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[1.]Viktor J. VOJNICH, [2.]Attila HÜVELY, [3.]Judit PETŐ

CULTIVATION AND APPLICATION OF *Euphorbia Lagascae* SPRENG

[1-3.]Pallasz Athéné University, Faculty of Horticulture and Rural Development, Kecskemét, HUNGARY

ABSTRACT: *Euphorbia lagascae*Spreng is native to south-eastern Spain and Sardinia. The plant is annual and important species of the family *Euphorbiaceae*. The seed oil content (48–52%) consisting of about 58–62% cis-12,13-epoxyoleic acid, also called vernolic acid with attractive applications for the oleochemistry. As a long-chain fatty acid, vernolic acid can have various utilizations, e.g. dyes, coatings and plasticizer-stabilizer. Polyvinyl chloride can be more resistant to degradation by temperature and light when epoxyoleic acid is used in its composition. Epoxydized oils or their esthers can also be important components of phenolic resins for the electronic industry. The seeds of *Euphorbia lagascae* are also reported to contain piceatannol (a type of phenolic compound), which can increase apoptosis in some cancer cell lines. The large-scale production of the species is hindered by severe seed shattering trait universal in wild accessions. For this reason, breeding and domestication work have been undertaken in Spain, Germany and the Netherlands in recent years.
Keywords: *Euphorbia lagascae*, cultivation, seed yield, vernolic acid, oil content

1. INTRODUCTION

Synthesis of seed oils is restricted to unsaturated fatty acids with the chain length of 18 carbon atoms, especially oleic and linoleic acids. However, some plant species are able to produce unusual fatty acids with chain length variations (short, very long, respectively), or with functional groups within the fatty acid molecule (conjugated double bonds, hydroxy or epoxy groups) [1]. Epoxidation is one of the most common additive reactions with double bonds of unsaturated fatty acids [2]. So far, six different epoxy fatty acids have been found in seed oils of over 60 species of 12 plantfamilies [3,4,5]. The majority of examined species supplied fatty acids of little value only, which could not be adapted for commercial application [1]. It is exclusively in *E. lagascae* and in *Bernardia pulchella* (family *Euphorbiaceae*) and several species of the genus *Vernonia* (*Vernonia galamensis* and *V. anthelmintica,* family *Asteraceae*), that vernolic acid (cis 12,13-epoxy oleic acid) was discovered in more than 50% to 79% of the seed oil [5,6,7,8,9].

Figure 1. *Euphorbia lagascae* Spreng

Anti-carcinogenic characteristics of some species from the *Euphorbiaceae* family are well-known. Quercetin in *Euphorbia hirta* was found to inhibit carcinogenesis in lab animals [10]. The milky latex of *Euphorbia tirucalli* contain tigliane, inganen, terpenic alcohol, taraxasterol and tirucallol [11]. Piceatannol is a natural stilbene occurring in *Euphorbia lagascae.*

Euphorbia lagascae can be annual, biennial and sometimes perennial. The species has been grown for very different purposes, it can be medicinal and ornamental. It is folkloristically called ”moleplant” for its capacity to repel rodents. Vernolic acid, representing the main fatty acid of *E. lagascae* had been characterized as an isomeric compound of the ricinoleic acid (11-hydroxy monoenoic acid) [12]. After the isolation of ricinoleic acid, Gunstone proved in 1954 that vernolic acid not being a hidroxy but an epoxy fatty acid. This was the first proof of an epoxy fatty acid occurring in seed oils [1]. *E. lagascae* apart from Vernonia spp. is considered to be the most promising natural source of vernolic acid.

The oil content and the anticancer characteristics of *E. lagascae* make this species a valuable raw material for both pharmaceutical and petrochemical industry. However, the large-scale production of the species is hindered by severe seed shattering common in wild accessions. For this reason, breeding and domestication work have been undertaken in Spain, Germany and the Netherlands in recent years.

Today, the range of main distribution of the species covers the southeastern parts of Spain (Valencia, Murcia and Andalusia) but the herb is also present in the arid southeast and the coastal region of Cadiz [13,14]. In Spain, the most northern distributional area of the species is Catalonia [15]. *E. lagascae* is also indigenous to Sardinia [16,17].

