INFRASTRUKTURA I EKOLOGIA TERENÓW WIEJSKICH
INFRASTRUCTURE AND ECOLOGY OF RURAL AREAS

Nr IV/2/2016, POLSKA AKADEMIA NAUK, Oddział w Krakowie, s. 1363–1372
Komisja Technicznej Infrastruktury Wsi

DOI: http://dx.medra.org/10.14597/infraeco.2016.4.2.100

MORPHOLOGICAL CHARACTERISTICS OF ACHENES AND FERTILITY PLANTS OF CUP PLANT (*SILPHIUM PERFOLIATUM* L.) OBTAINED FROM MICROPROPAGATION GROWING UNDER IRRIGATION

Anna Figas, Anna Katarzyna Sawilska, Roman Rolbiecki, Magdalena Tomaszewska-Sowa
University of Science and Technology in Bydgoszcz,

Abstract

A field experiment was carried out in two vegetation seasons in the years 2012 and 2013 on very light soil at Kruszyn Krajeński, in the vicinity of Bydgoszcz, Poland. The aim of the study was to characterize morphological achenes and determine the fertility potential and real of cup plant (*Silphium perfoliatum* L.) plants derived from micropropagation grown under irrigation. Low fertility real of cap plant resulted from the participation of infertile fruit-achenes it means devoid of the seeds. Due to the fact that this plant is entered on the list of invasive species, this property may be desirable during plantation establishment. As a result, the plant will not be so easy to penetrate into the ecosystem and will not pose a threat to native biodiversity.

Key words: micropropagation, fertility, achenes, seeds, irrigation.

INTRODUCTION

Representing family *Asteraceae,* cup plant (*Silphium perfoliatum* L.) in the natural environment it occurs in the central and eastern part of the United States as well as in the south of Canada. *Silphium perfoliatum* L can be grown for feed, ornamental, honey-producing and energy-generating purposes (Majtkowski 2007, Piłat et al. 2007, Decourtye et al. 2010, Ţîţei et al. 2013, Wróbel et al. 2013, Jasinskas et. al. 2014) and as it has low soil requirements, it can be

recommended as a pioneer plant for the recultivation of degraded areas (Klimont 2007). The herbage of cup plant is also a potentially precious material for food and pharmaceutical industries (El Sayed et al. 2002, Kowalski and Wolski 2003 ab, Kowalski and Wierciński 2004, Kowalski 2005, Jemiołkowska and Kowalski 2012). However, despite all these advantages of cup plant in Poland is entered on the list of potentially invasive plants. It is highly probable that this plant plantation will penetrate spontaneously into other ecosystems posing a threat to native biodiversity (Tokarska-Guzik et al. 2012).

The plant is a perennial reaching up to 2.5 meters height. In the first year of growth the plant creates a rosette, generative shoots develop in successive years (Stanford 1990, Kowalski and Wierciński 2004). In its natural habitat, *S. perfoliatum* blooms from early July to early October. The inflorescence of this species is typical of the family *Asteraceae* (Stanford 1990, Wróbel et al. 2013). Previous studies show a positive effect of irrigation on some parameters of growth of the plant including the number of inflorescences (Figas et al. 2011, 2015ab).

Numerous functional properties of the cup plant and climatic conditions similar to those it occurs naturally can enhance the cultivation of that species in the country. The technology which facilitates obtaining vegetative propagation material is the micropropagation of plants in cultures *in vitro*.

The aim of the study was to characterize morphological achenes and determine the fertility potential and actual of cup plant (*Silphium perfoliatum* L.) plants derived from micropropagation grown under irrigation.

