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Asclepias syriaca L. – an underexploited industrial crop for energy and chemical feedstock

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Abstract

Milkweed (Asclepias syriaca L.), un underexploited crop up to now, is considered a multiuse plant species of the future, due to its exquisite potential for industrial processing for producing fibers, oils, nematicides, and pharmaceuticals. The current interest centers especially on the organic-soluble extractives of the whole plant, considered a potential substitute for crude petroleum. Having in view the problems derived from the seed propagation of milkweed for the development of commercial crops, our research focused both on testing the influence of seed storage conditions on germination and plantlet establishment, as well as on experimenting the methods of in vitro cultures, in order to obtain plant biomass in the form of multiple shoots. The seeds germinated sooner and in a higher proportion after stratification for 2 months in humidified perlite, at 4°C. For in vitro cultures the somatic explants (shoot apices) were inoculated on 6 variants of the basal culture medium Murashige &Skoog (MS), supplemented by various concentrations of phytohormones: BAP (6-Benzyl-aminopurine), Kin (Kinetin), TDZ (Thidiazuron = N-phenyl-N'-1,2,3-thiadiazol-5-yl urea), 2iP (2-isopentenyl adenine). Superior results in the development of multiple shoots from every inoculated shoot apex and consequently in establishing long-term morphogenetic cultures were registered under the influence of TDZ (1-2 mg/l) and of BAP in high concentrations (3-5 mg/l).

Key words: common milkweed, petroleum plants, whole plant extractives, latex, phytohormones, morphogenetic cultures

Introduction

The global problems related to the energy insecurity from the perspective of exhaustion of the fossil oil reserves, revitalized the interest for identification of alternative sources of energy, among which the plants producing energy biomass enjoy a privileged place (4).

Some phanerogam plant species, belonging to different families, are able to convert a substantial proportion of photosynthetic products in latexes containing carbohydrate compounds that can be converted into superior fuels, like petroleum. These plants, also called petroleum plants or petroplants are members of several families, the most important being Euphorbiaceae, Apocynaceae, Asclepiadaceae, Sapotaceae, Urticaceae and Convolvulaceae [3].

Common milkweed (*Asclepias syriaca* L.) is part of the petroplant category by being a hydrocarbon yielding plant, but is underexploited so far as a new agronomic crop with important potential industrial uses. It is a perennial plant belonging to Order Gentianales, Family Asclepiadaceae, originating from the Eastern part of Nord America, from Southern Canada to Mexico, but centuries ago it was naturalized and cultivated in various zones in Europe.

Asclepias syriaca has many uses in traditional medicine. The leaves and latex are described as having anticancer effect, wound healing, diuretic, anti-asthmatic, being also used to treat bronchitis, pneumonia, rheumatism, and kidney stones. The latex is present in all vegetative organs, but is found in greater quantity in the leaves, especially at high temperatures in summer and dry soils.

Besides its ornamental qualities and medicinal virtues, the leaves of the plant are the preferred food source for monarch butterfly larvae which stores some secondary metabolites, mainly cardenolides, giving protection of the adult monarch butterfly from animal predators. The seeds are provided with a tuft of silky fiber (floss) which is used as hypoallergenic filler for pillows, comforters, life jackets, etc., while the fibers of stems are used for textiles.

The seeds contain 20-30% highly unsaturated oils that were transesterified to determine the biodiesel fuel performance properties. It was also found that the seed oils modified by epoxidizing the olefin bonds of the triglycerides have potential in non-food industrial applications as ideal moisturizer ingredients in cosmetics. Similarly, field trials of milkweed seed meal and seed pods have shown promise as a potent natural nematicide and a pesticide against fall army worms [5, 6, 7, 9].

Harvesting of the whole plant, above ground level, offers a potential source of biomass, fiber, protein, crude petroleum substitute, natural rubber and chemical intermediates. The interest centers especially on the organic-soluble extractives of the whole plant, because the whole plant oil is considered a potential substitute for crude petroleum, having cracking products similar to those from naphtha category [1, 2, 8, 10, 12].

