

Changes in Some Parameters in Mixtures of Sainfoin with Subterranean Clover

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Abstract

Changes in some morphological and physiological parameters (leaves/stems ratio, total plastid pigments content, amount of fixed nitrogen) of sainfoin (*Onobrychis* Adans.) and subterranean clover (*Trifolium subterraneum* ssp.), pure grown and in mixtures in ratio 50:50% were studied in filed experiment in the Institute of Forage Crops, Pleven (2011-2015). Local population of sainfoin and three subspecies of subterranean clover, i.e. *Trifolium subterraneum* ssp. *brachycalicinum* (cv. Antas), *Trifolium subterraneum* ssp. *yanninicum* (cv. Trikkala) and *Trifolium subterraneum* ssp. *subterraneum* (cv. Denmark) were used. Leaves/stems ratio of sainfoin in pure stands was 0.35 and it was found be enhanced in mixtures with *Trifolium subterraneum* ssp. *brachycalicinum* (0.39) by 12.8%, and with *Trifolium subterraneum* ssp. *yanninicum* (0.38) by 9.5%. Total plastid pigments in mixtures increased by 8.3% for *Trifolium subterraneum* ssp. *yanninicum* ssp. *brachycalicinum* and *Trifolium subterraneum* ssp. *yanninicum*, and by 9.8% for *Trifolium subterraneum* ssp. *subterraneum*, and decreased in sainfoin by 17.2% for mixture with *Trifolium subterraneum* ssp. *yanninicum*. There were from 2.20 kg N/da (sainfoin + *Trifolium subterraneum* ssp. *subterraneum*) to 2.88 kg N/da (sainfoin + *Trifolium subterraneum* ssp. *brachycalicinum*) more fixed nitrogen in comparison to the amount fixed from pure grown sainfoin. Sainfoin and subterranean clover in mixtures showed good mutual tolerance, morphological and physiological status.

Keywords: Leaves/stems ratio; Plastid pigments; Sainfoin; Subterranean clover; Mixtures

Introduction

Mixed crops between legumes and grasses have an essential role in building a system of sustainable and ecologically friendly farming [1,2]. They are more effective than pure grown in using environmental resources, better withstand adverse conditions and are more productive [3]. Mixtures involved several components, relationships between which are complex and depend on many factors. On the one hand species are competitors, on the other interact positively in which many morphological and physiological parameters changed [4]. The productivity and quality of forage from mixtures depends on the physiological status of the components involved.

One of the most important factors determining productivity is the photosynthesis process by which green plants accumulate organic matter and energy [5,6]. Ingestion and transformation of solar energy is achieved by photosynthetic pigments - chlorophyll a and b, and carotenoids. A major chlorophyll is chlorophyll a and it provides higher efficiency of the conversion of carbon dioxide and of water in the organic compounds. Carotenoids also have a protective function - prevent destructive photooxidation of organic compounds of protoplasm in the presence of free oxygen [7].

Sainfoin is perennial legume crops with high protein content, palatability and high nutritional value properties [8-10]. It is suitable for pasture use as it does not cause swelling of animals due to the condensed tannins content [11-13]. It can be grown both - pure and in mixtures. Leaves are richer in mineral nutrients than stems [10] and the proportion of leaves declines to maturity because of senescence of the lower leaves [14]. Leaves/stems ratio is an important factor in determining quality, diet selection, and forage intake of sainfoin [15].

Sainfoin as a legume crops has nitrogen fixing ability and nitrogen from biological nitrogen fixation is used directly by plants [16]. Many authors [17-22] found lower amounts of fixed nitrogen compared to other legumes (white and red clover, and alfalfa). As a possible reason

they point to the fact that sainfoin needs twice larger amount of CO₂ for 1 mol N₂ compared to other legumes included in the study. In addition, sainfoin has less ability to absorb carbon, as well smaller leaf surface. The root system of sainfoin has little major branches and more fine and numerous lateral roots, where the most nodules are located [23]. In addition to this were small nodule number, low specific nodulating activity and lower effectiveness of the process of nitrogen fixation [24,25].

Subterranean clover (*Trifolium subterraneum* L.) is widespread component in pastures of temperate areas of middle and northern Europe, and America [26]. Studies with subterranean clover during last years as a component of pasture mixtures showed that this crop is suitable for the climatic conditions of Bulgaria [27-29].