MATERIALS AND METHODS

Micropropagation: The initial research material involved the achenes of cup plant (*Silphium perfoliatum* L), which came from the Botanical Garden of the National Center of Plant Gene Resources of the Institute of Plant Breeding and Acclimatization (IHAR) in Bydgoszcz in Poland. The seeds isolated with the preparation needle were sterilized in 70% ethyl and calcium hypochlorite. To induce germination, the seeds were placed onto the Murashige and Skoog medium (MS) (Murashige and Skoog 1962). From 6-week sterile seedlings apical parts of shoots were isolated. The explants were put into the test-tubes on the MS regeneration medium, which next to mineral compounds, contained organic substances and growth regulators: 1 mg dm^{-3} NAA (1-naphthaleneacetic acid) and 5 mg dm^{-3} BAP (6-enzylaminopurine)**.** The medium was solidified with 0.7% agar. After about 6-8 weeks the shoots were isolated and transferred onto MS rooting medium without growth regulators. The culture was exposed to 16-hour photoperiod, light intensity of about 2500 lux, temperature of 25 ± 2 °C and air humidity of 70%. The rooted plants were transferred into the mixture of sterile soil and perlite (1:1) and acclimatized under greenhouse conditions and field conditions.

Field trial**:** The field experiment of the cup plants was carried out in the period of 2012-2013 at the very light soil (type – Mollisoil; texture – Fine sand) in Kruszyn Krajeński in the vicinity of Bydgoszcz (53°3´39″N, 17°52´52″E). The soil characteristic is shown in table 1.

Table 1. Physical properties of the soil

Genetic horizon	Depth [cm]	Texture		Bulk density		Porosity	Moisture
			Specific density	temp.	actual		
			$Mg \cdot m^{-3}$			% vol.	
Ap	0-33	slightly loamy sand	2.290	1.426	1.324	42.2	10.2
AC	33-60	loose sand	2.680	1.620	1.591	40.6	2.9
C	60-150	loose sand	2.740	1.691	1.653	39.7	3.8

Table 2. Air temperature and rainfall (°C) during the vegetation period of cup plant

Specification	Air temperature (°C)					
	Months					
	05	06	07	08	09	05-09
2012	14.5	15.2	18.8	17.6	13.3	15.9
2013	14.2	14.4	18.9	18.1	10.7	15.3
Mean for 2012-2013	14.4	14.8	18.9	17.9	12.0	15.6
Long period average 1981-2010	13.1	16.0	18.5	17,9	13.2	15.7
	Rainfall (mm)					
2012	25.4	133.8	115.6	51.8	25.1	351.7
2013	91.7	49.3	79.0	56.6	64.1	340.7
Mean for 2012-2013	58.6	91.6	97.3	54.2	44.6	346.3
Long period average 1981-2010	49.3	52.8	69.8	62.6	46.0	280.5

The water reserve to 1 m soil depth at field capacity was 87 mm and the available water quantity 67 mm. The soil was characterized by the low organic matter content (1.5%). In 2010 the cup plants were cultivated from the micropropagation seedlings. The agrotechnical treatment and fertilization adopted were the standard used across the country. The mineral fertilization was applied at the rates of 500 kg N:P:K $\cdot$ ha^{-1} at the ratio of 2:2:3. Doses of potassium (potash salt) and phosphorus (superphoshate) fertilization were dependent on the abundance of these nutrients in the soil. The nitrogen fertilization (ammonium nitrate)

was supplied at three single rates. The single experimental plot was 11 m^2. The seedlings were planted with a row spacing of 1.0 m. Plants of cup plant grew under drip irrigation. Drip irrigation was scheduled according to tensiometers indications, installed at 250 mm depth. The irrigation started when the soil water pressure was up to – 0.04 MPa. The irrigation water rates were strictly connected with the rain distribution and amounted to 116 and 108 mm for 2012 and 2013 respectively. The single rates of water ranged from 6 to 12 mm. Irrigation was done with the drip line 'T-Tape' with the distance among the dripers 30 cm.

Biometric analysis: Biometric analysis of fertility cup plant was taken in September. When the plants were in bloom counted density of cup plant shoots per unit area and calculated the number of inflorescences on a single shoot and the number of flowers per inflorescence. Also the achenes produced in one inflorescence and on the 1 shoot were calculated. Analyses were performed on 30 shoots. These analyzes were performed using an magnifying glass with a magnification of 5 diopters. Fertility potential defined as the effort to reproductive generative expressed in the average number of flowers in the basket. For fertility real effort to reproductive generative measured by the number of fruits in the basket. The share of produced fruit for all resulting in a basket of flowers allowed to determine fertility rate for a basket.

