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The potential of the new synthetic variety of Iranian sainfoin (Onobrychis viciifolia Scop.)

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Abstract

Sainfoin (*Onobrychis viciifolia* Scop.) is a perennial forage legume, widely distributed in the northern temperate region and has received renewed interest in the world. This experiment was carried out to proceed from the previously-produced Syn1 generation of a new synthetic variety to the Syn2 generation. The Syn2 generation was produced and evaluated during the 2015-2019 growing seasons. In the first two years, the seeds of the Syn2 generation and composite varieties were produced in completely isolated condition. Then, the Syn2 generation along with the Composite, Sarab, and Bostan-Abad varieties were evaluated in the field conditions for three years (2017-2019). Significant differences were observed for the fresh and dry matter yield and plant height among the studied sainfoin varieties. The Synthetic variety showed the highest annual fresh forage yield (36785 kg ha⁻¹) which was significantly different from other varieties. A similar result was obtained for the annual total dry matter yield, and the Synthetic variety showed the highest dry matter yield (11715 kg ha⁻¹). The superiority of the Synthetic variety over the average of the ecotypes of Sarab and Bostan-Abad was about 25% for the dry matter and fresh forage yield. The plant height of the Synthetic variety was 83 cm, which was higher than other varieties. Among the studied varieties, no significant differences were observed for the traits related to forage quality. The protein content, total ash, crude fiber, water-soluble carbohydrates, and acid detergent fiber in the Synthetic variety were 18.52, 7.40, 19.95, 44.70, and 19.6%, respectively. The results indicated that the improved new Synthetic variety has sufficient potential to be introduced as a new cultivar.

Keywords: dry matter; forage quality; fresh forage; sainfoin; synthetic variety

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Introduction

Sainfoin (*Onobrychis viciifolia* Scop.) is defined as the king of forage crops (Okcu and Şengül 2014) due to its superior agricultural characteristics which can be adapted to different ecological regions, and higher yield and quality (Jafari *et al.* 2014). Sainfoin is usually cut and used as fresh forage or hay or pellets, while grazing is restricted to dry areas with moderate irrigation (Wilman and Asiedu 1983). In addition, a high potential for silage production has also been reported (Copani *et al.* 2016). Sainfoin may be particularly adapted to specific environments based on physiological and morphological properties or especially for animal feed because of having valuable secondary metabolites. Sainfoin may be particularly suited for alkaline and drought-prone soils (Sölter *et al.* 2007) and due to climate change it is likely to gain more importance (Trnka *et al.* 2013). Recent research has demonstrated that sainfoin can fix up to 168 kg N ha⁻¹ via symbiosis (Malisch *et al.* 2017). In addition, promising concentrations of proanthocyanidins have been found in different sainfoin accessions and cultivars (Häring *et al.* 2007; Azuhnwi *et al.* 2011; Stringano *et al.* 2012),

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which if fed in sufficient concentrations, can significantly improve animal health by reducing bloat and diminishing gastro-intestinal parasites (Wang *et al.* 2006; Azuhnwi *et al.* 2013).

Sainfoin cultivation is not widespread today. The main reasons for the poor representation of sainfoin in productive systems are its lower yield when compared to alfalfa, its low competitive ability in early establishment, limited persistence, susceptibility to waterlogging (Häring *et al.* 2008; Liu *et al.* 2010), low tolerance to frequent harvesting (Malisch *et al.* 2017), and susceptibility to diseases such as powdery mildew or phytophthora root rot (Sears *et al.* 1975).

Despite a long history of cultivation of a relatively broad range of forage legume species including medics, clovers, and vetches, today's grassland agriculture largely depends on three main legume species, alfalfa, red clover, and white clover. These are either mainly grown in monoculture or mixtures (Annicchiarico et al. 2015). As a result, plant breeding attempts and achievements were focused on these species, while sainfoin has received little attention from plant breeders and plant scientists over the past decades. The choice of appropriate cultivars may substantially influence the sainfoin yield, as the Perly cultivar achieved higher yields and suppressed weeds better in both monocropping and mixtures than other cultivars (Malisch et al. 2017).

