AGRO BIOLOGICAL PECULIARITIES AND BIOMASS QUALITY OF LIQUORICE, *GLYCYRRHIZA GLABRA*, UNDER THE CONDITIONS OF MOLDOVA

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Abstract

Fabaceae species play a crucial role in protein production for human and animal diet, soil structure and environmentally friendly substitution for industrial N-fertilizers, valuable medicinal properties and multi-purpose use in various industries. The local ecotype of liquorice, *Glycyrrhiza glabra*, maintained in monoculture, served as object of study. The 3-year-old *Glycyrrhiza glabra* started vegetating 23 days later, the plants had moderate growth and development rates that allowed mowing them at the end of June, the green mass yield reached 4.38 kg/m^2, with moderate foliage (55 %), but reduced content of dry matter, protein (13.80 %), cellulose (29.40 %), minerals (5.40 %) and high amount of fats (3.65 %), nitrogen free extractive substances (47.75 %), essential amino acids (lysine, leucine, valine), in comparison with *Onobrychis viciifolia.* The liquorice forage had cell wall content 485 g/kg NDF and 323 g/kg ADF can be classified as good quality with relative feed value 122, dry matter digestibility 65.40 % and organic matter digestibility 56.70 %. The gas forming potential of *Glycyrrhiza glabra* (first mowing) reached 567 L/kg with 52.5 % methane, but biogas yields based on the concentration of acid detergent lignin and hemicellulose – 535 L/kg with 51.2 % methane, the potential methane production ranged from 3014 to 3278 m^3/ha, exceeding *Onobrychis viciifolia.* The briquettes of *Glycyrrhiza glabra* were distinguished by moderate specific density (873 kg/m^3), gross calorific value (18.7 MJ/kg) and ash content (1.7 %). The theoretical ethanol potential from structural carbohydrates dry biomass averaged 511 L/t for *Glycyrrhiza glabra*, compared to 485 L/t corn stalks.

Keywords: agro biological peculiarities, biochemical composition, bio fuel feedstock, forage value, *Glycyrrhiza glabra*

Future demographic projections, 9 billion world's population in 2050, suggest the need to produce more food and energy, together with negative impact of climate changes, they will necessitate effective utilization of land, water and plant resources. *Fabaceae* species play a crucial role where their capacity supports global protein production by partially replacing meat and dairy products in the human diet, improve forage quality and performance in animal production, symbiotic fixation of atmospheric nitrogen and can provide a more environmentally friendly substitution for industrial N-fertilizers, decrease the soil bulk density, improve land desalinization, lowering the groundwater levels, increase the humus and nutrient content of the soil, improve soil structure and its biological activity, bring improvements in resource efficiency and production costs. Many legumes have valuable medicinal properties and multi-purpose use in various industries, they are also excellent honey plants (Duke, J. A., 1981; Frame J., 2005; Luscher A. *et. al., 2013*; Stoddard F.L., 2013; Stinner P. W., 2015).

The genus *Glycyrrhiza* L. (syn. *Liquiritia* Medik.) includes 13 - 30 species, natively belongs to Europe, Asia, North and South America along with Australia. In Romania, in natural grasslands, there are 3 species (Marusca T., 1999). In the spontaneous flora of the Republic of Moldova, there are also 3 species: *Glycyrrhiza glabra* L., *Glycyrrhiza echinata* L.*; Glycyrrhiza foetidissima* Tausch. (Negru A., 2007), from these species, *Glycyrrhiza glabra* L. is the most widespread. Licorice, *Glycyrrhiza glabra* (syn. *G. glandulifera* Waldst. & Kit.; *G. hirsuta* Pall.; *G. pallida* Boiss. & Noe.; *G. violacea* Boiss. & Noe.; *Liquiritia officinarum* Medik.), a species in the family *Fabaceae* Lindl., has been used by physicians and herbalists since the earliest times for its edible roots and rhizomes which contain glycyrhizin, a

