Sugar Tech (2009) 11(3) : 307-310

SHORT COMMUNICATION

Effect of epimutagen Triton X-100 on morphological traits in sugarbeet

S.S. Kirikovich · E.V. Levites

Received: 17 February, 2009; Revised: 12 July, 2009; Accepted: 20 July, 2009

Abstract It was found out that non-ion detergent X-100 changed the leaf and root morphological traits in sugarbeet (*Beta vulgaris* L.). Appearance of induced changes in sugarbeet was observed as a result of Triton X-100 effect on the multi-dimensional system of inherited information coding. These changes could be retained for a longer time with large number of cell generations which suggests Triton X-100 to be used as an epimutagen of a new type.

Keywords epigenetics, epimutagen, Triton X-100, inherited information coding, sugarbeet.

In the recent research, it was revealed that Triton X-100, usually used as a non-polar detergent to affect the membrane structure and separate proteins from them (Barsukov, 2004), can also induce inherited changes of morphological traits in wheat (Makhmudova, 2007; Makhmudova *et al*., 2008, 2009). In these experiments, spring common wheat seeds were treated with Triton X-100 0.1 % solution during germination. Such treatment led to a 100 % variability of spike morphology and to appearance of denser and squareheaded spikes, also multispikelet and branchy spikes (Makhmudova, 2007; Makhmudova *et al*., 2008, 2009). The ability of Triton X-100 to change the interaction of nucleoproteins with the nuclear matrix may lay ground for the inheritance of such changes. Such an effect well accords with the model of multi-dimensiality of coding of plant inherited information (Levites, 2003, 2005, 2007). According to this model, the inherited information is coded not only by nucleotide sequences but also by a differential endoreduplication degree of certain chromosome sites. In this model, the processes of DNA chromosome interaction with the nuclear membrane and nuclear matrix play a considerable role.

According to the model, a cell under embryogenesis gets rid of excessive chromatid site copies; thereafter only the pair of copies of this or that chromatid site, which attached to the nuclear membrane or nuclear matrix (Levites, 2003, 2005, 2007), remain in the cell. The model was being elaborated on the basis of studying sugarbeet epigenetic variability (Levites *et al*., 1998; Levites and Kirikovich, 2003). In this connection, the focus was on analyzing the effect of Triton X-100 on sugarbeet. Changes in morphological and biochemical traits in experimental plants are in favour of the proposed model. The present research, that aims at investigating the effect of Triton X-100 on of morphological traits, sugarbeet (*Beta vulgaris* L.) is the first stage of the number of research activities including the effect of Triton X-100 also at physiological, biochemical and molecular levels of biological organization.

Seeds obtained by self-crosses from the sugarbeet agamospermous plant with laboratory number 8-3 were involved in the research. Control seeds were soaked in Petri dishes in a thermostat at 29^0C. Experimental seeds were soaked in the 0.1 % Triton X-100 solution for 18 h at 29^0C. Then they were washed in the running water and placed into the thermostat again. Thus, control and experimental seeds were

---

S.S. Kirikovich · E.V. Levites
Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 10 Lavrentyev Aven., Novosibirsk, Russia, 630090
email : levites@bionet.nsc.ru

germinated at the same temperature and humidity. In spring, 2008, shoots were planted in the hydroponic greenhouse under lightening 5000-10000 lx during 16 h a day with mineral nutrition according to Knopp.

Control and experimental progenies of plant No. 8-3 were taken from the greenhouse and planted into the open ground on the 11th of June, 2008 where they were growing till the 10th of October, 2008. Plant trait observation was arranged in two periods: on the day of planting into the open ground and harvesting.

Significance of differences of control and experimental plants was found using G-test (Weber, 1986).

Polymorphism on a number of leaf and root morphological traits was revealed in control and Triton X-100-treated sugarbeet plants. The most clearly identified traits were *leaf bending* (Fig. 1), *root bending* (Fig. 2), *leafed root head*, *short* and *small root*. Trait of *leafed root head* is characterized by the presence of a big number of small leaves at the base of big leaves. The trait of short root is typical of roots not more than 4 cm in diameter and 7 cm in length and having no narrowed lower parts. The trait of *small root* was typical of roots which could be considered as undeveloped being not more than 2 cm in diameter.

In total, 102 control (8-3c) and 199 experimental plants (8-3tr) were planted into the open field. By the harvesting time, 96 control (8-3c) and 189 experimental plants (8-3tr) survived and were considered for analysis.

The manifestation degree of a number of traits was typically dependent on the time of observation and was significantly changing within each plant group, both experimental and control (Table 1). Therefore, registration of traits *leaf bending*, *root bending*, *short root*, *small root* and *leafed root head* was arranged in two terms (Table 1, 2).

