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Content of Triterpene saponins and phenolics compounds in leaves of in vitro *Beta vulgaris* L. genotypes

O.L. Klyachenko[1], A.F. Likhanov[2]

[1]*University of Life and Environmental Sciences of Ukraine*
Kiev, 15 Heroev Oboronu str.
[2]*Institute for evolutionary ecology of the National Academy of Sciences of Ukraine*
Kiev, 37 Lebedeva str.
E-mail: Klyachenko@ukr.net, Tel.:+38 067-453-74-05
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An important element in plants adaptation to adverse environmental stress factors is the synthesis of secondary metabolites which involved in formation for the constitutional stability of plant organism. Saponins play an important role in the regulation of metabolic processes and the development of adaptive and defense plant reactions. Triterpene saponins have pronounced surface-active properties, increase activity of certain enzymes and perform antioxidant function, which in general determines their important role in formation of a common nonspecific plant resistance system. Sugar beet contains saponins of the triterpenoid type. Our experiments were shown that some vegetative parts of in vitro plants of sugar beet accumulate secondary metabolites including phenolic carboxylic acids, flavonoids and saponins, which play an important role in the formation for the constitutional resistance. The general condition of the plant organism and the survival strategy depends on activity of phenolic substances and saponins synthesis. Phenolic carboxylic acids in leaves of in vitro sugar beet plants were determined by thin layer chromatography method. Generally, in leaves of sugar beet varieties, hybrids were found saponins with Rf values of 0.21, 0.32, 0.35, and 0.51. Qualitative composition and quantitative indices of triterpene saponins and phenolic compounds content in sugar beet leaves during in vitro cultivation have sortspecific character, due to the peculiarities of their metabolism. Taking into account the absolutely identical composition of nutrient medium, same photo- and thermo-regimes for cultivation of in vitro sugar beet, triterpenoid saponins with Rf = 0,56 and 0,62 are biochemical markers which determine adaptive potential of plants-regenerants. This makes it possible to consider it expedient carrying out the researches of content of triterpene saponins as a marker for primary diagnostics and selection of plants-regenerants with a high adaptive potential and drought resistance at the initial stages of selection.
Key words: triterpenoid saponins; phenolic substances; in vitro; sugar beet; markers

Introduction

In the rapidly changing climate, irregular rainfalls and prolonged drough the study of plant resistance mechanisms to abiotic stresses becomes more urgent. During investigations for drought resistance of sugar beet plants special attention should be given to the study of relationship between physiological processes and dynamism in adapting to osmotic stress (Adkins et al., 1995) Long-term research have established patterns of changes in the metabolic processes of plant organism which is determined by genotype features and conditions of drought action (Genkel, 1982; Grigoryuk et al., 200**Ошибка! Источник ссылки не найден.**). Some vegetative parts of in vitro plants of sugar beet accumulate secondary metabolites including phenolic carboxylic acids, flavonoids and saponins, which play an important role in the formation for the constitutional resistance of plant organism and adaptability to adverse environmental factors. (Mohd, 2011).

Significant role in a regulation of metabolic processes and development of adaptive and defense reactions of plants play saponins which consisting of steroid or triterpene sapogenin and glycosidic glucan, with sugars (glucose, xylose etc.) (Vinken, 2007). The qualitative composition of saponins in sugar beet roots and leaves is different, having one common aglikon - oleanolic acid. They differ in a composition of sugar residues, which include D-glucuronic acid, D-glucose and D-xylose (Brezhneva, 2003). Saponins are able to integrate into cell membranes and regulate transmembrane ion transport with a lipophilic aglycon. (Ali Khan et all., 2012). The role of triterpene glycosides plants is various and underinvestigated. They have strong surface-active properties, increase the activity of certain enzymes and have antioxidant function. It is also known about antiviral and fungicidal function of saponins (Shimoyamada et al., 1990), which generally determines their essential role in general nonspecific resistance system formation in plants (Sparg, 2004).