The most important requirement for bioenergy production is to develop proper agrotechnology for the plants that produce oils and hydrocarbons of high energy value.

Having in view the problems derived from the seed propagation of milkweed for the development of commercial crops, the present research focused both on testing the influence of seed storage conditions on germination and plantlet establishment, as well as on experimenting the methods of *in vitro* cultures, in order to obtain plant biomass in the form of multiple shoots to be used as a suitable material for eventual testing and optimization of cropping trials and of biomass extraction and processing for the production of biofuels.

Materials and Methods

Asclepias syriaca seeds were purchased from Easyliving Wildflowers, of Willow Springs, Mo., USA, from the harvest of 2008.

a) Seed germination tests

Sowing was done on three different dates and in various conditions of seed storage:

- 3 April 2009 and 3 May 2009 in alveolar plates, maintained in the greenhouse at the Faculty of Horticulture, University of Agronomic Sciences of Bucharest; the seedlings were transplanted to experimental plots after the second true leaf was fully expanded. The seeds were divided into two groups: ¾ of the seeds were kept in a refrigerator at 2-3° C, in several variants and ¼ of the seed storage were kept in normal room temperature of 20-23° C (104 seeds/variant).

  The experimental variants of seed storage conditions are listed in Tables 1 and 2.

Table 1. Experimental variants of seed storage conditions for 3 April 2009 sowing

Variant	Seed storage conditions
V 1	seeds packed in paper bags and kept for 1 month in refrigerator
V 2	seeds soaked in water and kept for 1 month in refrigerator
V 3	seeds stratified in moist sand and kept for 1 month in refrigerator
V 4	seeds stratified in moist perlite and kept for 1 month in refrigerator
V 5	seeds packed in paper bags and kept at normal room temperature (20 -23°C)

Table 2. Experimental variants of seed storage conditions for 3 May 2009 sowing

Variant	Seed storage conditions
V 1	seeds packed in paper bags and kept for 2 months in refrigerator
V 2	seeds soaked in water and kept for 2 months in refrigerator
V 3	seeds stratified in moist sand and kept for 2 months in refrigerator
V 4	seeds stratified in moist perlite and kept for 2 months in refrigerator
V 5	seeds packed in paper bags and kept at normal room temperature (20 -23°C)

- 15 April 2009 sowing in the field at S.C. Frasinu S.A. Buzau; the seeds, after being maintained at 4°C in refrigerator for 6 months, were sown in rows, at 1 cm depth, on a chernozem soil type, the preparatory work of land consisting of plowing, double discs and harrowing. Distance between plants in the row was 30 cm and 70 cm between rows.

b) *in vitro* culture establishment

- *Explant sources:* experiments were carried out using as explants sources plantlets having approx. 20 cm in height, obtained by germinating common milkweed seeds in laboratory conditions, in a 2:1 mixture of compost and peat;
- *Explant types*: 1 cm nodal stem segments with axillary buds;
- *Surface sterilization* : 20 minutes soaked in 10% w/v commercial bleach (Domestos), followed by 3 rinses (5 minutes each) in sterile distilled water; the external sterilization, inoculations and the subsequent transfers were carried out under aseptic conditions, in a laminar flow cabinet;
- *Morphogenetic culture establishment:* the surface sterilized explants were placed on the surface of the culture media variants (Table 3), distributed in 5 cm ø Petri plates (containing 5 ml of sterile autoclaved culture medium solidified with 8 g/l agar) and the incubation was performed in the growth chamber, at 25± 2 °C, under a 16/8 h photoperiod, with a light intensity of 3000 lux. The periodical transfers on fresh culture media were performed at 3 week intervals.
- *Culture media:* full-strength Murashige and Skoog (1962) basal medium (MS), with the addition of 3% sucrose, 0.8% agar and with different concentrations and combinations of plant growth regulators (PGRs) - Table 3; plant growth regulator supplements were added prior to the media autoclaving, which was carried out for 20 min at 121° C and the pH was adjusted to 5.8 – 6, prior to autoclaving.