Sainfoin is generally considered to be an allogamous species, depending on insects for pollination. Breeding improved varieties is indispensable to increase farmers' acceptance of sainfoin and to promote its cultivation. Several sainfoin cultivars have been released for use in temperate regions but genetic improvement is relatively slow due to the absence of adapted germplasm and limited breeding effort (Bhattarai *et al.* 2016).

Synthetic cultivars are common in sainfoin breeding. At the end of the breeding process, new varieties are developed by open pollination among a larger number of selected plants to form an openpollinated variety, or by intercrossing a smaller number of chosen parents (four to ten) in a polycross to build up the synthetic varieties. The resulting varieties show a large genetic diversity, evoking a generally broad environmental adaptability. Toorchi et al. (2007) reported that in Iran not much research has been done about the sainfoinand the genetic specimens of the country have been named more as their place of origin. Iran is one of the most abundant sources of plant germplasm in the world and a wide range of forage plants are scattered throughout the country. Also, Iran is one of the sainfoin distribution centers and seems to have valuable gene diversity for this forage crop.

Monirifar *et al.* (2017) evaluated the traits related to forage yield and quality of 25 sainfoin ecotypes. They observed significant differences among the ecotypes for plant height, shoot fresh and dry weight, leaf fresh and dry weight, and fresh and dry matter yield. Also, dry matter digestibility, crude fiber, and crude protein of the studied sainfoin ecotypes were significantly different. They constructed selection indices by multiplying the crude protein by the fresh forage and dry matter yield and selected four superior ecotypes to be used for the development of the Syn1 generation of the new synthetic variety. This study aimed to evaluate the performance of the Syn2 generation of this synthetic variety along with three other sainfoin varieties.

Materials and Methods

Experiment site

This experiment was conducted at the East Azarbaijan Agricultural and Natural Resources Research and Education Center in the Tikmadash station (37° 45' N, 45° 55' E, 1800 m above sea level) located 80 km east of Tabriz, Iran. According to meteorological statistics, Tikmadash and its suburbs have mild summers and cold winters and are in the semi-arid region. The absolute minimum temperature in the winter reaches 25 °C below zero and the maximum in summer reaches 32 °C. The average 10-year rainfall is 386 mm per year. The area is covered with snow and frost for more than five months of the year, and 91 percent of the surface water flows occur after the snow melts. Some chemical and physical attributes of the experimental soil are shown in Table 1.

Plant materials

The four sainfoin varieties evaluated in this study were 'Synthetic', 'Composite', 'Bostan-Abad', and 'Sarab'. Previously, the Syn1 generation of this Synthetic variety was produced from four selected ecotypes. The Syn1 generation was planted in April 2015 to obtain the seed of the Syn2 generation. In the first and second years of the experiment (2015, 2016), the seed of the Syn2 generation was produced in the isolated conditions. Sarab and Bostan-Abad ecotypes were the best local ecotypes and the Composite variety was produced from open pollination of 28 sainfoin ecotypes (Monirifar *et al.* 2017).

Experimental design

Seeds were sown in four drilled lines with a 5 m length and 25 cm distance between the lines in the sward condition, using a randomized complete block design with three replications in April 2017. The experiment was arranged as the split-split-plot design in time. Irrigation was made according to the plant's requirements. Weeds were controlled mechanically and proper fertilizers were applied based on the crop's recommendations (Bhattarai *et al.* 2016).

Traits

The first year (2017) was considered the establishment year. Thus morphological traits were only measured later in the years 2018 and 2019. Forage quality traits were measured only in 2019. Ten plants of each plot were selected and their heights (in cm) were measured.

The fresh forage yield was determined by the yield of the experimental plots after removing the margins. Plots were harvested to a stubble height of 6 cm on June 17 and August 15 in 2018 and on June 17 and August 31 in 2019. All plants were harvested by hand. The dry matter yield of varieties was determined as follows: three samples (about 500 g) were selected from each experimental plot and their fresh weight was measured. The samples were then dried in an oven at 78 °C for 48 hours and then their dry matter yield was measured. About 500 g forage of each plot was selected and leaves and stems were separated and weighted to estimate the leaf-to-stem weight ratio. The dry

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Depth	Sand	Silt	Clay	Soil	EC	pН	CaCO3	N	P	K	Zn
(cm)	(%)	(%)	(%)	texture	(ds m ⁻¹)		(%)	(%)	(mg kg ⁻¹)	(mg kg ⁻¹)	(mg kg ⁻¹)
0-35	42	32	26	Clay loam	0.34	7.6	11.6	0.8	10.5	282	1.5

Table 1. Some chemical and physical attributes of the experimental soil.

weight of the separated leaves and stems was determined after drying at 78 °C for 48 hours and the dry leaf-to-stem weight ratio was calculated.

