DOI: 10.17707/AgricultForest.65.1.04

Oksana KLYACHENKO, Larysa PRYSIAZHNIUK [1]

EVALUATION OF GENETIC DISTANCES CORRELATIONS AMONG SUGAR BEET GENOTYPES (*BETA VULGARIS* L.)

SUMMARY

A search and a combination of different statistical methods for determining the similarity and difference between genotypes is a hot topic for breeding. In the formation of adaptive potential and appropriate protective mechanisms for the implementation of the adaptive potential of sugar beet, terpene compounds and saponins are involved.

To determine polymorphism based on DNA of sugar beet materials under investigation, RAPD analysis was performed. A cluster analysis of the hybrids affinity was carried out in terms of the qualitative biochemical state of the system and DNA polymorphism. As a result, it was found that hybrids that demonstrated high activity of the secondary metabolism and, consequently, showed different ability to form a high adaptive potential in the conditions of long-term cultivation *in vitro*, formed separate clusters. With the aid of RAPD markers, the authors also determined two clusters that were formed on the basis of genotype affinity. In order to determine the correlations between genetic distances obtained by RAPD markers and terpene compounds, the Mantel test (linear correlation for Pearson) was performed. The values of coefficients r = 0.517 were obtained at $\alpha = 0.05$. However, according to the data interpretation, the calculated value of p (0.088) was higher than the significance level of $\alpha = 0.05$, which indicates the absence of correlations between the matrices under investigation. Thus, the revealed genotypic features of the secondary metabolism and genetic distances are additional characteristics for evaluation of the ecological plasticity of sugar beet plants, which is important in the breeding process.

Keywords: terpene compounds, saponins, DNA markers, Mantel test.

INTRODUCTION

One promising way to increase sugar beet (*Beta vulgaris* L.) productivity is to create new hybrids featuring the best response to environmental changes that can be adjusted by cultivation technology and genetical resistance to uncontrollable environmental factors at certain ontogenetic stages (Roik, 2010; Srivastava *et al*., 2017; Pazuki *et al.*, 2018).

[1]Oksana Klyachenko, National University of Life and Environmental Sciences of Ukraine, UKRAINE; Larysa Prysiazhniuk (corresponding author: prysiazhniuk_l@ukr.net), Ukrainian Institute for Plant Variety Examination, UKRAINE
Paper presented at the 9th International Scientific Agricultural Symposium "AGROSYM 2018".
Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Increasing environmental problems and anthropogenic influences leads to narrowing borders of tolerance and reducing crops resistance to abiotic and biotic stress factors (Strausbaugh *et al.*, 2010; Kito *et al.*, 2017). Therefore, in recent years, the development of crop production is focused on creating agro-ecosystem capable of fast responding to stressful effects and subsequent self-regulation (Bray, 2002; Zhuchenko, 2008; Pazuki *et al.*, 2017).

A complex nature of the issue of plant resistance to stress factors, in particular, drought-tolerance and salt-resistance, requires new approaches to the solution (Chirkova, 2002; Kito *et al.*, 2017). Today, cell selection is a promising and highly efficient method that makes it possible to create new starting material resistant to adverse conditions. Selection in vitro is performed in regard to the signs that can be detected at the cellular level, in particular an increase in the expression of certain genes that control the metabolic pathways providing tolerance to stress factors (Serheeva *et al.*, 2001; Tsilke, 2002; Dubrovna and Morhun, 2009; Golovko and Tabalenkova, 2014). A cell reacts to all deviations from its normal state with a change in a number of physiological and biochemical characteristics. This fact allows in vitro selection aimed at stability based on these characteristics (Dolgih, 1993; Gubanova *et al.*, 2000; Kalashnikova, 2003; Sidorov, 2004; Shearman *et al.*, 2005; Dubrovna and Morhun, 2009; Chugunkova, 2009; Dubrovna *et al.*, 2012).