Table 1. Manifestation of morphological traits in control and Triton X-100 0.1 % solution-treated sugarbeet plants at different vegetation periods

Trait	Plant No.	11.06.2008	10.10.2008	G-test	*P*
Leaf bending	8-3c, th. (%)	23 (22.5)	42 (43.8)	5.1769	< 0.05
	8-3tr, th. (%)	80 (40)	100 (52.9)	2.3086	
Root bending	8-3c, th. (%)	11 (11)	26 (27.1)	6.0964	< 0.05
	8-3tr, th. (%)	21 (10.5)	99 (52.4)	46.1892	< 0.01
Leafed root head	8-3c, th. (%)	7 (6.9)	20 (20.8)	6.4906	< 0.05
	8-3tr, th. (%)	22 (11.1)	79 (41.8)	29.8734	< 0.01
Small root	8-3c, th (%)	19 (18.6)	13 (13.5)	0.6868	
	8-3tr, th. (%)	49 (24.6)	10 (5.3)	22.9676	< 0.01
Short root	8-3c, th. (%)	10 (9.8)	10 (10.4)	0.0164	
	8-3tr, th. (%)	11 (5.5)	4 (2.1)	2.9374	

Table 2. Effect of Triton X-100 0.1 % solution on morphological traits of sugarbeet plants No. 8-3

Trait	Observation date	8-3 c, th. (%)	8-3 tr, th. (%)	G-test	*P*
Leaf bending	11.06.2008	23 (22.5)	80 (40)	5.0008	< 0.05
	10.10.2008	42 (43.8)	100 (52.9)	0.7368	
Root bending	11.06.2008	11 (11)	21 (10.5)	0.003	
	10.10.2008	26 (27.1)	99 (52.4)	7.1952	< 0.01
Leafed root head	11.06.2008	7 (6.9)	22 (11.05)	1.1852	
	10.10.2008	20 (20.8)	79 (41.8)	6.676	< 0.01
Small root	11.06.2008	19 (18.6)	49 (24.6)	0.9006	
	10.10.2008	13 (13.5)	10 (5.3)	4.616	< 0.05
Short root	11.06.2008	10 (9.8)	11 (5.5)	1.5668	
	10.10.2008	10 (10.4)	4 (2.1)	7.8786	< 0.01
Hollows	10.10.2008	4 (4.2)	62 (32.8)	25.0975	< 0.01
Sorrel-like leaf	10.10.2008	7 (7.3)	35 (18.5)	5.4316	< 0.05

Leaf bending manifestation frequency (Fig. 1) was significantly higher by the autumn harvesting time in control plants compared to growth onset in the open ground, whereas high frequency of this trait was observed right after the moment of planting into the open ground in experimental plants, and it survived till autumn harvesting (Table 1). In this connection, differences between control and experimental plants were significant only at the time of planting into the open ground ($P < 0.05$), and they became insignificant by the time of autumn harvesting (Table 2).

During vegetation, there was a significant increase of plant number with the trait of *root bending* (Fig. 2) and *leafed root head* - both in experimental and control groups. However, in the experimental group, this increase was more considerable (Table 1). It is due to this that significant differences between control and experimental plant groups were revealed only at the moment of autumn harvesting (Table 2). It is noteworthy that a change of trait manifestation frequency in the population was accompanied by its change in a concrete plant during vegetation. Meanwhile, it was observed that the plant trait present at the initial observation period disappeared at the end of vegetation and vice versa.

The trait of *short root* had a stable manifestation in control and experimental plant groups at ontogenesis. Changes of this trait frequency during vegetation were nonsignificant within each group, but in different directions. Therefore, the differences between control and experimental groups in the frequency of the trait *short root* became significant by the time of autumn harvesting (Table 2).

The trait of *small root* had quite a stable manifestation in the control. However, it was unstable in the experimental plant group (Table 1). This trait frequency sharply decreased by the time of autumn harvesting in Triton X-100-treated plants, and that led to significant differences between control and experimental groups (Table 2).

Fig. 1 *Leaf bending* trait in sugarbeet plants.

It is noteworthy that manifestation frequency of the trait *short root* and *small root* significantly decreased in the experimental plant group by the time of autumn harvesting ($P < 0.01$ and $P < 0.05$, respectively), and the plant number with the traits *root bending* and *leafed root head* significantly increased by the time ($P < 0.01$) (Table 2).

Observation of such traits as presence of *hollows* (Fig. 3) and *sorrel-like leaf* was carried out only during autumn harvesting; significant differences between control and experimental plants being revealed with $P < 0.01$ and $P < 0.05$, respectively (Table 2).

