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Evaluation of New Alfalfa Genotypes for Forage, Quality and Seed Yield Potential under Different Field Trials

Mihaela Popa[1], Maria Schitea[*], Elena Petcu[1], Eugen Petrescu[2], Șerban Cătălin Dobre[2], Victor Petcu[1, 3*]

[1]National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călărași County, Romania
[2]Craiova University, Research and Development Station for Agriculture, Caracal, Olt County, Romania
[3]Centre of Study and Research for Agroforestry Biodiversity "Acad. David Davidescu", District 5, Bucharest, Romania
[*]Corresponding authors. E-mail: mariaschitea@yahoo.com; victor.petcu@incda-fundulea.ro

ABSTRACT

Alfalfa is the main perennial forage crop in Romania. The area cultivated with this specie is more 400.000 ha, this means around 50% from the total fodder crops.

In Romania the alfalfa breeding program started in 1962, in NARDI Fundulea. From the beginning to now (2023) were developed and registered in Official Catalog 33 cultivars.

In this paper are presented the results obtained with nine alfalfa varieties, tested in two locations: NARDI Fundulea in non irrigated land and in ARDS Caracal in irrigated land during 2017-2020. Two of them were registered in the Official Catalogue in 2023. These are synthetic varieties created by the polycross method, obtained by recombination and selection from Romanian and foreign germplasm.

These are the result of selection for high feed and seed yielding, very good feed quality and good adaptability to different biotic and abiotic environmental conditions.

This alfalfa varieties are characterized by a fast growth rate in spring, a fast regeneration after cutting, a good resistance to the most common diseases spread in Romania and have a very good level of resistance to winter. They achive large fodder yield, depending on the year and the crop system, between 11.5 and 18.8 t dry matter/ha, in irrigated land and exceeding the control varieties Catinca with outyielding of 10.3-17.3%.

They are suitable for early cutting (in the budding phase), offering a very good quality feed, with a content of 20-21.5% C.P. from the dry matter and with a very good nutritive value, 72-73 digestibility coefficient. Based on the obtained results, two alfalfa varieties, from this set, were tested in the official network and were registered in 2023 under the names Petra and Monica.

Keywords: alfalfa, synthetic cultivars, green matter, forage quality, adaptability.

INTRODUCTION

The alfalfa (*Medicago sativa* L.) is one of the most important perennial forage crops in the world for livestock production systems because of its high biomass yield and good nutritiv quality, as well as desirable agricultural traits (Annicchiarico, 2015; Putnam, 2021). The alfalfa crop has a number of advantages: it is a perennial legume, it is exploited for 3-5 years, it achieves high fodder yield (14-20 t/ha dry matter in intensive system), it has a high crude protein content (19-21% P.B. of the dry matter) (Varga et al., 1973, 1998; Schitea, 2010), in addition, it plays a very important role in soil improvement, leaving important amounts of nitrogen fixed in the soil in a symbiotic way (Moga et al., 2007; Schitea et al., 2015).

In Romania, alfalfa is the main fodder plant. The area cultivated with this specie was between 136,300-442,000 ha in the period 1938-2020 (Schitea, 2022) and now, according to the Statistical Yearbook on 2023, alfalfa occupied 422,200 ha, this means 49.8% of the total fodder crops or 62.5% of the area with legumes and perennial forage grasses.

Taking into account these considerations, in Romania great importance is given to scientific research in the field of alfalfa, a fact that was realized at National Agricultural Research and Development Institute from Fundulea (NARDI Fundulea) through the

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existence of an intensiv breeding program, which led to the creation and registration of 33 alfalfa varieties in the period 1963-2023 (Gumaniuc et al., 1984; Gumaniuc and Varga, 1985; Varga et al., 1998; Schitea et al., 2014).

Breeding new cultivars with superior yield and forage quality with satisfactory seed yield is now one of the biggest challenges for alfalfa breeders and seed producers (Julier et al., 2000; Bolanos-Aguilar, 2002; Tucak et al., 2017, 2023) as well as for Romanian breeders from NARDI Fundulea (Schitea et al., 2007, 2020a, 2020b).

În order to develop such varieties, in the alfalfa breeding program from NARDI Fundulea the pressure of selection is made of genotypes characterized by a high dry matter accumulation rate, expressed at the phenotypic level, by a rapid regeneration and growth after mowing, with a good distribution of yield on cuttings. The longevity of the selected forms, expressed by perennity and associated with resistance to biotic and abiotic stress factors, also contributes to the total yield per cycle of exploitation, in order allows them to continue to be kept in the official catalog and hold a top place on the Romanian seed market (Gumaniuc et al., 1984; Varga et al., 1998; Schitea et al., 2014; Petcu et al., 2019), to which is added the registration of the variety Mădălina in Russia, Belarus and Ukraine, Cezara in the Republic of Moldova and Daniela and Sandra in Turkey.

