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TESTING THE MUTAGENICITY OF SOILS FROM DIFFERENT AREAS OF THE IVANO-FRANKIVSK REGION (UKRAINE) USING HETEROZYGOUS *NICOTIANA TABACUM (SU/+)* PLANTS

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Abstract: In this work we used *Su/+ Nicotiana tabacum* plants to analyze potential mutagenecity of soils from three different zones in Ukraine, zone of chemical contamination, zone of enhanced radiological control and conditionally clean zone. Leaves of *Su/+* plants are pale green with occasional appearance of dark green spots indicating mutations at the *Sulfur* locus. We found that plants grown on soils from all three zones had shorter stem and smaller crown diameter as compared to plants grown on control greenhouse soil. For the mutagenecity assay, we analyzed the spot appearance, the number of spots per plant and the average area of spots per plant. The analysis showed that soil samples from most of the tested areas had higher mutagenecity as compared to control soil sample. Thus, our data showed that *Su/+* plants are effective indicators of soil pollution.

Keywords: transgenic *Nicotiana tabacum Su/+* plants, contaminated soil, mutation frequency, plant growth

1. INTRODUCTION

Based on soil and climatic conditions, the Ivano-Frankivsk region may be divided into three zones: 1. The Prednistrovska plain zone 2. The Precarpathian/Subcarpathian (premountainous) zone and 3. The Carpathian Mountains zone (Adamenko 2000). The composition of the topsoil is very diverse. Almost all types of topsoil characteristic of forest-steppe, pre-mountainous and mountainous zones of the Carpathian region can be found there.

Altogether, there are 22 different types of soil in the region. The largest area is covered by brown mountain-forest soils on which forests grow. In the premountainous zone, gleysols and podzols prevail; in the river valleys - fluvisols and histosols; and in the forest-steppe zone, cambisols, podzoluvisols and chernozems (classified as per World References Base for Soil Resources, 1998). In the south-east, one can observe large areas of podzolized and leached chernozems. Soils from Galych and Snyatyn areas being investigated here belong to the plain zone; soils from Kalush area and the town of Kolomyja – to the pre-mountainous zone; soils from the Verkhovyna area – to the mountainous soil-climatic zone (Adamenko 2000; Adamenko 2005).

The ecological situation in the Ivano-Frankivsk region can be characterized as a critical one that has resulted from a long-time of negligence in laws of the development and recovery of natural resources. The main part of contamination in the region comes from electric power, chemical and mining industries as well as from manufacturers of auto transport and food. According to ecological researches done by Adamenko (2000) and based on natural conditions, industrial infrastructure and atmospheric contamination, there are several safety zones in the Ivano-Frankivsk region: conditionally safe, zones of enhanced radiological control and ecologically dangerous zones (Adamenko, 2000).

Ecologically dangerous areas include the towns of Kalush and the Galych area. This is due to dispersion of pollutants and contaminants produced by the Burshtyn heat power plant and chemical and petrochemical plants in the town of Kalush (Adamenko, 2000; Adamenko, 2005). Moreover, soils from the Kalush area have the highest content of lead, copper and cadmium: an average content of lead in these soils is 15.7-16.6 mg/kg, while the highest one is 28.3-30.0 mg/kg; the highest content of cadmium is 0.6-1.0 mg/kg (the background content – up to 0.5 mg/kg). Since the Kalush and Galych areas have very high levels of contamination with toxic substances, they are considered to be zones of chemical contamination (Adamenko, 2005). It should be mentioned that in soils of the Kolomyja area, remnants of pesticides, mineral fertilizers and sewage water toxicants have been found, which allows us to consider the area as a chemically contaminated zone as well.

Zones of enhanced radiological control include Rusiv, Potichok and Stetsiv villages in the Snyatyn area. Soil monitoring data on contamination with radionuclides, heavy metals and pesticides provided by the Ivano-Frankivsk radiology&toxicology services have revealed that the Snyatyn area has the highest density of contamination with ^{137}Cs in soils – 2.51 Ci/km^2 (Jakymiv et al., 2011).

Mountain territories in the Verkhovyna area, including the town of Verkhovyna and villages Krasnyk and Iltsi, are considered to be conditionally ecologically safe (Adamenko, 2000).

Based on the ecological and geographical characteristics of the region, we have collected soil samples from different towns and villages from three above mentioned zones. We used heterozygous *Nicotiana tabacum (Su/+)* plants (Burk & Menser, 1964; Kawata & Cheung, 1990) to analyze physiological growth parameters and the level of genomic rearrangements upon exposure to soil samples from different areas of Ivano-Frankivsk region. We found that growing plants on soil samples from all three zones showed high mutagenecity of these soils as compared to control plants.

2. MATERIALS AND METHODS

2.1. Soil sampling and analysis

The subject of investigation have been samples of soil from various areas of the Ivano-Frankivsk region: ecologically dangerous zones (zones of chemical contamination) – the town of Kalush, the villages of Grabivka and Zaviy in the Kalush area, the town of Burshtyn and the Burshtyn heat power plant in the Galych area, the town of Kolomyja; zones of enhanced radiological control – Rusiv, Potichok and Stetseva villages in the Snyatyn area; conditionally clean zones as controls – the town of Verkhovyna the villages Krasnyk and Iltsi in the Verkhovyna area and commercial greenhouses (Fig. 1, Fig. 2). The selection of soil samples has been done based on requirements of state standards: GOST 26483-85, GOST 26490-85, GOST 2481-80, GOST 24849-81. Soil samples were taken by removing the 10 cm of the surface area, and then collecting 30 cm of the inner layer.

