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## Review – Szemle

### Distribution, utilization and cropping possibilities of *Euphorbia lagascae*

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#### SUMMARY

*Euphorbia lagascae* Spreng. is an annual spurge native to south-eastern Spain and Sardinia. The species is characterized by its valuable 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 esters 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*, *Euphorbiaceae*, vernolic acid, fatty acid, oilseed crop.

#### 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) (Vogel *et al.* 1993). Epoxidation is one of the most common additive reactions with double bonds of unsaturated fatty acids (Carlson and Chang 1985). So far, six different epoxy fatty acids have been found in seed oils of over 60 species of 12 plant

families (Morris and Wharry 1966, Krewson 1968, Earle 1970). The majority of examined species supplied fatty acids of little value only, which could not be adapted for commercial application (Vogel *et al.* 1993). 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 (Kleiman *et al.* 1965, Earle 1970, Campbell 1981, Perdue *et al.* 1989, Spitzer *et al.* 1996).

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 (Erlund 2004). The milky latex of *Euphorbia tirucalli* contain tigliane, inganen, terpenic alcohol, taraxasterol and tirucalol (Cataluna and Taxa 1999). 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) (Vidyarthi 1940). 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 (Vogel *et al.* 1993). *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.

## DISTRIBUTION

At the time of the first botanical characterization about *E. lagascae*, Sprengel (1821) was unable to determine the region of origin. The first accurate description originates from Nyman (1854–1855) who stated the species to be indigenous in southern Spain (Granada, Murcia). Willkomm (1893) specified the provinces of Granada and Valencia as the principal distributional area in Spain (Vogel *et al.* 1993).

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 (Krewson and Scott 1966, De Bolos and Vigo 1990). In Spain, the most northern distributional area of the species is Catalonia (Rovira 1987). *E. lagascae* is also indigenous to Sardinia (Tutin *et al.* 1968, Pignatti 1982) (Figure 1.).



Figure 1. Natural occurrence of *Euphorbia lagascae*

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 (Krewson and Scott 1966). *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 (Vogel *et al.* 1993). The species germinates in autumn after the first rains, flowers in March or April, and becomes ripe from April to May.

## BOTANICAL DESCRIPTION

### Taxonomy

Genus: *Euphorbia*

Family: *Euphorbiaceae*

Subfamily: *Euphorbioideae*

Tribe: *Euphorbieae*

Subtribe: *Euphorbiinae*

Taxon: *Euphorbia lagascae* Spreng.

*Euphorbia lagascae* Spreng. is an annual species containing  $2n = 16$  chromosomes (Perry 1943, Singh 1968). The species produces a central primary shoot, which can reach the height of 60–110 cm. On the base of the primary shoot, two vigorous cotyledonary shoots are connected. Leaves are inserted on the primary and the two secondary (cotyledonary) shoots. Shape of the leaves is oblong-lanceolate with a length/width ratio of 3–4:1. On the upper part of primary and cotyledonary shoots, the generative organ, called the pseudoanthium can be found, which in the genus *Euphorbia* is called cyathium. Cyathium is usually placed amongst a threefold pseudo-whorl. This whorl consists of three leaves, which extrude each one side branch. Each of these side branches bifurcate up to seven times carrying a cyathium in each bifurcation point (Troll and Heidenhain 1952). The whorl leaves of the cyathium, also called bracts, have an oval-lanceolate shape with a length/width ratio of 1–1,5:1 (Vogel *et al.* 1993). The cyathium itself is a reduced inflorescence, which resembles a solitary flower by interlacement of five bracts (whorl leaves) to form an involucre (Schmidt 1907, Weberling 1981). Four stuffed extranuptial nectaries functioning for insect attractions can be found at the upper edge of the involucre. In the central part of the cyathium is the female flower to be found consisting of a tripartite, uniovulate ovary. The ovary is surrounded by male flowers, (five groups with two male flowers each) each represented by one anther (Goebel 1931). Both male and female flowers are exempt from any perianth (Vogel *et al.* 1993)