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compound 50 times sweeter than sucrose, possesses different pharmacological properties such as anti-bacterial, antitumor, antioxidant, antimalarial, expectorant, antitussive, antispasmodic, anti-inflammatory and anti-hyperglycemic properties (Karkanis A *et. al., 2013*). Potential uses of *Glycyrrhiza glabra* in agriculture: forage, cover, medicinal crop and phytomeliorant (Shamsutdinov N. Z. 2002; Kushiev H. *et. al.,* 2005; Toderich K. *et. al.,* 2015; Kappas M. *et. al., 2016*). The root system, as in so many leguminous plants, is double, one part consisting of a vertical root or tap root, the other of horizontal rhizomes or stolons thrown off from the root below the surface of the ground. These runners are furnished with leaf buds and produce stems in their second year. The perennial downward-running roots as well as the long horizontal stolons are equally preserved for use. Each root, if unrestricted, can reach a depth of 90 to 120 cm and can extend to 10 m. The plants grow 90-180 cm tall, with pinnate leaves about 7-15 cm long, with 9-17 alternate oblong to lanceolate leaflets. Cross-pollinated entomophilous plant. It flowers in June-July; the flowers are 0.8-1.2 cm long, purple to pale whitish blue, produced in a loose inflorescence. The fruit is an oblong pod, 3-5 cm long, containing several seeds. The seeds are characterised by dark colour, reniform shape and small size, with a diameter of 2.5 mm and weight of a thousand seeds of 6.2 g. Chromosome number is 2n=16. Propagation – by seed and vegetative (cutting, division of the plants in spring or autumn). *Glycyrrhiza glabra* is sown in spring; seed germination is low and irregular, scarification of seeds is recommended.

This research was aimed at evaluating the biological peculiarities, the biochemical composition of the local ecotype of liquorice, *Glycyrrhiza glabra*, and the possibility to use biomass as fodder for animals and feedstock for bio fuel production.

MATERIALS AND METHODS

The local ecotype of liquorice, Glycyrrhiza glabra, maintained in monoculture, served as object of study, as control variant – common sainfoin, *Onobrychis viciifolia* Scop., (for fodder and biogas), wheat straw (briquettes) and corn stalks (bioethanol). Liquorice seeds were collected from the spontaneous flora and the experiments were performed on experimental land in the Botanical Garden (Institute) of the Academy of Sciences of Moldova, latitude 46°58′25.7″ and longitude N28°52′57.8″E. The growth and development of plants as well as their productivity were assessed according to methodical indications (Novoselov Y. K. *et al 1983*). The green mass was harvested in the flowering stage. The analyses were performed in the Laboratory of Nutrition and Feed Technology of the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine according to standard laboratory procedures for forage quality analysis (Petukhov E.A. *et al. 1989*). The content of neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), dry matter digestibility (DMD), organic matter digestibility (OMD) were evaluated using the near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research-Development Institute for Grassland Brasov, Romania. Relative feed value (RFV) was calculated according to standard procedures.

The carbon content of the substrates was obtained from data on volatile solids, using an empirical equation reported by (Badger C.M. *et. al., 1979*).

The biogas production potential (Yb) and specific methane yields (Ym) were evaluated by the parameter “content of fermentable organic matter”, according to (Weissbach F., 2008), also, they were calculated according to the equations of Dandikas V. et al. 2014, based on the chemical compounds - acid detergent lignin (ADL) and hemicellulose (HC) values:

$$Yb=727+0.25\ HC - 3.93\ ADL$$

$$Ym =371 + 0.13\ HC - 2.00\ ADL$$

The dry biomass was harvested in November. Automatic calorimeter LAGET MS-10A with accessories was used for the determination of calorific value, according to CEN/TS 15400. The cylindrical containers were used for the determination of bulk density, calculated by dividing the mass over the container volume. The briquetting was carried out by hydraulic piston briquetting press BrikStar model 50-12 (Briklis). The mean compressed (specific) density of the briquettes was determined immediately after removal from the mould as a ratio of measured mass over calculated volume.

Ethanol yields from structural material were calculated according to the equations of Goff B.M., et al., 2010 based on NDF, ADF and ADL values:

$$H = [\%Cellulose+(\%Hemicellulose \times 0.07)]\times 172.82$$

$$P = [\%Hemicellulose \times 0.93] \times 176.87$$

$$\text{Theoretical Ethanol Potential (L/t)} = [H + P] \times 4.17$$

H and P are theoretical ethanol production from the conversion of hexose and pentose sugars; cellulose is ADF minus ADL and hemicellulose is NDF minus ADF.

