2.51 \pm 1.18	4.06 \pm 2.40	51.20 \pm 5.14
	nr3	40		11.11 \pm 4.93	2.81 \pm 1.34	4.76 \pm 3.00	46.53 \pm 6.82
One-way ANOVA							
F_2 (Dikul \times nr3) (July 12, 2018)	wt	50	24.8 \pm 0.63	12.58 \pm 4.17	2.14 \pm 0.57	6.38 \pm 4.42	42.92 \pm 4.36
	nr3	50		13.75 \pm 4.88	2.16 \pm 0.35	6.20 \pm 2.71	38.96 \pm 5.24
One-way ANOVA							
F_2 (Bogatyr \times nr3) (July 13, 2018)	wt	30	25.0 \pm 0.35	11.48 \pm 3.84	2.70 \pm 1.10	5.13 \pm 3.22	35.17 \pm 2.96
	nr3	30		10.37 \pm 3.43	2.08 \pm 0.77	6.03 \pm 4.35	35.73 \pm 2.77
	det	30		9.73 \pm 4.45	2.47 \pm 0.73	4.13 \pm 2.06	38.03 \pm 3.13
	nr3 + det	30		10.38 \pm 3.81	2.21 \pm 0.88	5.17 \pm 2.37	37.23 \pm 3.02
Two-way ANOVA							
nr3				NS	$p < 0.02$	NS	NS
det				NS	NS	NS	$p < 0.001$
nr3 \times det				NS	NS	NS	NS

3.1.2 *nr3*-mutant

This single-gene mutation makes leaves narrow and reduces their area by 1.4 times, 38.4 ± 12.2 vs 27.1 ± 7.4 (mean \pm SD). Apparently, it should cause a shift within the “source-sink” balance toward a source deficiency. A priori, there can be two ways to compensate for such a shift. The first is a decrease in the number of seeds, i.e. a decrease in sink strength. The second is an increase in the intensity of photosynthesis per leaf area unit. In the experiments, we observed both types of effects (Table 1).

In two experiments carried out in different years on the same hybrid material, the same patterns were obtained in terms of both the changes in the number of seeds per plant and the gas exchange parameters. In both cases, the number of seeds on narrow-leaved plants was significantly less compared to plants with normal leaves. Photosynthesis rate in both cases was higher for the narrow-leaved plants, on average, and in one of the two experiments, the difference was significant. Water use efficiency (WUE) tends to slight growth when photosynthesis rate is significantly higher. Thus, this mutation can be considered as a model in which the sink potential is not fully realized due to the source insufficiency.

Since this work shows the *nr3*-mutant has insufficient leaf area resulting in the source deficiency, an essential aspect of its use for breeding commercial varieties should most likely be an increase in leaf size or/and an additional increase in photosynthesis rate due to the selection of some hypothetical modifiers that can be able to compensate the effect of the mutation.

3.1.3 *nr3 + det*

We have previously reported that determinate varieties manifest higher photosynthesis rates at the seed-filling stage than indeterminate varieties. However, the *det* mutation itself does not affect the gas exchange intensity. An experiment was conducted to evaluate the joint effect of *det* and *nr3* mutations on photosynthesis parameters.

The less number of grains per plant in the experiment compared to other ones in this work is due to the old low-yielding variety with normal leaves and indeterminate growth habit Bogatyr has been used in crosses with determinate plants with narrow leaves (genotype *det det/nr3 nr3*). The *det* mutation did not affect the rates of photosynthetic gas exchange and had a certain effect on the number of seeds. However, the difference revealed was at a low level of significance (Table 1). The photosynthesis rate did not differ significantly also between *nr3* mutants and plants with normal leaves. It should be noted that *nr3* homozygotes, both determinate and in-

determinate, showed a significantly lower transpiration intensity. However, it could not be considered as a trend since in the experiments with F_2 hybrids ‘*nr3* \times ‘*Dikul*’ the differences between mutants and non-mutants in transpiration intensity were not significant (Table 1). Water use efficiency also was not significantly affected by the mutations across all the experiments.