Figure 1. Map of the Ivano-Frankivsk region.
Red squares indicate where the samples were taken.

The values in Ci/km^2 of the mean density of soil pollution in the areas of sampling were The gamma-irradiation exposure rate (μR/h) in the air at ground level was determined using a SRB9-1 ship beta-gamma radiometer, a FD-5 field dosimeter and a DC9-04 dosimeter control signal (Kovalchuk et al., 1999). Soil samples from the experimental plots were assayed for ^{137}Cs and ^{90}Sr activity, using the β- and γ-spectrometry devices RUB – 91 and RUG - 91M, respectively. Chemical characterization of the soils was conducted on split samples. These were analyzed for mobile forms of major chemical elements, such as N, P, K, Ca and Mg as well as for pH of the soil salt extract. In additional, presence of heavy metals Cu, Zn, Cd, Cr, Ni and Pb were analyzed as reported before (Kovalchuk et al., 2000).

2.2. Plant growth

For the analysis, we used seeds of *Nicotiana tabacum* plants that were heterozygous for Sulfur gene (*Su*/+) (Burk & Menser, 1964; Kawata & Cheung, 1990). Heterozygous plants (*Su*/+) are able to carry on photosynthesis and have a yellow-green phenotype due to the semidominant nature of the mutation at the *Sulfur* locus. Wild-type tobacco plants (+/+) are green, whereas homozygous plants (Su/Su) are pale yellow and do not carry on photosynthesis (Fig. 3).

Figure 2. Maps of regions where samples were taken. A) Kalush area – samples from town of Kalush and villages Zaviy and Grabivka; B) Snyatyn area – samples from Rusiv, Stetseva, Potichok; C) - Verkhovyna area, villages Krasnyk and Iltsi. Red squares indicate where the samples were taken.

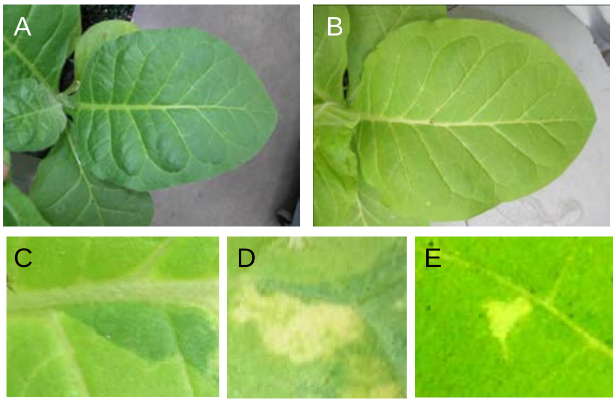

Figure 3. Representative images of wild type and *Su/+ Nicotiana tabacum* plants. A. Representative image of *Nicotiana tabacum* plants cultivar Big Havana. B. Representative image of *Su/+ Nicotiana tabacum* plants.
C. Representative image of dark green spot *(+/+)*.
D. Representative image of twin spot *(Su/Su / +/+)*.
E. Representative image of albino spot *(Su/Su)*.

The selfed progeny of (*Su*/+) plants segregates in a normal Mendelian manner: 25% of plants are wild-type (+/+), 50% are heterozygous (*Su*/+), and 25% are homozygous for mutations in the Sulfur gene (*Su*/*Su*). In the current study, we used heterozygous (Su/+) plants. Mutations at *Sulfur* locus typically lead to the appearance of three types of sectors: light green spots (albino), dark green spots against the background of yellow-green leaves, and a rare combination of both, called twin spots, presumably consisting of cells with *Su*/*Su* (albino) and +/+ (dark green) genotypes (Kawata & Cheung, 1990; Friedlender et al., 1996) (Fig. 3C,D,E).

Plants were germinated and grown on soil taken from the aforementioned town and village areas. Approximately 10 plants per each treatment were used. Each experiment was repeated three times. Plants were grown in standard pots under the following conditions: at a temperature of 22°C and under the light regime of 16h day and 8h night.

2.3. Analysis of dynamics of plant growth and mutations at the Sulfur locus

On average, five to ten plants were taken for the analysis. We analyzed the dynamics of stem elongation and the dynamics of the enlargement of crown diameter. The data points were taken every two weeks for approximately 4 months.

For the analysis of the appearance of dark and twin spots, the following parameters were measured: the time of sport appearance, the percentage of plants with spots, the average number of spots per plant, the average area that spots occupy per plant, and the index of mutation intensity. For the analysis of the average number of spots per plant, spots were counted at the last day of measuring stem height. The average area of spots per plant was calculated by measuring the size of each spot, summing it for each plant, and then averaging it for all plants grown on a particular soil sample. The index of mutation intensity (IMI) was measured by relating the average size of the total area that spots occupy in a single plant to the total area of leaves in a single plant.

2.4. Statistical analysis

Statistical analysis was performed using a Student's t-test and a Spearman's and Pearson rank correlation test.

