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SHORT COMMUNICATION

Effect of Triton X-100 on the Viability and Morpho-physiological Traits in Sugarbeet

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Abstract The effects of Triton X-100 (TX-100) on seed germination, pollen sterility manifestation, number of chloroplasts in stomata guard cells and the viability in sugarbeet (*Beta vulgaris* L.) were studied. It is was found that TX-100 decelerated seed germination of sugarbeet, changed pollen sterility manifestation, affected chloroplasts number in stomata guard cells and decreased the viability of sugarbeet seeds. The results of the present study indicated that detergent TX-100 may be considered as an epimutagene capable of changing plant genome functioning.

Keywords Epimutagene · Triton X-100 · Pollen fertility · Seed germination · Stomata guard cells · Viability · Sugarbeet

Immense attention of researchers on epigenetics makes it important to search for substances that induce epigenetic variability. Currently, 5-azacytidine is the most known epimutagene causing DNA chromosome demethylation and, as a consequence of this, activating earlier methylated genes (Jones 1985; Jablonka and Lamb 1989; Janousek et al. 1996; Maletskaya et al. 2006). Demethylated genes are preserved in a number of generations conditioning stable changes of different morphological traits. Changes in the degree of pollen sterility and also the character of branching in floret-bearing shoots, and manifestation of unianthy and synanthy were found in sugarbeet plant treated with 5-azacytidine (Maletskaya et al. 2002). It was also shown that 5-azacytidine decreased a number of cell organelles, chloroplasts in stomata guard cells in sugarbeet plants (Maletskaya et al. 2002). However, alongside with 5-azacytadine that modifies nucleotides in the DNA molecule, there is a principally new type of epimutagene which affects not DNA but the interaction process of DNA with nuclear membrane and nuclear matrix. Non-polar detergent Triton X-100 capable of aberrating protein-membrane bonds could be of such a substance.

It was revealed that TX-100 initiates the inherited changes of morphological traits when wheat and sugar beet seeds are treated with it during their germination (Makhmudova et al. 2009; Kirikovich and Levites 2009a, b). Moreover, it was revealed in these experiments that the effect of TX-100 decreased plant viability. Therefore, it was interesting to compare plants viability and the expression of morpho-physiological traits with the plant cell genome statement estimated on chloroplasts number. It is known that chloroplasts number correlates with the plant genome size and plant ploidy level (Savitsky 1966; Yudanova et al. 2002; Yuan et al. 2009). So measuring of chloroplasts number in plant cells gives the integral information about its genome statement.

The aim of the present research was to study the effect of epimutagene TX-100 on seed germination, pollen fertility manifestation, chloroplasts number in stomata guard cells and the viability of sugarbeet seeds.

Seeds obtained by self-reproduction of agamospermous sugarbeet plants under laboratory number 8-3 were used. Control seeds were soaked in Petri dishes in a thermostat at 29 °C. Experimental seeds were soaked in the 0.1 % TX-100 during 18 h at 29 °C. Then they were washed in the streaming water and placed into the thermostat again. Thus, control and experimental seeds were germinated at the same temperature and humidity. Shoots were planted in the

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Table 1 Sugarbeet plants viability during different periods of ontogenesis

	Soaked seeds number	Germinated seeds number and %	First vegetation, 2008		Second vegetation, 2009	
			June, number and %	October, number and %	May, number and %	August, number and %
8-3c	300	133 (44.3 %)	102 (76.7 %)	96 (94.1 %)	69 (71.9 %)	55 (79.7 %)
8-3tr	510	355 (69.6 %)	199 (56.1%)	189 (95 %)	118 (62.4 %)	47 (39.8 %)
Periods	I	II	III	IV	V	
P	< 0.001	< 0.05	> 0.05	> 0.05	< 0.01	

hydroponic greenhouse under lightening 5,000–10,000 lx during 16 h a day with mineral nutrition.