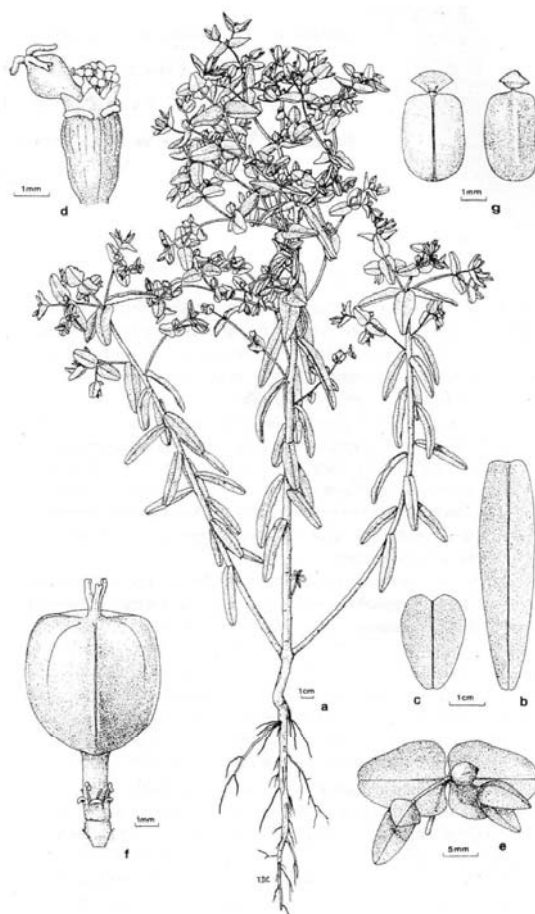
The cyathia of the genus *Euphorbia* usually behave as allogamous, but self-fertile. The stigma of the inflorescence is receptive before the pollen is viable (protogyny). According to Bodman (1937) the female flower of the genus *Euphorbiaceae* bend down geotropically towards the missing nectary prior to protrusion of the first anthers out of the involucre. At the end of the flowering, the anthers fall off and the ripening capsule assumes again to a standing-up position (Bodman 1937)

From the ovary a tripartite capsule is developed. At ripening, each of the three carpels split up from the bottom to the tip. Consequently, the included seeds will be shot away (Vogel *et al.* 1993). *E. lagascae* possess a loculicidal capsule. The capsule wall (pericarp) in wild plants differs remarkably from the one found in indehiscent cultures. In plants growing wild, the capsule wall usually consists of three pericarp layers: the epicarp made of schlerenchyma cells, the mesocarp with a palisade parenchyma, and the endocarp with a compacted collenchyma tissue.

During ripening, tensions are obviously built up between these three tissues, which evoke an opening of the capsule. The improved indehiscent variants do not include the mesocarp layer with the palisade parenchyma. As a result, they have significantly thinner fruit wall which results in lower fruit wall weight. Consequently, in absence of the mesocarp layer such tensions are not generated, which result in capsule indehiscence (*Pascual-Villalobos et al.* 1994).

Shape of the seeds is longish and rectangular, their surface is brownish-glossy. At their upper edge, a special formation, a yellow caruncle can be found. The caruncle supports the release of the seed from the placenta giving them super-fine energy to catapult away (*Pax and Hoffmann* 1931). Seed composition of the species is characterized by its high seed oil content of about 48–52%, consisting of about 58–62% vernolic acid.

The morphological characteristics of *Euphorbia lagascae* Spreng. is shown by *Figure 2*.



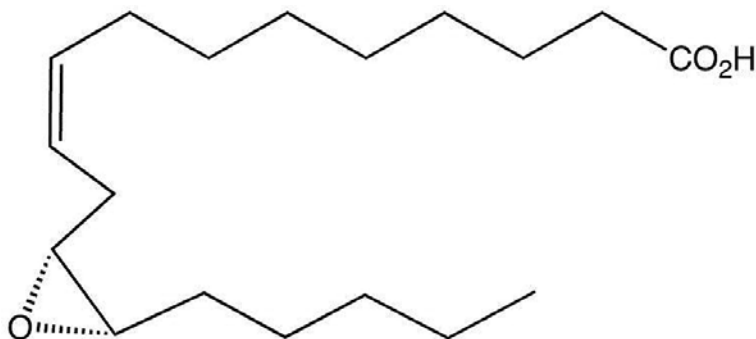
a – whole plant, b – leaf of primary and secondary (cotyledonary) shoots, c – whorl leaf (bract) of the cyathium, d – cyathium, e – capsule with bracts, f – ripe capsule, g – seed with caruncle (front and side view)