Related to improving forage quality as a main objective in the alfalfa breeding program, it is taken into account that one of the important morphological characteristics, which contributes to the improvement of quality, is the proportion of leaves from total aerial part. For this purpose, work done to increase the proportion of leaves by selecting forms with normal height, but with short internodes. At the same time, the selection of forms with fistulous shoots is made market (Gumaniuc et al., 1984; Varga et al., 1998; Schitea et al., 2015, 2020b).

These morphological characteristics contribute to the improvement of the feed quality by increasing the nutritiv value and feed consumption, by high values of the crude protein content, by increasing the digestibility coefficient, the amount of net energy and the energy value.

As the alfalfa plants develop and grow, the crude protein content, which ultimately determines the quality of the forage, decreases, due to, on the one hand, the increase in the proportion of stems from the total aerial plant mass at the expense of the leaves, and on the other hand, as a result of the gradual decrease in the protein content and the sharp increase in the cellulose content, respectively the change in the carbon/nitrogen ratio in the plant (Portillo et al., 2022).

The process of synthesis and accumulation of the dry matter, respectively of the protein, is strongly influenced, both by the stage of vegetation in which the alfalfa is harvested in the first harvest, as well as the interval between the following harvests.

Although alfalfa is not primarily bred for its seed, seed yield is a prerequisite for good forage production as well as commercial exploitation of the crop. Seed yield is an important character for the market success of a new forage vegetable cultivar (Varga et al., 1998; Ualiyeva et al., 2022).

The paper presents results regarding the estimation of fodder and seed yield of some newly developed alfalfa varieties under two test centers: NARDI Fundulea (2017-2022) and ARDS Caracal in three years of experimentation (2017-2019).

MATERIAL AND METHODS

The breeding methods of alfalfa take into account the reproduction characteristics of the species, as well as the genetic characteristics. Alfalfa is a typical allogamous, autotetraploid species which has a high genetic complexity at individual and population levels due to its autotetraploidy and cross-pollinate (allogamy) traits, therefore differentiated a high level of heterozygosity, and strong inbreeding depression (Julier et al., 2000; Shi et al., 2017; Tucak et al., 2017; Ünal, 2024).

In alfalfa using the heterosis effect is possible in two ways: by creating hybrids

based on cytoplasmic androsterility (Gumaniuc et al., 1984; Wiersma, 2001; Bhandari et al., 2007) or by creating synthetic varieties, a method unanimously used in most of alfalfa breeding centers in the world.

The synthetic variety is an artificial population, resulting from the sexual multiplication during a determined number of generations, of the progenies of a multiple natural crossing, between a certain number of constituents (clones, lines, families) selected for certain characteristics (Gallais, 1990).

In the present, the most useful methods to improve alfalfa cultivars is recurrent phenotypic selection and aims to collect frequently desired genes in the population (Li and Brummer, 2012; Shi et al., 2017).

In the alfalfa breeding program in NARDI Fundulea there are several phases involved in developed an new alfalfa variety, which include:

• the study and creation of the initial material (inbred lines, hybrids);

• the selection of valuable forms during two selection cycles;

• determining the general combinative capacity;

• verification of the effect of the selection.

The results obtained between 2017-2019, with the new alfalfa genotypes created within the framework of the alfalfa breeding program at the Department of Forage Crops Breeding at the NARDI Fundulea in Romania for fresh and dry forage yield, different traits, quality and seeds yield are presented in this paper.

The experiments for testing the forage yield capacity were carried out in two different geographical areas in Romania at NARDI Fundulea under not irrigated and at ARDS Caracal under irrigated conditions, in a field trial crop, with ten alfalfa varieties developed in Fundulea. The experiment was in four repetitions, arranged according to the method of randomized blocks. Depending of the year, two-four cuts were harvested and the surface of the harvestable plot was 15 m^2 in Fundulea and 10 m^2, in Caracal.

The sowing was performed a depth of 1.5-2 cm, with a planting rate of 1000 germinating seeds per square meter, in Fundulea and 1200 germinating seeds per square meter, in Caracal, in dens crop. The new alfalfa cultivars were tested in order to determining seed yield with 250 germinating seeds per square meter, in Fundulea.

At Fundulea no irrigation treatment was applied in the experiment while at Caracal it was irrigated in each year with 1-4 irrigations.

