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SCIENTIFIC SUBSTANTIATION FOR THE INTRODUCTION, ON ROMANIAN TERRITORY, OF *Lycium barbarum* L.: A SPECIES WITH SANOGENE PROPERTIES

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Abstract

In the year 2010, studies have been started at U.Ş.A.M.V. Bucharest in order to meet the requirements for the scientific substantiation of the development of Lycium barbarum L. plant cultivation on Romanian specific spreading area. The aforementioned studies focused on the biological, agronomical and health-promoting properties of this plant species. Our study presents interpretations, conclusions and a synthesis regarding biological characteristics such as: growth and development dynamics of the goji plants' vegetative and reproductive parts, between the years 2010 and 2012. The quantitative and qualitative aspects of the plants' fruit production were also part of the research, as fructification characteristics, bio-physical and bio-chemical properties of goji berries and the quantity and quality of the fruit yield were all studied. This work also considers the adaptability potential of the Lycium plants to the environmental and climatic conditions of the Northern Bucharest region by mentioning: the traversing of vegetative and fructifying phases and the plants' resistance to diseases and pests. So far, there have been certain differences, between the two studied Lycium barbarum L. varieties, regarding adaptability potentials and biological characteristics. The significance of our contribution is that it aims to provide scientific support for the cultivation of goji species in Romania.

Key words: *adaptability, fructification, Lycium barbarum L. (Goji), scientific substantiation, varieties.*

INTRODUCTION

Lycium barbarum L. (LB) is a decumbent deciduous perennial nanophanerophyte from the *Solanaceae* family which is native to Asia and S-E Europe. It is also called wolfberry or "goji" from the Chinese "gou qi", but these are terms which can also be used to denote its close relative: *Lycium chinense* Mill. (LC). Sometimes the distinction "Chinese wolfberry" for LC and "Ningxia goji" for LB is made, but in this study we refer to goji only as LB.

Though used for a long time by Chinese people, who have included this plant amongst the remedies used in the Traditional Chinese Medicine, it is only in recent years that the popularity of this species has started to grow in western countries, being associated with the idea of a "super fruit".

These exotic berries are highly valued for their: anti-ageing properties, anti-diabetic effects, antioxidant activity, cardiovascular benefits, for promoting eye health and for strengthening the immune system. Some of these health claims have been studied and it has been shown that the fruit's nutritional value and sanogene potential are notable (Amagase et al., 2011).

There have been many studies regarding this species' biological characteristics in China. Also, there is an ascendant trend in the number of studies focusing on the fruit's nutritional and sanogene properties.

In spite of this, in Romania, *L. barbarum* has only been regarded as a potentially invasive species (Anastasiu, 2005) and – to our best of knowledge – the possibility of introducing these plants towards cultivation has not been studied yet.

Moreover, there haven't been any studies regarding how the information existing in the speciality literature matches up to the agro-pedo-climatic characteristics of the Northern Bucharest region.

This is why this work aims to verify the information and hypotheses found in the consulted scientific studies (over 70 bibliographic titles have been consulted), by comparing it to the synthesised data from the experimental field.

MATERIALS AND METHODS

The period of this study was 2010 - 2012. Two phenotypes (V_1 and V_2), were planted in a non-random block experiment with 6 repetitions (3 for V_1 and 3 for V_2), within the research field at U.Ș.A.M.V.'s Campus in Bucharest.

In order to study the growth and development characteristics of plant individuals from the *Lycium barbarum* L. species, the following indicators were determined: plant height, number and length of shoots, number of leaves, flowering and fructifying dates, fruit yield, soluble dry substance in fruit (DS%) and fruit weight. Also, the volume and shape of the two years old goji shrubs were determined.

The volume of the plants was determined by using the mathematical formula for the truncated cone shape which bared the most similarity with the plants' natural architecture.

The harmful and the helpful arthropod species, for the *Lycium barbarum* shrubs planted in the experimental field, were determined by using yellow sticky traps. One trap per variety was installed in the shrubs' crowns. New traps were set up weekly, between July and October 2012.

The entomological probes were collected and sorted according to taxonomic groups. The probes were studied under a binocular eyeglass, and identified according to existing speciality determinants, described in literature.

The data from the experimental field was synthesised and analysed in order to verify the information and hypotheses found in the consulted scientific studies (over 70 bibliographic titles).