Sugar beet contains saponins of triterpenoid type. Derivatives of oleanolic acid are included into the structure of sugar beet aglycone saponins. Their allocation in tissues of sugar beet vegetative organs is inequal. The largest number of sugar beet saponins concentrated in the outer part of a beet-root, but a relatively large amount of triterpene glycosides is also found in leaves (Boeva et al., 2007). Taking into account the important role of secondary metabolites in the adaptation responses implementation, the aim of our research was investigation of characteristics of phenolic compounds and triterpene glycosides in tissues of sugar beet vegetative organs *in vitro* and to establish the possible relationship of their synthesis with adaptive capacity and the productivity of different sort and hybrids of the Ukrainian selection.

Materials and methods

In investigation diploid sort Yaltushkivskiy single-seeded 64 (standard), diploid hybrids Ukrainskiy MS 70, Uladovo-Verhnyatskiy MS 37, Ivanivsko-Veselopodilskiy MS 84, Atamansha and triploid Alexandriya were used. Aseptic seedlings of sugar beet were obtained by surface sterilization of seeds of concentrated sulfuric acid during 7-8 min with next three-time washing by sterile distilled water for 10 minutes. Aseptic seedlings were cultured on the non hormonal agarized MS nutrient medium at 24^0C temperature.

Plant regenerants were cultivated on the modified Murashige-Skoog (Murashige, Skoog, 1962) medium supplemented 1 mg/1 thiamine, 10 mg/1 glutamine, 0.2 mg 6-benzylaminopurine, 0.5 mg/1 naphthaleneacetic acid, 0.1 mg/1 indoleacetic acid, 2 mg/1 gibberelic acid, 30 g/l sucrose (Kliachenko, Krylovskaya, 2012).

Identification of secondary metabolites in plant regenerants tissues was made by TLC metod, using plates with the size 100x150 mm, sorbent – Silica gel G60 (Merck, Germany). For the phenol carbonic acids and flavonoids identification were used the next solvent systems: 1) chloroform : methanol : water (70 : 30 : 4) and 2) chloroform : glacial acetic acid : methanol : water (60 : 32 : 12 : 8). To enhance the fluorescence of substances in ultraviolet light (UV 365 nm) plates with chromatograms were treated with 5% EtOH solution of $AlCL_3$ with following heating (5 min at 105°C). Saponins were detected by the sequential treatment of plates with alcoholic solution of sulfuric acid and vanillin (Mironenko, 2011). Chromatogram was held for 7-10 minutes at 110°C until the indicative spots appearance. Photographic materials and digital experimental data processing were made in AxioVision 40V Carl Zeiss. Digital data processing was made in the program Image Pro Premier 9.0 (Trial version). Analysis of chromatograms was performed in the program Sorbflil TLC.

Results

Biotechnological methods play a significant role in the selection process. These methods allow to create different plant material with high efficiency, to obtain stable genotypes, to directly influence to genetic apparatus of plants, which reduces amount and duration of breeding schemes. The in vitro technologies significantly increase the efficiency of selection as a result of the expansion of the genetic basis, accelerated creation and selection of new plant materials with required traits and remain relevant at the present stage of scientific and selection research of crops. (Kliachenko, Kolomiets, 2013).

In vitro conditions the accumulation of secondary metabolites in vegetative parts of sugar beet plant regenerants, including phenolcarbonic acids, flavonoids and saponins, which has an important role in the constitutional stability formation, is triggered. General condition of the plant organism and its survival strategy depends on the activity of phenolic compounds and aponins synthesis. TLC in MeOH leaves extract revealed phenol carbonic acid (Fig. 1A, B). By the nature of fluorescence in the UV (365 nm), color reaction of $FeCl_3$ solution and R_f substance the most closely corresponds to chlorogenic acid (R_f 0.05).

In the chloroform : methanol : water (70 : 30 : 4) solvent system after the plate treatment with 5% EtOH solution of $AICl_3$ we have detected flavonoids and other polyphenolic substances with blue and blue-green fluorescence (Fig.1). It was also marked that *in vitro* conditions the most active synthesis of aromatic compounds was detected in diploid hybrids Uladovo-Verhniatskiy MS 37, Ivanivsko-Veselopodilskiy MS 84 and triploid hybrid Alexandriya and the lowest content of phenolic compounds was found in leaves of Yaltushkivskiy single-seeded 64.