RESULTS AND DISCUSSIONS

It can be noted that plantlets emerged non uniformly, some individual plantlets of liquorice appeared on the 5- 6th day after sowing, and in mass – on the 15th day. By the time of sprouting, the length of the main root reached 4-7cm and its diameter at the root collar was 1.3-1.5 mm. The first real leaf was simple, with an oval-round blade, was formed on the 8th -9th day after seedling, during the next 10-15 days, 3 more simple leaves developed. Two-three lateral roots, from 5.7 to 8.3 cm in length, formed at the end of May. At this time, on them, there are already noticeable root nodules of irregular shape. The first compound leaf appeared on the 4th-5th internodes, the height of the plants at that time was 4.5-6.5 cm. The first compound leaves were ternate**,** then, starting with the 7th-8th leaves, odd-pinnate with 2 pairs of leaflets, and in the 15-16th leaves, the number of pairs of leaflets increased to 4-5. In early July, the taproot grew faster than the aerial shoot. During this period, above-ground shoots reached 28-43 cm in height, in the axils of compound leaves, buds emerged and developed 4-7 lateral shoots. The root collar together with cotyledonary nodes and axillary buds were drawn into the soil to a depth of 1.5-2 cm. The horizontal stolons develop from the buds embedded in the axils of the cotyledons. At the end of the first growing season, the main shoot reached a height of 70 cm and lateral shoots – up to 25 cm, the length of the main root was 50 cm and the diameter of the root collar was 13-15 mm, the length of horizontal rhizomes was 45-55 cm with a diameter of no more than 2 mm.

In the second year, the buds from the basal part of last year's shoots started growing when the average air temperature was 17-20 °C, at the end of April, the first compound leaves had 2 pairs of leaflets, and the subsequent leaves developed up to 5 pairs of leaflets. Two-three shoots developed, they grew fast (15-23 mm/day) from the middle of May to the end of June, then the growth slowed down slightly until the end of August, reaching a height of 135 cm by the end of the growing season. At the end of May, in the middle part of the shoot, in leaf axils, first-order lateral shoots began to develop and second shoots and in June - third-order shoots. In the middle of July, the drying and falling of the lower leaves was noted.
The growth of the main root continued until the end of August, reaching 80 cm. Lateral roots, up to 40 cm long, were formed along the entire length of the main root. Of the axillary buds located on the basal part of last year's shoots, in July, 4-5 new horizontal rhizomes were formed and, by the end of the growing season, they grew 70-180 cm long and 2-3 mm thick. The base of the main root, by the end of the second year, was retracted into the soil to a depth of 5 cm.

In the 3rd year, the shoots appeared 5-7 days earlier than in the 2nd year of life, but 23 days later in comparison with *Onobrychis viciifolia*. The first leaves had by 3 pairs of leaflets; the subsequent leaves had up to 6 pairs of leaflets. By 4-6 main shoots grew, and by the end of May, they developed lateral shoots.

In June, liquorice produced flower buds and the shoots were up to 160 cm long. In late June, liquorice plants bloomed and the biomass was harvested. The local ecotype of liquorice, *Glycyrrhiza glabra*, was distinguished by moderate foliage (55 %) and higher productivity (4.38 kg/m^2), but lower content of dry matter in the harvested mass (25.10 %), as compared with *Onobrychis viciifolia*.

In other studies, it was mentioned that the productivity of liquorice under the climatic conditions of Lower Volga region, Russia, the green mass varied from 22 t/ha, on non irrigated land, up to 55 t/ha, on irrigated land (Astafyev S.V. *et. al. 2016*); in India, the shoot biomass of liquorice, harvested on different alkali soils, varied from 5.63 to 7.95 t/ha, in first year and 11.07-15.03 t/ha of dry matter in the next year, forage biomass production was better on soil with higher ph than normal soil (Dagar J.C *et.al. 20*15). In Moldova, the yield of fresh roots and rhizomes reached 31.7-43.9 t/ha (Muchnik, Z.S. 1973).