The purpose of this work was to study changes in some morphological and physiological parameters, i.e. leaves/stems ratio, chlorophyll a and b, carotenoids, total plastid pigments and amount of fixed nitrogen in plants of sainfoin and subterranean clover grown pure and in mixtures with direction of use for forage.

Materials and Methods

The trial was conducted at the experimental field (43° 23'N, 24° 34'E, 230 m altitude) of the Institute of Forage Crops, Pleven, Bulgaria (2011-2015) on podzolized soil subtype without irrigation. Long plot

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method was used and size of plots was 70 m². Sainfoin (*Onobrychis Adans.*) (local population) and three subclover subspecies, i.e. *Trifolium subterraneum* spp. *brachycalicinum* (cv. Antas), *Trifolium subterraneum* spp. *yanninicum* (cv. Trikkala) and *Trifolium subterraneum* spp. *subterraneum* (cv. Denmark) were the object of study. They were pure grown (100%) and in mixtures sainfoin:subterranean clovers (50:50%) as follows: sainfoin + *Trifolium subterraneum* spp. *brachycalicinum* (50:50%); sainfoin + *Trifolium subterraneum* spp. *yanninicum* (50:50%); sainfoin + *Trifolium subterraneum* spp. *subterraneum* (50:50%). All treatments were four replicated.

The sowing was done in autumn of 2011 and sowing rates were for sainfoin 12 kg/da and for subterranean clover 2.5 kg/da. The sowing rate in mixtures was half of that in pure stands. No fertilizers and pesticides were applied during the vegetation. One cut was obtained during the first year after sowing (2012), two cuts during the second and third year (2013 и 2014) and one cut during the fourth year (2015). Samples were taken before the cutting from all replications using 0.25 m² quadrat, separated into leaves and stems and weighted (g fresh weight). Leaves/stems ratio based on fresh weight was calculated.

In fresh plant samples plastid pigments content (chlorophyll a, chlorophyll b and carotenoids) was determined according Zelenskii and Mogileva [44]. For the mixtures samples were taken from every component. Ratios chlorophyll a/chlorophyll b, chlorophyll a+b, chlorophyll a+b/carotenoids and total plastid pigments content were calculated.

A formula of Carlsson and Huss-Danell [18] was used for roughly estimation of fixed amount of nitrogen in sainfoin (pure grown and in mixtures with subterranean clover). Data were averaged and statistically processed using [30].

Results and Discussion

The period of study was characterized as an unfavorable in regard to the agro meteorological conditions. Data for mean air temperature and cumulative rainfall was shown on the Table 1.

After the sowing long dry period occur. Small quantities and unevenly distributed rainfall in May of the second year marked the beginning of early spring drought lasts one month. The period is accompanied by average monthly temperatures with values above the norm for the area. After the first ten days of July lasting drought occur and continued in August and September. The average annual temperature in the third year had higher values (12.3°C), there are no extremely high temperatures and prolonged dry periods, and rainfall (856.8 l/m²) were greatest. These conditions impacted on the overall development of sainfoin and subterranean clover.

According Mohajer et al. [31] the variation in the morphological parameters is significant. Leaves/stems ratio is an important morphological characteristic. It is associated with quality and forage intake [32]. In our study, leaves/stems ratio in sainfoin has the lowest values in the first year after sowing in pure stands (0.34) as well in mixtures (0.35-0.36) with little variation (Table 2). Slightly higher values of this ratio in mixtures with subterranean clover were not statistically proven. This is understandable because of the unfavourable meteorological conditions. Similar results obtained Kallenbach et al. [33]. The age of the crop had effect on the value of the leaves/stems ratio, too.

The leaves/stems ratio in subterranean clover in pure stands strongly varied, i.e. *Trifolium subterraneum* spp. *brachycalicinum* (0.94) and *Trifolium subterraneum* spp. *subterraneum* (0.79). Morphological

feature of *Trifolium subterraneum* spp. *subterraneum* are small in size leaves.

The leaves/stems ratio in sainfoin retain unchanged in the first year after sowing only, when the agrometeorological conditions were very unfavorable. In subsequent years of study the leaves/stems ratio had higher values in the both components of mixtures as compared to pure stands. In the second year of study the leaves/stems ratio was found higher - to 16.6% in sainfoin and to 12.9% in subterranean clover. The tendency for the highest leaves/stems ratio in *Trifolium subterraneum* spp. *brachycalicinum* retained. Many authors found higher leaves/stems ratio led to higher both, quality and intake of forage [15,33,34].