The sainfoin whole plants harvested for the forage dry matter yield were used for determining the forage nutritive traits. Crude protein, water-soluble carbohydrates, acid detergent fiber, crude fiber, and total ash were measured using near-infrared spectroscopy in Seed and Plant Improvement Institute (SPII), Karaj, Iran. Details of the methodology and calibrations used are described by Jafari *et al.* (2014).

Data analysis

Before analysis of variance, the assumption of the normal distribution of deviations and incremental effect of block and treatments for all traits were tested and confirmed. A combined analysis of variance was performed for the plant height, fresh forage yield, dry matter yield, fresh leaf-to-stem weight ratio, and dry leaf-to-stem weight ratio. Analysis of variance was also performed for crude protein, water-soluble carbohydrates, acid detergent fiber, crude fiber, and total ash. For each trait, the means were compared using Duncan's multiple range test if the analyses of variance indicated significant differences at $p \le 0.05$.

Results

Fresh forage and dry matter yield

There were significant differences among varieties and years for the fresh forage yield and dry matter yield. The interaction of year \times harvest time was also significant for the fresh forage yield and dry matter yield. The harvest time and the interactions of variety \times year, variety \times harvest time, and variety \times year \times harvest time were not significant for the fresh forage yield and dry matter yield (Table 2).

The overall mean of the fresh forage yield and dry matter yield in 2018 was 12292 and 3930 kg/ha, and in 2019 it was 18458 and 5878 kg/ha, respectively. The highest fresh forage yield was obtained from H₁ (first harvest) in 2019, followed by H₂ in 2018, H₂ in 2019, and H₁ in 2018, respectively. Maximum fresh forage yield was achieved in 2019. Similar results were observed for the dry matter yield (Table 3).

A comparison of varieties for annual fresh forage yield and dry matter yield is presented in Table 4. The Synthetic variety showed significantly higher annual fresh forage yield and dry matter than other varieties. However, there was no significant difference among other varieties and they were grouped in one class.

Plant height

There were significant differences among varieties and years for the plant height. The harvest time and the interactions of variety \times year, variety \times harvest time, and variety \times year \times harvest time were not significant for the plant height. However, the year \times harvest time interaction was significant for this trait (Table 2). The mean of the plant height in the second year was higher than in the first year.

The overall mean of the plant height in the first and second years was 71 and 86 cm, respectively (Table 4). In this study, all sainfoin genotypes in the second year were taller, denser, and more vigorous than in 2018. The highest and lowest plant height was observed in the first cut in the second year and the first cut in the first year, respectively (Table 3). The highest plant height was observed in the Synthetic variety, followed by Bostan-Abad, Composite, and Sarab varieties (Table 4).

Leaf-to-stem ratio

The mean squares of variety, year, the interactions of variety \times year, variety \times harvest time, and variety \times year \times harvest time was not significant for

Table 2. Combined analysis of variance of morphological traits of sainfoin.

				Mean squares		
Source	df	FFY	DMY	PH	FLSR	DLSR
Block (B)	2	11275891ns	326125 ns	47 ns	0.015 ns	0.013 ns
Variety (V)	3	49925992**	4985417**	251*	0.042 ns	0.008 ns
Error 1	6	14933174	1491165	129	0.030	0.029
Year (Y)	1	456228506**	45557157**	2809**	0.003 ns	0.009 ns
$\mathbf{Y} \times \mathbf{V}$	3	6728337 ns	671863 ns	164 ns	0.055 ns	0.015 ns
Error 2	8	14933174	1491165	129	0.030	0.029
Harvest time (H)	1	3441658 ns	343677 ns	14ns	0.897**	0.010 ns
$V \times H$	3	2650028 ns	264620 ns	34 ns	0.013 ns	0.035 ns
$Y \times H$	1	1036383240**	103488982**	11569**	2.075**	0.075*
$V \times Y \times H$	3	11401910 ns	1138540 ns	67 ns	0.014ns	0.021
Error 3	16	11996474	1197922	89	0.014	0.013
CV†† (%)		18.76	18.62	10.96	14.08	17.01

ns, *, **: Not significant and significant at $p \le 0.05$ and $p \le 0.01$, respectively; FFY: Fresh forage yield; DMY: Dry matter yield; PH: Plant height; FLSR: Fresh leaf to stem weight ratio; DLSR: Dry leaf to stem weight ratio; ††CV was calculated based on Error 3.