Table 3. Variants of PGRs in culture medium for obtaining morphogenetic cultures from *Asclepias syriaca* nodal stem explants.

	Cytokinins – mg/l			
Var.	**BAP**	**2iP**	**Kin**	**TDZ**
A1	**3**	**-**	**-**	**-**
A2	**-**	**-**	**2**	**-**
A3	**-**	**2**	**-**	**-**
A4	**-**	**-**	**-**	**1**
A5	**-**	**-**	**-**	**2**
A6	**5**	**-**	**-**	**-**

- **Legend:** PGRs = Plant growth regulators; BAP = 6-benzyl-aminopurine Kin = Kinetin; TDZ = Thidiazuron (N-phenyl-N′-1,2,3-thiadiazol-5-yl urea); 2iP = 2-isopentenyl adenine

Results and discussions

Common milkweed can reproduce both vegetatively, by adventitious root buds and by seeds. After harvesting, the seeds of *A. syriaca* present a physiological dormancy which can be broken by scarification, treatments with gibberellic acid and kinetin, or by seed storage at low temperatures for several months (9).

The results recorded following our testing of the influence of various seed storage conditions (Tables 1 and 2) on germination are presented in Tables 4 and 5 and are illustrated in Fig.1. The dynamics of seed germination and seedling development was obviously influenced by the seed storage conditions. In both series of sowing experiments the highest percentage of seedling emergence were recorded in the case of storing the seeds in conditions of low temperature and moderate humidity (V2, V3 and V4), while the seeds stored in paper bags and kept at normal room temperature displayed the lowest percent of germination. The emergence of seedlings from the seeds stratified in moist perlite (V4) and kept for 1 or 2 months in refrigerator surpassed the results recorded in the other variants as regards the installing of the germination process and the dynamics of seedling emergence and development.

In the experiment of directly sowing in the field of the seeds stored for 6 months at 4° C in the refrigerator the percent of germination was of 78%, the germination starting after the temperature has risen above 15° C. After studying the production variables affecting follicle and biomass development in common milkweed, W.P. Phippen (2007) [9] concludes that for commercially producing milkweed biomass the sowing directly in the field of the seeds whose dormancy was broken is recommended as more cost effective, because transplanting the milkweed seedlings in the field though feasible but is not very practical.

Table 4. The dynamics of seedling emergence for the sowing at 3 April 2009, according to seed storage conditions

Variant	Seedling emergence (%)			
	17.04.2009 (2 weeks from sowing)	3.05.2009 (1 month from sowing)	17.05.2009 (1.5 months from sowing	3.06.2009 (2 months from sowing)
V 1	6.73	20.19	43.26	93.26
V 2	22.11	60.57	85.65	100.00
V 3	17.31	57.69	69.23	100.00
V 4	32.69	83.65	95.76	100.00
V 5	0.96	4.80	10.57	50.96

Table 5. The dynamics of seedling emergence in the sowing at 5 May 2009, according to seed storage conditions

Variant	Seedling emergence (%)	
	17.05.2009 (2 weeks from sowing)	3.06.2009 (1month from sowing)
V 1	37.50	72.11
V 2	55.76	100.00
V 3	51.92	100.00
V 4	60.57	100.00
V 5	1.92	7.69

Fig. 1. *A. syriaca* seedling development following germination of seeds stored in various conditions.

In the present study, the biotechnological approaches focused on establishing morphogenetic cultures from common milkweed somatic explants, useful both for cropping trials and for obtaining plant biomass in the form of multiple shoots to be used as a suitable material for eventual testing and optimization of extraction and processing of biomass for the production of biofuels. The data regarding in vitro culture of *A. syriaca* are scarce, probably because previous workers reported only limited success with the tissue culture of latex-producing plants [11].