Given that leaves of sainfoin are more palatable and retain higher digestibility over time than stems [31] it is desirable high and balanced leaves/stems ratio [35]. Improvement of leaves/stems ratio of sainfoin could therefore have a practical effect on animal preference. Improvement of leaves/stems ratio of sainfoin could therefore have a practical effect on animal preference. It has an even greater significance in view of the less leaves surface in this crop [36].

In subterranean clover, leaf size contributes to plant competitively in mixtures. Competition for major nutritive elements, as well as environmental factors such as light, heat etc. in mixture with *Trifolium subterraneum* spp. *subterraneum* was less and it was the reason for higher variation in values of that ratio. Cohen [32] found strong relationship between leaves/stems ratio and crude protein content in subterranean clover.

Data for leaves/stems ratio on averaged for the period (Figure 1) showed that in pure grown sainfoin the value was 0.35. Our results correspond to those of Mohajer et al. [37], who found 0.39 (min-max, 0.16-0.88). Compared with other legume crop (birdsfoot trefoil) leaves/stems ratio was significantly lower [38-40].

In mixtures with *Trifolium subterraneum* spp. *brachycalicinum* leaves/stems ratio in sainfoin (0.39) was found significantly increased (by 12.8%), as well in these with *Trifolium subterraneum* spp. *yanninicum* (0.38) (by 9.5%).

Leaves/stems ratio in subterranean clover was found be higher as compared to the same in pure stands, i.e. in *Trifolium subterraneum* spp. *brachycalicinum* (1.12 vs. 1.00) by 11.2% and in *Trifolium subterraneum* spp. *yanninicum* (0.99 vs. 0.91) by 8.7%, respectively.

Plastid pigments content

Chlorophyll a and b and carotenoids are the main photosynthetic pigments. For the degree of formation of the photosynthetic apparatus is judged by the ratio of chlorophyll a/chlorophyll b. This is related to the basic activity of chlorophyll a. It is relatively constant and is considered genetically determined value [41]. Obtained in our study value for chlorophyll a/chlorophyll b ratio in pure grown sainfoin was 1.56, and in mixtures with subterranean clover ranged from 1.51 to 1.59 (Table 3). The chlorophyll a/chlorophyll b ratio in pure stands of three subterranean clover subspecies ranged in the limits from 1.33 to 1.47 and in the mixtures with sainfoin from 1.37 to 1.45.

Chlorophyll a+b/carotenoids ratio is also important as a characteristic of the photosynthetic apparatus and respond to changes in environmental factors [41]. In our study values for pure grown crops are 5.45 for that ratio in sainfoin and from 5.38 to 5.67 in subterranean clover.

Values of this ratio in mixtures were lower for sainfoin (4.73-5.08) and in subterranean clovers were closed to its pure grown (5.28-5.41). The lower values of chlorophyll a+b/carotenoids of sainfoin in mixtures

Months	2012		2013		2014		2015	
	temperature °C	rains l/m2	temperature °C	rains l/m2	temperature °C	rains l/m2	temperature °C	rains l/m2
January	-5.2	17.8	0.5	19.0	0.9	41.8	2.0	14.4
February	8.5	7.6	3.9	60.9	2.6	3.2	2.3	39.2
March	14.8	46.3	6.3	39.6	9.7	76.9	6.8	68.4
April	17.4	85.2	14.0	50.8	12.2	139.8	12.1	43.6
May	24.1	40.3	19.5	63.7	16.7	83.0	18.7	30.4
June	27.8	1.4	21.3	112.4	20.6	54.3	20.8	95.7
July	25.8	35.6	22.7	105.8	23.1	71.8	24.9	21.5
August	21.1	21.0	24.9	20.2	23.7	23.9	24.4	30.2
September	15.0	56.0	18.4	15.8	17.9	142.6	20.0	130.1
October	8.1	4.0	12.2	59.2	12.1	79.1	10.9	92.7
November	-1.0	56.7	8.7	29.1	5.6	48.1	10.1	50.1
December	-5.2	17.8	0.2	2.3	2.0	92.3	5.5	0.9
Avd/summ	13.0	416.1	12.7	578.8	12.3	856.8	13.2	617.2

Table 1: Agro meteorological conditions for the period of study.