The species colonizes spontaneously on devastated cultivated lands and road margins with loamy soils rich in nitrogen and often of saline origin, however it avoids silicic soil types [13]. *E. lagascae* requires specific edaphic and climatic conditions for its permanent colonization of given locations. This is particularly true for the neutral to moderately alkaline soils with mainly clayish structure as well as semiarid climates with temperate but not too cool winters. At lighter soils, water might become a growth limiting factor, since seed ripening happens to occur during the low-rainfall period of the year [1]. The species germinates in autumn after the first rains, flowers in March or April, and becomes ripe from April to May.

2. MATERIAL AND METHODS

In Europe, the first plant performance tests were performed in Göttingen in 1986. In Spain, in the Murcia region first cultivations started in 1988 to collect data for agronomical techniques and potential of *E. lagascae* for future oil crop production [1]. *Breemhaar* and *Bouman* [18] performed experiments in the Netherlands on mechanical harvesting and cleaning *E. lagascae.* First field trials with *E. lagascae* in the region of Murcia, Spain were meant to explore the chances of cultivation of this species over winter. Under Spanish growing conditions with winter temperatures rarely dropping below zero degree of Celsius, sowing in autumn is accomplishable. In Murcia, sowing was performed on 12th of October (autumn trial), the second field experiment was sown on 3rd of March (spring trial). After spring sowing, flowering started after about 90–100 days, while after autumn sowing it took almost the double time [1].

In a trial conducted by *Breemhaar* and *Bouman* [18] about one hectare of *E. lagascae* was sown on 22nd of April at a rate of 12.5 kg/ha. Since the crop characteristics of Euphorbia are very similar to green peas, the trials performed in 1993 and 1994 were harvested with pea harvester type FMC 879 and FMC 979. Harvesting dates were 10th and 17th of August. The trial performed by Research Station for Arable Farming and Field Production of Vegetables (PAGV) showed that the most appropriate date to harvest *E. lagascae* is when the third branches start to shed their seeds [19]. According to *Breemhaar* and *Bouman* [18] seed shedding occurred at the second branch, however by the second harvest date (17th of August) it had just reached the third branch.

In another experiment performed by *Pascual-Villalobos et al.* [20] 14 accessions of Euphorbia were tested in 2 locations in both autumn and spring trials. Accessions included in the autumn trial were equal to the ones used in spring trial. Sowing dates were 18th of October 1990 for location 1 and 24th of October 1990 for location 2. Harvesting dates were the following: spring trial was harvested in July 1990, the autumn trial of 1990 was harvested during May–June 1991.

3. RESULTS

Harvesting was performed using the following three procedures:

= Uprooting the plants after the beginning of seed dehiscence
= Drying the plants in the field using black plastic cover
= Collecting the shattered seed from the plastic cover underneath [20]

According to the results of *Pascual-Villalobos et al.* [20] the average oil content was about 44%, 63% of it was vernolic acid, however some accessions could be highlighted for having higher contents (48% and 69%, respectively). Seed yield (kg/ha) was determined upon calculating from the number of capsules m^2 and thousand seed weight. The first herbicide tolerance tests were conducted in Spain at the end of 80s, as chemical weed control was felt to be compulsory before field tests. Therefore, a pre-test was accomplished under glasshouse conditions including the components described in Table 1.

Table 1.Test on herbicide tolerance of *Euphorbia lagascae* Spreng [1].

Herbicide	Application	Rate (kg/ha)	Crop damage*	Weedreduction (% of control)	Dicot. weeds (%) dead	Seed yield (rel.)
Dicuran[1]	Pre-emerg.	1.25	0	36.7	-	125
		2.5	0	71.0	-	60
		5	0	80.2	-	101
Pyramin[2]	Pre-emerg	2	0	57.8	-	94
		4	0	86.8	-	110
		8	0	94.7	-	113
Basagran[3]	Post-emerg	2	0	-	68.3	77
		4	0	-	90.8	86
		8	0	-	96.4	126
Goltix[4]	Post-emerg.	5	5	-	75.0	130
		10	7	-	83.3	97
		20	9	-	84.1	0
Tribunil[5]	Post-emerg.	1.5	2	-	77.8	158
		3	7	-	75.0	96
		6	7	-	88.2	95

