

Multifoliolate Alfalfa line with 23-24 leaves on a leaf stalk

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ABSTRACT

*Increasing the ratio leaves / stems is considered as one way to improve forage quality in alfalfa (*Medicago sativa* L.). A number of studies provided evidence that multifoliolate alfalfa genotypes had the potential to produce higher quality herbage than some trifoliolate ones. Multifoliolate alfalfa phenotype MF 23 was developed on the basis of self pollination of line AD-93 (possessing 3 to 7 leaflets per leaf). MF 23 is characterized by internodes shorter 3 cm than those of the standard trifoliolate varieties and possesses 23-24 leaflets per leaf stalk.*

Key words: Alfalfa, inbreeding, and multifoliolate

Alfalfa (*Medicago sativa* L.) named also Lucerne, is one of the most important legume fodder species, which is a valuable feed for many classes of livestock, because of high protein, minerals and vitamins content. Alfalfa varieties with multifoliolate leaves (ML) are being promoted as a re source of high quality forage.

Increasing the ratio leaves / stems is one of the ways to improve the quality of forage in alfalfa and multifoliolate genotypes are considered as a potential in this respect. Breeding for high expression of multifoliolate character could be an effective way to reduce the concentrations of neutral-and acid detergent fibres in green mass, by increasing the number of leaves without reduction of dry matter yield Nestor *et al.* (1993). Alfalfa genotypes with a very high proportion of multifoliolate character (> 60 - 70%) showed significantly higher quality of the green mass. Multifoliolate varieties and germplasms with 5, 7, 9 and 13 leaves of a leaf stalk have been reported by Bingham, (1964); Bingham and Murphy, (1965); Barnes and Hanson, (1967); Ferguson and Murphy, (1973); Lensen *et al.*, (1991); Petkova *et al.* (2007). Correlation between leaves quantity and protein content was detected by Mao Pei-chun *et al.*, (2006).

There is no information with respect to the inheritance of the multifoliolate character in tetraploid alfalfa. According to Bingham and Murphy (1965) in diploid alfalfa, this trait was controlled by a recessive gene (*mf*) and two more genes that influenced its expressivity. It is known that inbreeding aims at the development of homogenous crops population and that in cross-pollinated crops it results in inbreeding depression in a number of economically important biological characters such as productivity, tolerance and resistance to biotic and abiotic stresses, nutritive quality etc.

In some cases, however, on the basis of self-pollination in cross-pollinated crops new genotypes possessing valuable biological traits have been developed. In vine, for example, large-fruited table varieties have been developed using inbreeding (Todorov, 2009). Hence, the aim of the present study was the investigation of self-pollinated progenies of AD-93 alfalfa population characterized by internodes shorter than those in trifoliolate varieties and possessing 3-5-7 leaflets on a leaf stem. The yield of AD-93 was found to be similar to that of the Bulgarian standard variety Prista 2, but exceeding it in protein content.

MATERIALS AND METHODS

This study was conducted in Bulgaria at the Institute of Agriculture and Seed Science "Obraztsov Chiflik" – Rousse situated in the North climatic region of the Danubian plane (at 43° 48' northern latitude and 26 ° 03' eastern longitudes) at 152 m above sea level. The climate is continental. The average annual rainfall amounts to 587,5 mm. Soil is leached chernozem. In 2003 self-pollination of alfalfa AD-93 plants possessing 3-5-7 leaflets on a leaf stem and characterized by intensive purple color of the inflorescence, was executed. The flowers were opened with tweezers and put under insulator for 2-3 days. Plants of the trifoliolate varieties Prista 2, Prista 3 and Prista 4, developed at the IASS, exceed in height those of AD-93. The height of trifoliolate varieties was 70-80 cm and the same trait of AD-93 is 65 cm. AD-93 plants were used as a standard, because this genotype is the initial generation, and it is considered to be multifoliolate type. All plants were grown under uncontrolled greenhouse conditions. Ten plants survived from the S₁ seeds sowed in 2004 and among them plants characterized by high expression of multifoliolate character (over 85% according to the classification of Hauptvogel and Dobias, 1992) have been selected. The plants originating from self

pollination of AD-93 have been studied during the period 2004 – 2008.

The following economically important traits have been investigated: plant height, number of stems and weight of green mass. The rate of the expressivity of the multifoliate character was determined based on the number of leaflets on a leaf stem. The same traits were determined in the parents' population that was propagated by cloning.