To calculate the chloroplasts number in stomata guard cells from the leaves, plants of the first (field, August, 2008) and the second years (field, August, 2009) of vegetation were used. For preparations epidermis was taken from the basal side of leaves and the chloroplasts were stained with nitrogenous silver ($AgNO_3$) solution. Chloroplasts of 40–70 cells were counted on each slide.

Plant pollen sterility-fertility cytoanalysis was arranged in blooming period (27 July, 2009) with preliminary carmine-treated pollen.

Viability was estimated on the number of living plants during 5 periods. The first period (I) includes the time from the soaking to germination; the second period (II)—the time from shoots planting in the greenhouse (31st of March, 2008) to its replanting in the field (11th of June, 2008). The third period (III) includes the time of vegetation in the field (June–October, 2008); the fourth period (IV)—the time of vernalization (October, 2008–May, 2009) and the fifth period (V)—the time of the second vegetation in the field (May–August, 2009).

Significances in germination differences, fertile-sterile pollen ratio and the viability of control and experimental plants were found using *G* test (Weber 1986). Statistical analysis for each control and experimental stomata guard cell populations was carried out using *t*-distribution (Sokal and Rohlf 1995).

It was found that TX-100 affects seed germination process. Thus, 133 untreated seeds (44.3 %) out of 300 from sugarbeet plant no. 8-3 (8-3c) germinated, and 355 seeds (69.6 %) germinated out of 510 seeds of the same plant treated with TX-100 (8-3tr) (Table 1). *G* test analysis showed the significance in differences between the control and treated seeds at the first period (I) (G = 13.312). It was revealed that TX-100 significantly stimulates germination and viability this period ($P < 0.001$) (Table 1).

Differences were observed not only in the number of germinated seeds but also in their germination dynamics (Fig. 1).

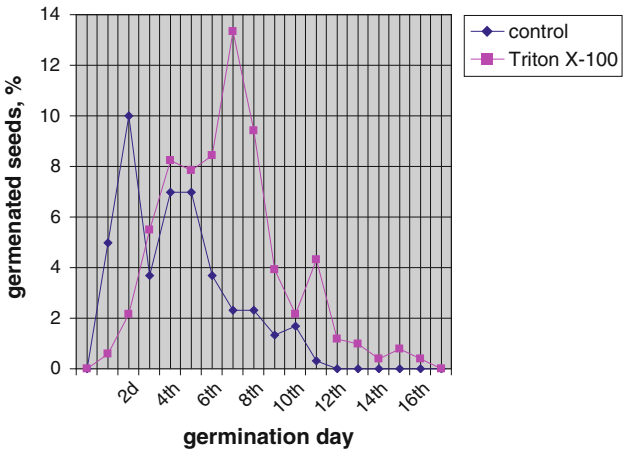

Fig. 1 Germination dynamics for control and 0.1 % TX-100 solution-treated seeds of plant no. 8-3

As is seen from Fig. 1, the seeds 8-3tr germination duration was 5 days longer than that of 8-3c, germination peak being on the 7th day in experimental seeds, whereas it was on the 2nd day in control. Prolongation of experimental seed germination is suggestive of the thing that TX-100 weakens germination energy, whereas germination ability in the seeds of this genotype becomes higher. Longer germination terms indicated that TX-100 may changed plant genome functioning.

Comparison of viability of control and experimental plants during different periods of vegetation is given in Table 1. Viability of treated shoots after planting in greenhouse significantly decreased: 102 (76.7 %) out of 133 planting control plants and 199 (56.1 %) out of 355 planting treated plants gave well-developed roots; differences between control and experimental plants are significant ($P < 0.05$) (Table 1). No differences in viability between control and experimental plants were revealed during the next period of the first year of vegetation (III) and vernalization (IV) (Table 1).

However, the differences in viability were revealed in the second year of vegetation (period V) ($P < 0.01$) (Table 1). This supports conclusions that differences

between control and experimental groups of plants may be observed not only at the initial, but also at later developmental stages separated by a big time span from the moment of TX-100 treatment and by a big number of cell generations. The high loss of treated plants points to the fact that TX-100 leads to the change of genome functioning and decreases plant viability.

