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Grasses Tolerant to Radionuclides Growing in Kazakhstan Nuclear Test Sites Exhibit Structural and Ultrastructural Changes – Implications for Phytoremediation and Involved Risks

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ABSTRACT

The vegetation of the study area in Kazakhstan represents steppes predominated by xerophytic grasses such as *Stipa capillata*, *Festuca valesiaca* and *Agropyron cristatum*. This region is contaminated by radionuclides, products of 357 underground nuclear explosions and tests carried out during 1949 to 1989. This area is being used as grazing land and partly for mining (coal and gold). Focussed radioecological and radiophytoremediation investigations on the migration of radionuclides from underground water into soils, translocation to plant aerial parts, the transfer factor and concomitant effects on the food chain viz., underground water → soils → plants → animals are rather scanty. This paper details the anatomical structural changes in the stems and leaves of the abundant grasses viz. *S. capillata*, *F. valesiaca* and *A. cristatum* due to radionuclides and the implications for radiophytoremediation and involved risks.

Keywords: anatomical and morphological changes, phytostabilization, radionuclides, radiophytoremediation, grasses, risks, tolerance

INTRODUCTION

The Republic of Kazakhstan (North-eastern part) consists of 470 nuclear test sites comprising of an area of 430 km^2 conducted during 1949-1989 (**Fig. 1**) of which 26 were surface, 87 air and 357 underground. The surroundings are polluted with radionuclides. The main contribution to radioactive pollution was made by aboveground nuclear tests conducted until 1963, after which radioactive pollution was caused by radionuclide-splinters of nuclear fuel fission, unreacted nuclear fuel and radionuclide-products of explosive devices activated by neutrons. Nuclear decay of the underground nuclear tests and the subsequent decay products eventually accumulated in these test sites (**Table 1**). Radionuclides, including dangerous ones such as $^{239, 240}$Pu, ^{90}Sr, and ^{137}Cs (^{137}Cs-has a half-life of 30 years; ^{90}Sr has a half-life of 28 years) leached to surface and underground waters (Freiling 1962; Izrael 1970; Killham 1995; Entry *et al*. 1996). ^{137}Cs acts similarly to potassium and ^{90}S behaves similarly to calcium (Killham 1995). Vertical and horizontal migration of these decay products of radionuclides enter plants and reach humans through the food chain and is thus a health hazard (Westnoff 1999; Aidossova 2003). Unauthorized activities of local people to recover cables and radioactive scrap (Akhmetov *et al*. 2000) further aggravated the situation. Presently this contaminated territory is grazing land, and is partly mined for coal and gold.

Fig. 1 Nuclear test in the Republic of Kazakhstan (1949).

Radioecological investigations of the territory, migration of radionuclides from underground water into soil, roots of plants, uptake and translocation to aerial parts, the transfer factor and concomitant effects on the food chain (underground water → soil → plants → animals) have not been investigated. Strategies for rehabilitation of ecosystems destroyed by nuclear tests have not been planned although radiophytoremediation is gaining considerable progress in other parts of the world (Vandenhove 2006).

It is known that low doses of ionizing radiation increases cell size and the tissue growth of plants. However, high doses inhibit cellular processes in different plant organs (Stoklasa 1932; Alexander 1950; Drobkov 1951; Sparrow 1955). But radiosensitivity can differ and depends on a plant's genetic background and the sensitivity of tissues and organs. For example *Lilium longiflorum* and *Tradeschantia paludosa* are highly radiosensitive plants while *Digitaria*, *Brassica oleracea* and *Gladiolus* are radioresistant plants (Sparrow 1955). Different cultivars of *Secale seriale* (rye) such as 'Iotun' and 'Maya' exhibit diverse levels of radiosensitivity (Kuzin 1957). Moreover, different organs of the same plant are reported to respond to radiation in different ways. Butenko's study (1954) showed that root tips and burgeons i.e., growing parts, are more sensitive than other parts of the plant. Cells of the division zone are more sen-

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Table 1 Radionuclide content in radical soil of Degelen nuclear test site (becquerel/kilogram).

plot	Soil horizon	Natural radionuclides			Technogenic radionuclides		
	cm	^{40}K	^{232}Th	^{266}Ra	^{137}Cs	^{90}Sr	$^{239, 240}$Pu
1	0-5	520	30	25	191	24633	8.52
	5-25	665	48	34	1969	2.96	8.5
1 control	0-3	150	30	14	120	-	-
	3-9	837	46	33	0.9	-	-
	9-15	916	46	40	0.9	-	-
	15-50	999	52	52	4	-	-
	50-70	1223	49	49	0.9	-	-
2	0-5	690	56	100	378	72594	-
	5-28	610	96	78	39	-	-
	28-45	1190	45	39	21	-	-
2 control	0-3	740	30	18	15	-	-
	3-12	724	27	28	9	-	-
	12-26	640	28	28	4	-	-
	26-35	1020	18	22	9	-	-
	35-75	360	9	4	5	-	-