When culturing in vitro, plants remain in conditions of partially heterotrophic nutrition. This causes changes in metabolic processes and plant adaptation to specific conditions of the environment. The ability of plants in vitro to synthesize terpene compounds and saponins is of great importance. These compounds perform important structural, constitutional, regulatory, and protective functions. Their production depends on the formation of assimilation organs and the activity of the photosynthetic apparatus. Along with the development of DNA technologies, new methods of direct genome analysis and the related search for molecular markers appear, in particular, RAPD analysis that allows differentiation, identification, and monitoring of various accessions independently of the stage of plant development and environmental conditions. The method is highly informative, technological, reproducible, and has a high potential of polymorphism (Havkin, 2003; Capistrano-Gossmann *et al.*, 2017; Ibrahim *et al.*, 2017; Wang *et al.*, 2018).

The issue of determining the relationship between DNA markers and the physiological state of plants is promising for the creation of new sugar beet lines based on the effect of heterosis. Research on the correlation of genetic distances of physiological parameters and DNA markers is an important field for the development of breeding in order to find and combine different statistical methods to determine the affinity and differences between genotypes that are involved in the breeding process and increase the efficiency of breeding.

The purpose of the research was to estimate the correlations in terms of the genetic distances of polymorphism of sugar beet genotypes based on RAPD analysis and metabolic profile of the biochemical status of the leaves.

MATERIAL AND METHODS

Five Ukrainian sugar beet genotypes were studied: variety Yaltushkivskyi monogerm 64, diploid hybrids Uladovo-Verkhniatskyi MS 37, Ukrainian MS 70, Ivanivskyi MS 33, and triploid hybrid Oleksandriia. The studies have been carried out for 2015–2017 in The Problem Laboratory Of Phytovirology and Biotechnology of National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

DNA isolation and PCR

The sampling within each genotype numbered 30 accessions. DNA was isolated from 100 mg of green leaves of 5-day sprouts using CTAB and dissolved in TE buffer (Velikov, 2013; Roik *et al.*, 2007). The authors used four RAPD markers (GEN 2-80-7: GCAGGTCGCG; GEN 1-80-5: ACCCCAGCCG; GEN 1-70-9/2: TGCAGCACCG; GEN 4-70-2: GGACCGACTG) (Smulders *et al.*, 2010; Lučić *et al.*, 2011; Klyachenko and Prysiazhniuk, 2016). The size of the alleles obtained by electrophoretic spectra was analysed in pairs of nucleotides using computer program TotalLab v.2.01. Genetic distances, the frequency of detected alleles, and the index of polymorphism of the locus (PIC) were determined using the allele presence/absence matrix (Roik *et al*., 2007; Fedulova, 2014).

Identification of triterpene glycosides

The identification of triterpene glycosides was carried out according to the thin-layer chromatography method on 100 mm by 150 mm plates using sorbent Silica gel G60 (Merck, Germany) (Ladyigina *et al.*, 1983). The chromatograms were exposed 7–10 min at a temperature of 110 °C until the characteristic bands appeared. Digital data processing was performed using the Image-Pro Premier 9.0 (Trial version) program and chromatographic analysis using Sorbflil TLC.

Statistical analysis of data

The genetic distances between the genotypes under study were determined using a computer program STATISTICA 12 (Trial version) based on cluster analysis. The grouping of genotypes in terms of RAPD analysis and the metabolic profile of the biochemical status of the leaves was carried out by the unweighted average method with the calculation of Euclidian distances (Fortin *et al*., 2002; Drozdov, 2010; Everitt *et al.*, 2011; Dong *et al.*, 2014).

Correlation by genetic distances of sugar beet genotype polymorphism based on RAPD analysis and metabolic profile of the biochemical status of leaves was calculated by Mantel test using the computer program XLSTAT 2018 (Trial version) (Legendre *et al*., 2010; Diniz-Filho *et al.*, 2013).