The weeds and pests were controlled using recommended pesticides when necessary.

Fresh forage yield was measured by cutting the whole plot area to a 5 cm stubble height using a forage plot harvester with an electronic weigh system (Haldrup). Plants were harvested at the first cut in the late budding or in beginning of flowering stage. Immediately before cutting, subsamples of 500 g of green matter were taken from the middle of each plot, weighed fresh, dried in a dryer at 105°C for 48 h and weighed dry to determine dry matter content in order to calculate dry matter yield. The yield of green and dry matter are presented over the three years of testing.

The main traits of the alfalfa varieties tested in the field trial were the onset of vegetation, vigor, fall dormancy, winter resistance, growth rate, regeneration after mowing (by grades of appreciation from 1 to 9, with 1 for starts very quickly in the vegetation and 9 for starts very hard in the vegetation) and plant height (cm), the leaf/stem ratio (%) as well as the number of internodes.

Samples for quality analyzes were collected at the beginning of flowering. Crude protein (CP %) was obtained multiplying the N content obtained by micro-Kjeldahl analysis by 6.25. Neutral detergent fiber (NDF, %) and acid detergent fiber (ADF, %) were determined by methods of Goering and Van Soest (1970), digestible organic matter (DOM, g/kg) and net energy (NE, kcal.) were determined by methods of Tilley and Terry (1963), meat fodder units (MFU) were determined by methods of Georgescu (1995).

The seed yield was determined from the second growth in the first and second year.

Climatic Conditions during the Study Period

From a climatic point of view, the three years were very different, thus in Fundulea, 779.1 mm rainfall were recorded, in the 2017 agricultural year, 200.1 mm more than the multi-annual average (578.9 mm), and in the years 2018, 2019, the amount of precipitation was lower than multi-annual average. The months of April and May 2018 were very dry, only 2.4 mm and 34 mm, respectively, were recorded, which affected the level of production and its quality during the first and second cutting (Table 1).

On the other hand, in Caracal in 2017, only 492 mm were recorded compared to the multi-year average of 541.7 mm, a situation in which it was necessary to apply five irrigations.

In Caracal, the 2018 agricultural year can be considered a favorable year for the cultivation of fodder plants. From a pluviometric point of view, the rainfall fell were higher by 301.9 mm compared to the multiannual average (843.6 mm compared to 541.7 mm). Water deficit was in the months of April (10.6 mm), August (30.6 mm) and September (6.8 mm). However, in order to ensure the plants' water needs, it was necessary to apply two irrigations, one for the first mowing and the other one for the fourth mowing. From the point of view of recorded temperatures, the warmest year in Fundulea was 2019, with an average temperature of 12.9°C, compared to 10.7°C (2.2°C more then the multiannual average), and in Caracal, the years 2018 and 2019 exceeded average (11.6°C) by 1.1°C.

Table 1. The monthly rainfalls and temperatures recorded at NARDI Fundulea and ARDS Caracal during 2017-2019

Year	Month												Sum/ average	Differences (±)
	X	Xl	Xll	l	ll	lll	lV	V	Vl	Vll	Vlll	lX		
Rainfall (mm)														
NARDI Fundulea														
2016-2017	74.4	48.8	0	35.4	50.5	47.5	118.4	65.8	96.4	135.2	94.4	12.2	779.0	200.1
2017-2018	111.6	49.2	27.8	36	58.6	40.6	2.4	34	120.6	47	2.8	28.6	559.2	-19.7
2018-2019	10.8	23	43	53.8	21.4	22.4	51.4	124.2	74.6	87.4	12.6	6.2	530.8	-48.1
Multi year average	40.5	43.5	44.1	33	31.1	37.5	44.6	59	72.3	72.2	51	50.1	578.9	
ARDS Caracal														
2016-2017	65	48	14	6.8	12.4	53	54	84.8	17.6	101.4	25.4	9.6	492.0	-49.7
2017-2018	144.6	73	63.8	33.8	56.4	86.4	10.6	55.6	134.2	147.8	30.6	6.8	843.6	301.9
2018-2019	7.4	46.8	53.4	38.6	14.2	25.2	44.4	69.0	285.8	60.0	1.0	2	647.8	106.1
Multi year average	46.0	37.0	39.1	30.8	26.3	34.2	47.8	58.6	69.7	62.1	46.6	43.5	541.7	
Temperature (°C)														
NARDI Fundulea														
2016-2017	10.3	5.7	-0.3	-5.5	-0.03	8.5	10.6	16.8	22.2	23.3	23.9	19	11.29	0.59
2017-2018	11.7	7	3.6	0.78	1.58	3.29	15.8	18.7	22.6	0.78	25	19.1	10.83	0.13
2018-2019	13.4	5.2	-0.55	-1.16	3.81	9.33	11.22	22.98	23.6	22.97	24.72	19.28	12.90	2.2
Multi year average	11.2	5.1	-0.2	-2.4	-0.3	4.7	11.1	16.9	20.6	22.5	22	17.2	10.70	
ARDS Caracal														
2016-2017	10.7	5.5	0.7	-6.1	0.0	8.8	10.6	16.9	23.5	24.2	25.2	19.4	11.60	0
2017-2018	12.3	6.5	3.1	0.8	1	3.8	16.4	19.6	22.1	22.7	24.3	19.6	12.70	1.1
2018-2019	13.8	5.3	0.2	0.5	3.4	9.4	12.1	17.1	22.8	23.1	25.0	20.0	12.70	1.1
Multi year average	11.7	5.4	0.3	-1.3	0.8	6.0	12.0	17.7	21.6	22.8	23.5	18.1	11.60	