Treatments	2012	+,-, %	2013	+,-, %	2014	+,-, %	2015	+,-, %
Sainfoin (100%)	0.34		0.37		0.35		0.35	
Sainfoin +	0.36	+5.9	0.43	+16.6	0.39	+11.1	0.40	+14.1
<i>Tr. sub. ssp. brachycalicinum</i> (50:50)	1.05	+11.7	1.12	+12.9	1.17	+9.7	1.12	+10.9
Sainfoin +	0.35	+2.9	0.41	+10.8	0.38	+9.7	0.39	+11.0
<i>Tr. sub. ssp. yannicum</i> (50:50)	0.94	+9.3	0.97	+7.9	1.02	+9.5	1.03	+8.3
Sainfoin +	0.35	+2.9	0.40	+8.4	0.36	+3.3	0.37	+4.4
<i>Tr. sub. ssp. subterraneum</i> (50:50)	0.84	+6.3	0.93	+8.9	0.97	+0.8	1.00	+0.8
<i>Tr. sub. ssp. brachycalicinum</i> (100%)	0.94	-	0.99	-	1.07	-	1.01	-
<i>Tr. sub. ssp. yannicum</i> (100%)	0.86	-8.5	0.90	-9.1	0.93	-13.1	0.95	-5.9
<i>Tr. sub. ssp. subterraneum</i> (100%)	0.79	-16.0	0.85	-14.1	0.96	-10.3	0.99	-2.0
SE (P=0.05) sainfoin	0.03		0.02		0.03		0.02	
SE (P=0.05) subclover	0.02		0.02		0.02		0.02	
±SD sainfoin	0.01		0.03		0.02		0.02	
±SD subclover	0.09		0.09		0.09		0.06	

Table 2: Leaves/stems ratio in sainfoin and subterranean clover (pure and in mixtures).