RESULTS AND DISCUSSIONS

Ecologic area of Lycium barbarum L.

Climatic zones and distribution areas. Goji shrubs prefer temperate or sub-tropical climatic zones. They are sun-loving plants and the quantity and quality of their fruit production heavily depends on the quantity of solar exposure they get. So, even if they will tolerate growing in partly shaded environments, the recommended planting spot would be that which ensures the plants get around 6 hours of sunlight per day.

The plants are quite resistant to cold as they can tolerate temperatures as low as -15 °C, and even -23 °C, but only for short periods of time (Doroftei, 2009). Also, this species can easily tolerate maritime exposure and strong winds.

Lycium barbarum L. originates from the region spanning from south-eastern Europe to south-western Asia (Harrison et al., 1997). But, nowadays, the main cultivator and producer of goji plants and derived products is China (especially in the Ningxia Hui Autonomous Region). Same as the whole genus it belongs to, this species presents a disjunction between the northern and southern hemispheres (Levin et al., 2005).

Along with *Lycium barbarum* L. (LB), 10 more species are mentioned as being native to the Euro-Asian zone (*L. europaeum, L. chinense, L. rhutenicum*). The richest diversity of Lycium species can be found in South America where the presence of almost 30 species of this genus has been recorded (*L. cestroides, L. chilense, L. elongatum*). North American (*L. andersonii, L. californicum, L. pallidum*) and South African (*L. afrum, L. ferocissimum, L. cinereum*) zones each have around 20 different Lycium species while, Australia (*L. australe*), The Galapagos Islands (*L. minimum*) and other Pacific Islands (*L. sandwicense*) each have only 1 Lycium species (Fukuda et al. 2001).

Nowadays, the goji shrub has been introduced to most parts of the world due to its ornamental or commercial value. It can be found in almost all regions of the U.S.A., in Canada and in some parts of south-eastern Australia. It has been observed even in New Zeeland, but it is very rare (usually found in manmade habitats) as it cannot fruit freely in this region (Webb et al., 1988).

One of the first European countries it has been introduced to is Great Britain. Usually the shrubs are used as hedges, especially in the southern parts of the country, such as the Suffolk region, or in coastal areas.

Regarding this species' distribution over European territory, its presence has been signalled in states like: Austria, Belgium, Bulgaria, Luxembourg, Italy, Greece, the Czech Republic, Slovakia, France, Germany, Switzerland, Holland, Spain, Hungary, Portugal, Poland, Russia and Turkey. It has even been recorded in northern countries such as: Denmark, Sweden and Norway.

In Romania, *Lycium barbarum* L. has been signalled in the eastern part of the country: in Dobrogea and Moldova regions. It has also been signalled in the western part of Romania, in the city of Timişoara.

The climatic characteristics of the experimental field, where the two LB varieties have been planted, are those of a temperate-continental region. Slight thermal excesses tend to occur due to the high number of constructions, car traffic and industrial activities in this urban area. So, while at the outskirts of the city, the annual average temperature is 10.5 °C, at the centre the average value is 12 °C.

In normal years, the summers are hot, even torrid, with frequent draught episodes and winters are cold, with large snow falls. Springs are short, with ample temperature fluctuations from one month to the other and with notable day-night variations. Autumns are characterised by thermal moderation as they ensure a slow transition towards the cold season.

The usual sum of the minimum active temperatures from September to April is 148.1 °C and the sum for the maximum active temperatures reaches 1,477.4 °C. The number of days without frost spans between 178 and 205, while the vegetation period tends to be around 245 days.

Annual precipitation ranges from 550 to 600 mm, with a peak in the May-July interval. Showers and heavy rains are frequent. Dominant circulation of air is from the east and northeast in winter and from the west in the rest of the year. The maximum wind speed is 3.5-5 m/sec.

It can be concluded that the climatic conditions in the experimental field are generally favourable to the cultivation of grains, vegetables and also to different fruit-growing or ornamental shrub and tree species.

Soil requirements. The LB shrub is a rather undemanding species when it comes to its soil requirements. It prefers sandy, loamy or even clayey soils as long as they are well drained. The optimum pH of the soil is around 7 (neutral), but the plants may get used to more acid or alkaline soils too.

Soils with high salinity are especially well tolerated by this species. This is due to a special biochemical mechanism regarding the structure of glycoproteins at the cell surface of LB leaves. Under salt stress, the glycoprotein layer gets thinner, while in low salt conditions it thickens (Juan et al., 2004).