RESULTS AND DISCUSSION

Forage yield, fodder quality, resistance to biotic and abiotic environmental factors are the result of the interaction of the genotype with the environment, it being known that genes and gene systems are involved in complex traits, which depending on the genetic dosage allow their expression in the new genotypes (Rotili et al., 1999; Julier et al., 2000; Riday and Brummer, 2002). In alfalfa, winter resistance, autumn growth (fall dormancy) and the start of vegetation in the spring are generally negatively correlated.

That's why for Romania's conditions, new varieties must be created that simultaneously

have a good winter resistance and a good growth rate in the spring (Schitea et al., 2007). The new alfalfa genotypes studied had a good vigor, the fastest when starting vegetation was the F 2609-17 and F 2611-17 cultivars (2.5) and the slowest was the standard cultivar Catinca (control) with note by 2.9 (Table 2). Also, it can be highlighted a good regeneration capacity after mowing, of 1.9-2.4 and a medium to high height, between 69.6 cm (standard cultivar) and 75.9 cm (F 2612-17) (Table 2).

Table 2. The main morphophysiological properties of new alfalfa cultivars, NARDI Fundulea, average 3 years (2017-2019)

Genotype	Fall dormancy*	Vigor (notes 1-9)**	Regeneration after cutting** (notes 1-9)	Height of plants in the 2th year (cm)	Disease resistance**	leaves/stem ratio %
F 2609-17	2.3	2.5	2.3	72.2	2.0	49.8
F 2610-17	2.2	2.7	2.0	72.2	2.0	49.7
F 2608-17	2.0	2.7	2.4	74.4	2.0	49.5
F 2613-17	2.1	2.8	1.9	75.5	2.0	49.3
F 2614-17	2.1	2.7	2.1	73.6	2.2	49.2
F 2615-17	2.2	2.7	2.1	72.3	2.0	49.1
F 2616-17	2.2	2.8	2.2	72.9	2.1	49.0
F 2611-17	2.5	2.5	2.1	71	2.0	47.3
F 2612-17	2.7	2.7	2.0	75.9	2.0	47.1
Catinca (control)	2.8	2.9	2.6	69.6	2.5	46.7
Average	2.3	2.7	2.2	73.0	2.1	48.5

** Fall dormancy 1-11; 1 = very dormant, 11 = very non-dormant.*
*** Vigor notes 1-9; 1 = starts very quickly in the vegetation, 9 = starts very hard in the vegetation.*
Notes 1-9; 1 = very good, 9 = very low.

Concerning the forage yield of the new cultivars, in Fundulea in the testing period, in average on three years, the yield of green matter was between 51.9 t ha^{-1} of the control variety Catinca and 54.3 t ha^{-1} in the new alfalfa variety F-2613, which achieved a production increase of about 4.6% under non irrigated conditions (Table 3).

Table 3. Fodde yield achieved by new alfalfa cultivars developed in NARDI Fundulea during 2017-2019

Genotype	Green matter (t ha^{-1})				% from control
	2017	2018	2019	Average	
F 2613-17	30.6	58.9	73.3	54.3	104.6
F 2608-17	31.2	59.9	71.4	54.2	104.4
F 2615-17	30	60.0	71.7	53.9	103.9
F 2611-17	30.4	59.9	71.2	53.8	103.7
F 2609-17	31.2	58.1	71.8	53.7	103.5
F 2616-17	31.4	58.1	71.4	53.6	103.4
F 2614-17	30.4	59.2	70.8	53.5	103.0
F 2610-17	30.5	58.0	71.7	53.4	102.9
F 2612-17	30.8	58.8	70.3	53.3	102.7
Catinca (control)	29.8	57.8	68.1	51.9	100.0
Average	30.6	58.9	71.2	53.6	103.2
LD 5%	*1.6*	*1.8*	*2*	*1.8*	*103.1*

In terms of dry matter yield, the new alfalfa cultivar produced, in average, 7.7 t ha^{-1}, in the first year, 12.5 t ha^{-1} in the second year and 16.9 t ha^{-1} in the third year.