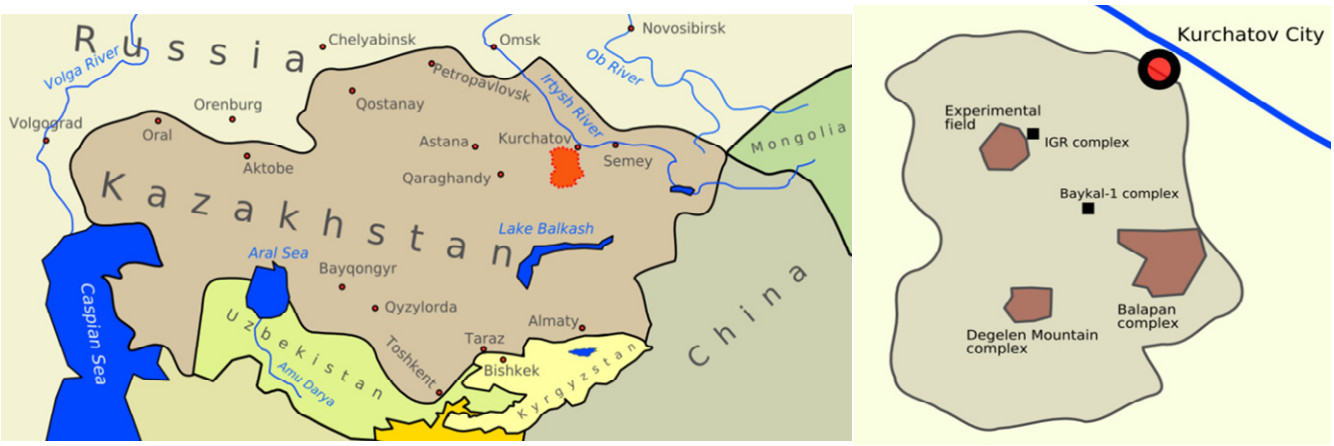

Fig. 2 Four test sites (right) located in the north-east of the Republic of Kazakhstan (left).

sitive than those of the distention and differentiation zones. Consequently meristematic tissues are the most radiosensitive organs (Butenko 1954).

Investigations of the Chernobyl nuclear disaster revealed many morphological anomalies in winter wheat (*Triticum vulgare* L.) such as sterile corn in ears, additional ears and short-cut ears (Grodzinskii 1991). In conifers the leaf blade increased three-fold and the color of leaves changed. For instance, the color of Scotch pine (*Pinus sylvestris*) needles became flax, while the needles of spruce fir (*Picea excelsa*) became crimson (Kozubov 1991). In oak trees (*Quercus robur* L.) leaves changed form by forming palm-shaped suckers (Uvarova 1991). Thus, the changes in anatomical and morphological structure of plants in radionuclide-contaminated and/or polluted areas would serve as a convenient approach for environmental monitoring and assessment of radionuclides (Aidossova 2003).

"Radiophytoremediation" is emerging as a method for the clean-up of radionuclide-contaminated environments using radionuclide-tolerant and -accumulator plant species. Radionuclides are removed from the contaminated substrate by harvesting the phytomass, after which it is processed to either recover the useful material or further concentrate it via chemical and biological treatments which would facilitate safe disposal (Vandenhove 2006).

MATERIALS AND METHODS

The study area was located in the mountain-steppe ecological zone in the Semipalatinsk region, Republic of Kazakhstan. This territory includes dry steppes on chestnut soils and deserted sagebrush-feather-grass steppes on light chestnut soils. Humus content is up to 1.5% in light chestnut and 3.0-4.5% in livery-chestnut soils (**Table 2**). Swampy meadows form on the river valleys of Semipalatinsk nuclear test site. The vegetation of the region represents steppes predominated with xerophytic grasses viz. *Stipa capillata*, *Festuca valesiaca*, and *Agropyron cristatum* (Tuleubaev *et al*. 2000). The mean average temperature in January is 16-20°C, and in July 20.3-23.84°C. Annual precipitation is 250 mm. Plants were collected from the Degelen test site of the Semipalatinsk nuclear test site (49°45′04″N, 78°00′28.2″E and 49°45′13.7″N, 78°02′56″E in case of contaminated plots; 49°44′29.3″N, 78°00′96.1″E and 49°40′08.8″ N, 78°06′47.8″ E of control plots). During the period 1949 to 1989, about 307 nuclear weapon tests were conducted by the former Soviet Union government on the Degelen test site which is the only mountain range in Semipalatinsk nuclear testing area. The trinitrotoluene equivalent rate of each explosion was more than 50 kilotons. As a consequence of these explosions the structure and contents of rocks were changed and streams with radioactive pollutants have spewed to the crust of the earth. Hence, at the Degelen test site 4 plots were chosen, two contaminated and two relatively clean (control) (according to Standards of Radiation Safety of Republic of Kazakhstan the maximum permissible dose of equivalent dose of γ radiation is 0.2 microsievert per hour, or μSv/h).

Table 2 Soil characteristics of Semipalatinsk nuclear test site.

Soil	Humus %	pH	Gradation characteristics of soil
Light chestnut	1.2-1.5	7.0-7.50	Sandy, loamy
Livery-chestnut	3.0-4.5	7.0-7.65	Sandy, loamy

1. Contaminated plot 1 – spring of Tokhtakushuk stream (49° 45′04″N, 78°00′28.2″E). α particle's dose rate <0.2 particles/min ×cm^2, β particle's dose rate is 1000 particles/min×cm^2 Equivalent power dose of γ-radiation for 3 cm above ground (h_0) – 0.7 μSv/h, for 1 m above ground (h_1) – 4.8 μSv/h (**Table 3**).

2. Control plot 1 for the contaminated plot 1 – 1 km from Tokhtakushuk stream (49°44′29.3″N, 78°00′96.1″E). α particle's dose rate <0.2 particles/min×cm^2, β particle's dose rate is 10 particles/min×cm$^{2.}$ Equivalent dose rate of γ-radiation for 3 cm above ground (h_0) – 0.16 μSv/h, for 1 m above ground (h_1) – 0.14 μSv/h.

Table 3 Radioactive dose rates of Degelen's plots.