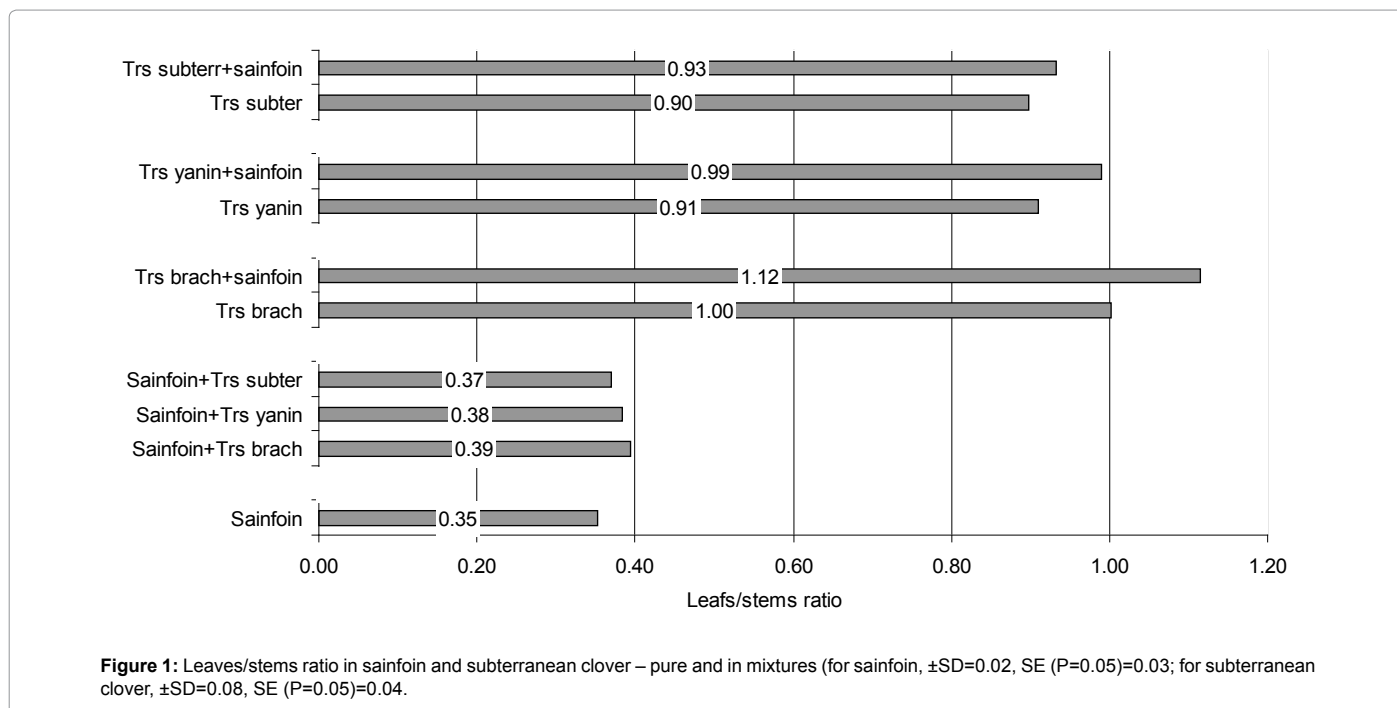


Figure 1: Leaves/stems ratio in sainfoin and subterranean clover – pure and in mixtures (for sainfoin, ±SD=0.02, SE (P=0.05)=0.03; for subterranean clover, ±SD=0.08, SE (P=0.05)=0.04).

Treatments	Chl. a	Chl. b	Chl. a/b	Chl. a+b	Carotenoids	Chl. a+b/ carotenoids
	mg/100 mg FW					
Sainfoin (100%)	145.74	93.31	1.56	239.05	43.89	5.45
Sainfoin +	139.77	92.61	1.51	232.38	45.76	5.08
Trs brach (50:50)	165.81	114.51	1.45	280.32	53.05	5.28
Sainfoin +	118.62	74.74	1.59	193.36	40.92	4.73
Trs yanin (50:50)	161.35	117.88	1.37	279.23	51.98	5.37
Sainfoin +	146.32	94.36	1.55	240.68	49.57	4.86
Trs subter (50:50)	159.14	115.84	1.37	274.98	50.81	5.41
Trs brach (100%)	149.65	112.13	1.33	261.78	46.17	5.67
Trs yanin (100%)	153.65	104.24	1.47	257.89	47.96	5.38
Trs subter (100%)	145.78	105.79	1.38	251.57	45.09	5.58
SE (P=0.05) sainfoin	6.50	4.68	0.02	11.14	1.81	0.15
SE (P=0.05) subclover	2.84	2.36	0.03	4.62	1.27	0.06
±SD sainfoin	13.00	9.37	0.03	22.29	3.62	0.31
±SD subclover	6.36	5.28	0.06	10.33	2.85	0.14

Table 3: Total plastid pigments content in sainfoin and subterranean clover, grown pure and in mixtures.

with *Trifolium subterraneum* spp. *brachycalicinum* and *Trifolium subterraneum* spp. *subterraneum* associated with the increasing content of carotenoids and in mixture with *Trifolium subterraneum* spp. *yanninicum* - by reducing the contents both, chlorophylls and carotenoids.

Total plastid pigments content in sainfoin pure grown was 282.94 mg/100 mg FW (Figure 2). The values of total plastid pigments content in subterranean clover were closed -*Trifolium subterraneum* spp. *subterraneum* (296.66 mg/100 mg FW), *Trifolium subterraneum* spp. *yanninicum* (305.85 mg/100 mg FW) and *Trifolium subterraneum* spp. *brachycalicinum* (307.95 mg/100 mg FW).

Total plastid pigments content in mixtures of sainfoin with *Trifolium subterraneum* spp. *brachycalicinum* and *Trifolium subterraneum* spp. *subterraneum* retain almost unchanged in sainfoin and increased by 8.3% in subclovers compared to pure stands. Total plastid pigments content in sainfoin in mixture with *Trifolium subterraneum* spp. *yanninicum* decreased by 17.2%.

Photosynthetic pigments increased in subterranean clover in three mixtures studied, i.e. by 8.3% for *Trifolium subterraneum* spp. *brachycalicinum* and *Trifolium subterraneum* spp. *yanninicum*, and by 9.8% for *Trifolium subterraneum* spp. *subterraneum*.

Changes in the total plastid pigments content in leaves of sainfoin and subterranean clover subspecies are also related to the leaves/stems ratio. The coefficient of correlation between leafes/stems ratio and total plastid pigments content was found $r = 0.81$.

The amount of fixed nitrogen in mixtures was changed and depends on the competition between the components. It is evident that amount of fixed nitrogen obtained from mixtures on average for the period was from 2.20 kg N/da (sainfoin + *Trifolium subterraneum* spp. *subterraneum*) to 2.88 kg N/da (sainfoin + *Trifolium subterraneum* spp. *brachycalicinum*) more compared to the amount from pure grown sainfoin (Figure 3). Competition for soil nitrogen in mixtures can have a beneficial effect on the nitrogen fixing process, as in the case sainfoin and subterranean clover have the same type nitrogen metabolism. They fixed nitrogen from the atmosphere through nitrogenase in the nodules. According Sebastia et al. [42] legumes effectively regulate processes nodulation and nitrogen fixation in mixtures.