Good results were given by five new varieties (F 2615-17, F 2613-17, F 2611-17, F 2608-17, F 2609-17) which, produced 16.9-17.6 t ha^{-1} dry matter, in the third year of the testing period, or 12.4-12.7 t ha^{-1}, in average on three years, an increase of 4.5-6.4% compared to the control variety Catinca (Table 4).

Table 4. Forage production (dry matter) achieved by new alfalfa cultivars developed in NARDI Fundulea during 2017-2019

Genotype	Dry matter (t ha^{-1})				% from control
	2017	2018	2019	Average	
F 2615-17	8.0	**12.8**	17.2	12.7	106.4
F 2613-17	7.7	**12.6**	17.6	12.6	106.2
F 2611-17	7.8	**12.6**	17.1	12.5	105.0
F 2608-17	7.8	**12.6**	16.9	12.4	104.5
F 2609-17	7.6	**12.5**	17.2	12.4	104.5
F 2614-17	7.5	**12.6**	17.0	12.4	103.9
F 2616-17	7.8	**12.4**	16.9	12.4	103.9
F 2610-17	7.8	**12.3**	16.9	12.3	103.6
F 2612-17	7.8	**12.6**	16.6	12.3	103.6
Catinca (control)	7.4	12.3	16.0	11.9	100.0
Average	7.7	12.5	16.9	12.4	104.2
LD 5%	*0.3*	*0.5*	*0.7*	*0.5*	*4.2*

In the testing period, the new alfalfa varieties created at INCDA Fundulea responded very well at the intensification conditions. Thus tested under irrigation conditions at SCDA Caracal, in the second year of vegetation, eight of the varieties achieved between 80.5 and 91.5 t ha^{-1} green matter (Table 5), respectively, 16.2-18.5 t ha^{-1} dry matter (Table 6).

Table 5. Forage production (green mass) achieved by new alfalfa cultivars in ARDS Caracal (irrigated) during 2017-2019

Genotype	Green matter (t ha^{-1})				% from control
	2017	2018	2019	Average	
F 2613-17	56.8	81.5	89	75.8	112.7
F 2611-17	56.3	83.8	86.3	75.5	112.3
F 2608-17	58.8	91.5	74	74.8	111.3
F 2609-17	56	88.8	79.5	74.8	111.3
F 2612-17	55.8	84.3	83.5	74.5	110.9
F 2615-17	58.3	80.5	84.3	74.4	110.7
F 2610-17	55.5	85.8	78.3	73.2	108.9
F 2616-17	54.5	79	84.3	72.6	108.0
F 2614-17	54.3	83	78.3	71.9	106.9
Catinca (control)	52	76.3	73.3	67.2	100.0
Average	55.8	83.5	81.1	73.5	109.3
LD 5%	*3.5*	*4.9*	*4.7*	*4.4*	*6.5*

Table 6. Forage production (dry matter) achieved by new alfalfa cultivars in ARDS Caracal (irrigated) during 2017-2019

Genotype	Dry matter (t ha^{-1})				% from control
	2017	2018	2019	Average	
F 2613-17	11.8	16.3	18.8	15.6	117.3
F 2611-17	11.7	16.9	18.1	15.6	116.8
F 2608-17	12.2	18.5	15.2	15.3	114.8
F 2609-17	11.5	17.8	16.4	15.2	114.3
F 2612-17	11.6	16.9	17.2	15.2	114.3
F 2615-17	11.3	16.2	17.7	15.1	113.0
F 2610-17	11.4	16.9	16.3	14.9	111.5
F 2614-17	12	15.8	16.3	14.7	110.3
F 2616-17	11.1	15.5	17.5	14.7	110.3
Catinca (control)	10.4	14.8	14.8	13.3	100
Average	11.5	16.6	16.8	15.0	112.2
LD 5%	*0.6*	*0.9*	*0.9*	*0.8*	*6.0*

On the average of the second and third years of vegetation, the new alfalfa varieties achieved between 66.8 and 75.5 t ha^{-1} of green mass, respectively 13.3-15.6 t ha^{-1} of dry matter (Tables 5 and 6). The production of the control variety Catinca was thus exceeded by 8.0-12.7% at green matter and by 10.3-17.3% in the dry matter yield. This proves a very good water use capacity of the new alfalfa varieties (Table 7).