*Figure 2.* Morphological characteristics of *Euphorbia lagascae* Spreng. (*Vogel et al.* 1993)

## UTILIZATION

*Euphorbia lagascae* has been grown for very different purposes. The seed oil is a marketable product however its industrial use is still little less known. Along with medicinal uses, studies dates back to 1976 by Nobel Prize M. Calvin suggested to use it for biofuels, oil composition of wild spurge being very close to that of diesel oil. Ten years later, *Hirsinger* (1989) corroborated this hypothesis.

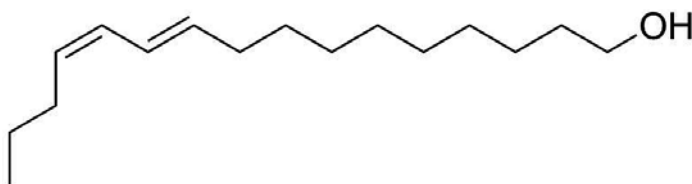
Vernolic acid (*Figure 3.*) is the most important natural epoxy fatty acid in the seed oil *Euphorbia lagascae*. The oil is of significant interest in lubricant and polymer industries as a biodegradable replacement of petrochemical oils or as an oil with new properties (*Verdolini et al.* 2004). The seed oil consists of over 60% vernolic acid allowing a relatively easy recovery of this versatile starting material for the synthesis of a wide range of (fine) chemicals (*Cuperus and Derksen* 1996). *Cuperus and Derksen* (1996) examined several synthesis routes for the production of selected special chemicals in which the vernolic acid epoxy group serves as a key functionality.



*Figure 3.* Structural formula of vernolic acid

### *Utilization in the chemical industry*

Bombykol ((10E,12Z)-hexadeca-10,12-dien-1-ol) (*Figure 4.*) has been identified as a sex pheromone of the silkworm (*Bombyx mori*) and can be used in pest control. Bombykol can be synthesized starting from vernolic acid. First the epoxy group was ring-opened, resulting in a diol product.



*Figure 4.* Structural formula of bombykol



This diol could be selectively cleaved to the aldehyde without affecting the carbon-carbon double bond and leaving hexenal as a side-product, that in itself may find useful applications in the flavor industry. Protecting the carboxylic acid group and isomerization of the double bond yielded a reactive intermediary product [12-oxo-10(E)-dodecenoic acid] which contained a carboxylic ester and also an aldehyde functionality in conjugation with the carbon-carbon double bond. Subsequent Wittig olefination reaction leads to the 10(E), 12(Z)-product, which is selectively reduced to bombykol (Cuperus and Derksen 1996). Traumatic acid (Dodec-2-enedioic acid) (Figure 5.) is a wound healing agent in plants ("wound hormone") that stimulates cell division near a trauma site to form a protective callus and to heal the damaged tissue. Traumatic acid can be used as an intermediate in prostaglandine synthesis. The first part of the synthesis is identical to the synthesis of bombykol. Mild oxidation of the reactive aldehyde 12-oxo-10(E)-dodecenoic acid leads to the formation of a carboxylic acid functionality while leaving the carbon-carbon double bond unaffected. Traumatic acid can be utilized as a base for certain pharmaceuticals. (Cuperus and Derksen 1996).

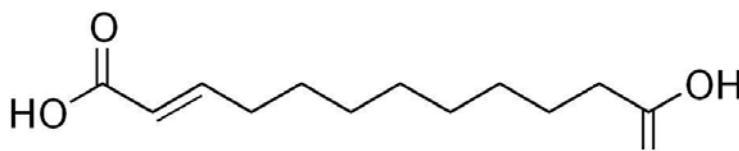


Figure 5. Structural formula of traumatic acid

#### **Pharmaceutical utilization**

Piceatannol (Figure 6.) is a stilbenoid, considered to be an anti-carcinogenic compound.