Fig. 2. *Root bending* trait in sugarbeet plants.

Thus, all the traits under investigation changed and enhanced their manifestation affected by Triton X-100; the most significant differences between control and experiment accumulated by autumn harvesting, except for *leaf bending* (Fig. 1), where the differences between control and experiment were found already during planting into the open ground. This can be explained by the fact that Triton X-100 stimulated manifestation of *leaf bending* at the early vegetation period and, normally, its manifestation was gradually developing and was equal to that of experimental forms only by the end of autumn vegetation. Notable is also the fact that the changes affected by Triton X-100 are very diverse. For instance, if *leaf bending* (Fig. 1) and *root bending* (Fig. 2) can be considered as the result of different cell division velocity on different sides of a given organ, then the increased percentage of *hollows* (Fig. 3) on roots of Triton X-100-treated plants is indicative of the thing that the processes of differentiation, qualitative rearrangement in genome functioning are affected. Moreover, a big number of versatile changes is suggestive of the thing that not a certain gene, but most part of plant genome was Triton X-100-affected.

Fig. 3. *Presence of hollows* in sugarbeet plants.

The findings prove the fact that Triton X-100 induces clearly identifable changes in morphological trait of sugarbeet. They are observed not only at the initial, but also at later developmental stages separated by a long time span from the Triton X-100 treatment to a large number of cell generations. As a non-polar detergent, Triton 100-X is capable of separating proteins from membranes and induces changes of structure and function of membranes (Barsukov, 2004). Preservation of morphological changes for a long time allows us to hypothesize the thing that the changed membrane states can survive in a number of cell generations. Moreover, the data on the Triton X-100 effect on wheat seeds (Makhmudova, 2007; Makhmudova *et al.*, 2008, 2009) are indicative of the thing that the Triton X-100-affected membrane state is preserved in a number of sexual generations. Therefore, the obtained data indicates not only that Triton X-100 can be considered as an epimutagen, but also the thing that biological membranes and, first, membranes of cell nucleus, are an important element of genome structure, determining its functioning state and variability. Studying the mechanism for preservation of changed membrane states in cell and sexual states is of great interest. It is clear that, by some common mechanism of genome functioning, chromosome interaction with nuclear membrane and nuclear matrix is affected. Appearance of induced changes in sugarbeet can be considered as a result of Triton X-100 effect on the multi-dimensional system of inherited information coding.

Acknowledgement The research was financed by Grant N 99 on the SB RAS Integrative Project for the period of 2009-2011 ‘’Induction of epigenetic changes as a new effective method for developing initial plant breeding forms”. The authors would like to express their gratitude for the English version of this article to Alexander V. Zhuravlev, SB RAS Executive Interpreter.

References

Barsukov LI (2004) How to assemble a membrane (membrane solubilization and reconstruction) // Soros Educational Journal, 8 (1) : 10–16. (in Russian)

Levites EV (2003) Theoretical and practical aspects of studies in epigenetic variability in sugarbeet. Sugar Tech., 5 (4) : 209–211.

Levites EV (2005) Sugarbeet plants produced by agamospermy as a model for studying genome structure and function in higher plants. Sugar Tech., 7 (2&3) : 67-70.

Levites EV (2007) Marker enzyme phenotype ratios in agamospermous sugarbeet progenies as a demonstration of multidimensional encoding of inherited information in plants. http://arxiv.org/abs/q-bio/0701027

Levites EV, Kirikovich SS (2003) Epigenetic variability of unlinked enzyme genes in agamospermous progeny of sugar beet. Sugar Tech., 5 (1&2) : 57-59.

Levites EV, Shkutnik T, Ovechkina ON, Maletskii SI (1999) Pseudosegregation in agamospermic progeny of male sterile plants of the sugar beet (*Beta vulgaris* L.). Repots Biol. Sciences. 365 : 182-184.

Makhmudova K Kh (2007) Inheritance of epigenetic changes in common wheat (*Triticum aestivum* L.) // The thesis on competition of scientific degree of the candidate of biological sciences according to specialty genetics. Almaty, 26 p. (in Russian)

Makhmudova K Kh, Bogdanova ED, Levites EV (2008) A way of epigenetic variability induction in common wheat. RF Invention Patent N 2322801. Application N 2006130357. Invention priority date 22.08.2006. Published in Bulletin No. 12. 27.04.

Makhmudova K Kh, Bogdanova ED, Levites EV (2009) Triton X-100 induces heritable changes of morphological characters in *Triticum aestivum* L. // Russian Journal of Genetics. 45 (4) : 495–498.

Weber E (1986) Grundriss der biologischen statistic. Stuttgart: Gustav Fischer Verlag. 652 p.