The ratio irrgated/non-irrigated the tasted cultivars were between 118.9-124.5% more than Catinca (112%) (Table 7).

We mention the fact that the potential of the alfalfa species and varieties developed in Fundulea is around 20 t ha^{-1} and more, under conditions of intensive technology (sown in autumn and optimally irrigated), and the ratio irrgated/non-irrigated exceeds the values 150-160 (Schitea et al., 2018).

The alfalfa varieties tested showed a good adaptability, the correlation between the yield of dry matter in the two different conditions being statistically ensured (r = 0.872***) at a very significantly level (Figure 1).

Table 7. Forage yield achieved by new alfalfa cultivars in NARDI Fundulea and ARDS Caracal, average 2017-2019

Genotype	Fundulea (non irrigated land) (t ha^{-1})	Caracal (irrigated land) (t ha^{-1})	Average (t ha^{-1})	% from control	Irrigated/non-irrigated ratio (%)
F 2611-17	12.5	15.6	14.1	111.5	124.5
F 2613-17	12.6	15.6	14.1	111.9	123.7
F 2612-17	12.3	15.2	13.8	109.1	123.5
F 2608-17	12.4	15.3	13.9	109.9	123.1
F 2609-17	12.4	15.2	13.8	109.5	122.5
F 2610-17	12.3	14.9	13.6	107.9	120.5
F 2615-17	12.7	15.1	13.9	110.3	118.9
F 2616-17	12.4	14.7	13.6	107.5	118.9
F 2614-17	12.4	14.7	13.6	107.5	118.9
Catinca (control)	11.9	13.3	12.6	100.0	112
Average	12.4	15	13.7	108.7	120.2

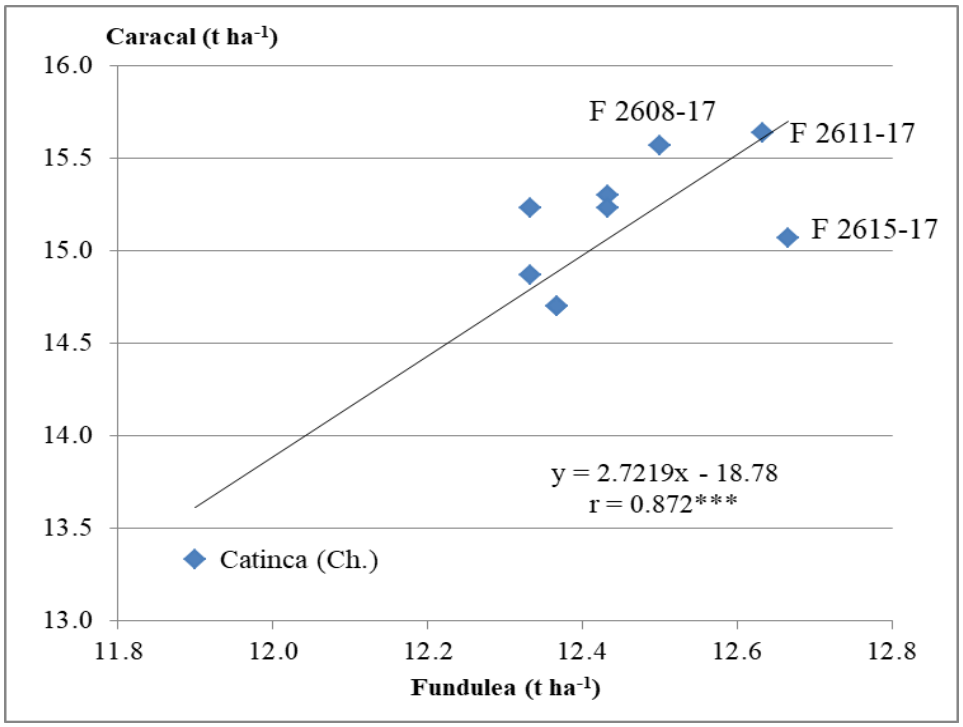

Figure 1. The relationship between the forage yield achieved by the new alfalfa varieties at Fundulea and Caracal

Concerning the content in crude protein a series of morphological traits are involved, this are the percentage of leaves and the empty stems, respectively the number of internodes per shoot. Knowing that leaves are formed at each node, a greater number of nodes, respectively short and numerous internodes, enrich the forage in leaves, thus increasing the crude protein content (Varga et al. 1998; Schitea et al., 2007). The empty shoots inside determine a low cellulose content and increase the digestibility of the forage. Concerning the content in crude protein, in Table 8 are presents the results obtained in 2017 and 2019 compared to 2018. In 2018, the drought in spring, from April and the first part of May was also found in the quality of the fodder, because there was a senescence of the plants much faster, although the first mowing took place on May 15, 2018. In this conditions, in average, the content was only 17.77% crude protein from dry matter, comparatively with 2017 and 2019, when at the second cuting were registered 21.10 and 21.86%, respectively.