Plot	α particle's dose rate particle/min×cm^2	β particle's dose rate particle/min×cm^2	Equivalent dose rate of γ radiation μSv/h	
			h_0=3cm	h_1=1m
1 contaminated	<0.2	1000	0.7	4.8
2 control	<0.2	10	0.16	0.14
3 contaminated	<0.2	2000	369	190
4 control	<0.2	70	0.33	0.22

3. Contaminated plot 2 – spring of Baiteles stream (49°45′ 13.7″N, 78°02′56″ E). α particle's dose rate <0.2 particles/min ×cm^2, β particle's dose rate is 2000 particles/min×cm^2. Equivalent dose rate of γ-radiation for 3 cm above ground (h_0) – 369 μSv/h, for 1 m above ground (h_1) – 190 μSv/h.

4. Control plot 2 for the contaminated plot 2 – the end of Tokhtakushuk stream (49°40′08.8″ N, 78°06′47.8″ E). α particle's dose rate <0.2 particles/min×cm^2, β particle's dose rate is 70 particles/min×cm^2. Equivalent dose rate of γ-radiation for 3 cm above ground (h_0) – 0.33 μSv/h, for 1 m above ground (h_1) – 0.2 μSv/h.

Equivalent dose rate of γ-radiation was determined by using RKSB-104 (2001) equipment by research workers of the National Nuclear Centre of the Republic of Kazakhstan. Three species of grasses (Poaceae family), *Agropyron cristatum* (L.) Gaerth, *Festuca valesiaca* Gaudin., and *Stipa capillata* L. were chosen as these are the most abundant grasses in the selected test sites. Plant species were identified by the "Flora of Kazakhstan" vols. I-IX (1961-1966) and IV (1956-1966). Collected plant material (stems, leaves, roots) were cut to small pieces (15-20 mm) in the middle part of each organ. Materials were fixed in 70° ethanol for anatomical studies following Permiakov (1988) and Barykina (2004). Anatomical transverse sections of stems, leaves and roots were carried out by using freezing microtome TOS-2. Sampling was 15 sections of each organ of one plant species per site. Examination of stems, leaves and roots' transverse sections were made with MBI-6 microscope fixed with a Zorki camera. Anatomical measurements of anatomical parameters were carried out with help of PhotoM 1.31 software (Lotova 1989). The results were subjected to statistics by using descriptive statistics function MS Excel. Fifteen samples were measured for each anatomical parameter of plant tissue.

RESULTS

Anatomical structural changes in the stem

Agropyron cristatum (L.) Gaertner

The epidermis, primary cortex and hollow internodes were conspicuously determined in the transverse section of *A. cristatum*'s stem. The epidermis consists of allied, clypeate cells. Ring-shaped sclerenchyma is situated behind the epidermis. There are slices of chlorenchyma in the pits of the sclerenchyma. They fail to function and are destroyed in time. All the vascular bundles have sclerenchyma (**Fig. 3A, 3B**). Combined sclerenchyma girders are present (with all the primaries, the rest with adaxial girders or strands only). Closed collateral bundles are situated in tessellated order and form two rings. Hollow internodes are without the medullar parenchyma. All anatomical parameters of the stem decreased in the contaminated plots. For instance, the thickness of the sclerenchyma in polluted plots were 64.74 μm (1) and 87.17μm (3); in control plots 119.37μm (2) and 113.83μm (4), respectively. Likewise, the area of conducting bundles in polluted plots was 42.24 μm^2 (1) and 27.22 μm m^2 (3); in control plots 77.91 μm^2 (2) and 69.64 μm^2 (4), respectively (**Table 4**).

Festuca valesiaca Gaudin

There is typical structure of monocotyledonous plants in the transverse section of stem. Stem with hollow internodes, cylindrical in section. Epidermis, primary cortex and hollow internodes are clearly determined. Epidermis consists of allied, clypeate cells. Primary cortex is strongly reduced. Sclerenchyma's pericycle has changed into a row of subepidermal cells. All the vascular bundles are accompanied by sclerenchyma and form two rings of bundles. Main conducting bundles are bordered with cells of subepidermal sclerenchyma and minor bundles are sided with a row of chlorenchyma cells (**Fig. 4A, 4B**).

Hollow internode is without medullar parenchyma. Secretary cavities, cork cambium and secondary thickening are absent. Xylem has vessels. If we compare the polluted and control plots the following picture becomes evident. The thickness of the epidermis decreases (in the polluted plots (1, 3): it was 8.97 μm and 7.76 μm, respectively; in the control (2, 4) 9.44 μm and 9.88 μm, respectively). At the same time the thickness of sclerenchyma (in plots 1 and 3 – 49.81 μm and 48.86 μm, respectively; in plots 2 and 4 – 39.72 μm and 40.15 μm, respectively) and the area of conducting bundles (in plots 1 and 3 – 30.51 μm^2 and 30.11 μm^2, respectively; in plots 2 and 4 – 25.33 μm^2 and 24.27 μm^2, respectively) increased. No changes occurred in the areas of xylem vessels except in the area of xylem vessels on the contaminated plot 2 with very high radiation dose (**Table 4**).

Stipa capillata L.

The anatomical structure is the same as that of monocotyledonous plants. A ring-shaped sclerenchyma is situated behind the epidermis. It provides the stem with flexibility and strength. Slices of chlorenchyma are located in the pits of the sclerenchyma. Closed collateral bundles are situated in

Table 4 The morphological measurements of the leaves.