[1] 700 g/l Chlortoluron; [2] 65% Chloridazon; [3] 480 g/l Bentazon; [4] 70% Metamitron;
[5] 70% Methabenzthiazuron, * 0 = no damage; 9 = severe damage

The field test was conducted in Göttingen in 1986. The herbicides were applied at preemergence 2 days after sowing or at post-emergence when plants had reached 4–8 leaves. Concentrations were applied as specified by the producer and in addition with the half and double dose, respectively. Controls remained unsprayed and unweeded. As shown in *Table 1,* Dicuran (700 g/l Chlortoluron) and Pyramin (65% Chloridazon) exerted no damage to the crop, however with 20 kg/ha Goltix (70% Metamitron) all crop plants perished [1].

During another trial conducted by *Breemhaar* and *Bouman* [18], 0.6 l Stomp (pendimethalin) and 3.2 l Propachlor (propachlor) ha–1 at pre-emergence were applied. Weeds were controlled mechanically with rear-mounted tractor. During flowering the crop was sprayed against botrytis with Ronilan (vinclozolin) when necessary [18].

4. CONCLUSIONS AND RECOMMENDATIONS

Breeding trials

The major difficulty that makes this species hard to commercialize is the severe capsule dehiscence, which is a disadvantageous trait common to all accessions. That means improving this plant by breeding is inevitable. For this reason, *Pascual-Villalobos et al.* [20] applied a useful technique called induced mutagenesis. Previously, some mutants had already been selected by *Vogel* and *Röbbelen* [21] after chemical mutagen treatment. Non-shattering genotypes have been selected frequently as spontaneous mutants from natural populations, e.g. shattering-resistant *Lupinus luteus* [22], *Vicia faba* [23], *Vicia sativa* [24] and *Onobrychis viciifolia* [25]. Mutation breeding has been successful to induce shattering-resistant mutants of *Brassica juncea* [26] and soybean [27].

Induced mutagenesis

After choosing the appropriate accession, 500 g of ungerminated seeds were presoaked for 12 h at room temperature (20–22 °C) in a thin layer on wet filter paper. Seed lots of each 50 to 100 g were submersed in solutions of ethyl methanesulphonate or EMS ($CH_3SO_3C_2H_5$) for 2 to 6 h using a concentration between 0.4 and 1% EMS at pH 7. Seeds were thoroughly post-washed in running tap water for 12 h and surface-dried. The second mutagenic treatment was applied in essentially the same way. In both cases, controls were used, i.e. equal amounts of seeds were treated the same way except for the use of EMS [28].

M1 generation (the first generation after chemical treatment) was grown from treated seed in the glasshouse at Göttingen (Germany) and bulk harvested. M2 and subsequent generations were grown in the open field at Torreblanca Experimental Station (Murcia, Spain). Screening for indehiscence was done by visual observation at ripening in M2 and M3 generations.

M3 and M4 generations were sown in the open-field in spring at Torreblanca Experimental Station two locations and two sowing dates (autumn and spring) were used [28].

Diseases and pests

During the cultivations performed by *Vogel et al.* [1] in Spain, the only disease invariably found was rust caused by *Melampsora euphorbiae* (Schub.) Castagne. Occurrence of *Melampsora euphorbiae* on *E. lagascae* has been reported for the first time by *Pascual- Villalobos* and *Jellis* [29]. The symptoms appeared first on the adaxial surface of the leaves, where the yellowish uredinia were surrounded by a chlorotic area. Under favourable conditions for disease spread, defoliation occurs and finally blackish telia appear on stems and older leaves. Chemical control was successful with spraying a 0.2% solution of Plantvax-EC (active ingredient: oxycarboxin) once a week at outburst of the disease and later every 10–12 days. *Vogel et al.* [1] observed *Phytium ssp.* on the roots of some plants as a possible cause of the disease, however no treatment against this agent was tried out. Among pests the following most common ones were observed: leaf bugs *(Nezara viridula),* the black bean louse *(Aphis fabae),* a green louse species of the genus Acirtosiphum (possibly *A. pisum*), and the Euphorbia moth *(Celerium euphorbiae).* Because of low grades of infestation, no treatment was applied.

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