Goji plants thrive in soils with rich or moderate trophicity, but they can tolerate poor soil as well. According to some sources, this species can grow even in very dry soil conditions (Harrison et al., 1997). It is also capable of resisting moderate drought episodes, though it is advisable for the relative soil humidity to be over 50 % at all time, in order for the production not to be affected.

On the other hand, the *Lycium* shrubs shouldn't be planted in very humid soil, or in soils which tend to form puddles, otherwise the forming of fungus might set in. Some studies have shown that the appropriate monthly irrigation quota for this species, in terms of saving water, is 900 m^3 x hm (-2) (Zheng et al., 2010).

There have been studies stating that Ningxia is the optimal place for producing high-quality *L. barbarum* fruit (Zheng G.Q., Zheng Z.Y. et al., 2010). Possible favourable factors for the quality of these respective soils are the mineral-rich deposits (loess) from the Huan He River.

From an ecological point of view, this species is resistant to pollution (Möllerová, 2005) and there have even been studies attesting the fact that the goji shrubs may be used for aiding the conversion of croplands into forests, especially in lower mountain regions (Guoxiong, 2007).

In a similar way, LB can also have the ecological function of fixing soil or preventing erosions. And in some regions, especially where it is grown as a hedge, it can also act as an ecological barrier (buffer strip).

The experimental field, where we have chosen to plant the two LB varieties, is situated in the northern part of Bucharest. This city is situated in Vlăsiei Plain, the middle section of the Romanian Plain which is bordered by the Titu-Gherghiţa Plain in the north, by the Bărăganul Mostiştei Plain in the east and south-east, by the Ialomiţa River in the northwest and by the lower valley of the Argeş in the west and southwest.

This is a typical tubular plain, with wide and flat interfluves. The foundation consists of Proterozoic crystalline deposits belonging to the Vlach Platform. On top of these, there are sedimentary deposits of Paleozoic and Mesozoic age, topped by Quaternary surface

materials represented by 2 to 12 m thick layers of loess on which numerous coves have formed through compacting and suffusion processes.
Most of the relief is that of a smooth plain. The native vegetation of this plain consists of deciduous forests with *Quercina* species, remains of the Vlăsiei Woods and with tree steppe meadows.
From a pedological point of view, the predominant soil class is that of the red preluvosoils which have a clayish texture, a 6.2 pH and a humus content between 2.17% and 2.64%. As a conclusion, the relief and soil requirements of the LB species is met by the experimental field's characteristics.

Plant architecture

In most works, *Lycium barbarum* L. has been described as a decumbent, sometimes climbing, perennial nanophanerophyte which can grow up to 6 m tall and 3 m wide, if left unattended (Missouri Botanical Garden, 1932). Usually the cultivated shrub isn't allowed to grow beyond 2 m high so that its fruit can be easily harvested.
The species has a moderate growth rate which is somewhere around 0.5 m/year. The plants are usually resistant to cuttings as they tend to regenerate easily.
The two years old *Lycium* shrubs planted in the experimental field, have displayed similar growth tendencies as described in the aforementioned scientific works. In the first year from planting (2011), the average plant height for the first goji variety (V_1) was 52 cm. The next year (2012), the average plant height for V_1 plants was 108 cm, thus, the plants' growth rate was 0.56 m/year.
The second variety, however, displayed a more luxuriant growth rate: 1.23 m/year. In the first year from planting, the V_2 plants had reached an average height of 53 cm, being mostly as tall as the V_1 plants. The second year, however, the plants from the second variety had reached an average of 178 cm, being almost twice as tall as those of the first variety.
Goji plants have a large number of curved, flexible branches which tend to bend if not supported. Their colour is either pale brown or white-greyish, with a glabrous surface. It is possible for the branches to also have small thorns, approximately 1 cm long, which sometimes develop into a proper branch, capable of bearing leaves, flowers and fruit.
These characteristics have been displayed by the shrubs from the experimental field. Some of the plants' branches, and also their shoots, have 1-3 cm long thorns which sometimes develop into proper branches. This characteristic has been observed mostly on V_1 plants.
Because of the goji's decumbent nature, in the second year from planting, the shrubs have been assisted in their growth using tutors (bamboo canes). This has been done taking into account the plants' natural growth tendencies (Figure 1).