Plant species	Plot	Height of plant (cm)	Length of leaves (cm)	Length of internode (cm)
Agropyron cristatum	1 contaminated	33.56 ± 1.47*	18.13 ± 1.03*	11.37 ± 0.67*
	2 control	30.76 ± 1.27	14.86 ± 1.19	9.29 ± 0.63
	3 contaminated	35. 32 ± 2.02*	20.05 ± 1.74*	12.73 ± 0.54*
	4 control	31.13 ± 1.49	14.82 ± 1.46	8.34 ± 0.87
Festuca valesiaca	1 contaminated	23.96 ± 0.79*	16.27 ± 1.02*	17.70 ± 0.56*
	2 control	19.17 ± 0.89	10.99 ± 0.77	12.13 ± 0.88
	3 contaminated	24.87 ± 0.71*	16.18 ± 0.88*	19.28 ± 0.43*
	4 control	18.74 ± 0.79	14.39 ± 1.30	11.73 ± 0.56
Stipa capillata	1 contaminated	70.03 ± 2.03*	25.91 ± 1.42*	11.74 ± 0.77*
	2 control	43.44 ± 1.29	22.86 ± 2.17	9.52 ± 0.37
	3 contaminated	69.57 ± 4.48*	25.72 ± 0.91*	11.75 ± 0.76*
	4 control	41.38 ± 3.69	23.04 ± 1.11	9.57 ± 0.34

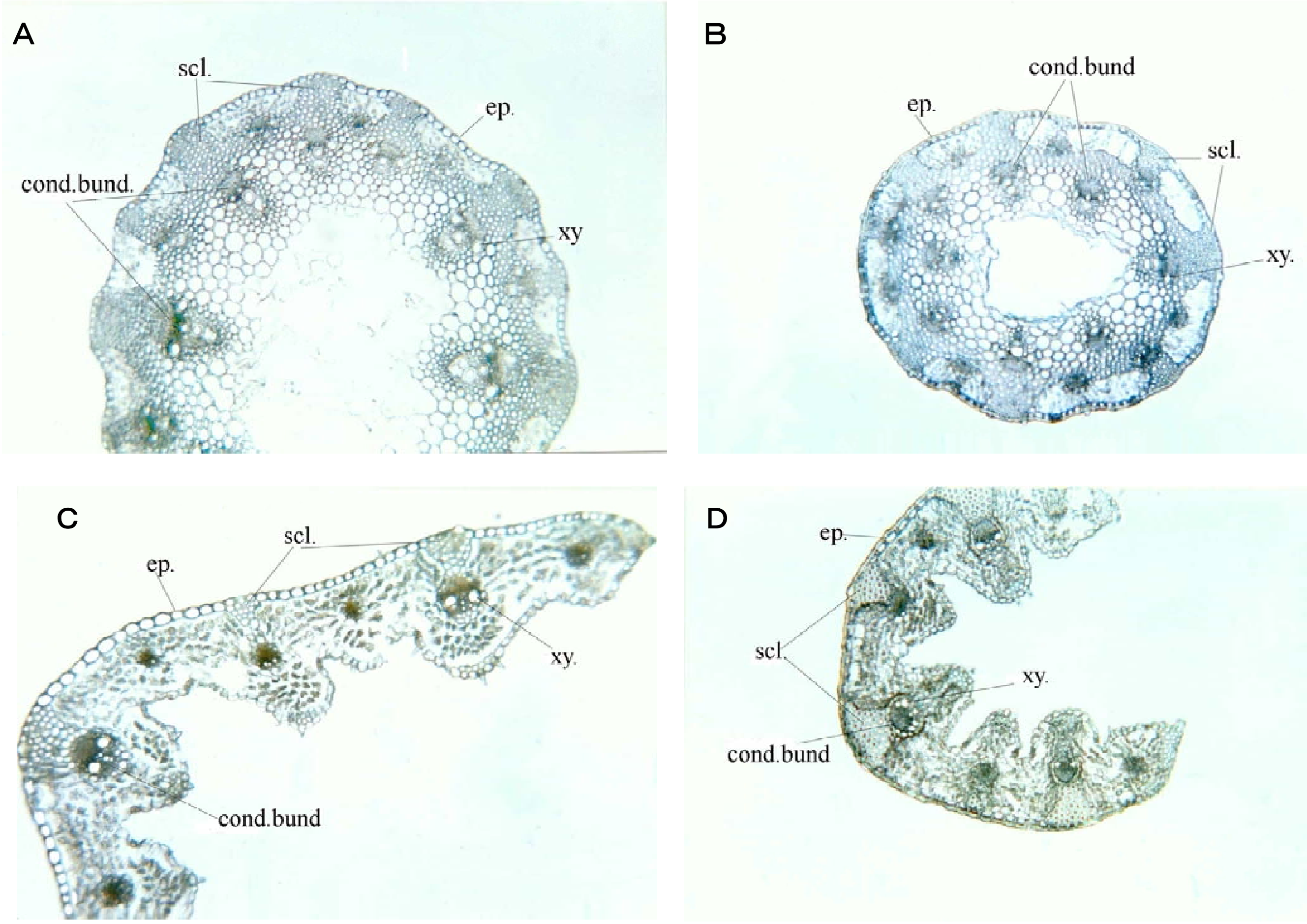

Fig. 3 Anatomical variations in stem (**A**, control; **B**, polluted) and leaf (**C**, control; **D**, polluted) of *Agropyron cristatum*. ep. – epidermis; scl. – sclerenchyma; cond. bund. – conducting bundle; xy. – xylem).