In this connection, it is interesting to study some traits of these plants characterizing genome statement. The chloroplasts number in the stomata guard cells are concerned as those integral characteristics. It is known that this trait correlates with plant genome size and ploidy level (Savitsky 1966; Yudanova et al. 2002; Yuan et al. 2009). In Tables 2 and 3 the chloroplasts number in stomata guard cells of control and treated plants are compared. It was shown that vernalization leads to the evidence of an increasing chloroplasts number in both groups of plants (Table 2).

Distinct differences were revealed in the number of chloroplasts between control and experimental plants for the first year of vegetation (Table 3). However, no differences were revealed in the viability between control and treated plants during this period (III) (Table 1). Significant differences in viability were revealed between control and experimental plants during the second year of vegetation (period V) (Table 1). However, they had equal number of chloroplasts (Table 3).

These results can be explained by the fact that TX-100 induced changes conserve under normal plant viability, but at the critical period of ontogenesis, such as the reproductive period (V) (Table 1), plants carrying arising changes die and plants similar with control survive. This fact supports Darwin's conclusions on the point that natural selection keeps organisms corresponding to the environment (Darwin 1868).

However, for some changes of traits, which were not considerably affected by natural selection, there is still a possibility to survive even after a considerable death of part of sample. Pollen fertility turned to be such a trait.

Control and experimental plants were, in some extent, sterile, but treated plants had more fertility than control plants ($G = 26.3597$; $P < 0.001$), (Table 4). Thus, TX-100 increased pollen fertility in this plant form. Validity of differences was revealed in big samplings of pollen grains: 8-3c 5604 (p. g.) from 28 plants and 8-3tr 4508 (p. g.) from 23 plants (Table 4). It indicates that TX-100 effectively changes physiological processes in treated plants.

Table 2 Chloroplasts number in stomata guard cells of control and experimental plants in the first and second years of vegetation

	Vegetation	*n*	Min–max	X ± m	*t*	*P*
8-3c	2008	825	5–21	13.72 ± 0.08	13.64	<0.001
	2009	477	10–26	15.65 ± 0.12		
8-3tr	2008	909	2–25	14.43 ± 0.09	9.45	<0.001
	2009	288	10–22	15.93 ± 0.13		

Table 3 Influence of environment and TX-100 on changing the chloroplasts number in stomata guard cells and viability of sugarbeet plants during of III and V periods of ontogenesis

	8-3c	8-3tr	*P*
First vegetation year (August, 2008)			
Chloroplasts number	13.72 ± 0.08	14.432 ± 0.09	<0.001
Number (%) of survived plants	96 (94.1 %)	189 (95 %)	>0.05
Second vegetation year (August, 2009)			
Chloroplasts number	15.65 ± 0.12	15.93 ± 0.13	>0.05
Number (%) of survived plants	55 (79.7 %)	47 (39.8 %)	<0.01

Table 4 Statistical estimation of differences in pollen grain (p. g.) fertility-sterility level in control and experimental sugarbeet plants (field, 2009)

	27 July, 2009	
	Number of fertile p. g. (%)	Number of sterile p. g. (%)
Control plants	628 (11.21 %)	4,976 (88.79 %)
Experimental plants	660 (14.64 %)	3,848 (85.36 %)
G test	26.3597	
P	<0.001	

The obtained data proved that detergent Triton X-100 affects plant genome function changing DNA chromosome interaction with the nuclear membrane and nuclear matrix. As these changes are observed not only at the initial developmental stages, but also during blooming which is separated from the germination stage by a big span of time and a large number of cell generations, it is possible to hypothesize that, on membranes, there are certain sites carrying the information on interaction with chromosomes, and they are capable of not only changing under Triton X-100 treatment but also preserving this changed state in a number of cell divisions.

Thus, the obtained data are indicative of the thing that Triton X-100 may also be considered as an epimutagene. Moreover, they are in favour of the suggested model for multidimensional inherited information coding (Levites 2003, 2005) whose important point is chromosome interaction with the nuclear membrane and nuclear matrix.

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