In average on three years, regarding the crude protein content, most of the varieties tested were superior compared to the official witness, the Catinca variety. The best performer was the variety F 2609-17, which on the average of the scythes at budding had values between 18.21% in 2018 year and 23.15% in the year 2019, or on the average of the 3 years, 20.71%, exceeding the control by 4.5% (Table 8). In addition, new varieties F 2610-17, F 2608-17, F 2613-17, F 2614-17 had a high protein content (20.28-20.66% C.P., in average over 3 years), under the conditions that the control variety is a genotype that produces a very good fodder quality (Table 8).

Tabelul 8. Crude protein content of new synthetic alfalfa cultivars created at NARDI Fundulea in 2017-2019

Genotype	Crude protein (% from D.M.)				% from control
	2017 (second cut)	2018 (first cut)	2019 (second cut)	Average	
F 2609-17	20.76	18.21	23.15	20.71	104.5
F 2610-17	22.31	17.65	22.02	20.66	104.2
F 2608-17	22.09	18.5	21.19	20.59	103.9
F 2613-17	20.53	18.26	22.14	20.31	102.5
F 2614-17	21.00	17.59	22.24	20.28	102.3
F 2615-17	22.17	17.11	21.25	20.18	101.8
F 2616-17	20.73	17.95	21.77	20.15	101.7
F 2611-17	20.76	17.35	21.49	19.87	100.2
F 2612-17	20.76	17.11	21.67	19.85	100.1
Catinca (control)	19.87	17.93	21.66	19.82	100.0
Average	21.10	17.77	21.86	20.24	104.5

During the last two decades, improving the forage nutritive value became the main objective in alfalfa breeding program in Romania (Schitea et al., 2007).

The quality of the fodder is expressed by the crude protein and cellulose content.

Alfalfa forage consists of structural components that differ dramatically in forage quality. Leaves are much more digestible and lower in fiber than stems, and can have 2-3 times the protein. Within the cell, soluble components are 100% digestible whereas the cell wall is only partially digestible. Since the cell wall is the plant part most difficult to digest, it is the focus of chemical analysis.

NDF approximates total cell wall and ADF approximates the most difficulty-digested portions of the cell: cellulose and lignin. Total plant quality is determined to a large extent by leaf-stem components, and development of the cell wall; both are affected by plant maturity (Putnam et al., 2000). Concerning of this, new Romanian alfalfa cultivars, based on the analyzes regarding the nutritive value of the forage carried out at The National Research - Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA Balotești) (Tables 9, 10), they had NDF values between 35.85-40.04%, ADF 23.89-25.34% with 72-73% digestibility coefficient.

Figure 2. Phenotypic appearance of alfalfa plant (improved type compared to wild type) and CP, NDF and ADF content (Schitea et al., 2018)

Table 9. Fodder quality of new alfalfa cultivar developed in NARDI Fundulea

Genotype	Neutral detergent fiber (NDF)	Acid detergent fiber (ADF)	Digestibility coefficient	Organic digestibility substance	Net energy milk (NEM)	Net energy meat (ENC)	Milk nutritiv units (MNU)	Meat nutritiv units (MNU)
	%	%	%	S.O.D. g/kg D.M.	Kcal/kg S.U.	Kcal/kg S.U.	N.U.	N.U.
F 2614-17	39.33	23.89	73	662	1149	1126	0.80	0.76
F 2610-17	36.46	24.31	73	663	1165	1129	0.81	0.77
F 2615-17	37.54	24.8	73	659	1153	1116	0.80	0.76
F 2608-17	37.23	24.15	73	657	1135	1085	0.79	0.74
F 2616-17	40.04	25.08	72	654	1142	1092	0.80	0.74
F 2612-17	37.06	25.34	72	652	1140	1090	0.79	0.74
F 2613-17	38.76	25.14	72	651	1127	1090	0.79	0.74
F 2609-17	38.12	24.04	72	652	1141	1091	0.80	0.74
F 2611-17	38.68	24.9	72	651	1142	1091	0.80	0.74
Catinca (control)	35.85	24.56	73	664	1161	1124	0.81	0.76
Average	37.91	24.62	73	657	1146	1103	0.80	0.75