RESULTS AND DISCUSSION

In vitro cell selection aimed at increased complex tolerance to drought and salinity usually consists of two main steps: the very selection of resistant cell lines in the appropriate selective media and plant regeneration. Complex cell selection for drought- and salt-tolerant lines was carried out using selective agents PEG 6000 (20 %) and Na2SO4 (2.0 %). The stability of the signs of tolerance of the resulting lines to the complex of stress factors was determined through the alternation of transplantations to nutrient media containing / without stress factors. Obtaining of the drought- and salt-tolerant lines was performed through a long-term (up to seven passages) in vitro culturing of both callus and produced regenerated plants. The highest regeneration capacity was demonstrated by the callus lines of variety Yaltushkivskyi monogerm 64 (77 %), triploid hybrid Alexandria (60 %), and diploid hybrid Ukrainian MS 70 (50 %).

As a result of RAPD analysis, 14 alleles with a frequency varied from 0.2 to 0.8 were detected. The average PIC value was 0.45. According to the obtained data, the vast majority of the genotypes under investigation had an allele measuring 50 bp (at a frequency of 0.8). Noteworthy, that the allele of 176 bp (at a frequency of 0.2) was identified only in Yaltushkivskyi monogerm 64.

The analysis of the metabolic profile of the biochemical status of the leaves allowed to find out the peculiarities of the genotypes under study in respect of synthesis of terpene compounds and saponins. Using the method of photodensitometric analysis of chromatogram, the authors determined the ratio of individual compounds to the corresponding indices of electrophoretic mobility (Rf) and their occurrence in the examined sugar beet samples (Klyachenko and Likhanov, 2017).

In order to analyse the polymorphism based on the ratio of terpene compounds to saponins and RAPD analysis, a cluster analysis was performed, and genetic distances between genotypes were calculated. Shown in Table 1 are the genetic distances between sugar beet genotypes in terms of the ratio of terpene compounds to saponins.

Table 1. Euclidean distances between sugar beet genotypes based on the metabolic profile of triterpene compounds and saponins.

Variable	Uladovo-Verkhniatskyi MS-37	Ukrainian MS-70	Oleksandriia	Yaltushkivskyi monogerm 64
Ivanivskyi MS-33	2.83	2.65	2.45	2.65
Uladovo-Verkhniatskyi MS-37	-	1.73	2.00	1.73
Ukrainian MS-70	-	-	1,00	2.00
Oleksandriia	-	-	-	1.73

Given that objects with zero digital expression of genetic distances are considered to be identical, according to cluster analysis results, the least value of genetic distance (1.00) was found between triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70. Diploid hybrids Uladovo-Verkhniatskyi MS 37 and Ukrainian MS 70, Yaltushkivskyi monogerm 64 and triploid hybrid Oleksandriia had a fairly close distance (1.73). The diploid hybrids Uladovo-Verkhniatskyi MS 37 and Ivanivskyi MS 33 appeared to be the most remote (2.83) by the metabolic profile of triterpene compounds and saponins.

In the conditions of long-term cultivation in vitro, hybrids Oleksandriia and Ivanivskyi MS 33 demonstrated high activity of secondary metabolism and, accordingly, a potentially high adaptive potential. Hybrids Uladovo-Verkhniatskyi MS 37 and Ukrainian MS 70, as well as variety Yaltushkivskyi monogerm 64, demonstrated the least activity in the synthesis of triterpene compounds and saponins. Thus, according to the results of the biochemical test of the metabolic profiles of terpenes and triterpene saponins in vitro, genotypic features that serve as additional signs for assessing the ecological plasticity of sugar beet plants were identified, which is very important for breeding work.

According to the distribution of the RAPD analysis results, genetic distances between genotypes ranged between 2.45 and 3.46 (Table 2).