Using the information about the average number of flowers in the basket and the average number of the resulting obtained from them achenes on each trial of cup plant calculated fertility real for a single shoot.

The experiment also determined the weight, length, width, perimeter and area of achenes, thickness and aspect ratio seeds. The length, width, perimeter and area of achenes, the aspect ratio of the fruits of cup plant, ScanDisk program were analyzed (Moraczewski 2005). The thickness of the achenes was measured using electronic callipers.

Ability to germinate: Research of cup plant fruit from field crops consisted of verifying their ability to germinate. In order to check the germination, the achenes of cup plant were sterilized in 70% ethyl alcohol for 30 seconds and then in 5% calcium hypochlorite with 2-3 drops of Tween 20 added for 7 minutes and rinsed with sterile bidistilled water three times. Then they were placed in petri dishes with filter paper, moistened with sterile bidistilled water. Petri dishes the seeds were stratified by placing them at + 4° C for a period of 30 days. After this period the calculated percentage of germinated achenes. The experience taught 50 seeds to 5 dishes.

Statistical analysis: The results of fertility and morphological characteristics achenes of cup plant are expressed as the Mean±Standard Deviation (SD). The data was analysed using one-way analysis of variance applying 'Statistica for Windows Pl'.

RESULTS AND DISCUSSION

In 2012-2013, the average number of generative shoots per plant of cup plant cultivated under irrigation was 17.70 (Table 3). According to Wróbel et al. (2013), their number may amount to 10-20, and increases with the age of the plant. In our study and Figas et al. (2015a) with one shoot generative collected 54.71 inflorescences. A thorough assessment of their construction showed that they consist of 58.86 flowers on average.

Table 3. Fertility potential of cup plant (*Silphium perfoliatum* L.) on the basis of measurements in the months in the IX in 2012 and 2013 years

Specification	2012	2013	Mean in the years 2012-2013
Number of generative shoots per plant	17.33±2.04	18.00±3.06	17.70±2.02
The number of inflorescences on 1 shoot	15.67±3.30	93.75±6.11	54.71±9.76
The number of flowers per inflorescence	59.96±11.03	57.76±8.56	58.86±9.33

Results are mean ± SD (standard deviation)

Table 4. Fertility real of cup plant (*Silphium perfoliatum* L.) on the basis of measurements in the months IX in 2012 and 2013 years

Specification	2012	2013	Mean in the years 2012-2013
The number of achenes in one inflorescence	14.23±3.24	14.90±4.01	14.57±44.22
The number of achenes 1 on shoot	145.85±30.30	465.63±85.50	305.74±209.00
Weight achenes with a single inflorescence [g]	0.10±0.03	0.11±0.02	0.11±0.03
Weight of 1000 achenes [g]	7.15±1.22	11.25±2.05	9.20±5.90
Fertility rate [%]	23.73±4.17	25.80±4.83	24.78±4.95

Results are mean ± SD (standard deviation)

With a single inflorescence obtained 14.6 pc. achenes on average and per rush an average generative reproduction was 305.74 pc. (Table 4). The resulting measurements are much lower than those reported in the literature. According to Stanford (1990) with inflorescences of plants obtained in the United States naturally collected up to 20-30 fruits on average that is 37-105% more than in the experiment. Similarly 1000 seed weight was 9.20 g and was lower by comparison with the literature data (Niqueux 1981), according to which it may be 23 g on average or even 150% more than that obtained in the experiment.

Table 5. Morphological characteristics achenes of cup plant (*Silphium perfoliatum* L.) on the basis of measurements in the month IX in 2012 and 2013 years

Specification	2012	2013	Mean in the years 2012-2013
Length [mm]	8.53±0.73	9.14±0.61	8.84±0.80
Width [mm]	6.15±0.87	6.49±0.94	6.32±1.03
Circuit [mm]	30.00±1.23	28.73±1.08	29.37±1.30
Area [mm]	33.02±3.99	37.56±4.01	35.29±4.18
Thickness [mm]	0.05±0.01	0.08±0.02	0.06±0.02
Achenes the aspect ratio (ratio of length to width)	1.39±0.09	1.41±0.87	1.40±0.88

Results are mean ± SD (standard deviation)

The length, width, circuit, area, thickness and aspect ratio of fruit averaged in the studies respectively 8.84 mm, 6.32mm, 29 37mm, 35.29mm, 0.06mm and 1.40 (Table 5). According to Stanford (1990) in the climatic conditions of South America and Canada of achenes of cup plant may be 9-15 mm long, 6-9 mm wide and about 1 mm thick.