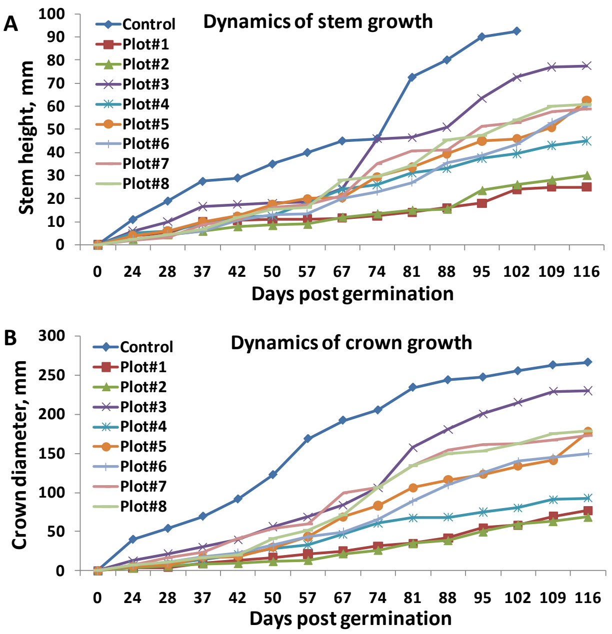

Figure 4. Dynamics of stem and crown growth in plants grown on soil from the zone of chemical contamination
A. Dynamics of stem elongation in plants germinated and grown on soils from the zone of chemical contamination. Y axis shows the stem height, whereas X axis shows days post germination.
B. Dynamics of increase in crown diameter in plants germinated and grown on soils from the zone of chemical contamination. Y axis shows the crown diameter, whereas X axis shows days post germination.

3. RESULTS

3.1. Analysis of stem and crown growth

The analysis showed substantially slower growth of the stem and the crown in plants grown on samples from all three testes areas as compared to control soil (Fig. 4 and Fig. 5).

Plants grown on samples from chemically contaminated areas from Plot 1 and 2 as well as conditionally safe area from Plot 13 scored well below all other plants in both, stem growth and crown expansion.

Comparably small stem and crown were found in plants grown near the Burshtyn power plant (Plot 4). Poor performance of plants grown on soil samples from conditionally safe area (Plot #13) could be due to the type and structure of soils (brown mountain-forest) and the absence of fertilizers. The best growth rates were observed in plants grown on soil samples from the town of Kolomyja in the vicinity of water pumping station (Plot 3); stem size and crown diameter were 78 mm and 230 mm, as compared to 93 mm in 266 mm in controls, respectively.

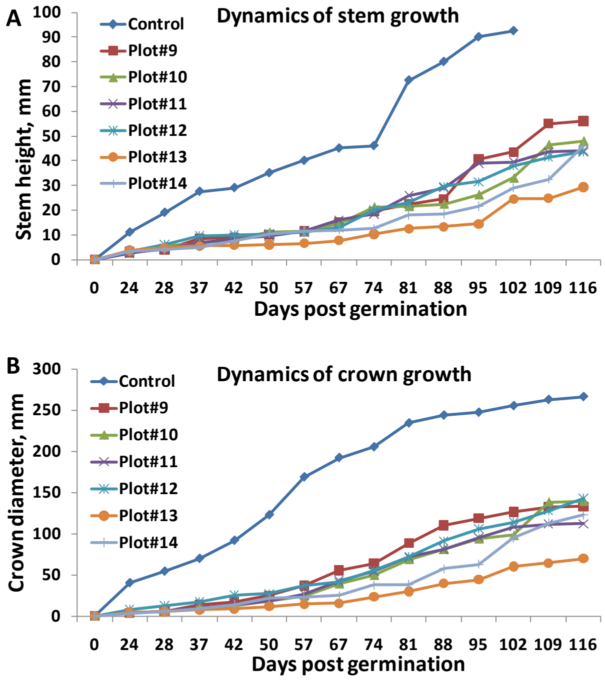

Figure 5. Dynamics of stem and crown growth in plants grown on soil from the zone of enhanced radiological control and conditionally clean zone
A. Dynamics of stem elongation in plants germinated and grown on soils from the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Y axis shows the stem height, whereas X axis shows days post germination.
B. Dynamics of increase in crown diameter in plants germinated and grown on soils from the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Y axis shows the crown diameter, whereas X axis shows days post germination.

3.2. Analysis of mutagenecity of soils from different ecological zones

We studied the intensity of mutagenic background of soils in terms of time, frequency and peculiarities of spot occurrence on the leaves of *Su/+* plants (Table 1). The earliest occurrence of mutations at *Sulfur* locus was observed in plants grown on samples from radioactively contaminated soil from the village of Rusiv, Plot 11; first mutation was scored on day 48 post germination, as compared to day ~95 post germination in control.

Table 1. The areas from which the soil samples were taken and the sample labels

SAMPLE LOCATION	SAMPLE LABEL
Soil from greenhouse	Control
Zone of chemical contamination	
Near Kolomyja river	Plot 1
Near Kolomyja lake	Plot 2
Near Kolomyja water pumping station	Plot 3
Burshtyn power plant	Plot 4
Burshtyn town	Plot 5
Kalush	Plot 6
Zaviy	Plot 7
Grabivka	Plot 8
Zone of enhanced radiological control	
Stetseva	Plot 9
Potichok	Plot 10
Rusiv	Plot 11
Conditionally clean zone	
Iltsi	Plot 12
Krasnyk	Plot 13
Verhovyna	Plot 14

Spots on plants cultivated on soil samples from the village of Grabivka (Plot 8) were reported in 70.33±10.33 days of vegetation; in the town of Kalush (Plot 6) – in 72.33±4.24 days and in the village of Zaviy (Plot 7) – in 74±6.43 days of vegetation (Fig. 6). Thus, plants cultivated on soil samples from Kalush area belonging to the zone of chemical contamination had spots 1.3-1.4 times earlier than plants cultivated on control soil.