Table 1

Agro biological peculiarities of *Glycyrrhiza glabra*

Indicators	*Onobrychis viciifolia*	*Glycyrrhiza glabra*
Resumed vegetation up to:		
- budding, days	75	63
- flowering, days	99	85
- seed ripening, days	133	124
Plant height, cm		
- at the end of April	35.90	3.70
- at flowering period	85.50	165.50
The yield 1st mowing:		
- fresh mass, kg/m^2	3.95	4.38
- dry matter, kg/m^2	1.03	1.10
The leaf content, %	39	55

Table 2

Biochemical composition and digestibility of dry matter of *Glycyrrhiza glabra*

Indicators	*Onobrychis viciifolia*	*Glycyrrhiza glabra*
Dry matter, g/kg	261	251
Raw protein, %	17.44	13.80
Raw fats, %	3.39	3.65
Raw cellulose, %	33.50	29.40
ADF, %	-	32.30
NDF, %	-	48.50
ADL,%	-	5.90
Cellulose, %	-	26.4
Hemicellulose, %	-	16.7
NFE, %	39.43	47.75
Minerals,%	6.24	5.40
DMD, %	-	65.40
OMD, %	-	56.70
Relative feed value	-	122

The biochemical composition of harvested mass is presented in Table 2. It was found that the dry matter of *Glycyrrhiza glabra* contained a lower amount of protein (13.80 %), cellulose (29.40 %), minerals (5.40 %) and high amount of fats (3.65 %), nitrogen free extractive substances (47.75 %), in comparison with *Onobrychis viciifolia*.

Other important quality parameters for forages are cell wall content and digestibility of dry matter. The concentrations of NDF, ADF, ADL, cellulose and hemicellulose of *Glycyrrhiza glabra* whole plant, harvested in the flowering stage, were 485 g/kg, 323 g/kg, 59 g/kg, 264 g/kg and 167 g/kg respectively, dry matter digestibility was 65.40 % and organic matter digestibility 56.70 %. The liquorice forage obtained from the first mowing, with calculated Relative feed value 122, can be classified as class 2 (good quality). Stavarache M. *et al., 2012*, reported that the quality of alfalfa forage obtained from the first and third harvests can be classified as prime class (0), while the forage from the second harvest can be classified as class 2, as a criterion of American Quality standards of grasses, legumes and grasses-legumes mixtures.

Some authors mentioned various findings about the quality of liquorice fodder. Alekseeva T.B., 2007, remarked that the biomass of *Glycyrrhiza glabra* ecotypes, in the conditions of Kalmykia, Russia, contained 6.80-11.50 % sugars, 15.67-25.67 % protein, 12.80-21.40 % cellulose, 5.67-17.40 % ash and 1.30-1.70 % flavonoids. According to Toderich K. *et. al., 2014*, in Kyzylkesek, Uzbekistan, the chemical composition and gross energy value of air dried matter of liquorice (fruit maturation stage) was: 20.7 % protein, 4.2 % fat, 33.4 % cellulose, 33.3 % nitrogen-free extract, 7.51 % ash and 18.4 MJ/kg, but alfalfa (flowering stage) – 16.1 %, 1.6 %, 11.6 %, 60.8 %, 9.1 % and 17.4 MJ/kg, respectively. Astafyev S.V. et. al., in 2016, reported that liquorice forage, in Lower Volga region, Russia, contained 8.2 % protein, 4.8 % fat, 25.4 % fibre, 53.3 % nitrogen-free extract and 33.94 mg/kg carotene. Kamalak A., 2006, reported that the nutritive values of the leaves of *Glycyrrhiza glabra* L. ranged from 16.19 to 26.93 % crude protein, from 20.74 to 29.07 % acid detergent fibre and from 1.57 to 10.83 % condensed tannin.

Analyzing the results on the amino acid content in the fodder *(tab.3)*, it was found that the species *Glycyrrhiza glabra* was distinguished by an optimal content of both essential and nonessential amino acids. Comparing each amino acid separately, we could mention that the content varied in comparison with *Onobrychis viciifolia*. We could mention that the first deficient essential amino acid, methionine, of the species *Glycyrrhiza glabra* reached 0.82 g/kg dry matter; thus, it was lower than in *Onobrychis viciifolia*. The second limiting amino acid for protein biosynthesis, lysine, was higher – 7.62 g/kg. We found that *Glycyrrhiza glabra* fodder was very rich in leucine and valine, rich in glutamine and glycine, but contained very low amounts of phenylalanine, arginine, histidine and tyrosine in comparison with control forage crops.