Figure 6. Structural formula of piceatannol

Duarte *et al.* (2008) successfully isolated piceatannol, two coumarins and two 12-deoxyphorbol diterpene esters by chromatographic methods, from the methanol extracts of the defatted seeds of *Euphorbia lagascae*. The structures of these compounds were elucidated by a combination of physical and spectral data (IR, MS and high-resolution (1)H-NMR and (13)C-NMR spectroscopy utilizing COSY, HMBC, HMQC and NOESY

experiments). The stilbene, piceatannol, was screened for its antileishmanial activity against promastigotes as an extracellular form of *Leishmania donovani*, *Leishmania infantum* and *Leishmania major* and amastigotes of *Leishmania donovani* as an intracellular form. Piceatannol was moderately active against the extracellular forms of the three tested *Leishmania* species, and more active than the reference compound against the intracellular form of *Leishmania donovani* (Duarte *et al.* 2008)

## CROPPING POSSIBILITIES

### *Domestication*

First field tests were accomplished in the USA (White and Wolff 1968, White *et al.* 1971). 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 (Vogel *et al.* 1993). Broomhaar and Bouman (1995) performed experiments in the Netherlands on mechanical harvesting and cleaning *E. lagascae*.

### *Sowing and harvesting*

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 12<sup>th</sup> of October (autumn trial), the second field experiment was sown on 3<sup>rd</sup> of March (spring trial). After spring sowing, flowering started after about 90–100 days, while after autumn sowing it took almost the double time (Vogel *et al.* 1993).

In a trial conducted by Broomhaar and Bouman (1995) about one hectare of *E. lagascae* was sown on 22<sup>nd</sup> of April at a rate of 12.5 kg ha<sup>-1</sup>. 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 10<sup>th</sup> and 17<sup>th</sup> 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 (Borm and van Dijk 1993). According to Broomhaar and Bouman (1995) seed shedding occurred at the second branch, however by the second harvest date (17<sup>th</sup> of August) it had just reached the third branch.

In another experiment performed by Pascual-Villalobos *et al.* (1992) 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 18<sup>th</sup> of October 1990 for location 1 and 24<sup>th</sup> 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.

Harvesting was performed using the following three procedures:

1. Uprooting the plants after the beginning of seed dehiscence (Figure 7.)
2. Drying the plants in the field using black plastic cover
3. Collecting the shattered seed from the plastic cover underneath

(Pascual-Villalobos *et al.* 1992)

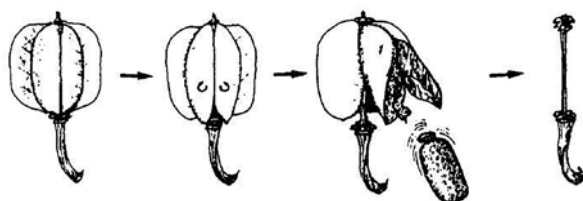


Figure 7. Process of capsule (seed) dehiscence in *E. lagascae* (Röbbelen *et al.* 1994)

According to the results of Pascual-Villalobos *et al.* (1992) 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 ( $\text{kg ha}^{-1}$ ) was determined upon calculating from the number of capsules  $\text{m}^{-2}$  and thousand seed weight.

### Plant protection tests

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. (Vogel *et al.* 1993)

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

<sup>1</sup> 700 g/l Chlortoluron; <sup>2</sup> 65% Chloridazon; <sup>3</sup> 480 g/l Bentazon; <sup>4</sup> 70% Metamitron; <sup>5</sup> 70% Methabenzthiazuron  
\* 0 = no damage; 9 = severe damage

The field test was conducted in Göttingen in 1986. The herbicides were applied at pre-emergence 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 (Vogel *et al.* 1993).

During another trial conducted by *Breemhaar* and *Bouman* (1995), 0.6 l Stomp (pendimethalin) and 3.2 l Propachlor (propachlor) ha<sup>-1</sup> 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 (*Breemhaar* and *Bouman* 1995).

### **Diseases and pests**

During the cultivations performed by *Vogel et al.* (1993) 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* (1992). 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.* (1993) 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.