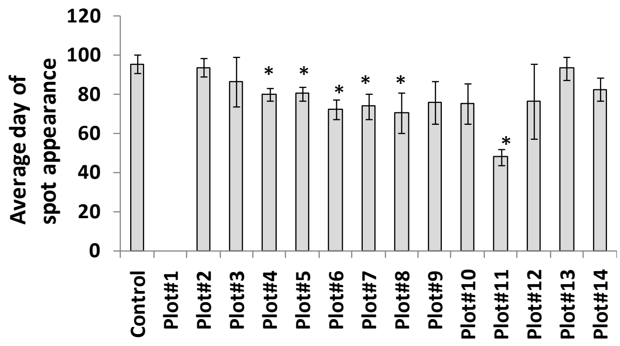

Figure 6. Time of spot appearance in *Su*/+ plants
Plants were germinated and grown on soils from the zone of chemical contamination (Plot 1-8), the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Spot appearance was monitored daily. The Y axis shows the average time (days) of spot appearance (average of three experiments with SD), whereas X axis shows the place of sampling (Plot 1-14). Asterisks show significant from control differences (p<0.05).

Plants grown on soil from the zone of enhanced radiological control, Plot 9 and 10, had mutations of the *Su* gene visualized at days 75-76 of growth, i.e., 20 days earlier than in control plants.

In contrast, tobacco plants cultivated on soil samples from the town of Kolomyja had spots appeared at days 86.5±12.5 after planting in the vicinity of water pumping station and at days 93.67±4.7 after planting near the lake (Plot 3 and 2, respectively). Also, the appearance of spots at days 93.25±5.94 in plants grown on soils from conditionally clean area (Plot 13) was similar to control. Therefore, according to the time of appearance of mutations at *Sulfur* locus, soil samples from the town of Kolomyja (Plot 2 and 3) and the village of Krasnyk in the Verkhovyna area (Plot 13) appeared to be the closest to the corresponding values in control plants. Plants cultivated on soil samples from the village of Rusiv in the Snyatyn area had the spots appear the earliest.

Next, we compared the percentage of plants with spots. Plants grown on control soil had spots on every plant (100%) (Table 2). Similar results were observed for plants from Plots 3-9 and Plot 12; thus every plant grown on soil samples from zone of chemical contamination had spots. Curiously, plants grown on soil samples from conditionally clean zones (Plot 13 and 14) had spots in only 2/3 of all plants. To our surprise, the soil sample from radioactively contaminated area (Plot 11) that triggered the earliest appearance of spots on plants also showed the lowest percentage of plants with spots, just 25%.

Next we analyzed the number of spots per plant. This analysis has been regularly done in past for variety of transgenic plants carrying GUS or LUC recombination substrates (Ilnytskyy et al., 2004; Filkowski et al., 2003; Boyko et al., 2006).

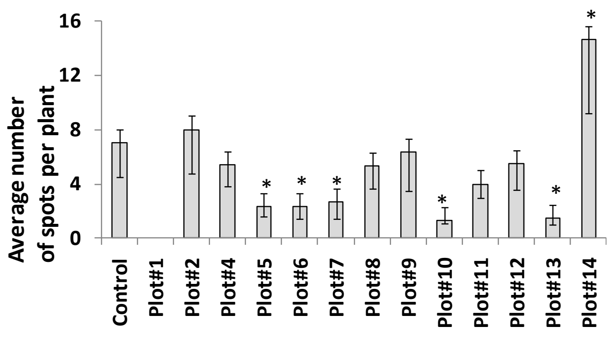

Figure 7. Average number of spots in *Su*/+ plants. Plants were germinated and grown on soils from the zone of chemical contamination (Plot 1-8), the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Spots were counted at 4 month after germination. The Y axis shows the average number of spots per plant (average of three experiments with SD), whereas X axis shows the place of sampling (Plot 1-14). Asterisks show significant from control differences (p<0.05).

Table 2. The analysis of dark green and twin spots in leaves of *Nicotiana tabacum Su/+* plants cultivated on soil samples from different areas of the Ivano-Frankivsk region

Sample location	Sample label	Plants with spots, %	Day of appearance of first spot	The number of spots per plant	Area of spots per plant, mm²	Index of mutation intensity, %
Greenhouse soil	Control	100	95.33±4.67	7.00 ±2.52	8.33±2.46	0.05±0.00
Near Kolomyja river	Plot 1	-	-	-	-	-
Near Kolomyja lake	Plot 2	75*	93.67±4.7	8.0±3.21	20.17±9.2*	2.11±0.82*
Near Kolomyja water pumping station	Plot 3	100	86.5±12.5	507±493	657.7±642	7.59±7.43
Burshtyn power plant	Plot 4	100	80.0±3.27*	5.43±1.6	13.57±5.8	0.8±0.35*
Burshtyn town	Plot 5	100	80.33±3.69*	2.33±0.71*	4.25±2.19	0.08±0.03
Kalush	Plot 6	100	72.33±5.24*	2.33±0.88*	1.83±0.88*	0.07±0.01
Zaviy	Plot 7	100	74.0±6.43*	2.67±1.2*	1.67±0.6*	0.08±0.05
Grabivka	Plot 8	100	70.33±10.33*	5.33±1.67	4.67±1.86	0.06±0.01
Stetseva	Plot 9	100	75.67±8.95	6.33±2.25	7.73±4.77	0.14*
Potichok	Plot 10	85.71	75.33±10.38	1.33±0.21*	1.25±0.38*	0.21±0.07*
Rusiv	Plot 11	25*	48±24*	4±2	23.5±11.2*	2.96±1.1*
Iltsi	Plot 12	100	76.25±19.21	5.5±1.94	184.0±103*	9.17±5.25*
Krasnyk	Plot 13	66.67*	93.25±5.94	1.5±0.5*	0.75±0.25*	0.11±0.03
Verhovyna	Plot 14	71.43*	82.6±5.62*	14.6±5.35	323.5±200*	5.7±2.41*

The soil samples from Plot 1-8 belong to the zone of chemical pollution, the soil samples from Plot 9-11 belongs to the zone of enhanced radiological control, and the samples from Plot 12-14 – conditionally clean zone. The data are shown as averages calculated from three independent experiments (± SD). Asterisks indicate significant differences from control (p≤0.05).