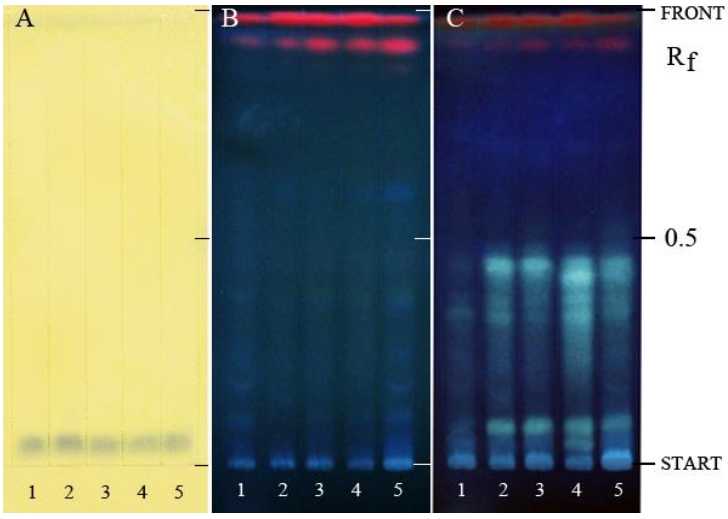

Fig.1. Chromatogram of sugar beet leaves methanol extracts *in vitro* (solvent systems: chloroform: methanol : water (70 : 30 : 4): demonstration of simple phenol compounds with 10% $FeCl_3$ solution; B – autofluorescence of phenol compounds and

chlorophyll (A and B); C – fluorescence of phenols after treatment with EtOH solution of $AlCl_3$. 1 – Yaltushkivskiy single-seeded 64; 2 – Uladovo-Verhniatskiy MS 37; 3 – Ukrainskiy MS 70; 4 – Ivanivsko-Veselopodilskiy MS 84; 5 – Alexandriya.

During studying of solvent systems better separation of phenolic compounds in sugar beet tissues was in; chloroform : glacial acetic acid : methanol : water in the ratio 60 : 32 : 12 : 8. In these conditions it is possible to divide more than 10 aromatic compounds in the R_f range from 0.1 to 0.72. After chromatograms treatment with 5% EtOH sulfuric acid solution and 1% vanillin solution in the plate purple-violet adsorbent-coated glass strip appeared, this is character for saponins (Fig. 2.A).

In addition in leaves of plant regenerants of Uladovo-Verhniatskiy MS 37 and Atamansha hybrids we have identified compounds with R_f = 0.56 and 0.62. In the UV with wavelength 365 nm substance with R_f = 0.62 and 0.67 had a bright turquoise color luminescence (Fig. 2.B). According to the published data, an organic compound with Rf = 0.62 can be defined as oleanolic acid - basic derivative substance for the synthesis of sugar beet triterpene saponins (Mironenko, 2011). In contrast, a spot with Rf = 0.56 strongly absorbed UV (365 nm).