Changes in morphological and physiological parameters of crops studied are related to the metabolic role of nodules which represent a powerful acceptor for carbon assimilates from leaves, mainly in the

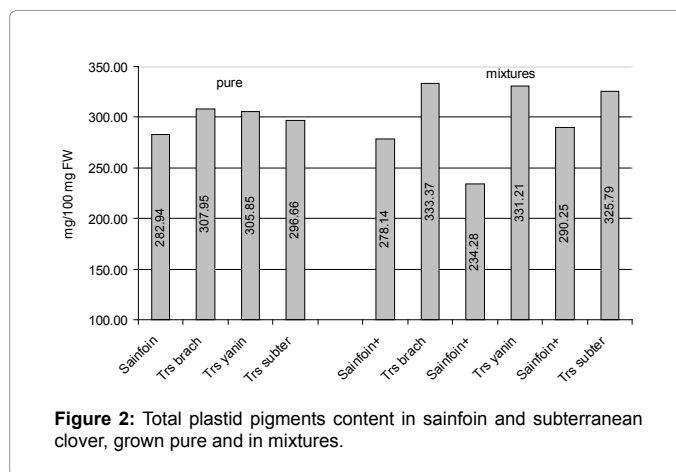


Figure 2: Total plastid pigments content in sainfoin and subterranean clover, grown pure and in mixtures.

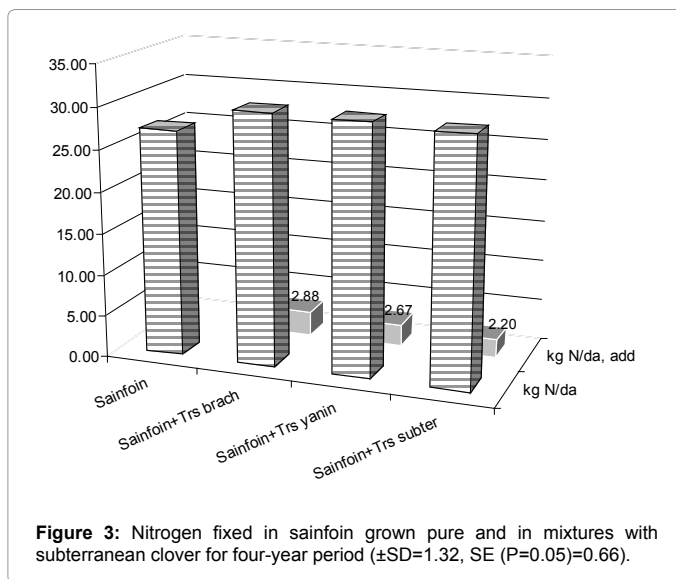


Figure 3: Nitrogen fixed in sainfoin grown pure and in mixtures with subterranean clover for four-year period (±SD=1.32, SE (P=0.05)=0.66).

form of transport amino acids and amides [43].

Based on the morphological and physiological parameters studied could be summarized that sainfoin and subterranean clover grown in

mixtures in ratio 50:50% showed good mutual tolerance, morphological and physiological status [44,45].

Conclusions

Leaves/stems ratio of sainfoin pure grown was 0.35 (0.34-0.37) and increased by 9.5% in mixtures with *Trifolium subterraneum* spp. *yanninicum* (0.38) and by 12.8% in mixtures with *Trifolium subterraneum* spp. *brachycalicinum* (0.39). There were from 2.20 kg N/da (sainfoin + *Trifolium subterraneum* spp. *subterraneum*) to 2.88 kg N/da (sainfoin + *Trifolium subterraneum* spp. *brachycalicinum*) more fixed nitrogen obtained in comparison to the amount from pure grown sainfoin. Total plastid pigments in mixtures increased by 8.3% for *Trifolium subterraneum* spp. *yanninicum* and *Trifolium subterraneum* spp. *brachycalicinum* and *Trifolium subterraneum* spp. *yanninicum*, and by 9.8% for *Trifolium subterraneum* spp. *subterraneum*, and decreased in sainfoin by 17.2% for mixture with *Trifolium subterraneum* spp. *yanninicum*. Coefficient of correlation between leaves/stems ratio and total plastid pigments was found be 0.81.

Sainfoin and subterranean clover in mixtures showed good mutual tolerance, morphological and physiological status.

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