Influence of some *rol* genes on sugar content in *Nicotiana* and *Vaccinium*

Tatiana Matveeva[1,*], *Ekaterina* Berezina[2], *Irina* Isaeva[1], *Alina* Dymo[1] and *Sofia* Sokornova[3]
[1]Saint Petersburg State University, 199034 St. Petersburg, Russia
[2]N. I. Lobachevsky State University of Nizhny Novgorod, 603950, Nizhny Novgorod, Russia
[3]All-Russian Institute of Plant Protection, 196608 St. Petersburg, Russia

Abstract. In natural conditions, insertion of Agrobacterium T-DNA into the plant genome and its subsequent transfer via sexual reproduction has been shown for several dozens of species, including species from genera *Nicotiana* and *Vaccinium.* In the framework of investigation of possible function of cT-DNA in naturally transgenic species we have shown, that increasing of expression of *rolC* in *Nicotiana tabacum* is associated with increase of amount of glucose and total sugar content. Similar trend was observed for *rolB*/*C*-like gene in *Vaccinium.*

1 Introduction

Agrobacterium-mediated transformation is the key method of genetic engineering of plants aimed to introduce novel genes into their genomes. At the same time in natural conditions, horizontal transfer of T-DNA genes into the plant genomes has been shown for several dozens of species, including species from genera *Nicotiana* and *Vaccinium* [1-4]. Such T-DNA was termed as «cellular T-DNA» (cT-DNA). One of the most conserved genes in cT-DNA is *rolC*. Its expression has been shown in *Nicotiana* and *Linaria* [5-6]. It is related somehow to sugar content [7]. Gene *rolC* belongs to the *plast* genes family [8]. The *plast* genes are identified by their common ancestry. They are mostly described in T-DNAs. The *plast* genes can modify plant growth in different ways, but the molecular basis of their function remains largely unknown. Just a few agrobacterial *plast* genes, including *rolB*, *rolC*, *orf13* and *6b*, have been studied in detail. The list of *plast* genes was significantly expanded due to the discovery of new types of cT-DNA. Some *plast* genes have a significant potential for applied biology, agriculture and may be used to modify the growth of crops [8]. It is believed that the natural transformation of sweet potato with *plast* genes contributed to its domestication [3]. An interesting *plast* gene resembling *rolB* and *rolC* features was found in *Vaccinium macrocarpon* [4]. It is worth noting that all previously studied by us samples of *V. macrocarpon* contain intact gene while all samples of *V. oxycoccos* contain a deletion within the *plast* gene. *Nicotiana tabacum* contains intact

*Corresponding author: radishlet@gmail.com

rolC. Transgenic lines, containing an additional copy of *rolC* under the control of a dexamethasone-inducible promoter, were obtained based on Samsun cultivar of *N. tabacum*. It is possible to increase gene expression (as compared with the control) by adding the inductor to the cultural medium. Previously, the total sugar content and transport were studied in such plants [7].

In this study we compared the carbohydrates patterns in tobacco plants, differing in expression of the *rolC* gene and in *Vaccinium* plants, carrying intact *plast* gene and deleted one.

2 Materials and methods

Plant material was represented by 2 lines of cranberries*:* line GVo of *Vaccinium oxycoccos* L. and line GVm of *Vaccinium macrocarpon* Aiton; and three genotypes of tobacco: *N. tabacum*, cv. Samsun and two transgenic lines, derived from this cultivar and containing *rolC* of different origin under *Pdex*: *A. rhizogenes rol C* (*Pdex-A4-rolC*) and *N. tabacum rol C* (*Pdex-trolC*) correspondingly [7].