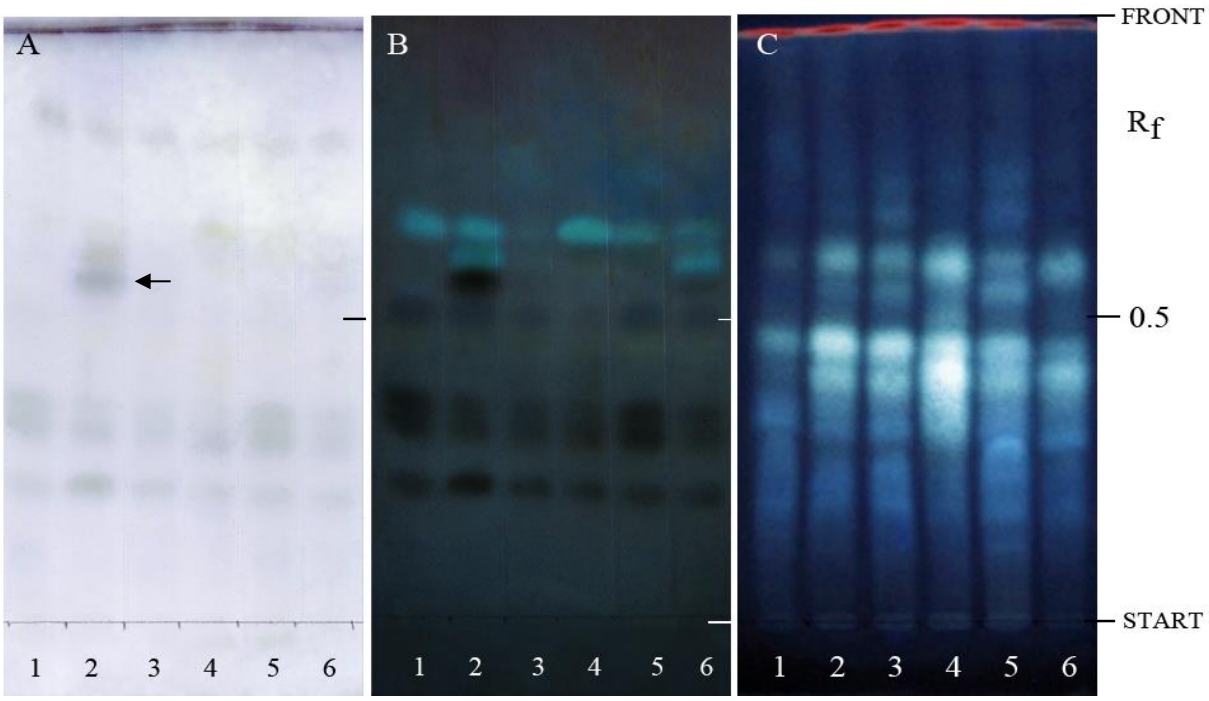

Fig. 2. Chromatogram of sugar beet leaves methanol extracts *in vitro* (solvent systems: chloroform : glacial acetic acid : methanol : water (60 : 32 : 12 : 8): A – saponins identification (revealing agent – 5% EtOH solution of H_2SO_4 and 1% vanillin solution solution); B – plate in UV (365 nm); C – fluorescence of phenolic compounds after treatment with EtOH solution of $AlCl_3$. 1 – Yaltushkivskiy single-seeded 64, 2 – Uladovo-Verhniatskiy MS 37, 3 – Ukrainskiy MS 70, 4 – Ivanivsko-Veselopodilskiy MS 84, 5 – Alexandriya; 6 - Atamansha.

In leaves of investigated sugar beet plant regenerants of Ukrainian selection of each selected variety we have detected saponins with R_f indexes 0.21, 0.32, 0.35, 0.51 (table 1).

Table 1. Chromatographic separation of triterpene saponins of sugar beet plant regenerants

№	Sort, hybrid	Значення R_f						
1	Yaltushkivskiy single-seeded 64	0.21	0.32	0.35	0.51	-	-	0.67
2	Uladovo-Verhniatskiy MS 37	0.21	0.32	0.35	0.51	0.56	0.62	0.67
3	Ukrainskiy MS 70	0.21	0.32	0.35	0.51	-	-	-
4	Ivanivsko-Veselopodilskiy MS 84	0.21	0.32	0.35	0.51	-	-	0.67
5	Alexandriya	0.21	0.32	0.35	0.51	-	-	0.67
6	Atamansha	0.21	0.32	0.35	0.51	0.56	0.62	0.67

According to literature data, an organic compound with Rf = 0.62 can be identified as oleanolic acid. This is the main derivative substance which is necessary for the synthesis of triterpene saponins. (Murakami et al., 1999).