Figure 1. LB V1 shrub which has been tutored

The tutored plants' shape has been determined by interpreting their biological measurements, and was found to be the most similar to a truncated cone or to a reversed truncated cone.
Leaves. The leaves of this species form on the shoot either in an alternating arrangement or in bundles of up to three. Their shape is symmetrical, but it tends to vary from: ovate to lanceolate or even to elliptical, depending on the cultivar or on the plants' individual biological characteristics. The surface of the leaves is glabrous, with their upper side being deep green, and their back being paler, glaucescent. Somewhat fleshy and with

inconspicuous lateral veins, they gradually pass into the petiole.

The plants in the experimental field showed similar biological characteristics as in scientific literature. However, there were some shape differences between V_1 and V_2 plants' leaves. Also, V_2's leaves were a paler shade of green than those on V_1's plants (Figure 2).

Figure 2. The leaves of a V2 LB individual

Figure 3. The leaves of a V1 LB individual

The V_1 leaves were broader, usually ovate or elliptical (Figure 3). The V_2 leaves were mostly lanceolate. It can also be noted that V_1 plants manifested variability for the shape of their leaves. The leave shape was differed between individuals, but also at individual level.

Flowers. *Lycium* flowers grow solitary, from leaf axils, or in fascicles of 2-6. Their corolla is infundibuliform, with 5 petals. Their colour can vary from light violet to dark purple in early stages, while flowers in late stages lose their colour becoming white-yellow.

In its centre, the flowers are lighter in colour and are traversed by darker-coloured nerves which lead to dense tufts of long hairs at the base or a little above it. The stamens, usually around 5, are longer than the corolla, but slightly shorter than the style.

The *Lycium* shrub flowers from June to September, depending on its environmental conditions. According to some sources, the shrub continues to flower until November (Fleischman, 2006).

These characteristics were displayed by the plants in the experimental field. There were no notable differences between the V_1 and V_2 flowers characteristics. Still, V_2 plants bore far less flowers than V_1 plants.

In 2011, V_1 individuals flowered towards the end of June and V_2 individuals started flowering in august. Both varieties continued flowering until late November. The next year, both phenotypes started flowering in May and continued this process until the beginning of December. Thus, it appears that the plants in the experimental field have a wider flowering interval than mentioned in scientific literature.

Fruit. Goji fruit are orange-red glabrous berries. Their shape may vary, depending on cultivar or individual biological characteristics, from oblong-ovoid to obtuse, or even to apiculate. They are approximately 1 cm long and 0.5-1 cm broad, again depending on cultivar or on the plants' own biological characteristics. The berries contain around 10-20 seeds inserted into the septum. They are brownish-yellow, globose-reniform, 2.5-3 mm long and 2.25-2.5 mm broad. The goji shrubs start fruiting from July until October in the northern hemisphere.

The plants from the experimental field displayed very similar characteristics. The average weight of a goji berry varied between 0.2 g and 0.4 g. V_2 fruit were slightly heavier, with an average of 0.4 g, while V_1 fruit had an average of 0.3 g. The average length of the

berries varied between 0.9 cm and 1.5 cm, and the average diameter was between 0.6 and 0.8 cm. Again, V_2 fruit were bigger than V_1 fruit, with an average length of 1.5 cm and an average diameter of 0.8 cm (Figure 4), as opposed to an average length of 1.2 cm and an average diameter of 0.7 cm (Figure 5).