Table 10. Seed yield of the alfalfa cultivars, average of 2 years (2017-2018)

Genotype	Seed yield (kg ha^{-1})			% from control
	2017	2018	Average	
F 2609-17	427	493	460.0	110.7
F 2610-17	421	479	450.0	108.3
F 2616-17	416	466	441.0	106.1
F 2612-17	419	457	438.0	105.4
F 2611-17	403	462	432.5	104.1
F 2613-17	409	455	432.0	104.0
F 2614-17	392	458	425.0	102.3
F 2608-17	394	451	422.5	101.7
F 2615-17	388	451	419.5	101.0
Catinca (control)	404	427	415.5	100.0
Average	405	456	460.0	110.7
LD 5%	*20*	*25*	*22.5*	*5.4*

From the data presented in the Table 10, it can be seen that the varieties F 2609-17, F 2610-17, F 2616-17 and F 2612-16 obtained the best results in seed yield, 438-460 kg ha^{-1}. Without exception, all genotypes studied, the seed yield was superior, or equal to the control variety (Table 10).

Based on the results presented, and the UPOV descriptions, from the new alfalfa varieties tested between 2017-2019, two new varieties were promoted:

1. **Petra** (F 2626-17) from the combination F 2610-17 x F 2616-17;
2. **Monica** (F 2629-17) from the combination F 2615-17 x F 2611-17.

These were tested in Fundulea during 2020-2022, and the results obtained in the II and III years of vegetation are presented in Table 11.

Based on the results obtained in the official ISTIS network, they were registered in 2023.

The varieties Petra and Monica favorably combine properties that contribute to a high yield of fodder, 5-11% higher than the control, with a high content in crude protein, simultaneously with a high yield of seed, a fact that will allow to extend and spread them on large surfaces.

The Petra and Monica varieties have a high capacity to recover after the disappearance of the period of water stress, this being a priority in the research currently being carried out in order to reduce the effects of climate change, (Petcu et al., 2009; Schitea et al., 2014, 2018; Popa et al., 2023); this results were confirmed by testing the new alfalfa varieties, in an ecological farming system, when, in the drought conditions of 2020 in Fundulea, they appeared to be very tolerance, compared to the control, the Catinca variety.

Table 11. Production of dry matter, seed and crude protein by the new alfalfa varieties Petra and Monica, during 2021-2022 at INCDA Fundulea

Genotype	Dry matter				Crude protein			Seed yield				Pretability for ecologycal sistem
	2021	2022	Average t ha^{-1}	%	% from D.M.	C.P. kg ha^{-1}	% from control	2021	2022	Average kg ha^{-1}	% Mt.	
Monica	17.02	12.7	14.86	111.1	21.58	3207	109.7	547	590	568.5	113.7	Very good
Petra	16.22	12	14.11	105.3	22.3	3147	107.7	610	549	579.5	115.9	Very good
Catinca (control)	15.06	11.9	13.48	100.0	21.68	2922	100	500	500	500	100.0	Good
LD 5%	*0.75*	*0.59*	*0.67*	*5*	*x*	*175*	*6*	*35*	*35*	*35*	*7*	*x*

CONCLUSIONS

In the alfalfa breeding program at INCDA Fundulea, there is a rich germplasm for the complex of morphophysiological traits that contribute to the production of fodder and seed, forage quality and improving adaptation to biotic and abiotic stress factors.

The strategy of the improvement program allows a multidisciplinary approach to research problems that allowed the creation and selection of genotypes with a high capacity to exploit thermal and especially water resources, fact that is found in the production and quality of the new genotypes and implicit in the knock-on effect for other crops that come into the rotation, through the nitrogen that remains in the soil.

Alfalfa varieties created at NARDI Fundulea represents a progress of improvement works; are finalized results of selection for improved feed quality, high forage and seed yield and good adaptability to biotic and abiotic environmental conditions.

The varieties of alfalfa Petra and Monica are synthetic genotypes, developed by polycross method, registered in 2023.

They are made up of predominantly Romanian germplasm, phenotypically uniform, with superior adaptability and quality, but genetically different from one variety to another in order to prevent genetic vulnerability.

The new alfalfa cultivars achieved large fodder yield, depending on the year and the crop system, between 11 and 18 t dry matter ha^{-1},

surpassing the control varieties Daniela and Catinca with production increments of 7-12%.

They are ready for early mowing (in the budding phase), offering a very good quality fodder, with a content of 20-21.5% P.B. from the D.M. and with a very good nutritional value, 72-73 digestibility coefficient.

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