Plants of genera *Vaccinium* and *Nicotiana* were cultivated in aseptic culture on MS medium at 24h photoperiod at 22°C. Transgenic *N. tabacum* plants, containing *rol C* under dexamethasone-inducible promoter and control non-transgenic plants were cultivated for 7 days on MS media supplied with 10 μM of dexamethasone or on MS0 media [9]. Shoots were ground in liquid nitrogen, ethanol-extracted (80°C, 30 min) and analyzed by HPTLC (high-performance thin-layer chromatography). The sugar standards and extracted samples were processed on the automated HPTLC system (CAMAG, Muttenz, Switzerland). The TLC plate was developed in chloroform-acetone-methanol-acetic acid-H_2O 50:20:10:10:5 v/v. The developed plate was carried out with the aniline reagent in methanol under heating. All tracks in the plate were scanned at 454 nm wavelength and individual Rf values of peaks were obtained. These data were matched with the standard. The experiment was done in three replicates. Statistical processing was performed by analysis of variance (ANOVA).

3 Results and discussion

Samples of *Vaccinium* and *Nicotiana* differed in total carbohydrate content and ratio. ANOVA have shown statistically significant differences in total carbohydrate content, glucose and sucrose composition among samples of *Vaccinium* ($p \leq 0.05$) (fig. 1A). Analysing *N. tabacum* lines we have shown statistically significant effect ($p \leq 0.05$) of both studied factors (genotype and cultural medium composition) on the sugar content (fig 1B). Difference in sugar levels of three studied tobacco genotypes without dexamethasone treatment can be partly explained by the effect of the localization of T-DNA in the genomes. In addition, dexamethasone itself increases sugar content of tobacco plants. However, the portion of the influence of dexamethasone on the level of sugars is higher in transgenic lines. For the *Pdex-trolC* line, Fischer intra-class correlation coefficient is 74%, for the *Pdex-A4rolC* line it is 49%, and in non-transformed plants it is only 17%. The carbohydrates patterns in tobacco plants, differing in expression of the *rolC* gene and in *Vaccinium* plants, carrying intact *plast* gene and deleted one, have shown some common features. Firstly, the sugar levels in the induced transgenic *N. tabacum* (*Pdex-trolC* and *Pdex-A4rolC*) plants were higher than in *N. tabacum* cv. Samsun and transgenic plants

without induction of *rolC* expression ($p \leq 0.05$). In *V. macrocarpon* total sugar content is higher, than in *V. oxycoccos.* Secondly, extracts from induced transgenic plant and extracts from *V. macrocarpon* show an increased level of glucose comparing to control plants. Earlier in the literature there was an idea, that the products of the *rolC* and *rolB* genes are beta-glucosidases [11-12]. However, later it was criticized [8]. Our results can be explained by an increase of glucosidase activity in case of the expression of *rolC* and *rolB/C*- like gene. However, we cannot say whether this is a direct effect of studied genes, or an indirect one.