The type of explants used in the present study for *in vitro* culture initiation, consisting of one bud and a portion of internode of approx. 1 cm in length, proved to express a pronounced morphogenetic capacity, following the inoculation on the culture medium variants supplemented with cytokinins (Table 3). The observations performed after 3 weeks from inoculation regarding the effect of phytohormones on the evolution of milkweed explants revealed that all tested cytokinins stimulated, in various degrees, the elongation of the preformed main shoot up to 5 – 10 cm in size, and the development of multiple buds and shoots was initiated. The process was more pronounced on the A1, A4, A5 and A6 variants which had BAP and TDZ as phytohormonal supplements and was inferior on the A2 and A3 variants, in the presence of Kinetin and 2-iP as plant growth regulators.

After several periodic transfers on fresh media, made every 3 weeks, a pronounced morphogenesis in the form of multiple shoots was recorded on the variant A1 and A6 (Fig. 2, Fig.3), under the effect of high concentrations of BAP (3mg/l and respectively 5 mg/l). A very good proliferation was also registered on the variant A4, in the presence of TDZ, a high potent cytokinin, which stimulated the development of numerous shoots, more vigorous than those developed under the influence of BAP (Fig. 4). On the variant A5, a higher concentration of TDZ (2 mg/l) proved to be less beneficial, because it encouraged the development of callus on the expense of shoot development (Fig.5). Consequently, based on our preliminary results, we may recommend the use of BAP (3 or 5 mg/l) and of TDZ (1 mg/l) for obtaining highly proliferating *in vitro* cultures in common milkweed, in the form of multiple shoots. Some of the elongated shoots, fragmented in new nodal explants and transferred on the proliferation media contributed to establishing new morphogenetic cultures, thus enhancing the shoot biomass production.

Though rhizogenesis induction was not an objective of this study, the development of roots at the base of *in vitro* developed shoots was achieved both on basal medium without growth regulators and on MS basal medium supplemented with 1.8 mg / l indole-3- acetic acid (IAA) plus 0.44 mg / l kinetin. The development of roots was achieved in both cases in a small proportion, requiring additional testing to determine optimum formulations for in vitro induction of rhizogenesis in common milkweed.

Fig. 2. *Asclepias syriaca* morphogenetic culture on the variant A1 (3 mg/l BAP).

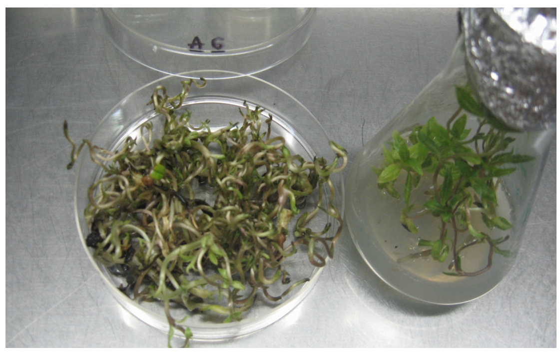

Fig.3. *Asclepias syriaca* morfhogenetic culture on the variant A6 (5 mg/l BAP) and detached shoots transferred on rooting medium

Fig. 4. *Asclepias syriaca* morphogenetic culture on the varianta A4 (1mg/l TDZ)

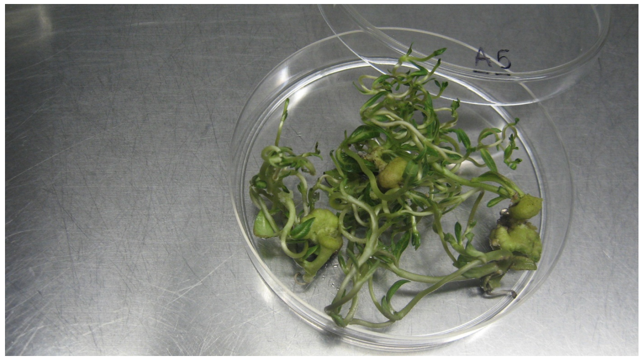

Fig. 5. *Asclepias syriaca* morphogenetic culture on the variant A5 (2 mg/l TDZ).

