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# PHOTOSYNTHETIC CHARACTERISTICS OF MULTIFOLIOLATE ALAFALFA LEAVES

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**Abstract**

Forage productivity and quality are the most significant traits of alfalfa (*Medicago sativa* L.). The objective of that study was to compare the photosynthetic properties of some trifoliolate and multifoliolate alfalfa genotypes. Plants were selected at the Institute of Agriculture and Seed Science "Obraztsov chiflik" – Ruse. MF23 multifoliolate alfalfa line and the two trifoliolate ones with high and low productivity and standard variety Prista 2 were investigated. The studied fluorescence parameters indicate the lack of differences between low and high yield alfalfa trifoliolate populations irrespective of their different productivity, while MF23 shows enhanced PSII down-regulation, which relates to a higher potential to adjust MF23 to environmental changes. Better adjustment to the environment is one of the factors that determine higher productivity of MF23 in comparison to multifoliolate alfalfa plants studied previously.

**Key words:** multifoliolate alfalfa, photosynthesis, chlorophyll *a* fluorescence

**Introduction.** Alfalfa or Lucerne (*Medicago saliva* L.) is one of the most important species among forage crops. Forage productivity and quality of the alfalfa (*Medicago sativa* L.) leaves are the most important traits of the crop [1]. Multifoliolate (MF) alfalfa cultivars characterized by four or more leaflets per leaf rather than by three, have been marketed for greater nutritive value and intake potential than standard trifoliolate (TF) alfalfa cultivars [2, 3]. Alfalfa forage quality might be improved by increasing the proportion of leaves to stems [4, 5]. According to these authors [4, 5], the higher leaf/stem ratio in multifoliolate plants has not been consistently associated with improved forage quality. A MF genotype developed for superior herbage quality had leaf concentration, forage quality, and intake potential similar to TF genotypes. Multifoliolate alfalfa genotypes have the potential to produce higher quality herbage than some TF types.

The productivity of MF alfalfa is an object of discussion and requires improvement in the breeding practice. Herbage yield of multifoliolate populations has previously been equal to or less than that of trifoliolate cultivars under space-planted conditions [6]. The authors indicate that the slight yield reduction of multifoliolate plants might be due to inbreeding depression associated with population development. In Bulgaria the first multifoliolate alfalfa variety was created in 1999 [7] and the breeding work with native multifoliolate genotypes was conducted at the Institute of Agriculture and Seed Science "Obraztsov chiflik" – Ruse.

The productivity of plants is closely related to their photosynthetic characteristics. The objective of the study was the photosynthetic properties of some trifoliolate and multifoliolate alfalfa genotypes with different yield potential to be compared.

**Material and methods.** ***Plant materials.*** Standard Prista 2 trifoliolate variety and populations B-19 and 5R-82 with higher and lower forage productivity respectively and MF23 multifoliolate alfalfa plants were used. The plants were grown at the Institute of Agriculture and Seed Science "Obraztsov chiflik" – Ruse in 2012 without irrigation. MF23 self-pollinated line with 23–24 leaflets per leaf originating from selection of trifoliolate and multifoliolate parents with 3 to 7 leaflets [7].

***Chlorophyll a fluorescence measurements.*** In vivo modulated Chl fluorescence was measured at room temperature using a PAM fluorometer (model PAM 101–103) (H. Walz, Effeltrich, Germany).

In vivo modulated chlorophyll (Chl) fluorescence was measured by a pulse amplitude modulation fluorometer on leaf discs (H. Walz, Effeltrich, Germany, model PAM 101–103). Details about experimental protocol and data analysis are provided elsewhere [8]. The following parameters were used to calculate maximum quantum yield of PSII, $\mathrm{F_v/F_m = (F_m - F_0)/F_m}$; actual quantum yield, $\Phi_{\mathrm{PSII}} = (\mathrm{F'_m - F})/\mathrm{F'_m}$, photochemical fluorescence quenching coefficient, reflecting the rate of electron flow to intersystem electron transport chain and PS1, qP = $(F'_m - F)/F'_v$; non-photochemical fluorescence quenching, reflecting heat dissipation, NPQ = $(F_m - F'_m)/F'_m$ and light energy used neither for photochemistry nor for heat dissipation, excess, E = $(F - F'_0)/F'_m$.

***Statistics.*** Statistical analysis of the data was performed. The statistical significance between the means of each pair of the fluorescence parameters (Fig. 2) for lucerne plants was assessed using $t$-test at $p \leq 0.01$.

**Results.** MF23 line consists of 23–24 leaflets per leaf which is opposite to the normal alfalfa trifoliate leaves with 3 leaflets per leaf (Fig. 1).

Fig. 1. Trifoliate alfalfa Prista 2 variety (left) and multifoliolate form, MF23 (right)

Fluorescence measurements to probe the functionality of photosystem II (PSII) were estimated by maximal yield ($F_v/F_m$) of dark-adapted MF23 and TF plants, actual PSII quantum yield in the light, $\Phi_{PSII}$ and photochemical fluorescence quenching coefficient, qP (Fig. 2*A*).