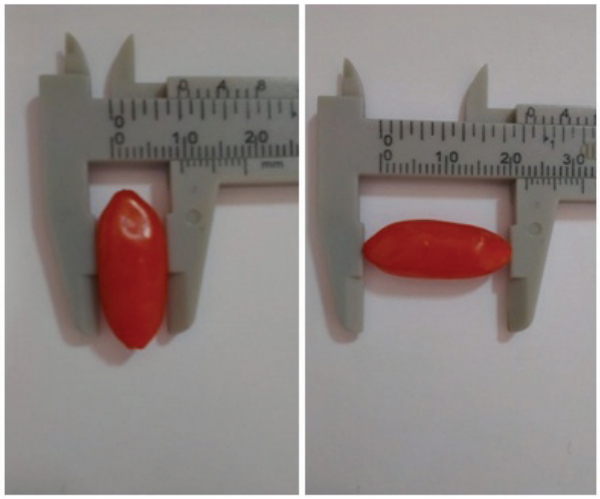

Figure 4. Broadness and length of LB V2 fruit

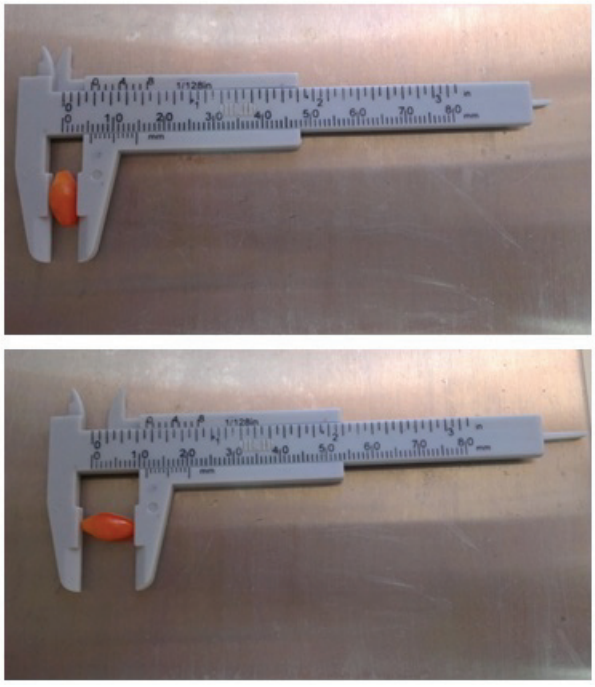

Figure 5. Broadness and length of LB V1 fruit

In 2011, the goji shrubs started fructifying towards the end of June and continued until the end of November. The next year, fructifying started earlier, towards the middle of May, and continued until December. We can conclude that the goji shrubs in the experimental field have shown precocity in their fructifying.

Pests and diseases

In England, Japan and Holland, there have been records of a certain eriophyoid mite, called goji gall mite or Kishida (*Aceria kuko*) which have infested goji plants. Although the plants have been labelled as being *Lycium barbarum*, specialists are of the opinion that this is a confusion induced by the fact that both species: *Lycium barbarum* L. and *Lycium chinense* Mill. are commercially known as goji. This opinion is backed by the fact that *A. kuko*, which has its reported range in Asia, has not been recorded on the *L. barbarum* species. Instead, one of its hosts is the aforementioned *L. chinense* (Ostojá-Starzewski, 2009).

The same author has mentioned that *Aceria* species associated with LB are: *Aceria eucricotes* (Nalepa), also known as A. *lycii* (Canestrini) and *Aceria kendalli* Baker, also known as A. *eucricotesmultistriata* (Kendall). Also, there have been studies reporting that *Lycium barbarum* can be an additional solanaceous host for *Candidatus Liberibacter*, a bacterium associated with the zebra complex disease of potato (Wen et al., 2009).

The material collected from the sticky traps placed on both of the *Lycium* varieties, in the experimental field, comprised a great variety of taxonomical groups. Both arthropod pests and auxiliary arthropod fauna were identified.

Though numerous arthropod groups were identified, none of these species were specific pests of the goji shrubs. The identified pests are, mostly, polyphagous species which are directly linked with the flora species from the immediate vicinity of the *Lycium* shrubs, rather than with this species itself.

The most representative data was that recorded in August and September (Table 1). The helpful fauna, identified consisted of parasites from the *Hymenoptera* group and predators from the: *Araneae*, *Dermaptera*, *Hemiptera*, *Coleoptera* and *Diptera groups* (Table 2).

The species with the highest frequency were the *Hymenoptera* parasites, the *Coleoptera* and *Neuroptera* groups and the bugs from the *Hemiptera* order.

We can conclude by pointing out the wide variety of recorded taxonomical groups and the high frequency of entomophagous species within all the systematic groups. Factors contributing to these numbers may be the

influence of the cultivated and wild flora surrounding the goji shrubs, as well as the lack of treatments with pesticides for this culture.