It should be noted that the distinguishing feature of hybrids Uladovo-Verhnyatskiy MS 37 and Atamansha is a high crop yield and sugar content of beet-roots, furthermore, the last hybrid has a high resistance to drought. According to densitograms (Fig. 3.a-d) the average content of saponin leaf extracts of sacchariferous hybrid Uladovo-Verhnyatskiy MS 37 in average was 2-3 times higher than in Yaltushkivskiy single-seeded 64. It also should be noted that the relative content triterpene saponins with R_f = 0.32 and R_f = 0.35 for all of investigated samples was stable and practically independent of the varietal identity.

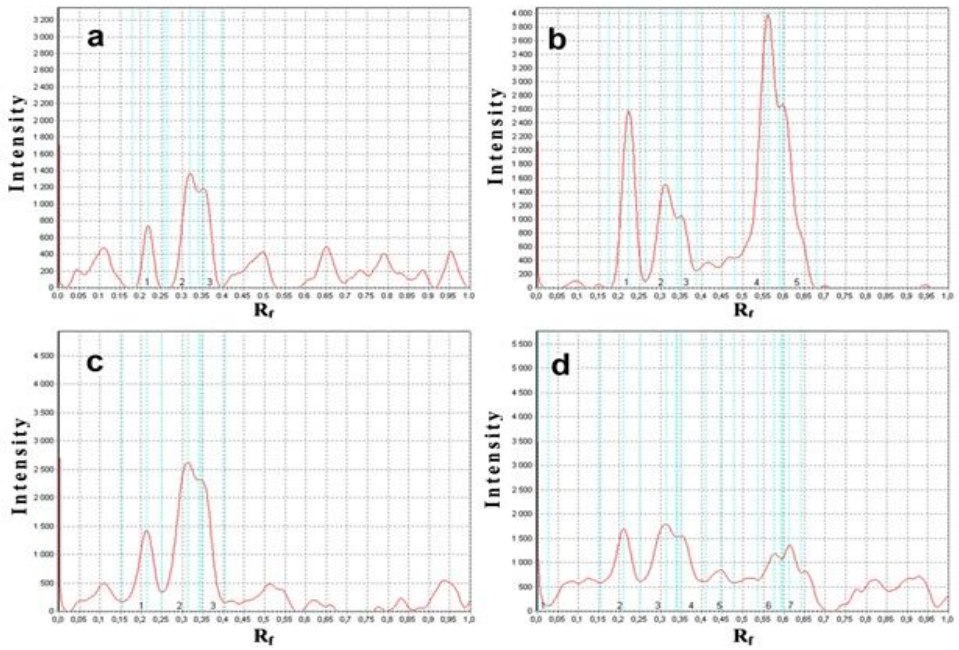

Fig. 3. Densitograms of triterpene saponins in sugar beet leaves, separated by TLC: a) sort Yaltushkivskiy single-seeded 64; b) hybrid Uladovo-Verhnyatskiy MS 37; c) hybrid Alexandriya; d) hybrid Atamansha

Thus, taking into account absolutely identical composition of nutrient media, the same photo- and thermal regime of cultivated *in vitro* plants, triterpenoid saponins with R_f = 0.56 and 0,62 can be biochemical markers for hybrids hybrids Uladovo-Verhnyatskiy MS 37 and Atamansha. The average content of saponins in leaf extracts of sacchariferous plant regenerants Uladovo-Verhniatskiy MS 37 and drought-tolerant Atamansha hybrid at the average rate was 2-3 times higher than in etalon sort Yaltushkivskiy single-seeded 64.

Conclusions

It was shown that the qualitative and quantitative composition of triterpene saponins and phenolic compounds content in leaves of sugar beet plants cultivated *in vitro* at the same photo- and thermal regime has varietal specificity, which is due to metabolism peculiarities. Was determined that at the stage of sugar beet plant regenerants formation in vitro culture, triterpene saponosides with Rf = 0.56 and 0.62 can be an important diagnostic markers in the solvent system: chloroform - glacial acetic acid - methanol - water (60 : 32 : 12 : 8). There is a reason to consider reasonable researches on usage of isolated by us compounds in primary diagnostics and plant regenerants breeding on valuable agronomic character, including potentially high drought tolerance.

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