4 DISCUSSION

There are many evidences suggesting the photosynthetic gas exchange intensity is a function of the source-sink interaction. The source potential is not realized at full capacity, and the value of photosynthesis rate per unit of leaf area can be increased if the leaves area of the plant is reduces by any way. Thus, soybean having leaves with smaller surface areas manifests a higher photosynthesis rate per unit of leaf area than those with larger leaves (Sung, Chen, 1989). Similar effect can be observed on a rice mutant *NAL1* with more narrow leaves compared wild type (Takai et al., 2013). In addition, the excision of several leaves from trees of *Eucalyptus globulus* Labill. resulted in the growth of photosynthesis rate in the remained leaves (Eyles et al., 2013).

Since photosynthesis rate usually does not reach the maximum possible values, there are some factors restricting it. Matsuda et al. (2011) discussed the hypothesis for sink-limitation suggesting the photosynthesis rate is limited by the demand for assimilates. This hypothesis was tested on two varieties of tomato and was not fully confirmed (Matsuda et al., 2011). Thus, when several fruits at the early developmental stage were removed from plants, the remaining ones became larger. On the other hand, on intact plants all the fruits were smaller. It suggests rather a lack of source ability in this case. The examples when assimilate demand was increased due to experimental manipulation also are known. So, nitrogen application can provide higher sink strength (Pissolato et al., 2019; Chen et al., 2022). Inoculation with nodule bacteria also resulted in a higher photosynthesis rate which could be explained in two ways: 1) it is able to provide additional nitrogen and 2) growing nodules requires additional assimilates (Kaschuk et al., 2012).

On potatoes it was shown the possibility to increase both source and sink ability using transgenic manipulations: it sufficiently increased the yield of starch in tubers (Jonik et al., 2012). Also, it was revealed the genetically controlled mechanisms influencing the translocation of assimilates toward developing seeds (Phung et al., 2019). In addition, it was hypothesized the buckwheat varieties with determinate growth habit manifest higher pho-

tosynthesis due to optimizing the assimilates logistics (Amelin et al., 2020).

We have analyzed the effect of well-distinguishable morphological mutations of two types on the photosynthetic gas exchange parameters. One of them, the *dfc* mutation, reduces the number of flowers within cyme (i.e., partial inflorescence) by 4-5 times. It can be assumed it forms some tendency to reduce sink potential. However, our experiment with this mutation shows the demand for assimilates is not different between non-mutant and *dfc*-mutant plants. The number of formed seeds on mutant and non-mutant plants also did not differ significantly. Thus, the increasing proportion of flowers setting seeds compensates for reducing flowers number on *dfc*-plants and allows them to form sufficient number of seeds and maintain the demand for assimilates.

Mutation *nr3* reduces leaf area by 1.4 times and increases photosynthesis rate, sometimes significantly. It can be interpreted as compensation for the decrease in leaf area. Also, it allows us to understand that in buckwheat, the physiological processes associated with photosynthesis are not at full capacity, and there are some possibilities to increase its intensity. Since the mutants typically produced fewer seeds compared to the wild type, it can be concluded that the compensatory increase in photosynthesis rate is insufficient to reach the wild type source levels.

Our experiments to assess the effects of mutations on the intensity of photosynthesis, both presented in this article and previously published, make it clear that buckwheat plants have a particular source limit per unit of leaf area which, however, is usually not reached, i.e. the source ability is not fully realized. It is due to certain limitations in the sink ability, an example of overcoming which, however, exists. This is a significant increase in the photosynthesis intensity within varieties with determinate growth habit at the stage of seed filling. The *det* mutation *per se* does not affect photosynthesis characteristics. Therefore, the higher photosynthesis rate of the determinate varieties is due to the accumulation of some additional genes, the role of which in physiology we do not yet understand (Amelin et al., 2020).

There are no commercial varieties based on the *nr3* and *dfc* mutations yet. If (or when) such varieties appear, it will be possible to evaluate their difference from varieties that do not carry these mutations, and, accordingly, it is possible to obtain additional cases of modifying the regulation of photosynthesis. This is especially true for the *nr3* mutation, which application for buckwheat breeding can be successful only with the accumulation of certain modifiers.

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