Table 2. Euclidean distances between sugar beet genotypes based on RAPD analysis

Variable	Uladovo-Verkhniatskyi MS-37	Ukrainian MS-70	Ivanivskyi MS-33	Oleksandriia
Yaltushkivskyi monogerm 64	3.46	2.83	2.65	2.83
Uladovo-Verkhniatskyi MS-37	-	2.83	2.65	2.83
Ukrainian MS-70	-	-	2.65	2.45
Ivanivskyi MS-33	-	-	-	2.65

Triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70 were found to be the most affine, which also coincided with the results of cluster analysis of the profile of triterpene compounds and saponins. According to the obtained distribution, most genotypes were at the same distance from each other. However, it should be noted that according to the results of RAPD analysis, the most remote genotypes proved to be Yaltushkivskyi monogerm 64 and diploid hybrid Uladovo-Verkhniatskyi MS 37 (3.46). Consequently, based on RAPD analysis, the molecular genetic polymorphism of sugar beet genotypes was found, specific alleles were identified, and their genetic affinity was determined.

The research identified specific and non-specific qualitative and quantitative changes in the regenerants of different sugar beet genotypes in the conditions of long-term cultivation in vitro on the basis of the content of triterpene compounds and saponins, which production varies in different genotypes. Owing to RAPD analysis, which allows involving methods of DNA analysis for the analysis of the varietal and hybrid material of sugar beet, it was possible to determine the genetic affinity of the sugar beet genotypes under study.

Noticeable, that cluster analysis on the basis of individual indicators shows a different distribution of genotypes and, accordingly, different degree of their affinity. However, effective breeding work requires involving modern methods of integrated genotype assessment in order to determine the most effective schemes.

The Mantel test (linear correlation by Pearson) was used to determine correlations between genetic distances obtained with the aid of RAPD analysis and the metabolic profile of triterpene compounds and saponins. As a result, p-value and r (AB) values at α = 0.05 were determined, which, according to the test interpretation, allowed accepting one of the hypotheses: presence (Ha) or absence (H0) of correlation.

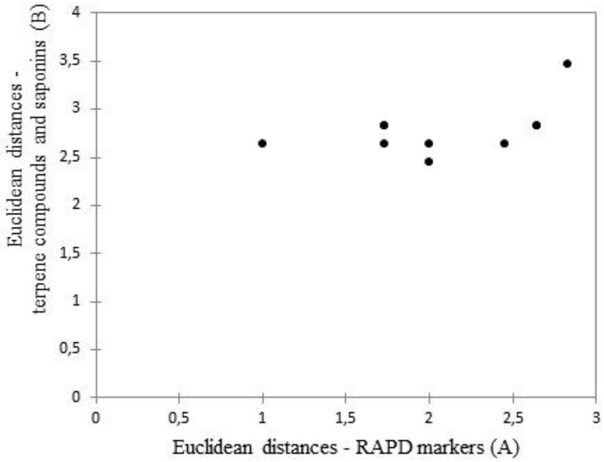

Fig. 4. Interactions between genetic distances of sugar beet genotypes by four RAPD markers and the metabolic profile of triterpene compounds and saponins.

It is known that the H0 hypothesis of the absence of correlation can be assumed given that p > α. In this research, the calculated value of p (0.088) was higher than the significance level α = 0.05, therefore the zero hypothesis H0 should be accepted and the alternative Ha hypothesis of the existence of correlation should be rejected (Burstin *et al.*, 1997; Diniz-Filho *et al.*, 2013). Shown in Fig. 5 are the correlation coefficient and normality of data distribution by matrixes of genetic distances.