Table 1. Listing and frequency of arthropod pests collected from Lycium barbarum shrubs

Taxon	August	September
ACARI Ord.	23	16
THYSANOPTERA Ord.		
	7	4
HEMIPTERA Ord.		
Cicadellidae Fam.	35	20
Membracidae Fam.	2	-
Cixiidae Fam.	3	-
Delphacidae Fam.	15	21
Flatidae Fam.	7	4
Iassidae Fam.	3	-
Cercopidae Fam.	8	5
Aphididae Fam.	14	27
Triozidae Fam.	4	2
HETEROPTERA Subord.		
Tingidae Fam.	2	-
Miridae Fam.	3	6
Pentatomidae Fam.	3	5
Gerridae Fam.	1	3
Pyrrhocoridae Fam.	5	-
Lygaeidae Fam.	3	2
Piesmatidae Fam.	2	-
COLEOPTERA Ord.		
Elateridae Fam.	5	-
Halticidae Fam.	7	2
Bruchidae Fam.	2	-
Curculionidae Fam.	2	1
Bostrichidae Fam.		-
LEPIDOPTERA Ord.		
Gracillariidae Fam.	2	1
Noctuidae Fam.		1
Pieridae Fam.	3	-
Papilionidae Fam.	1	-
DIPTERA Ord.		
Brachycera Subord.	9	16
Nematocera Subord.	6	3
TOTAL	**177**	**139**

The choice for an integrated management of our *Lycium* agro-system is backed by the fact that it is an efficient form of conservation of the helpful fauna which, in turn, makes it possible to maintain and observe the unaltered trophic relations that form within this system.

Production

The biggest producer and exporter of goji berries and other goji products is China. These products are exported every year to countries such as Australia, Canada, the U.S.A. and the European states. It has been estimated that nearly 100,000 t of berries were produced in 2004 in regions such as Xinjiang, Shaanxi or Gansu alone. The most productive of all the regions in China is Ningxia, where goji production varied between 20,001-25,000 t in the year 2002, according to the Chinese Ministry of Agriculture.

Table 2. Listing and frequency of helpful arthropod species collected from Lycium barbarum shrubs

Taxon	August	September
ARANEAE Ord.	4	9
THYSANOPTERA Ord.		
Aeolothripidae Fam.	5	4
DERMAPTERA Ord.		
Forficulidae Fam.	2	-
HEMIPTERA Ord .		
Anthocoridae Fam.	5	7
Nabidae Fam.	2	1
Miridae Fam.	7	2
NEUROPTERA Ord.		
Chrysopidae Fam.	11	6
Hemerobiidae Fam.	4	3
HYMENOPTERA Ord.		
Chalcidoidea Suprafam.	18	23
Ichneumonoidea Suprafam.	6	3
Formicoidea Suprafam.	42	30
COLEOPTERA Ord.		
Coccinellidae Fam.	12	18
Staphylinidae Fam.	3	7
DIPTERA Ord.		
Syrphidae Fam.	6	4
TOTAL	**127**	**117**

In the experimental field, the total fruit yield for 2011 was 1,343.16 g. In 2012, the total fruit yield was 6,512.13 g, nearly 5 times bigger than in the first year. The average fruit yield, per plant, for V_1 was 88.33 g in 2011 and 317.74 g in 2012. For V_2, the average fruit yield, per plant, was 2.68 g in the first year and 50.77 g the second year from planting.

Judging by the second year's productions, the average fruit yield per hectare (ha), for V_1 was almost 795 kg. For V_2, the average fruit yield/ha was 127 kg. These values have been calculated considering that there are 2,500 goji shrubs grown on 1 ha of land (4 m^2 planting space per plant).

In 2012, the average value for dry substance within V1's fruit was 15.84%. For V_2's fruit, this indicator was 17.26%, demonstrating a superior dry substance concentration.

The values have shown that the goji shrubs are still developing and they haven't reached their normal production capacity.

CONCLUSIONS

Having compared the information from the scientific literature with our own results from the experimental field in Bucharest, we can conclude that most of the data were consistent with each other.

The plants in the experimental field have shown a greater flowering and fructifying precocity, than mentioned in the speciality studies. Also, they have had an extended period for these two stages. The first variety (V_1) showed a greater conformity with the literature in its characteristics. The second variety (V_2) had a more luxuriant growth rhythm and a lower adaptability potential.

We weren't able to find any specific pests or diseases for this *Lycium* species, but a wide variety of entomophagous species has been reported on the shrubs, due to the lack of pesticides and to the influence of the cultivated and wild flora surrounding the goji plants.

We can conclude that the agro-pedo-climatic conditions at U.Ş.A.M.V.'s campus, as well as the whole ecosystem existing in the experimental field, have proved to be a favourable context for planting and growing the *Lycium barbarum* shrubs, especially the ones from the first variety.

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