RESULTS AND DISCUSSION

Based on the expression rate of the trait multifoliate, the ten plants grown from the S₁ seeds have been divided into three groups. The four plants from the first group MF 23 (No 114/04, 115/04, 116/04 and 117/04) possessed 23-24 leaflets on a leaf stalk, (Figure 1 and 2). This trait was observed from 1 to 5 cuts per year and throughout the five years of investigations. The five plants (No: 1, 3, 4, 5, 6) from the second group were characterized by relatively low expression of the multifoliate trait - 5 or 7 leaflets in 20-30% of the leaf mass. One of the plants (No: 2) was trifoliate (third group). It was found that plants from the first group had short internodes (4.5 cm), purple colour of the racemes and flowering initiation taking place later than that of the control. Plants from the second group were characterized by blue-white colour of the inflorescence and flowering initiation taking place earlier than that of the control plants. In 2004 50% of all plants exceeded the standard – AD-93 in green mass yield, while the 4 multifoliate plants – (MF 23 group) were strongly depressed regarding the main biological traits (Fig. 3). During the second year increase in green mass yield in MF 23 was observed. This tendency became stronger in 2006, and more precisely in N 116/04, that exceeded the control in green mass yield and in number of stems (Fig. 4). Plant 6/04 from the second group also exceeded the control concerning these same traits (Fig. 4). During the second year one of the plants from the first group, and during the third year of investigations – four plants from the second group, died.

In 2006 and 2007 the yield of plants 6/04 (second group) and 116/04 (first group) was higher than that of the control (435 g/plant in 6/04; 335 g/plant in 116/04 and 322.2 g/plant in the control).

Significant variability (VC=56%) in plant height was observed within the self-pollinated progeny of multifoliate population AD-93: plants

5/04 and 6/04 were higher than the plants of the control line and on the contrary, control plants exceeded in height the plants from the first group (MF 23), (Fig. 5).

The correlation analysis between the three traits investigated in MF 23 plants showed low correlation ($r=+0.26$) between green mass yield and plants height; moderate ($r=+0.55$) between green mass yield and number of stems and above the average ($r=+0.61$) between the height and the number of stems.

The results presented in Table 1 showed that in the first group of self-pollinated progeny of AD-93 there was a significant percentage of plants characterized by high rate of expression of the trait 23-24 leaflets on a leaf stalk. In AD 93 the number of leaves per stem varied from 161 to 190 and the number of leaflets per stem – from 900 to 1118, while in selected plants from the S₁ progeny these characteristics varied between 229-249 and 2302-2503, respectively.

The results obtained suggest that AD-93 possessed recessive genes controlling multifoliate character and that in some of the S₁ plants, being homozygous for such gene/s, high expression of multifoliate character was observed.

Study of the self-pollinated generation (S₁) of the multifoliate population AD-93 resulted in the development of a multifoliate alfalfa line MF 23 (including 114/04, 116/04 and 117/04) possessing higher number of leaves than the multifoliate alfalfa varieties and lines described in the literature. The MF 23 plants are characterized by internodes 2.5 - 3 cm shorter than those of the standard trifoliate varieties and possess 23-24 leaflets per leaf stalk (Table 1). Green mass yield of MF 23 was found to be 4.2% lower than that of the control plants.

The comparative results for some economically important traits of MF 23 S₁ and variety Mnogolistna 1, the latter being the only one multifoliate variety in Bulgaria (Petkova, 2003), are shown in Table 2. The self-pollinated line MF 23 S₁ is 12 cm lower than Mnogolistna 1, but it has relatively higher number of internodes. The number of stems slightly surpasses Mnogolistna 1, while the number of leaves on a stem is considerably higher. These results are the base for involving MF 23 S₁ in the selection process for creating a multifoliate variety, with good productivity and high forage quality.

Table 1: Biological traits of multifoliolate alfalfa genotype MF-23

Year	AD-93- St					MF 23 -S ₁				
	Height (cm)	No. of stems (pcs)	No. of leaves / stems (pcs)	No. of leaflets / stem (pcs)	Yield (g)	Height (cm)	No. of stems (pcs)	No. of leaves / stem (pcs)	No. of leaflets / stem (pcs)	Yield (g)
2004	61.0	7.5	189.0	1118.0	56.0	36.0	8,1	229.0	2302.0	43.0
2005	86.0	21.0	163.0	1002.0	123.0	77.0	22.0	248.0	2482.0	91.0
2006	70.0	21.6	182.0	1017.0	125.0	50.0	20.3	240.0	2420.0	132.0
2007	68.0	27.4	190.0	1003.0	322.0	52.0	28.7	249.0	2503.0	307.0
2008	86.0	30.7	161.0	900.0	46.0	40.0	12.3	244.0	2368.0	78.0
Average	74.20	21.40	177.00	1008.00	134.40	51.00	18.30	242.00	2415.00	130.20
LSD(0.05)	6.40	9.20	14.00	70.00	24.50	3.00	10.30	7.00	112.00	47.00

Table 2: Comparative performance of the standard variety Mnogolistna 1 with self-pollinated line MF 23