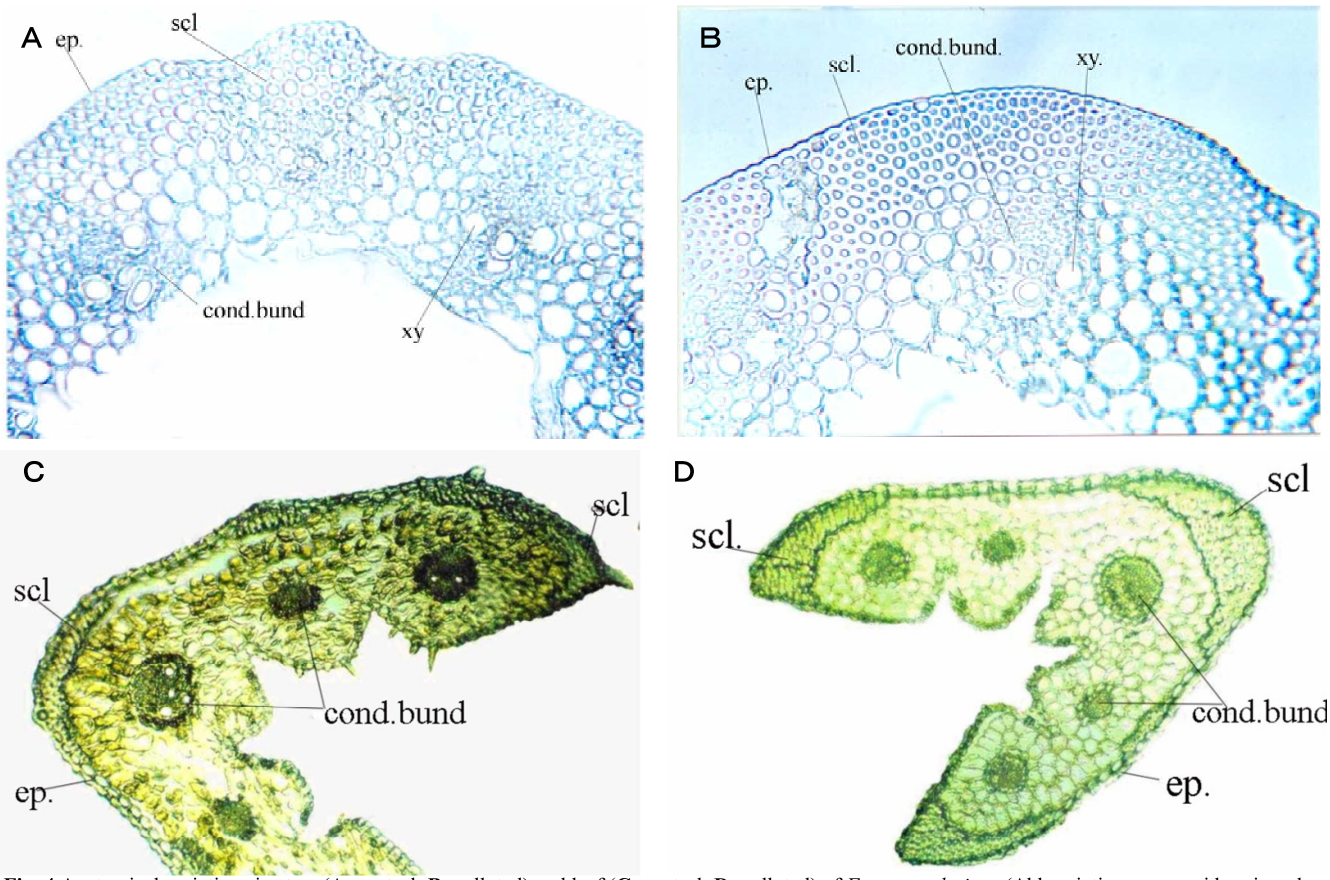

Fig. 4 Anatomical variations in stem (**A**, control; **B**, polluted) and leaf (**C**, control; **D**, polluted) of *Festuca valesiaca* (Abbreviation: ep. – epidermis; scl. – sclerenchyma; cond.bund. – conducting bundle; xy. – xylem).