Table 3. Mean of the sainfoin varieties for the traits measured in different years.

				2		
Year	Harvest time	FFY (kg/ha)	DMY (kg/ha)	PH (cm)	FLSR	DLSR
2018	H1	7913 d	2546 d	56 d	1.21 a	0.67 ab
	H_2	16671 b	5313 b	86 b	0.52 d	0.75 a
	Total	12292 b	3930 b	71 b	0.86	0.71
2019	H_1	23373 a	7431 a	102 a	0.77 c	0.73 a
	H_2	13544 c	4325 с	70 c	0.92 b	0.62 b
	Total	18458 a	5878 a	86 a	0.84	0.67

Means with different letters in each column are significantly different at $p \le 0.05$; FFY: Fresh forage yield; DMY: Dry matter yield; PH: Plant height; FLSR: Fresh leaf to stem weight ratio; DLSR: Dry leaf to stem weight ratio.

Table 4. Co			

	Annual total fresh forage yield	Annual total dry matter yield	PH	FLSR	DLSR
Variety	(kg/ha)	(kg/ha)	(cm)		
Synthetic	36785 a	11715 a	83 a	0.79	0.67
Sarab	29030 b	9264 b	72 b	0.81	0.68
Composite	27801 b	8875 b	77 ab	0.92	0.72
Bostan-Abad	29387 b	9376 b	80 ab	0.89	0.70

Means with different letters in each column are significantly different at $p \le 0.05$; PH: Plant height; FLSR: Fresh leaf to stem weight ratio; DLSR: Dry leaf to stem weight ratio.

the fresh leaf to stem weight ratio. However, the mean squares for harvest time and the year \times harvest time interaction were significant for this trait. For the dry leaf-to-stem weight ratio, the year \times harvest time interaction was the only significant source of variation (Table 2).

The ratio of fresh leaf-to-stem ratio in the first year was 1.021 and 0.52 and in the second year was 0.77 and 0.92, respectively. For dry leaf-to-stem ratio, the values were 0.67 and 0.75 in the first year, and 0.73 and 0.62 in the second year, respectively (Table 3). The highest value of the fresh leaf-tostem weight ratio (1.21) was observed in the first harvest in 2018 but decreased in the subsequent cu) (Table 4). The highest dry leaf to stem weight ratio was obtained from the second harvest in 2018. However, it was not significantly different from the first cuts in 2019.

Forage quality traits

There was no significant difference among sainfoin varieties for forage quality traits (Table 5). All varieties showed almost similar contents of crude protein, water-soluble carbohydrates, acid detergent fiber, crud fiber, and total ash (Table 6). source of variation (Table 2). The maximum fresh leaf-to-stem weight ratio was achieved in the first cut of the first harvesting year. The highest dry leaf-to-stem weight ratio was obtained from the second harvest time in 2018 and the first harvest time in 2019 (Table 3).

Discussion

A phenotypic variation among sainfoin accessions is valuable for developing varieties with high forage yield, high seed yield, and good nutritive value. Several studies on sainfoin revealed large variations among populations for a wide range of agronomic traits (Delgado et al. 2008; Nakhjavan et al. 2011; Mohajer et al. 2013; Zarrabian et al. 2013; Jafari et al. 2014). Delgado et al. (2008) reported significant variation among 44 Spanish sainfoin accessions that formed two distinct clusters based on regrowth rate and flower number in the seeding year. Zarrabian et al. (2013) grouped 56 sainfoin accessions (46 Iranian accessions and 10 accessions around the world) into three clusters using 13 agro-morphological traits. Nakhjavan et al. (2011) also observed wide genetic variation among 34 sainfoin populations from Iran and grouped them into four clusters based on forage dry matter yield and nutritional traits. Monirifar et al. (2017) evaluated 25 sainfoin ecotypes and found significant variation among the ecotypes for various yield-related and forage-quality traits. They selected four superior ecotypes and developed the Syn1 generation, from which we produced and evaluated the Syn2 generation in our study.