The largest number of spots (504±493) was observed in *Nicotiana tabacum* plants grown on soil samples from the town of Kolomyja (the water pumping station, Plot 3), which was over 70-fold higher than in control 7±2.52 spots per plant (Fig. 7; Table 2). The next highest number of spots per plants was observed in plants grown on soil samples from the town of Verkhovyna, Plot 14, the frequency of mutations was 14.6±5.35, which exceeded 2 times the control values. It is difficult to define the cause of such situation as the Verkhovyna area is considered to belong to the so-called “conditionally clean” territories. In all the other samples the frequency of spot per plant was smaller than in control.

While counting the spot number we noticed that some of the spots had very large surface area. Thus, we decided to calculate what area spots occupy per single plant. This analysis showed that samples from Plot 2, 3, 4, 12 and 14 had larger area occupied by spots than control samples (Fig. 8).

In contrast, several samples from the zones with chemical contamination had spot area of smaller size than control. Thus, it can be suggested that the spot area per plant is not a good indication of potential soil mutagenecity.

We thus decided to relate the total area occupied by spots to the total leaf area. We named this index as the index of the mutagenic intensity (IMI). This index takes into consideration not only the area occupied by cells with mutations but actually the proportion of cells with mutations to all cells in leaves. The analysis showed that IMI was the lowest in plants grown on soil samples from the greenhouse in the city of Ivano-Frankivsk (control) – 0.05%.

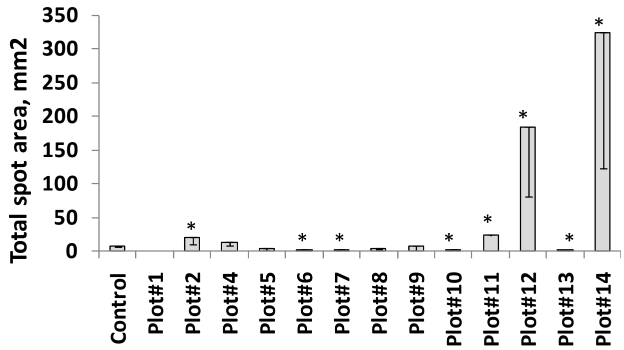

Figure 8. Total spot area in plants grown on different soils. Plants were germinated and grown on soils from the zone of chemical contamination (Plot 1-8), the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Each spot area was measured and total spot area (in mm^2, average of three experiments with SD) was shown on Y axis. X axis shows the place of sampling (Plot 1-14). Asterisks show significant from control differences (p<0.05).

The highest values of the index were observed in plants cultivated on soil samples from the village of Iltsi (9.17±5.25%) and in the town of Kolomyja (the water pumping station) –7.59±7.43%, which exceeds by 183.4 and 151.8 times the control values, respectively. The mutagenic intensity of soils from the town of Verkhovyna was 5.7±2.41% per plant, i.e., it was 114 times higher than the control value. The intermediate position as to this index was observed in soil samples from the village of Rusiv – 2.96% and the town of Kolomyja (the lake) – 2.11±0.82%, which exceeds by 59.2 and 42.2 times the control value, respectively. The IMI in plants grown on soil samples from Plots 5-8 was only slightly but not significantly different from control. Since Plots 5-8 represent soil samples from chemically polluted zone, it can be hypothesized that chemical mutagens induce mutations of the *Sulfur* gene at later stages of leaf development, and thus the plants do not have very large leaf area occupied by spots. In contrast, soil samples collected from radioactively contaminated areas (Plots 9-11) had the IMI substantially larger than control. This possibly indicates that radionuclides trigger mutations earlier during the development of plants. In general, IMI appeared to be the best indicator of possible environmental pollution; six out of 14 soil samples tested triggered higher mutagenecity in *Su/+* plants as compared to control plants (Fig. 9).

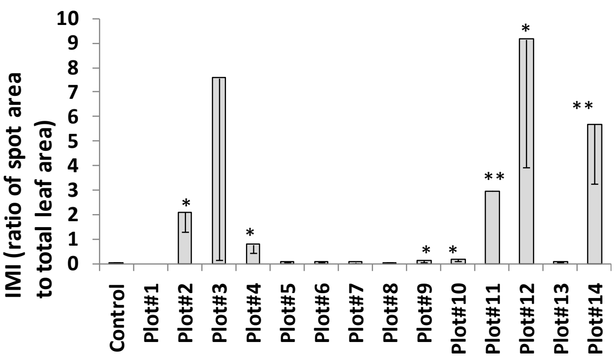

Figure 9. Average ratio of spot area to the total leaf surface in plants grown on different soils. Plants were germinated and grown on soils from the zone of chemical contamination (Plot 1-8), the zone of enhanced radiological control (Plot 9-11) and conditionally clean zone (Plot 12-14). Each spot area was measured and total spot area (in mm^2, average of three experiments with SD) was related to the total leaf surface (in mm^2). Y axis shows the index of mutation intensity (IMI) expressed in % (with SD). X axis shows the place of sampling (Plot 1-14). Asterisks show significant from control differences ($p<0.05$).