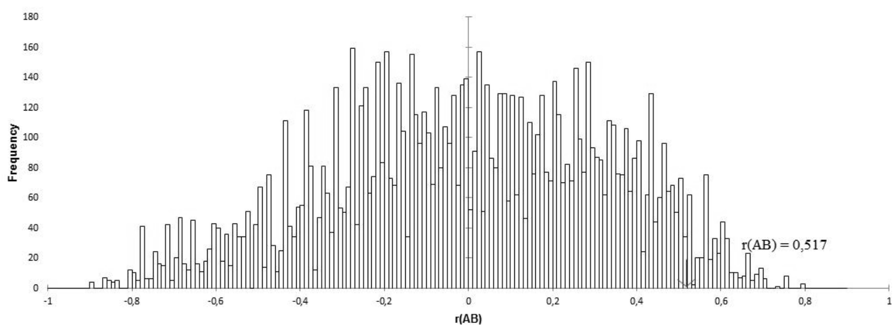

Fig. 5. Normality of Mantel test results distribution for sugar beet genotypes on the basis of genetic distances.

As a result of the analysis, it was found that there was no correlation between the genetic distances of five sugar beet genotypes by four RAPD markers and the metabolic profile of triterpene compounds and saponins. The shape of a diagram showing the normality of the distribution also indicated this fact. Consequently, it was found that a system consisting of four RAPD markers is effective for evaluation of genetic relationships of different sugar beet genotypes and analysis of their metabolic profile that reflects genotype ability to synthesize secondary metabolites, which is an indicator of adaptive potential.

CONCLUSIONS

As a result of the research, the molecular-genetic polymorphism of five sugar beet genotypes was investigated by four RAPD markers and the metabolic profile of triterpenes and saponins at long-term cultivation in vitro.

Based on cluster analysis and in accordance with genetic distances, it was found that triploid hybrid Oleksandriia and diploid hybrid Ukrainian MS 70 appeared to be the most affine ones, whereas the most remote genotypes were found to be variety Yaltushkivskyi monogerm 64 and diploid hybrid Uladovo-Verkhniatskyi MS 37. The results of this research are recommended to use in planning crossing schemes in breeding work.

According to the metabolic profile of triterpene compounds and saponins, diploids Uladovo-Verkhniatskyi MS 37 and Ivanivskyi MS 33 are the most remote hybrids, whereas the smallest distance is observed between the triploid hybrid Oleksandriia and the diploid hybrid Ukrainian MS 70.

It was proved that prolonged in vitro vegetation of sugar beet caused adaptive changes in metabolic processes, specifically in the synthesis of secondary metabolites. The changes in the metabolic processes are involved in the development of adaptive potential and appropriate protective mechanisms of sugar beet plants.

Based on the Mantel test results, there is no correlation between genetic distances on the basis of RAPD analysis and the metabolic profile of triterpene compounds and saponins. Therefore, in breeding work aimed at obtaining plants with complex resistance to drought and salinity, when selecting start material, it is necessary to consider not only genetic distances between genotypes but also their distribution in terms of adaptive potential.

REFERENCES

Bray, E. A. (2002). Classification of genes differentially expressed during water-deficit stress in Arabidopsis thaliana: an analysis using microarray and differential expression data. Annals of botany, 89(7), 803-811.

Burstin J., Charcosset A. 1997. Relationship between phenotypic and marker distances: theoretical and experimental investigations. Heredity, 79 (5): 477.

Capistrano-Gossmann, G. G., Ries, D., Holtgräwe, D., Minoche, A., Kraft, T., Frerichmann, S. L. M., ... & Varrelmann, M. (2017). Crop wild relative populations of Beta vulgaris allow direct mapping of agronomically important genes. Nature communications, 8, 15708. https://doi:10.1038/ncomms15708

Chirkova, T. V. (2002). Physiological basis of plant resistance. Publishing St. Petersburg University, St. Petersburg, Russian Federation. (in Russian).

Chugunkova, T. V. (2009). Use of cell selection to create resistant forms beets. Physiology and biochemistry of cultivated plants, 41(6), 509-515. (in Ukrainian).