The results of this study showed that the Synthetic variety has the highest fresh forage (36785 kg/ha) and dry matter yield (11715 kg/ha), and plant height (83 cm). The superiority of the Synthetic variety for fresh forage and dry matter yield was 25% compared to the second highestyielding variety (Bostan-Abad). Increasing the fresh forage yield and dry matter yield is important in a sainfoin breeding program. Also, it has been reported that the plant height is directly related to the forage yield. In addition, by increasing the

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	Mean squares							
Source	df	СР	Ash	CF	WSC	ADF		
Block	2	0.092 ns	0.104 ns	6.834 ns	217.75 ns	1.482 ns		
Variety	3	0.512 ns	0.125 ns	4.490 ns	47.58 ns	4.692 ns		
Error	6	0.686	0.072	1.654	76.707	11.35		
CV (%)		4.6	3.6	7.0	17.7	16.5		

Table 5. Analysis of variance for forage quality traits measured on the sainfoin varieties.

ns Not significant

Table 6. Comparison of sainfoin varieties for forage quality traits.

		U			
Variety	CP (%)	ASH (%)	CF (%)	WSC (%)	ADF (%)
Synthetic	18.52	7.40	19.95	22.35	19.60
Sarab	17.99	7.43	17.68	25.24	20.26
Composite	17.52	7.04	17.73	24.12	22.14
Bostan- Abad	17.87	7.50	17.23	27.09	19.40
CD. Cando masteine A.	h. Total ash. CE. C.	ada fiham WCC. Wata	a coluble comb obriduct	an ADE: Anid datamant f	ih ou

CP: Crude protein; Ash: Total ash; CF: Crude fiber; WSC: Water soluble carbohydrates; ADF: Acid detergent fiber.

plant height, the photosynthetic capacity improves, which results in better forage performance (Carpici and Celik 2010).

The dry leaf-to-stem ratio of the Synthetic variety (0.68) was not significantly inferior to other varieties in this study and its value was very close to the second high yielding variety, the Bostan-Abad ecotype (0.70). Irani *et al.* (2015) by evaluating 30 half-sib families of sainfoin, reported an average of leaf-to-stem ratio of 1.10 in their study. A high leaf-to-stem ratio is usually desirable because leaves are more palatable and retain higher digestibility over time than stems.

There was no significant difference among the sainfoin varieties for the traits related to the forage nutritional value. Therefore, the forage quality of the Synthetic variety was comparable to other tested varieties. The crude protein, water-soluble carbohydrates, acid detergent fiber, crude fiber, and total ash of the Synthetic variety were 18.52, 22.35, 19.60, 19.95, and 7.40%, respectively.

In addition to the dry matter yield, forage quality is important for efficient animal production (Arzani et al. 2004). Higher water-soluble carbohydrates, dry matter digestibly, and crude protein percent coupled with low fiber content have higher priority for weight gain and dairy production (Wilkins and Humphreys 2003). Fresh sainfoin is also suitable for silage and watersoluble carbohydrates are important factors in its production for silage. Decreasing the percentage of soluble sugars before silage will increase the pH of the silage, thereby reducing the quality of the silage (Van Soest *et al.* 1991).

Ash percentage indicates the total nutrients in the plant and affects the quality of forage. Also, the amount of ADF affects energy or total digestible nutrients in the forage. It has a negative impact on the forage quality and its higher ratio makes it difficult for the animals to digest it (Hackmann *et al.* 2008; Kamalak *et al.* 2011, Jafari *et al.* 2014).

Mohajer *et al.* (2013) studied quality traits in 12 sainfoin populations. They reported maximum values of dry matter digestibly and crude protein in the Urmia population as 74.27% and 27.31%, respectively. Majidi and Arzani (2009) by studying 10 sainfoin populations, reported the values of 17.11%, 24.86%, and 22.28% for the crude fiber, dry matter, and crude protein, respectively.