Genotype	Height (cm)	No. of stems (pcs)	No. of internodes (pcs)	No. of leaves / stem (pcs)	Yield (g)
Mnogolistna 1- St	65	13	11	27	113
MF 23 - S ₁	53	16	11	36	90
LSD (0.05)	9.00	0.90	NS	4.0	17.00

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Fig. 1

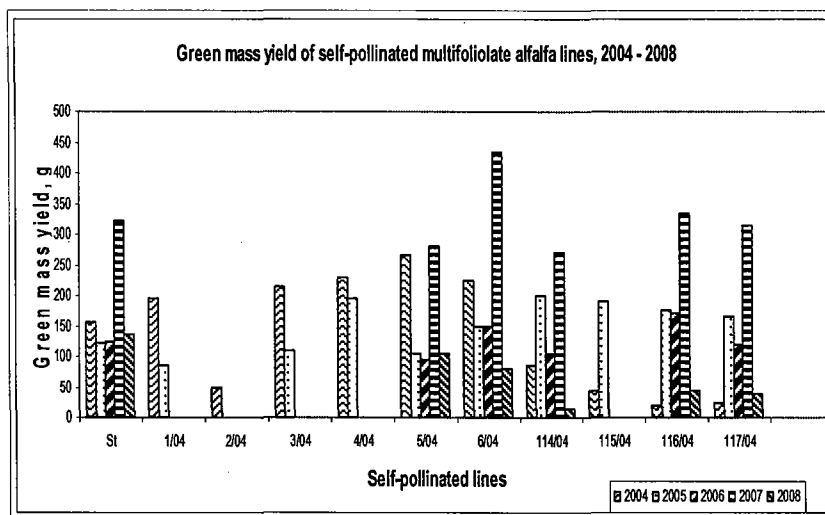


Fig. 2

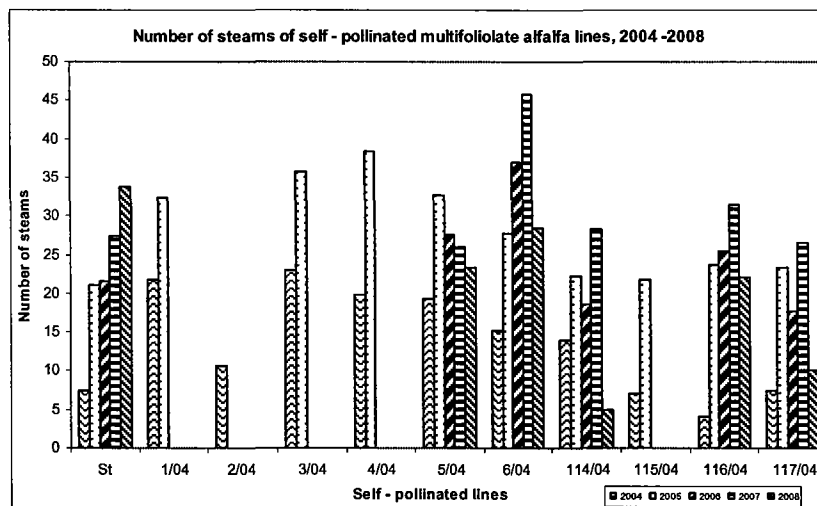
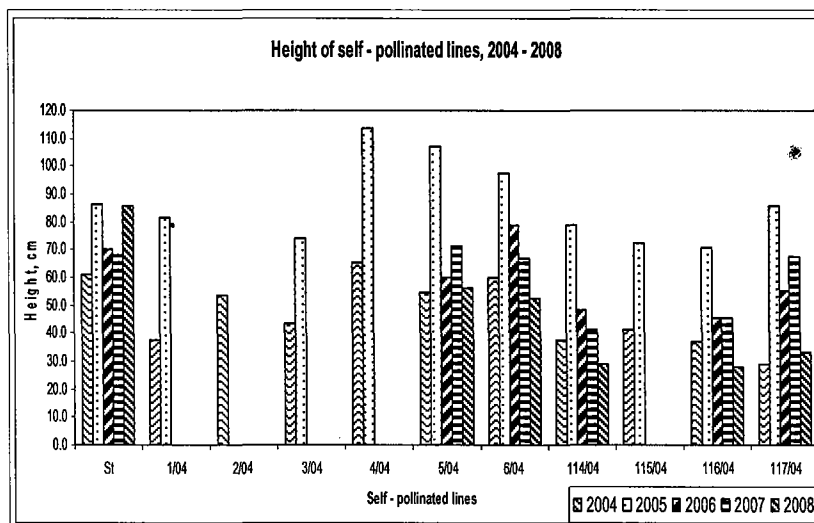


Fig.3





Plant characteristics of AD-93-St and MF23-S₁