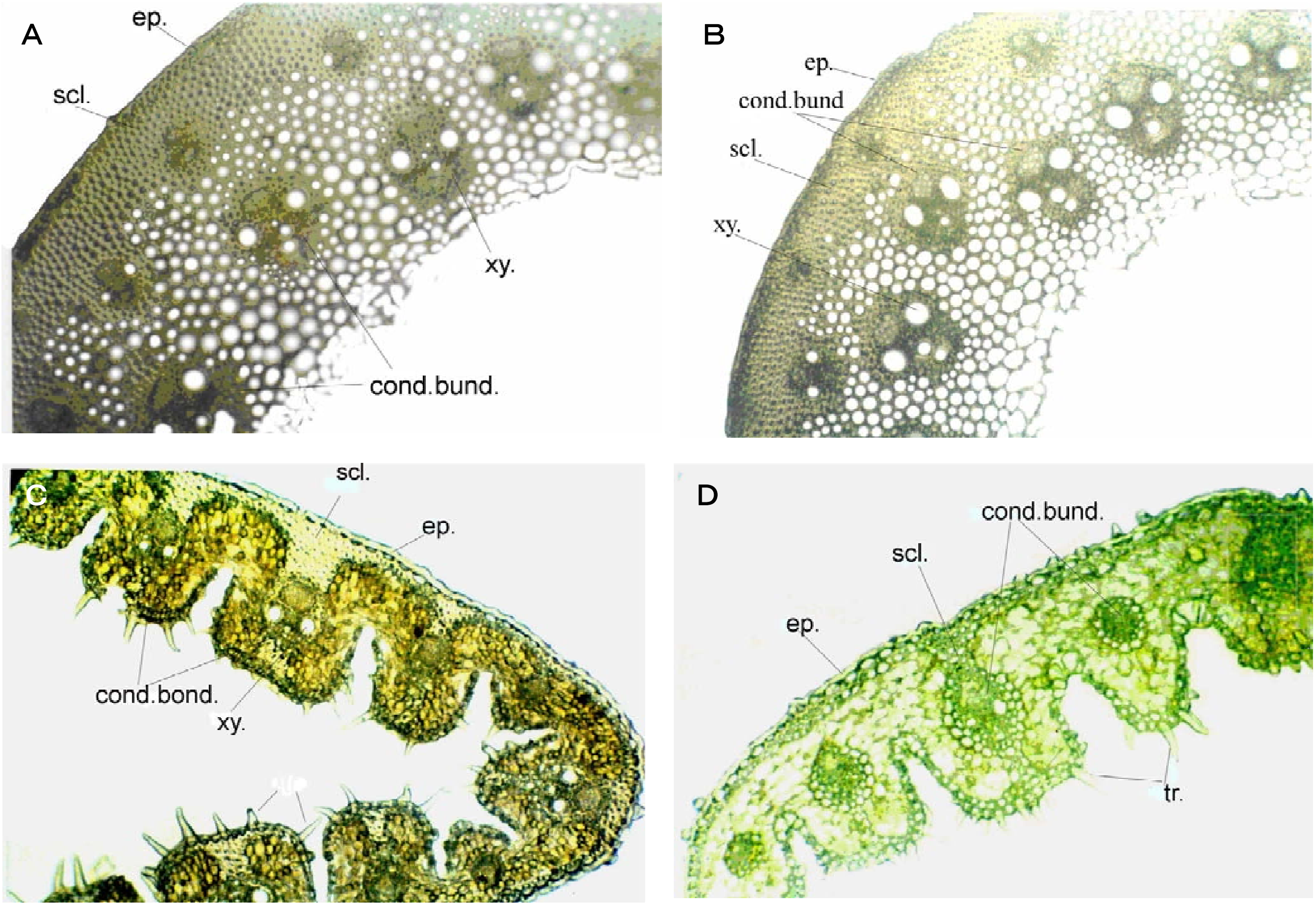

Fig. 6 Anatomical variations in stem (**A**, control; **B**, polluted) and leaf (**C**, control; **D**, polluted) of *Stipa capillata* (abbreviation: ep. – epidermis; scl. – sclerenchyma; cond.bund. – conducting bundle; xy. – xylem).

tessellated order and form two rings. The external ring's conducting bundles are bordered with cells of sclerenchyma and internal bundles are located in parenchyma cells. All the vascular bundles have sclerenchyma. The hollow internode has no medullar parenchyma (**Fig. 5A, 5B**).

Comparatively with control plots the thickness of epidermis in polluted plots thickened (in the polluted plots (1, 3) it was 10.09 µm and 11.5 µm, respectively; in the controls (2, 4) 7.2 µm and 8.88 µm, respectively). The thickness of the sclerenchyma ring (in plots 1 and 3 – 48.04 µm and 50.93 µm, respectively; in plots 2 and 4 – 56.82 µm and 62.24 µm, respectively) and area of conducting bundles (in plots 1 and 3 – 36.49 μm^2 and 34.36 μm^2, respectively; in plots 2 and 4 – 46.8 μm^2 and 49.62 μm^2, respectively) decreased. The area of xylem vessels changed only in the plot of very high radiation dose (**Table 4**).

The anatomical structural changes in leaf

Agropyron cristatum (L.) Gaertner

The epidermis conspicuously differentiated into 'long' and 'short' cells, vesiculous cells and stomata; the leaf blade had distinct, prominent adaxial ribs (**Fig. 3C, 3D**). Stomata were situated under the adaxial ribs and were distinctly grouped into long rows; their stomatic clefts are faintly visible. Vesiculous cells are in the pits of adaxial ribs of the leaf blade.

Closed collateral conducting bundles consist of 1-2 protoxylem and 2 big metaxylem, sieve vessels of phloem and companion cells. There are big and small conducting bundles which are situated in turn and accompanied by sclerenchyma. Sclerenchyma is presented as subepidermal cells.

All anatomical parameters of the leaves decreased comparatively in the contaminated plots. For example, on the plots which were exposed to ionizing radiation the thickness of leaves were 143.64 µm (1) and 142.6 µm (3); in the control plots 172.89 µm (2) and 165.63 µm (4) (**Table 5**).

Festuca valesiaca Gaudin

The leaf blade is rolled-up in a tubule in which prominent adaxial ribs are found. The epidermis conspicuously differentiated into 'long' and 'short' cells. Stomata are confined to the adaxial surface, paracytic, with faintly visible or invisible stomatic clefts. Vesiculous cells are in the pits of adaxial ribs of the leaf blade (**Fig. 4C, 4D**).

The mesophyll consists of identical, globular or multiangular cells, which are situated disorderly. Collateral conducting bundles have 1-2 protoxylems, 3 large metaxylem vessels, sieve vessels of phloem and companion cells. Main and minor bundles are situated alternately. Sclerenchyma is found in the ribs of the leaf blade. Leaf thickness increased on the plots with a high radioactive dose, whereas the area of conducting bundles and xylem vessels decreased (**Table 5**)

Stipa capillata L.

The leaf blade is rolled-up twice in the tubule in which prominent adaxial ribs are found. The leaf blade consists of epidermis, mesophyll and conducting bundles. The epidermis is conspicuously differentiated into 'long' and 'short' cells, vesiculous cells and stomata; Paracytic stomata are confined to the adaxial surface and situated under adaxial ribs. Trichoblasts are presented as long fuzzes (hairs). The mesophyll consists of identical, globular or multiangular cells, which are situated disorderly (**Fig. 5C, 5D**).