Diniz-Filho, J. A. F., Soares, T. N., Lima, J. S., Dobrovolski, R., Landeiro, V. L., Telles, M. P. D. C., ... & Bini, L. M. (2013). Mantel test in population genetics. Genetics and molecular biology, 36(4): 475-485. http://dx.doi.org/10.1590/S1415-47572013000400002

Dolgih, O. I. (1993). Principles of screening cells in vitro for the purpose of obtaining forms of plants resistant to abiotic stresses. 2nd Russian Symposium «New methods of plant biotechnology», May, 18-20 1993 г., Pushchino, Russian Federation. (in Russian).

Dong D., Fu X., Yuan F., Chen P., Zhu S., Li B., ... Zhu D. 2014. Genetic diversity and population structure of vegetable soybean (Glycine max (L.) Merr.) in China as revealed by SSR markers. Genetic Resources and Crop Evolution, 61 (1): 173-183. http://doi:10.1007/s10722-013-0024-y

Drozdov, V. I. (2010). Instructions for using the Statistica 6.0. (in Russian).

Dubrovna, O. V., & Morhun, B. V. (2009). Wheat Cell Selection for Resistance to Stressful Environmental Factors. Physiology and biochemistry of cultivated plants. (in Ukrainian).

Dubrovna, O. V., Chuhunkova, T. V., Bavol, A. V., & Lialko, I. I. (2012). Biotechnological bases for the creation of plants resistant to stress. Logos, Kyiv, Ukraine. (in Ukrainian).

Everitt, B.S., Landau, S., Leese, M., &Stahl, D. Cluster. (2011). Analysis 5th Edition.

Fedulova, T. P. (2014). DNA markers for identification of sortotypes in the gene pool of beet root. Sugar beet, (5), 14.

Fortin, M. J., Dale, M. R., & Ver Hoef, J. M. (2002). Spatial analysis in ecology. Wiley StatsRef: Statistics Reference Online. http://onlinelibrary.wiley.com/doi/10.1002/9781118445112.stat07766.pub2/full.

Golovko, T., & Tabalenkova, G. (2014). Pigments and productivity of the crop plants. Photosynthetic pigments: chemical structure, biological function and ecology.

Gubanova, N. Ya., Dubrovnaya, O. V., & Chugunkova, T. V. (2000). Selection and comparative analysis of salt-resistant callus cultures of fodder beet obtained from explants of different ploidy. Physiology and biochemistry of cultivated plants, 32(5), 362-369. (in Russian).

Havkin, E. E. (2003). Molecular selection of plants: DNA technology for creating new varieties of crops. Agricultural Biology, (3), 26-41.

Ibrahim, H. M., El-Leel, O. F., & Emam, K. A. (2017). Molecular Profiling for Geneti c Variability in Petroselinum crispum Based on ISSR and RAPD Markers. Middle East J, 6(1), 67-75.

Kalashnikova, E. V. (2003). Cell selection for resistance to fungal diseases (Cand. Agric. Sci. Diss). Moscow, Russian Federation. (in Russian).

Kito, K., Tsutsumi, K., Rai, V., Theerawitaya, C., Cha-um, S., Yamada-Kato, N., ... & Takabe, T. (2017). Isolation and functional characterization of 3-phosphoglycerate dehydrogenase involved in salt responses in sugar beet. Protoplasma, 254(6), 2305-2313. https://doi.org/10.1007/s00709-017-1127-7

Kito, K., Yamane, K., Yamamori, T., Matsuhira, H., Tanaka, Y., & Takabe, T. (2018). Isolation, functional characterization and stress responses of raffinose synthase genes in sugar beet. Journal of Plant Biochemistry and Biotechnology, 27(1), 36-45. https://doi.org/10.1007/s13562-017-0413-y

Klyachenko, O. L., & Likhanov, A. F. (2017). Content of Triterpene saponins and phenolics compounds in leaves of in vitro Beta vulgaris L. genotypes. Ukrainian Journal of Ecology, 7(4), 564-568. https://doi:10.15421/2017_161

Klyachenko, O. L., & Prysiazhniuk, L. M. (2016). Differentiation and identification of different genotypes of sugar beet (Beta vulgaris L.) using DNA markers. Scientific reports of NULES of Ukraine, (4 (61)).