As we mentioned above there could be three different types of spots detected in *Su/+* plants, dark green +/+, light-yellow (nearly albino) *Su/Su* and twin spots, which is a combination of both. Dark green spots were predominant in numbers. The analysis of dark green spots showed that plants grown on control soils did not have spots larger than 1.5 mm^2. Plants grown on soil from Plot 3 had the greatest number of these small spots, over 500 spots per plant on average. Plants grown on other soils had much lower number of spots, typically not more than 10 per plant. We also observed sectors, spots that were larger than 3 mm^2 in size; sectors were only found on plants from Plot 4 and 14 with sizes of 35, 106 and even 180 mm^2. These sectors represent early mutation events.

Light-yellow albino *Su/Su* spots were observed in great number in plants cultivated on soil samples from Plot 3, 11 and 14. The largest number of these spots was recorded in plants form Plot 3; for example, a single leaf had about 100 *Su/Su* spots of 0.5-1.5 mm^2 in size. In contrast *Su/Su* sectors found in plants from Plot 14 differed in size from 3 to 311 mm^2.

The frequency of occurrence of *Su/Su* spots was substantially lower than that of +/+ spots. This could be simply due to the fact that dark green spots are easier to score on pale green background, as compared to albino spots. Light-yellow spots could frequently be mistaken for hypersensitive response i.e. necrotic lesion.

Twin mutations were less frequent in occurrence. In total, there were only 9 twin spots observed in plants from all experimental groups. Twin spots were observed in plants from Plot 2, 4, 8, 9, 12 and 14. Curiously, we found in the most cases the sizes of dark green and albino spots (as constituents of twin spots) to be more or less the same: 1 mm^2 x 1 mm^2, Plot 8; 2 mm^2 x 2 mm^2, 3 mm^2 x 3 mm^2, both Plot 4; 6 mm^2 x 5 mm^2, Plot 9. Some twin spots had different size of dark green and albino sectors, such as in the plant from Plot 12, 340 mm^2 x 68 mm^2 and Plot 4, 12.5 mm^2 x 4 mm^2. The similar size of sectors in twin spots suggests that the appearance of these twin spots is the result of a single, rather than dual mutation event. Indeed, previous analysis of twin sectors suggests that these are the result of recombination events altering two loci simultaneously (Friedlender et al., 1996).

Thus, the analysis of all parameters characterizing the specificity of mutations in the *Sulfur* gene showed the highest mutagenecity in plants grown on soil samples from the town of Kolomyja (the water pumping plant, Plot 3), the village of Rusiv (Plot 11), the village of Iltsi (Plot 12) and the town of Verhovyna (Plot 14). Whereas Plot 3 and Plot 11 belong to zone of chemical and enhanced radiological control, Plot 13 and 14 belonged to conditionally safe zone.

3.3. Correlation analysis between the physiological parameters and mutagenic parameters in plants grown on soil from different areas

A high level of interdependence ($r \geq 0.84$, $p<0.001$) was identified between the dynamics of stem and crown growth of plants cultivated on soil samples from all regions. Significant positive correlations were established between the number of spots and the spot area per plant in plants from grown on all soil samples ($r \geq 0.9$, $p \leq 0.01$). It is noteworthy that IMI correlated negatively with the total spot area of on the leaves ($r = -0.88$, $p<0.05$) in control plants, but correlated positively in plants grown on soil from all tested zones ($r \geq 0.89$, $p \leq 0.01$).

Positive correlation was found between the number of spots and IMI of leaves with spots in plants from all zones but not in control plants ($r \geq 0.85$, $p<0.05$). Also, positive correlation was found between the time of mutation occurrence and the number of spots on leaves in plants from all zones but not in control plants ($r \geq 0.72$, $p \leq 0.05$).

To find out whether there is a correlation between soil composition and physiological growth parameters, we analyzed soils for the presence of various nutrients (N, P, K, Ca etc.), heavy metals and radionuclides. The analysis showed that control soil had higher level of most nutrients as compared to all 14 Plots (Table 3). Control soil also had much lower concentration of heavy metals as compared to all 14 Plots (Table 4). Radionuclide analysis showed that only soils from Plots 9-11 had high levels of ^{137}Cs (Table 5). Positive correlation was found between nutrient composition and plant growth (r=0.52) and negative correlation was found between presence of heavy metals and plant growth (r= -0.68). Correlation analysis performed between the number of spots in control group and 14 different plots showed that there was a positive correlation between the concentration of heavy metals and spot number (r>0.75) and negative correlation between the nutrient composition and spot number (r= - 0.73). When control group was excluded from the analysis, no correlation between chemical composition and plant growth or spot number was observed. We did however found positive correlation between radionuclides concentration and IMI, further confirming a trend between the presence of radionuclides and the time of occurrence of mutagenic events.

4. DISCUSSION AND FUTURE PERSPECTIVES

Our research showed that *Nicotiana tabacum Su/+* plants are effective sensors of potential soil pollution. In past, these plants have been used for the analysis of the influence of radiation, chemicals and even pathogen infection on the occurrence of DNA damage (Friedlender et al., 1996; Kovalchuk et al., 2003).