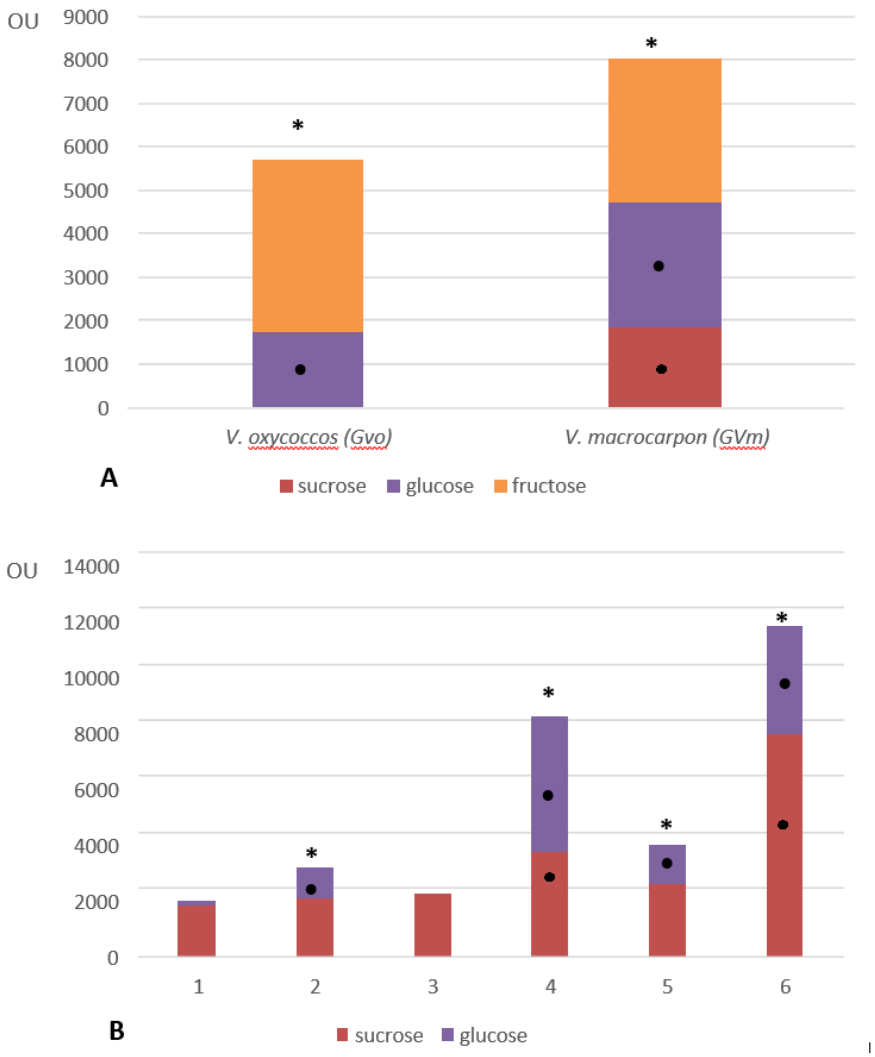

Fig. 1. Sugar content of *Vaccinium* (**A**) and *Nicotiana* (**B**) plants. Significant difference ($p \leq 0.05$) for total sugar content is shown by *, for glucose and sucrose - ●

1 - *Nicotiana tabacum*, cv. Samsun (MSO medium), 2 - *N. tabacum*, cv. Samsun (MS medium+dexamethasone), 3 - *N. tabacum*, *Pdex-trolC* (MSO medium), 4 - *N. tabacum Pdex-trolC* (MS medium+dexamethasone), 5- *N. tabacum Pdex-A4rolC* (MSO medium), 6 - *N. tabacum Pdex-A4rolC* (MS medium+dexamethasone)

This study was supported by a grant to TM from the RFBR 18-016-00118. Transgenic seeds [7] were kindly represented by Prof. L. Otten (IBMP, France).

References

1. F.F. White, D.J. Garfinkel, G.A. Huffman, M.P. Gordon, E.W. Nester, Nature, **301,** 348–350 (1983)
2. T.V. Matveeva, D.I. Bogomaz, O.A .Pavlova, E.W. Nester, L.A. Lutova, Mol. Plant. Microbe Interact. **25,** 1542–1551 (2012)
3. T. Kyndt, D. Quispe, H. Zhai, R. Jarret, M. Ghislain, Q. Liu, G. Gheysen, J.F. Kreuze Proc Natl Acad Sci U S A, **112(18),** 5844–5849 (2015)
4. T.V. Matveeva, L. Otten, Plant Mol. Biol. **101(4–5),** 415–437 (2019)
5. T. Matveeva, Curr. Top. Microbiol. Immunol. 421-441 (2018)
6. T. Matveeva, S. Sokornova Russ J Plant Physiol. **64(5),** 635-64 (2017)
7. H. Mohajjel-Shoja, B. Clement, J. Perot, L. Otten, MPMI **24,** 44–53 (2011)
8. L. Otten Curr, Top. Microbiol. Immunol. 418 (2018)
9. T. Murashige, F. Skoog, Physiol. Plant. **15,** 165–170 (1962)
10. J.A. Bailey, R.S. Burden, G.G. Vincent, Phytochem, **14(2),** 597−582 (1975)
11. J.J. Estruch, D. Chriqui, K. Grossmann, J. Schell, A. Spena, EMBO J. **10(10),** 2889-2895 (1991)
12. J.J. Estruch, J. Schell, A. Spena, EMBO J. **10(11),** 3125-3128 (1991)