In this experience in assessing the viability of fruit of cup plant germination rate averaged 3%. After careful observation, it was found that such a low level was due to participation infertile achenes it means devoid of the seeds (Fig. 1). Theoretically, the resulting plants under conditions of *in vitro* culture after transferring to the environment in the field should be developed as well as plants by conventional methods of breeding. Reduction in fertility actual can be explained by the fact that the plants as well as their organs and tissues during the acclimatization must change the metabolism of heterotrophic or mixed on autotrophic. Concentration in stress associated with the conditions at the last passage in the glass during the acclimation and leads to specific biochemical reactions that may be coded in the memory of the plant and thereby reduce the effort of the energy generative reproduction. Therefore, it is recommended further follow-up studies on how to acclimatize plants from *in vitro* cultures of cup plant in order to reduce stress factors.

Creating of infertile achenes by *Silphium perfoliatum* it can also be explained as a variable strategy in plants life strategies (Grime 1985, Stearns 1992). The species tested is a perennial plant of the Asteraceae family, that was reproduced in the natural conditions generatively or by achenes containing seed or vegetatively. Progeny plants, obtained vegetatively, are in the first period of life associated with the mother plant, which defines a much better chance of survival, and getting your own offspring, or reference reproductive success. Based on years of observation of the population of clonal plants it was stated that the

weather conditions (mostly rain) have a significant impact on the reproduction process (Kleunen et al. 2002, Assefa et al. 2015). Another clonal plant of family Asteraceae *Helichrysum arenarium* (L.) Moench, under optimal conditions for the growth and development produces numerous propagules, which is dedicating a large part of the biomass on vegetative propagation. However, in stress conditions, increasing competition or environmental disturbance it takes place mainly generative reproduction – there are created numerous and small achenes (Sawilska 2004, 2006, 2008, 2015). The impact of environmental conditions, like the origin of the plantles of *S. perfoliatum* from micropropagation growing and their subsequent cultivation in the field, may therefore decide about reduction of biomass invested in the fruits and seeds for the intensive growth of vegetative organs.

A.

B.

Figure 1. Achenes of cup plant (*Silphium perfoliatum* L.) with seeds used for tests (A) and without seeds derived from plants obtained in the micropropagation (B)

CONCLUSIONS

Plants of cup plant (*Silphium perfoliatum* L.) from micropropagation growing under irrigation issued infertile fruit-achenes. Due to the fact that this plant is entered on the list of invasive species, this property may be desirable during plantation establishment. As a result, the plant will not be so easy to penetrate into the ecosystem and will not pose a threat to native biodiversity. In order to confirm this relationship is necessary to continue the research.

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dr inż. Anna Figas, dr inż. Magdalena Tomaszewska-Sowa
Department of Plant Genetics, Physiology and Biotechnology
University of Science and Technology in Bydgoszcz
Bernardyńska 6, 85-029 Bydgoszcz
e-mail: figasanna@utp.edu.pl
e-mail: magda@utp.edu.pl

dr hab. Anna Katarzyna Sawilska
Department of Botany and Ecology
University of Science and Technology in Bydgoszcz
Prof. S. Kaliskiego 7, Building 3.1, 85-789 Bydgoszcz
e-mail: sawilska@utp.edu.pl

Prof. dr hab. Roman Rolbiecki
Department of Land Reclamation and Agrometeorology
University of Science and Technology in Bydgoszcz
Bernardyńska 6, 85-029 Bydgoszcz
e-mail: rolbr@utp.edu.pl

Received: 08.07.2016
Accepted: 11.10.2016