Table 3

The content of amino acids per kg dry matter *Glycyrrhiza glabra*

Amino acids	*Onobrychis viciifolia*	*Glycyrrhiza glabra*
asparagine, g	17.51	15.52
threonine, g	5.65	5.20
serine, g	6.85	5.50
glutamine, g	13.98	16.72
proline, g	11.54	9.06
glycine, g	5.57	6.76
alanine, g	6.72	5.64
valine, g	6.54	8.40
methionine, g	0.91	0.82
isoleucine, g	4.59	4.57
leucine, g	9.20	12.49
tyrosine, g	4.91	3.24
phenylalanine, g	9.37	5.24
histidine, g	3.71	2.76
lysine, g	7.06	7.62
arginine, g	5.87	4.68

The content of mineral elements in fodder is variable, depending on species. *Glycyrrhiza glabra* fodder is characterized by optimal level of potassium (15.67 g/kg), but very low – of magnesium (1.08 g/kg), low content of calcium (9.05 g/kg) and phosphorus (6.16 g/kg) in comparison with *Onobrychis viciifolia*.

Table 4

The content of minerals per kg dry matter *Glycyrrhiza glabra*

Minerals	*Onobrychis viciifolia*	*Glycyrrhiza glabra*
Calcium, g	11.20	9.05
Phosphorus, g	7.53	6.16
Magnesium, g	3.28	1.08
Potassium, g	15.17	15.67
Sodium, mg	366.20	121.41
Iron, mg	343.20	221.50
Manganese, mg	91.55	60.21
Zink, mg	26.15	28.03
Copper, mg	6.75	11.15
Strontium, mg	34.53	22.57

It was determined the content of trace elements in the dry matter of *Glycyrrhiza glabra*, so, the fodder contained large amounts of copper and zinc, but was poor in sodium, iron, manganese and strontium. Under the conditions of Kalmykia, Russia, depending on the cenotic populations (ecotypes) of liquorice, the vegetal biomass contained 9.61-16.30 g/kg calcium, 0.14-0.43 g/kg phosphorus, 5.27-9.05 g/kg magnesium, 3.20-15.25 mg/kg copper, 18.90-50.62 mg/kg zinc, 22.80-104.10 mg/kg manganese, 112-486 mg/kg iron (Alekseeva T.B., 2007).

Biorefining offers a way for combining feed and bioenergy production. Biomass based raw materials can be converted into the more valued energy forms using biochemical methods such as ethanol fermentation, methane fermentation and thermochemical methods such as direct combustion, pyrolysis, gasification, liquefaction.
The use of legumes as feedstock for multiple energy purposes increase the potential of bioenergy and reduction of greenhouse gas emissions, through symbiotic nitrogen fixation and compensates inorganic N fertilizer in conventional farms, if the digestate is applied as a fertilizer to the non-legume crops (Stoddard F.L., 2013; Durak H., 2014; Stinner P. W., 2015; Toderich K. *et. al.,* 2015).

The stability and productivity of anaerobic digestion is mostly influenced by the content of organic matter, biochemical composition, biodegradability and ratio of carbon and nitrogen(C/N). The biomass of the crop investigated in the present study revealed C/N ratios in a wide range, on average 19-23*(tab.5)*. In general, a C/N ratio of 20/1 to 30/1 is regarded as optimal for methanogenesis.