### **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.* (1992) applied a useful technique called induced mutagenesis. Previously, some mutants had already been selected by *Vogel* and *Röbbelen* (1989) after chemical mutagen treatment. Non-shattering genotypes have been selected frequently as spontaneous mutants from natural populations, e.g. shattering-resistant *Lupinus luteus* (*Von Sengbusch* and *Zimmermann* 1937), *Vicia faba* (*Sirks* 1931), *Vicia sativa* (*El-Moneim* 1993) and *Onobrychis viciifolia* (*Knoll* and *Baur* 1942). Mutation breeding has been successful to induce shattering-resistant mutants of *Brassica juncea* (*Rai* 1959) and soybean (*Humphrey* 1954).

**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 ( $\text{CH}_3\text{SO}_3\text{C}_2\text{H}_5$ ) 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 (Pascual-Villalobos *et al.* 1994)

M<sub>1</sub> generation (the first generation after chemical treatment) was grown from treated seed in the glasshouse at Göttingen (Germany) and bulk harvested. M<sub>2</sub> 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 M<sub>2</sub> and M<sub>3</sub> generations.

M<sub>3</sub> and M<sub>4</sub> generations were sown in the open-field in spring at Torreblanca Experimental Station two locations and two sowing dates (autumn and spring) were used (Pascual-Villalobos *et al.* (1994). Table 2. shows the genetic gain for indehiscence in *E. lagascae*.

Table 2. Genetic improvement in *Euphorbia lagascae* as a result of mutation breeding (Pascual-Villalobos *et al.* 1994)

Generation	Progenies tested (number)	Indehiscent plants (%)		Indehiscent capsules per plant <sup>a</sup> (%)		Total plants scored <sup>b</sup> (number)	Plants selected (number)
		mean	range	mean	range		
M <sub>2</sub>	–	2.2 x 10 <sup>-5</sup>		100.0	–	45384	1
M <sub>3</sub>	1	3.5		100.0	–	113	6 <sup>c</sup>
M <sub>4</sub>	6	6.5	0–17.5	100.0	–	1047	134
M <sub>5</sub>	19	38.5	0–100	50.5	11–100	1222	128
M <sub>6</sub>	76	52.4	0–100	77.8	7.3–100	4225	515
M <sub>7</sub>	386	82.3	0–100	67.4	4.8–100	15795	23

<sup>a</sup> Only in indehiscent plants; <sup>b</sup> Including dehiscent and indehiscent plants; <sup>c</sup> Four plants from the evaluated progeny and two new unrelated indehiscent mutant phenotypes

## **Az *Euphorbia lagascae* elterjedése, hasznosítása és termesztési lehetőségei**

GAÁL RICHÁRD – MÁTHÉ ÁKOS – SZŰCS ÁDÁM – VOJNICH VIKTOR JÓZSEF

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Környezettudományi Intézet  
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### ÖSSZEFOGLALÁS

Az *Euphorbia lagascae* Spreng. egy egyéves kutyatejféle, mely Délkelet-Spanyolországban és Szardínián őshonos. A faj magjának mintegy 48–52% olajtartalmára jellemző, hogy 58–62%-ban egy értékes, *cisz*-12,13 epoxi-olajsavat, ún. vernolsavat tartalmaz. A vernolsav a vegyipar területén széleskörű hasznosítási lehetőségekkel rendelkezik. Hosszú szénláncú zsírsavként festékek, bevonatok, lágyító-stabilizáló szerek gyártása során alkalmazható. A polivinil-klorid (PVC) ellenállóbb a hőmérséklet és fény általi degradációnak, amennyiben epoxi-olajsav szerepel az alkotórészei között. Az epoxidált olajok és észterek fontos komponensei lehetnek az elektronikai iparban alkalmazott fenolgyantáknak is. Ismereteink szerint az *Euphorbia lagascae* magja egy értékes fenolgyantát tartalmaz, mely néhány rákos sejtvonalnál megnövelheti az apoptózis mértékét. A biztató eredmények ellenére a nagyüzemi termesztési lehetőségek a fajra jellemző magpergés következtében korlátok közé szorúlnak. A hátrányos tulajdonság minimalizálásának céljából az utóbbi években Spanyolországban, Németországban és Hollandiában nemesítési és honosítási munka kezdődött el.

**Kulcsszavak:** *Euphorbia lagascae*, *Euphorbiaceae*, vernolsav, zsírsav, olajnövény.

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