Collateral conducting bundles are as the same as in the stems. They consist of 1-2 protoxylem and 2 big metaxylem, sieve vessels of phloem and companion cells. There are big and small conducting bundles which are situated alternately and accompanied by sclerenchyma. The water-bearing ca-

Table 5 Anatomical parameters of stem.

Plant species	Plot	Thickness of epidermis (µm)	Thickness of sclerenchyma (µm)	Area of conducting bundles ($\times10^{-3}$ mm^2)	Area of xylem vessels ($\times10^{-3}$ mm^2)
Agropyron cristatum	1 Contaminated	15.38 ± 1.12*	64.74 ± 5.93*	42.24 ± 1.68*	1.39 ± 0.13*
	2 Control	19.31 ± 0.63	119.37 ± 2.67	77.91 ± 5.22	2.78 ± 0.25
	3 Contaminated	15.89 ± 0.86*	87.17 ± 2.59*	27.22 ± 1.57*	1.03 ± 0.09*
	4 Control	19.57 ± 0.82	113.83 ± 5.81	69.64 ± 4.73	2.60 ± 0.23
Festuca valesiaca	1 Contaminated	8.97 ± 0.42	49.81 ± 1.58*	30.51 ± 2.34*	0.81 ± 0.04
	2 Control	9.44 ± 0.86	39.72 ± 2.37	25.33 ± 3.82	0.74 ± 0.11
	3 Contaminated	7.76 ± 0.42	48.86 ± 2.58*	30.11 ± 3.79*	1.87 ± 0.38*
	4 Control	9.88 ± 0.65	40.15 ± 2.58	24.27 ± 1.58	0.58 ± 0.08
Stipa capillata	1 Contaminated	10.09 ± 0.39*	48.04 ± 1.5*	36.49 ± 2.06*	1.25 ± 0.12
	2 Control	7.20 ± 0.42	62.82 ± 3.67	46.80 ± 3.85	1.15 ± 0.06
	3 Contaminated	11.50 ± 1.05*	50.93 ± 3.58*	34.36 ± 4.07*	2.82 ± 0.18*
	4 Control	8.89 ± 0.51	62.24 ± 7.21	49.62 ± 6.18	1.83 ± 0.15

Note: Values after "±" are standard deviation (n=15), " * " - indicates significant difference from the control (P<0.05).

Table 6 Anatomical parameters of leaf.

Plant species	Plot	Thickness of leaf (µm)	Area of conducting bundles ($\times10^{-3}$ mm^2)	Area of xylem vessels ($\times10^{-3}$ mm^2)
Agropyron cristatum	1 Contaminated	143.64 ± 3.65*	32.88 ± 3.93*	1.18 ± 0.08*
	2 Control	172.89 ± 1.08	44.68 ± 2.59	2.25 ± 0.23
	3 Contaminated	142.61 ± 5.04*	30.39 ± 1.93*	1.47 ± 0.12*
	4 Control	165.63 ± 4.74	41.35 ± 2.59	2.29 ± 0.11
Festuca valesiaca	1 Contaminated	155.14 ± 4.9*	15.84 ± 4.93*	0.52 ± 0.1*
	2 Control	123.19 ± 4.1	17.61 ± 2.44	0.71 ± 0.05
	3 Contaminated	159.07 ± 4.12*	15.54 ± 2.07*	0.59 ± 0.07
	4 Control	111.73 ± 3.53	18.24 ± 2.77	0.75 ± 0.06
Stipa capillata	1 Contaminated	97.57 ± 0.95*	31.51 ± 3.08*	1.45 ± 0.12
	2 Control	86.92 ± 3.12	22.09 ± 1.91	1.18 ± 0.14
	3 Contaminated	105.80 ± 3.91*	29.41 ± 2.48*	0.76 ± 0.07*
	4 Control	86.17 ± 2.7	21.41 ± 1.89	1.71 ± 0.14

Note: Values after "±" are standard deviation (n=15), " * " - indicates significant difference from the control (P<0.05).

nals which come from destroying protoxylem vessels are large in the main bundles. The sclerenchyma is as confluent layer.

Anatomical parameters such as areas of conducting bundles of leaves increased in the contaminated plots. For example, on the plots which were exposed to ionizing radiation the areas of conducting bundles (in plots 1 and 3 – 31.51 µm^2 and 29.41 µm^2, respectively; in plots 2 and 4 – 22.09 µm^2 and 21.41 µm^2, respectively). An effect of radiation was an increase in the thickness of leaves: 97.57 µm (1) and 105.8 µm (3); in the control plots 86.92 µm (2) and 86.17 µm (4). The area of xylem vessels decreased only on the plot of very high radiation dose (**Table 5**).

DISCUSSION AND CONCLUSIONS

Ionizing radiation influences plant growth (Grodzinskii 1991; Lee 1951; Nishita 1958). Growth inhibition is caused by disturbing cell fission in radiosensitive apical meristems that in turn limits the number of elongating cells (Grodzinskii 1991). Changes in morphological processes due to radiation depend on ionization doses and are characterized by speeding-up of growth processes (radiostimulation) under low radiation and inhibited formation under high doses.