There was no significant difference in the Fv/Fm ratio among MF23 and TF leaves, while all of the studied accessions were characterized by a decrease in both photochemical parameters, $\Phi_{PSII}$ and qP, in comparison to standard variety,

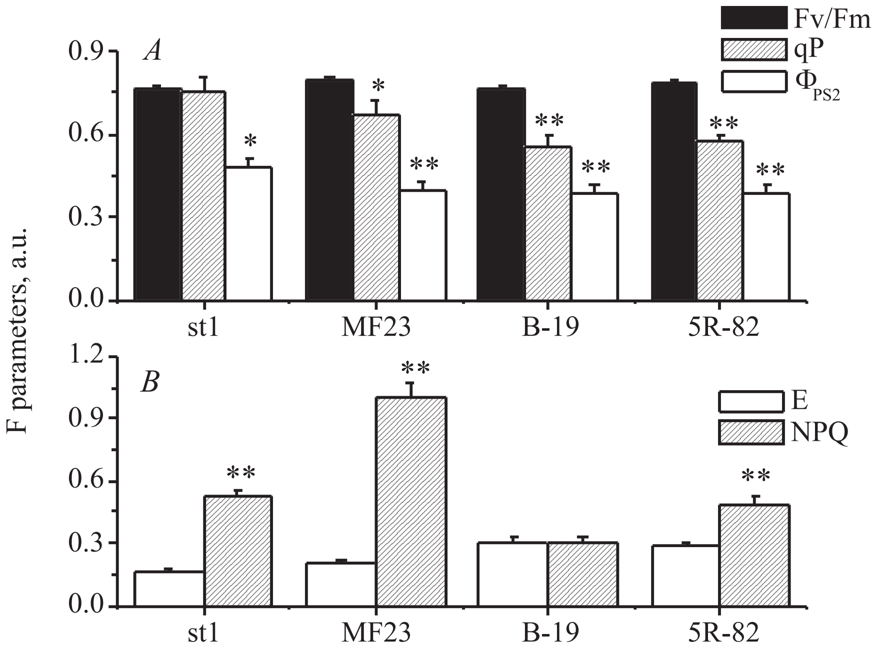


Fig. 2. Photochemical ($A$) and nonphotochemical and excess ($B$) fluorescence, F, parameters of trifoliate standard (st1) variety Prista 2 and populations (high yield, B-19 and low yield, 5R-82) and multifoliolate (MF23) lines. The fluorescence parameters determining PSII efficiency are shown: ($A$) maximal PSII efficiency in dark-adapted leaves, $F_v/F_m$, photochemical fluorescence quenching, qP, and actual PSII quantum yield in illuminated leaves, $\Phi_{PSII}$; ($B$) light energy neither used for photochemistry nor for heat dissipation – excess, E, and dissipation of light non-radiatively as nonphotochemical fluorescence quenching, NPQ. Means ± SD ($n = 6$) are given. Student's $t$-test was carried out and samples with $p \leq 0.01$ are considered statistically significant. $^*$ means $p \leq 0.05$; $^{**}$ means $p \leq 0.01$. For details about fluorescence parameters see Material and methods

Prista 2 (Fig. 2$A$). The index of energy dissipation as thermal energy, NPQ, showed significant differences between MF23 and all TF populations. NPQ was highest in MF23 leaves (Fig. 2$B$). The excess parameter was higher in both B-19 and 5R-82 populations in comparison to standard variety, while MF23 was similar to Prista 2 (Fig. 2$B$).

**Discussion.** Unique MF genotype, MF23, was created in Bulgaria [7]. It is known that multifoliolate forms up to 11 leaflets, while MF23 consists of 23–24 leaflets per leaf (Fig. 1). The size and number of leaflets are important traits for the alfalfa crop. Inbred MF lines reveal a tendency to decrease in leaf size and increase in leaflet numbers. The MF lines often cause a decrease in plant yield: from among those lines, however, MF23 expresses normal growth and higher yields when compared to other MF genotypes. Photosynthesis is one of the main processes that enable plant production features. The fluorescence parameters studied did not show differences between low and high yield alfalfa populations, B-19 and 5R-82 respectively. MF23 line is closely related to the standard Prista 2 variety. The index of energy dissipation as thermal energy, NPQ, was used to indicate the level PSII down-regulation. NPQ was highest in MF23 leaves (Fig. 2*B*), indicating high flexibly responses to environmental changes and higher potential to avoid stress influence of deleterious environmental factors. Such characteristics could be related to the observed low level of excess fluorescence parameter that reflects the level of damage in the photosynthetic apparatus [9]. Consequently, one of the main factors determining the retained plant productivity of MF23 line should be a better adjustment of MF23 alfalfa plants to the environment.

**Conclusion.** The new selected multifoliolate MF23 form of alfalfa posseses genetic potential for future theoretical and applied studies not only in areas of breeding science for higher productivity in multifoliolate alfalfa, but should be an object for investigation of MF23 physiology.

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