Fermentable organic matter represents the proportion of organic matter which can be biologically degraded under anaerobic conditions and, thus, can be potentially utilized in biogas facilities (Weissbach F., 2008). The calculated content of fermentable organic matter and its gas forming potential of *Glycyrrhiza glabra* biomass reached 709 g/kg VS and 567 L/kg VS, respectively, being higher than in the control species, but it had similar content of methane (52.5 %). The estimation of biogas and methane yields based on the concentration of acid detergent lignin and hemicellulose in total solids of *Glycyrrhiza glabra* reached values of 535 L/kg and 274 L/kg, respectively. The potential methane yield per ha of *Glycyrrhiza glabra* (first mowing) ranged from 3014 to 3278 m^3/ha, exceeding *Onobrychis viciifolia.*

Table 5

Gas forming potential of the fermentable organic matter *Glycyrrhiza glabra*

Indicators	*Onobrychis viciifolia*	*Glycyrrhiza glabra*
Ratio carbon/nitrogen	19	23
FOM, g/kg VS	658	709
Biogas, litre /kg VS	526	567
Methane, litre /kg VS	276	298
Methane productivity, m^3/ha	2843	3278

It was found that, in autumn, when temperatures below 0 °C were recorded, the leaves were falling and the stems of *Glycyrrhiza glabra* were drying fast, in November, the stems were already dry and could be harvested and chopped directly in the field. The yield of chopped biomass reached 0.65-1.07 kg/m^2, while the bulk density was of 153 kg/m^3. The briquettes of *Glycyrrhiza glabra* were distinguished by moderate specific density (873 kg/m^3), gross calorific value (18.7 MJ/kg) and ash content (1.7 %), but wheat straw – low density (704 kg/m^3) and calorific value (17.0 MJ/kg) and high content of ash (5.1 %). The potential of energy production constituted 200 GJ/ha.

The bioethanol yields are influenced by several factors, including biomass yield and its tissue composition (ratios of cellulose, hemicellulose and lignin). Analyzing the cell wall composition of dehydrated stems of local ecotype of liquorice, *Glycyrrhiza glabra*, we could mention that the concentrations of NDF, ADF, ADL, cellulose and hemicellulose of *Glycyrrhiza glabra* were 819 g/kg, 590 g/kg, 114 g/kg, 476 g/kg and 229 g/kg, respectively. The estimated content of structural sugars: 85.0 g/kg pentose and 35.6 g/kg hexose, in comparison with corn stalks – 75 g/kg and 41 g/kg, respectively. The theoretical ethanol potential from structural sugars per unit of dry biomass averaged 511 L/t for *Glycyrrhiza glabra*, compared to 485 L/t for corn stalks. For sorghum crop, the theoretical ethanol potential ranged from 560 to 610 L/t of dry biomass (Goff B.M. *et. al.*, 2010).

CONCLUSION

Glycyrrhiza glabra seeds germinate very slowly, need scarification and high soil temperature in comparison with sainfoin.

The 3-year-old *Glycyrrhiza glabra* plants have moderate growth and development rates that allow mowing them at the end of June. The green mass yield reaches 4.38 kg/m^2, but the content of dry matter is low, as compared with *Onobrychis viciifolia*.

The dry matter of *Glycyrrhiza glabra* is characterized by lower protein content (13.80 %), raw cellulose (29.40 %), minerals (5.40 %) and high amount of fats (3.65 %), nitrogen free extractive substances (47.75 %), it is rich in essential amino acids (lysine, leucine, valine) and copper in comparison with *Onobrychis viciifolia.*

The liquorice forage obtained from the first mowing can be classified as good quality with Relative feed value 122, dry matter digestibility 65.40 % and organic matter digestibility 56.70 %.

The gas forming potential of *Glycyrrhiza glabra* reached 567 L/kg with 52.5 % methane, but biogas yields based on the concentration of acid detergent lignin and hemicellulose – 535 L/kg with 51.2 % methane. The potential methane yield per ha of *Glycyrrhiza glabra* (first mowing) ranged from 3014 to 3278 m^3/ha, exceeding *Onobrychis viciifolia.*

The briquettes of *Glycyrrhiza glabra* were distinguished by moderate specific density (873 kg/m^3), gross calorific value (18.7 MJ/kg) and ash content (1.7 %). The theoretical ethanol potential from structural carbohydrates of dry biomass averaged 511 L/t for *Glycyrrhiza glabra*, as compared with 485 L/t for corn stalks.

The local ecotype of *Glycyrrhiza glabra* could be used for restoring degraded and salt-affected land, and is also a promising non-edible source of fodder and energy biomass converted to solid, liquid and gaseous products.

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