Plants exposed to ionizing radiation suffer anomalies and deformities by losing their capacity to divide. These changes alter normal developmental processes. Radioecological damage of a forest ecosystem in the vicinity of the Chernobyl disaster site exhibited several morphological changes. In the areas with mean level of radioactive pollution (300-1000 rad) morphological anomalies such as an increase in bud number, bud scale coalescence, inhibition of seedling growth, enlargement of needles, and buds with an underdeveloped apex were detected (Grodzinskii 1991; Uvarova 1991). Under the exposure of chronic and acute ionizing radiation the changes in internal structure of plants in well reported (Drobkov 1951; Dushenkov 2003). For instance in the stems of conifers exposed to radiation, the size of aerial parenchyma, xylem, phloem, and wood core cells decreased leading to retardation of tissue and plant development (Gudkov 1993).

Investigations of *Betula pendula* Roth. leaves' morphological and anatomical structures showed alterations in leaf blade size and in the number of palisade mesophyll layers (Uvarova 1990). The influence of gamma-radiation on leaf anatomical structure of *Quercus robur* L. invoked alterations in the number of mesophyll layers and enhanced xeromorphic features like a reduction of leaf blade thickness, and augmentation of the adaxial epidermis. Due to these morphological and anatomical changes the authors suggested the use of *Betula* and *Quercus* as indicators of radioactive pollution (Zhirina 1998).

Our investigations show that couch-grass (*Agropyron cristatum*), feather grass (*Stipa capillata*) and fescue (*Festuca valesiaca*) are sensitive for detecting chronic ionizing radiation. Radiosensitivity occurs at all levels of organization: organism, organ and tissue. Radioactive pollution of Semipalatinsk nuclear test site stimulated plant height, length of leaves and internode (**Table 6**), there enhanced the growth and dry matter a desirable feature for phytoremediation.

The same tissues of different organs responded to ionizing radiation in a different way. In polluted plots the mechanical (sclerenchyma) and vascular tissue (conducting bundles and xylem vessels) of stem were inhibited in *A. cristatum* and *S. capillata*. In contrast, the stem of *F. valesiaca* radiation stimulated growth of the area of vascular bundles and thickness of the sclerenchyma. Under the effects of radioactive ionization the anatomical parameters of leaves were reduced in *A. cristatum* and *F. valesiaca* and were enlarged in *S. capillata*. Size of conducting bundles as well as xylem became larger in feather grass when exposed to radiation-polluted sites but got smaller in couch-grass and fescue.

The three investigated species of plants are not only tolerant to radionuclides of the nuclear test site but are also widely distributed in various parts of the world (**Table 7**). Some of the species of the chosen genera are known as accumulators of radionuclides ^{90}Sr and ^{137}Cs and are useful in phytostabilization of readionuclide-contaminated sites

Table 7 Global distribution and symptoms exhibited by the selected grasses at the radionuclide contaminated plots.

Distribution in world	Symptoms of radionuclide exposure
***Agropyron cristatum* (L.) Gaertner**	
Dry mountain slopes, dry meadows, steppes, stony steppe slopes. Japan, Korea, Mongolia, Pakistan, Russia; SW Asia, Europe; introduced in North America. This species provides good forage (Flora of Kazahkstan. IV, Alma-Ata, 1956-1966).	In the plots with high ionizing radiation doses the height of plants, length of leaves are increased. For example, length of leaves of the 1 and 3 contaminated plots – 18.13 cm and 20.05 cm in accordance, in the control plots (2, 4) – 14.86 cm, 15.82 cm (**Tables 4-6**).
***Festuca valesiaca* Gaudin**	
Grassy mountain slopes, subalpine meadows, grasslands, roadsides; 1000-3700 m. Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tajikistan, Turkmenistan; SW Asia, Europe	Height of plants, length of leaves have been increased. For example, length of leaves of the 1 and 3 contaminated plots – 16.27 cm and 16.18 cm, in the control plots (2, 4) – 10.99 cm, 14.39 cm in accordance. The average height of plant in contaminated plot 1 was 29.23 cm, and in control 25.38. Consequently the height has been increased in the areas with high level of ionizing radiation (**Tables 4-6**).
***Stipa capillata* L.**	
Mountain valleys, plains, rocky slopes; 500-2300 m. Kashmir, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Tajikistan, Turkmenistan, Uzbekistan; SW Asia, Europe	Height of plants, length of leaves and internode are increased. Consequently chronic ionizing radiation has stimulating effect on morphology of *Stipa capillata.* (**Tables 4-6**).

Table 8 Grasses reported to hyperaccumulate ^{137}Cs, ^{90}S as probable candidates for radiophytostabilization of contamination soils.

Plant species	Radionuclides	Reference
Festuca arundinacea	^{137}Cs	Dahlman *et al.* 1969
Festuca/Agrostis	^{137}Cs	Coughtery *et al.* 1989
Lolium perenne	^{137}Cs	Salt *et al.* 1992
Festuca rubra	^{137}Cs	Salt *et al.* 1992
Lolium perenne	^{137}Cs, ^{90}Sr	Veresoglou *et al.* 1995
Paspalum notatum	^{137}Cs, ^{90}Sr	Entry *et al.* 2001
Sorghum halpense	^{137}Cs, ^{90}Sr	Entry *et al.* 2001
Panicum virginatum	^{137}Cs, ^{90}Sr	Entry *et al.* 2001

and are considered as indicators of radioactive pollution (**Table 8**). Entry *et al.* (1999) found that the mycorrhizal fungi *Glomus mosseae* and *G. intraradices* are associated with grasses such as bahia lovegrass (*Eragrostis bahiensis*), Johnsongrass (*Sorghum halepense*), and switchgrass (*Panicum virgatum*) which synergize the accumulation of ^{137}Cs in a relatively short period of time (**Table 8**). Therefore, additional studies would confirm the role of mycorrhizal fungi and natural attenuation of radionuclides in the chosen grasses and pave a way for radiophytoremediation (Dushenkov 2003; Vandenhove 2006).

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