Our results demonstrate the superiority of the

new Synthetic variety over other varieties for the fresh forage and dry matter yield, and plant height in this experiment. The superiority of the Synthetic variety over the average of two local ecotypes (Sarab, Bostan-Abad) was about 25% for the fresh forage and dry matter yield. Development of the synthetic varieties is the most effective breeding method for outcrossing perennial forage species (Vogel and Pedersen 1993).

Conclusion

Significant differences were observed among the sainfoin varieties for fresh forage and dry matter yield, and plant height. The highest annual yield of fresh forage belonged to the Synthetic variety which was significantly different from other varieties. The Synthetic variety had also the highest plant height among the four tested varieties. No significant differences were observed among the studied varieties for the measured traits related to forage quality and, therefore, the new Synthetic variety was not inferior to the Composite variety, and the Sarab and Bostan-Abad ecotypes. The results showed that the improved Synthetic variety has sufficient potential to be introduced as a new sainfoin cultivar.

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Conflict of interest

The authors declare no interest concerning authorship or publication of this article.

Availability of data and plant material

The plant material and improved variety are available.

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پتانسیل رقم جدید سنتتیک اسپرس ایرانی (.Onobrychis viciifolia Scop)

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چکیدہ

اسپرس (.Onobrychis viciifolia Scop) از لگومهای علوفهای چند ساله است که به طور گسترده در مناطق معتدل شمالی کشت میشود و اخیرا کشت و کار آن در دنیا مورد توجه قرار گرفته است. این پژوهش برای اصلاح و معرفی رقم جدید سنتیک اسپرس در مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان آذربایجان شرقی از سال ۱۳۹۵ تا سال ۱۳۹۹ اجرا شد. در دو سال اول اجرای آزمایش، واریتههای کمپوزیت و سنتیک ۲ در شرایط کاملا ایزوله تولید و سپس ارزیابی شدند. واریتههای مورد ارزیابی شامل اکوتیپهای بستان آباد و سراب و ارقام سنتیک ۲ و کمپوزیت به دست آمده از ۲۸ اکوتیپ بودند. بین واریتههای سپس ارزیابی شدند. واریتههای مورد ارزیابی شامل اکوتیپهای بستان آباد و سراب و ارقام سنتیک ۲ و کمپوزیت به دست آمده از ۲۸ اکوتیپ بودند. بین واریتههای اسپرس مورد بررسی از نظر عملکرد علوفه تر و ماده خشک اختلاف معنی دار مشاهده شد. بیشترین عملکرد سالانه علوفه تر برابر با ۲۵۸۵ کیلوگرم در هکتار متعلق به واریته سنتتیک بود که به طور معنی دار متفاوت از سایر واریتهها بود. از نظر مجموع سالانه عملکرد مالانه علوفه تر و این بایی حاصل شد و واریته سنتیک با تولید ۱۱۷۱۵ کیلوگرم ماده خشک در هر هکتار بیشترین تولید را نشان داد. برتری واریته سنتیک از نظر عملکرد علوفه تر و ماده خشک نسبت به متوسط دو اکوتیپ سراب و بستان آباد حدود ۲۵ درصد بود. میانگین ارتفاع بوته واریته سنتیک ۲۸ سانتیمتر بود که بیشتر از ارتفاع بوته سایر واریتههای مورد بررسی از نظر صفات مربوط به کیفیت علوفه اختلاف معنی داری مشاهده نشد. میزان پروتئین، خاکستر کل، فیبر خام، کربوهیدراتهای محلول در آب و فیبر نامحلول در شوینده اسیدی در واریته سنتیک به ترتیب ۱۸/۵، ۱۹/۹، ۱۹/۹۰ و ۱۹/۹ درصد بود. به طور کلی نتایچ نشان داد که واریته سنتیک اصل نامحلول در شوینده اسیدی در واریته سنتیک به ترتیب ۱۵/۵، ۱۹/۹۰، ۱۹/۹۰ و در ۱۹ درصد بود. به طور در مان در مور کلی نتایج نشان داد که واریته سنتیک اصلاح

واژههای کلیدی: علوفه تر؛ کیفیت علوفه؛ ماده خشک؛ واریته سنتتیک