Table 3. Agrochemical characteristics of the soil samples

SAMPLE LOCATION	SAMPLE LABEL	N, mg/kg	P, mg/kg	K, mg/kg	Ca, mg-eq/kg	Mg, mg-eq/kg	pH
Soil from greenhouse	Control	112.4	123.4	45.1	64.2	3.3	7.14
Zone of chemical contamination							
Near Kolomyja river	Plot 1	98.3	98.1	33.6	44.7	2.3	6.98
Near Kolomyja lake	Plot 2	84.9	92.5	31.2	49.2	1.9	7.32
Near Kolomyja water pumping station	Plot 3	101.3	90.9	38.4	49.9	2.8	7.15
Burshtyn power plant	Plot 4	64.2	54.7	21.9	28.5	1.5	7.23
Burshtyn town	Plot 5	62.1	52.1	33.8	48.2	1.2	7.19
Kalush	Plot 6	59.8	59.3	29.6	34.4	2.7	6.91
Zaviy	Plot 7	64.3	58.5	31.4	22.9	2.5	7.39
Grabivka	Plot 8	59.4	55.7	22.1	40.4	1.6	7.41
Zone of enhanced radiological control							
Stetseva	Plot 9	55.2	53.1	30.2	44.8	2.2	7.34
Potichok	Plot 10	49.4	55.7	32.0	43.9	2.9	7.22
Rusiv	Plot 11	58.4	68.4	38.3	51.5	2.1	7.18
Conditionally clean zone							
Iltsi	Plot 12	88.3	89.2	42.5	54.8	2.2	7.35
Krasnyk	Plot 13	105.3	75.8	32.2	44.9	2.6	7.42
Verhovyna	Plot 14	45.2	43.3	23.6	37.2	0.9	7.12

Table 4. Concentration of heavy metals

SAMPLE LOCATION	SAMPLE LABEL	Cu, mg/kg	Zn, mg/kg	Cr, mg/kg	Cd, mg/kg	Pb, mg/kg	Ni, mg/kg
Soil from greenhouse	Control	4.4	18.9	1.2	0.25	2.3	2.4
Zone of chemical contamination							
Near Kolomyja river	Plot 1	63.1	53.5	3.9	2.7	24.1	32.4
Near Kolomyja lake	Plot 2	44.3	42.6	3.2	2.9	19.4	29.5
Near Kolomyja water pumping station	Plot 3	62.3	53.1	4.4	3.9	22.5	47.4
Burshtyn power plant	Plot 4	100.2	108.7	7.9	4.4	31.5	87.3
Burshtyn town	Plot 5	82.2	72.9	5.4	3.9	21.9	69.3
Kalush	Plot 6	129.2	99.2	6.2	4.2	14.4	69.2
Zaviy	Plot 7	64.3	48.5	5.5	3.9	2.5	21.2
Grabivka	Plot 8	79.2	59.2	4.6	3.4	3.2	37.4
Zone of enhanced radiological control							
Stetseva	Plot 9	18.5	38.2	2.5	2.3	12.8	4.6
Potichok	Plot 10	16.3	34.8	2.8	3.1	11.4	7.22
Rusiv	Plot 11	28.9	35.2	3.3	2.6	15.2	7.18
Conditionally clean zone							
Iltsi	Plot 12	17.1	33.1	2.3	1.7	8.9	2.8
Krasnyk	Plot 13	12.9	27.6	3.1	2.1	9.9	2.9
Verhovyna	Plot 14	10.5	24.2	1.4	1.5	11.2	2.2

Table 5. Radioactive characteristics of the soil samples

SAMPLE LOCATION	SAMPLE LABEL	Contamination (Ci/km2)	Specific activity, Bq/kg, ^{137}Cs	Specific activity, Bq/kg, ^{90}Sr
Soil from greenhouse	Control	<0.1	18.3	<30
Zone of chemical contamination				
Near Kolomyja river	Plot 1	<0.1	21.3	<30
Near Kolomyja lake	Plot 2	<0.1	22.4	<30
Near Kolomyja water pumping station	Plot 3	<0.1	31.6	<30
Burshtyn power plant	Plot 4	0.3	18.8	<30
Burshtyn town	Plot 5	<0.1	21.4	<30
Kalush	Plot 6	0.2	19.8	<30
Zaviy	Plot 7	<0.1	24.2	<30
Grabivka	Plot 8	0.2	23.1	<30
Zone of enhanced radiological control				
Stetseva	Plot 9	2.4	412.1	<30
Potichok	Plot 10	1.4	127.2	<30
Rusiv	Plot 11	3.0	464.8	<30
Conditionally clean zone				
Iltsi	Plot 12	<0.1	21.5	<30
Krasnyk	Plot 13	<0.1	17.9	<30
Verhovyna	Plot 14	<0.1	23.5	<30

These plants were also recently successfully used to show genotoxicity of soils from various areas of Ivano-Frankivsk city (Jastrebova et al., 2011). In this work we analyzed the mutagenecity of soils sampled from areas with enhanced radiological control, samples from villages Stetseva, Potichok and Rusiv, Plots 9-11. In past, we analyzed the mutagenecity of these soils with the use of transgenic *Arabidopsis thaliana* assay and with *Alium cepa* chromosome aberration test. Both assays showed substantial genotoxicity of these soils (Kovalchuk et al., 1998; Kovalchuk et al., 1999).