Conclusions

Asclepias syriaca L. is a perennial crop rich in hydrocarbons with several market opportunities, the most important being at present the exploiting of the whole plant biomass as a renewable source of fuel and of chemical feedstock. Harvesting of the whole plant, above ground level, offers a potential source of biomass, fiber, protein, crude petroleum substitute, natural rubber and chemical intermediates. The interest centers especially on the organic-soluble extractives of the whole plant, considered as a potential substitute for crude petroleum, by having cracking products similar to those from naphtha category.

A basic requirement for having enough available milkweed biomass to be used as alternative source of fuel and chemical feedstocks is to develop reliable and cost effective agrotechnology for the cultivation of this species. Among the many production variables which affect the biomass development in common milkweed, seed germination is a major limiting factor. Based on our results, three efficient alternatives of seed storage for braking the physiological seed dormancy are recommended, leading to 100% emergence of seedlings.

The biotechnological approaches outlined the parameters of *in vitro* cultures in common milkweed shoot apices, for establishing the morphogenetic cultures in the form of multiple shoots. Though some authors reported that the latex containing plants are rather refractory to *in vitro* conditions, our preliminary results with *Asclepia syriaca* demonstrated that it is easily amenable for establishing morphogenetic cultures. The obtained milkweed biomass in the form of multiple shoots may be used as a suitable material for cropping trials and for the eventual testing and optimization of biomass extraction and processing for the production of biofuels.

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References

1. ADAMS R.P., 1982 – Production of liquid fuels and chemical feedstock from milkweed. In: Energy from Biomass and Wastes VI, Symposium papers. Institute of Gas Technology, D.L. Klass, Ed., Chicago, Il., 1113 – 1128;
2. BUCHANAN R.A., CULL I.M., OTEY F.H., RUSSELL C.R., 1978 – Hydrocarbons and rubber producing crops. Econ. Bot., **32**, 131 – 145;

3. CALVIN M., 1979 – Petroleum plantations for fuels and other materials. Bioscience, **29**, 533 – 538;
4. DOBRINOIU R., JURCOANE S., DANAILA-GUIDEA S., MORARU M., DUMBRAVA M., ROSU A., 2011 - The impact of new technological approaches upon establishing production components and yield randament in *Carthamus tinctorium* L. culture. Romanian Biotechnological Letters, **16 (2)**, 6125- 6134;
5. HARRY-O'KURU R. E., 2005 - 4-hydroxy-3-methoxycinnamate esters of milkweed oil: synthesis and characterization. Lipids.**40**:1179-1183;
6. HOLSER R.A., HARRY-O'KURU R.E., 2006. Transesterified milkweed (*Asclepias)* seed oil as a biodiesel fuel. ScienceDirect: www.sciencedirect.com/science;
7. HARRY-O'KURU R. E., 2008 –The common milkweed (*Asclepias syriaca*): A new industrial crop. Association for the Advancement of Industrial Crops Conference, p.50;
8. LAIDING G.L., KNOX E.G., BUCHANAN R.A., 1984 – Underexploited crops. In: Handbook of Plant Cell Cultures, Volume 3, Crop Species. Phillip V. Ammirato, David A. Evans, William R.Sharp, Yasuyuki Yamada Eds., Macmillan Publishing Company, New York, 38 – 64;
9. PHIPPEN W. B., 2007. Production variables affecting folicle and biomass development in common milkweed. In: Issues in new crops and new uses, J. Janick and A. Whipkey (eds.), ASHS Press, Alexandria, VA, 82-88;
10. RUSAN V., CASCAVAL C.N., ROSU D., POPA V., 1984 – Valorificarea complexa si integrala a plantei laticifere *Asclepias syriaca L.* Lucrari Stiintifice, **28,** Seria Agronomie, Inst. Agron, Iasi, ISSN 0379-8364, p.107-111;
11. TIDEMAN J., HAWKER J.S., 1982 – *In vitro* propagation of latex-producing plants. Annals of Botany, **49,** 273 – 279;
12. SIMIONESCU C.I., RUSAN V., CASCAVAL C.N., ROSU D., 1987. Complex and integral processing of *Asclepias syriaca* L. latex – bearing plant. Cellulose Chemistry and Technology, 21:113-120;