Ladyigina, E. Ya., Safronich, L. N., & Otryashenkova, V. E. (1983). Chemical analysis of medicinal plants. Vyisshaya shkola. Moscow. Russian Federation. (in Russian).

Legendre, P., & Fortin, M. J. (2010). Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Molecular ecology resources, 10(5): 831-844. https://doi:10.1111/j.1755-0998.2010.02866.x

Lučić, A., Isajev, V., Rakonjac, L., Ristić, D., Kostadinović, M., Babić, V., & Nikolić, A. (2011). Genetic divergence of Scots pine (Pinus sylvestris L.) populations in Serbia revealed by RAPD. Archives of biological sciences, 63(2), 371-380. http://doi:10.2298/ABS1102371L

Pazuki, A., Aflaki, F., Gürel, E., Ergül, A., & Gürel, S. (2017). A robust method for haploid sugar beet in vitro proliferation and hyperhydricity reduction. Folia Horticulturae, 29(2), 241-250. https://doi:10.1515/fhort-2017-0022

Pazuki, A., Aflaki, F., Gürel, S., Ergül, A., & Gürel, E. (2018). Production of doubled haploids in sugar beet (Beta vulgaris): an efficient method by a multivariate experiment. Plant Cell, Tissue and Organ Culture (PCTOC), 132(1), 85-97. https://doi:10.1007/s11240-017-1313-5

Roik, M. V. (2007). Determination of molecular genetic polymorphism of the genus BETA L. with polymerase chain reaction. К.: TOV «PolihrafKonsaltynh».

Royk, N. V. (2010). Combinative ability of sugar beet pollinators of different genetic structure by elements of productivity. Encyclopedia of the genus Beta: Biology, genetics and selection of beets, 525-541. (in Russian).

Serheeva, L. E. (2001). Changes in cell culture due to stress. Logos, Kiev, Ukraine (in Russian).

Shearman, V. J., Sylvester-Bradley, R., Scott, R. K., & Foulkes, M. J. (2005). Physiological processes associated with wheat yield progress in the UK. Crop Science, 45(1), 175-185.

Sidorov, V. A. (2004). Biotechnology of plants. Cell selection of plants. Naukova Dumka, Kiev, Ukraine. (in Russian).

Smulders, M. J., Esselink, G. D., Everaert, I., De Riek, J., & Vosman, B. (2010). Characterisation of sugar beet (Beta vulgaris L. ssp. vulgaris) varieties using microsatellite markers. BMC genetics, 11(1), 41. https://doi.org/10.1186/1471-2156-11-41

Srivastava, S., Pathak, A. D., Kumar, R., & Joshi, B. B. (2017). Genetic diversity of sugar beet genotypes evaluated by microsatellite DNA markers. Journal of Environmental Biology, 38(5), 777. http://doi.org/10.22438/jeb/38/5/MS-141

Strausbaugh, C. A., Eujayl, I. A., & Foote, P. (2010). Sugarbeet cultivar evaluation for bacterial root rot. Journal of Sugarbeet Research, 47, 51-64.

Tsilke, R. A. (2002). Some aspects of genetic engineering in plants. Improving the efficiency of plant selection and seed production of agriculture crops: of scientific articles, 17-23. (in Russian).

Velikov, V. A. (2013). Molecular biology. A Practical Guide. Saratov:«Saratovskiy istochnik ».–2013.–84 c.

Wang, M., Xu, Y., Wu, Z., Wang, H., & Zhang, H. (2018). High-Density Genetic Map Construction in Sugar Beet (Beta vulgaris L.) by High-Throughput Technology. Sugar Tech, 20(2), 212-219. https://doi.org/10.1007/s12355-017-0550-6

Zhuchenko, A. A. (2008). Adaptive plant growing (ecological and genetic basis): theory and practice. Agrorus, Moscow, Russian Federation. (in Russian).