Our analysis showed that soils from all three zones were mutagenic. All tested soils had higher concentration of various heavy metals as compared to control soil, thus it is possible that the reason for higher recombination frequency in plants grown on these soils is due to the presence of heavy metals.

Interestingly, soil samples from conditionally safe zone (Plot 14) showed second highest level of mutagenecity among 14 tested soil samples. Analysis of soil composition showed that soil from this region was lowest in nutrients, including low concentration of N, P and K (Table 3). So it is possible that high recombination frequency in this group is due to combination of poor nutrients and heavy metal presence. Previously published research also showed that concentrations of Cu, Cd and Zn in soils from mountainous area of Verhovyna regions was substantially lower than those from plain regions of Galych and Snyatyn (Basalytska & Nechytajlo, 2010).

As a control we used the soil from the commercial greenhouse. Plants grown on this soil grew much larger; they had longer stem and larger crown diameter (Fig. 4 and 5). Greenhouse soil has a balanced level of nutrients and thus it is not surprising that these plants performed much better than plants grown on soils sampled in different areas of Ivano-Frankivsk region. Indeed, positive correlation between the level of N, P, K or Ca and the number or size of the recombination events was found for control and experimental plants. Similarly, there was negative correlation between the level of heavy metals and spot number and spot size. As we mentioned above, when the data were analyzed only for the samples from Plot 1-14, no such correlation was found. It would be interesting however to perform a reconstruction experiment to test whether there is dose dependence between the concentration of nutrients (nitrates, phosphates etc.) and the frequency of occurrence of dark green (albino or twin) spots. Also, it remains to be shown whether there is a dose-dependent response in the frequency of occurrence of these spots in response to a chemical mutagene.

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REFERENCES

Adamenko, O.M., 2000. *Regional ecology and natural resources (example of Ivano-Frankivsk region).* Eds. Adamenko, O. and Pryhod'ko, M., p. 278. Ivano-Frankivsk, Ukraine.

Adamenko, O.M., 2005. *Agrochemical and agroecological characteristics of soils of Ivano-Frankivsk region.* Ivano-Frankivsk, Ukraine.

Basalytska, I.S. & Nechytajlo, L.Y., 2010. Analysis of Zn and Cu concentrations in soils of Precarpathian region. Proceedings of 79th scientific applied conference for students and young investigators. Ivano-Frankivsk, Ukraine, 197-198.

Boyko, A., Filkowski, J., Hudson, D. & Kovalchuk, I., 2006. *Homologous recombination in plants is organ specific.* Mutat Res., 595, 145-155.

Burk, L.G., Menser, H.A., 1964 *A dominant aurea mutation in tobacco.* Tob. Sci., 8, 101-104.

Filkowski, J., Besplug, J., Burke, P., Kovalchuk, I. & Kovalchuk, O., 2003. *Genotoxicity of 2,4-D and dicamba revealed by transgenic Arabidopsis thaliana plants harbouring recombination and point mutation markers.* Mutat. Res., 542(1-2), 23-32.

Friedlender, M., Lev-Yadun, S., Baburek, I., Angelis, K., Levy, A.A., 1996. *Cell divisions in cotyledons after germination: localization, time course and utilization for a mutagenesis assay.* Planta, 199, 307-313.

Ilnytskyy, Y., Boyko, A. & Kovalchuk, I., 2004. *Luciferase-based transgenic recombination assay is more sensitive than beta-glucoronidase-based.* Mutat. Res., 559, 189-197.

Jakymiv, M.M, Seredjuk, B.M., Bulavinetz, V.M., Agapova, O.G., 2011. *Radiecological monitoring of agricultural soils*. Visnyk Precarpathian University, Biology series, 15, 25-28.

Jastrebova, O., Kovalchuk, I. & Kovalchuk, L., 2011. *Analysis of soil genotoxicity in the city of Ivano-Frankivsk using Nicotiana tabacum Su/+ plants.* Carpath J Earth Environ Science, 6(2).

Kawata, E.E. & Cheung, A.Y., 1990. *Molecular analysis of an aurea photosynthetic mutant (Su/Su) in tobacco: LHCP depletion leads to pleiotropic mutant phenotypes.* EMBO J., 9, 4197-4203.

Kovalchuk, O., Kovalchuk, I., Arkhipov, A., Telyuk, P., Hohn, B. & Kovalchuk, L., 1998. *The Allium cepa chromosome aberration test reliably measures genotoxicity of soils of inhabited areas in the Ukraine contaminated by the Chernobyl accident.* Mutat. Res., 415(1-2), 47-57.

Kovalchuk, O., Kovalchuk, I., Titov, V., Arkhipov, A. & Hohn, B., 1999. *Radiation hazard caused by the Chernobyl accident in inhabited areas of Ukraine can be monitored by transgenic plants.* Mutat. Res., 446(1), 49-55.

Kovalchuk, O., Arkhipov, A., Barylyak, I., Karachov, I., Titov, V., Hohn, B. & Kovalchuk, I., 2000. *Plants experiencing chronic internal exposure to ionizing radiation exhibit higher frequency of homologous recombination than acutely irradiated plants.* Mutat. Res., 449(1-2), 47-56.

Kovalchuk, I., Kovalchuk, O., Kalck, V., Boyko, V., Filkowski, J., Heinlein, M. & Hohn, B., 2003. *Pathogen-induced systemic plant signal triggers DNA rearrangements.* Nature, 423, 760-762.

World References Base for Soil Resources, 1998. 84 *World Soil Resources Reports*. Food and Agriculture Organization of the United Nations. M-41 ISBN 92-5-104141-5.

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