Botanica Pacifica. A journal of plant science and conservation. 2025. 14(1):69–76
DOI: 10.17581/bp.2025.14105

RESEARCH PAPER

The effect of elevation gradient on cytogenetic characteristics of *Plantago major* L. (Plantaginaceae)

Marianna B. Malaeva[1], Nina V. Reutova[1]*, Fatima R. Dreeva[1], Tatiana V. Reutova[1], Petimat M. Dzhambetova[2]

Marianna B. Malaeva [1]
e-mail: malaevamarianna@yandex.ru

Nina V. Reutova [1]*
e-mail: reutova371@mail.ru

Fatima R. Dreeva [1]
e-mail: f.dreeva@mail.ru

Tatiana V. Reutova [1]
e-mail: reuttat@yandex.ru

Petimat M. Dzhambetova [2]
e-mail: petimat-lg@rambler.ru

[1] Kabardino-Balkar Scientific Center RAS, Nalchik, Russia

[2] Chechen State University, Department of Cell Biology and Microbiology, Grozny, Russia

* corresponding author

Manuscript received: 15.06.2024
Review completed: 25.12.2024
Accepted for publication: 24.01.2025
Published online: 27.01.2025

ABSTRACT

We aimed to study the effect of elevation gradient on the cytogenetic characteristics of *Plantago major* L. We determined the mitotic index, the frequency of chromosomal aberrations, micronuclei and chromosome numbers in the cells of the apical root meristem of the seed progeny of plants inhabit altitudes of 200, 600, 2000 and 2700 m above sea level. The frequency of chromosomal aberrations increased at altitudes more than 2000 m above sea level, which indicates the presence of a genetic effect. There was no similar increase in the frequency of micronuclei in interphase cells. There were no trends in cell proliferation (mitotic index) and the ratio of mitosis phases in accordance with the elevation gradient. Plants from populations inhabit different altitudes are diploids. No changes in chromosome numbers along the altitude gradient were detected.

Keywords: Central Caucasus; altitude gradient; mitotic index; micronuclei; chromosomal aberrations; polyploidy

РЕЗЮМЕ

Малаева М.Б., Реутова Н.В., Дреева Ф.Р., Реутова Т.В., Джамбетова П.М. Влияние высотной поясности на цитогенетические характеристики *Plantago major* L. (Plantaginaceae). Был изучен ряд цитогенетических характеристик подорожника большого (*Plantago major* L.) в высотном градиенте под влиянием экстремальных условий высокогорья. Мы определяли митотический индекс, частоту хромосомных аберраций, микроядер и хромосомные числа в клетках апикальной корневой меристемы семенного потомства растений, обитающих на высотах 200, 600, 2000 и 2700 м над уровнем моря. У растений, обитающих на высотах более 2000 м над уровнем моря, возрастает частота хромосомных аберраций. Аналогичного повышения частоты микроядер в интерфазных клетках не выявлено. Изменений в пролиферации клеток (митотический индекс) и соотношении фаз митоза в соответствии с высотным градиентом не выявлено. Растения из популяций, обитающих на разных высотах, являются диплоидами. Изменения плоидности по высотному градиенту не выявлено.

Ключевые слова: Центральный Кавказ; высотный градиент; митотический индекс; микроядра; хромосомные аберрации; полиплоидия

A large number of studies have been devoted to the influence of high mountains on plants. The study of this problem began in the late 19th – early 20th century. Many works were devoted to the problem of species diversity (Körner 1995, Bowman & Damm 2002, Körner & Spehn 2002, Parisod et al. 2022) and distribution of species in mountainous areas (Elumeeva et al. 2013, Onipchenko et al. 2014). Much attention was paid to the study of changes in morphological features of vegetative (Cordell et al. 2012, Halbritter et al. 2018, Kieltyk 2021) and generative (Blionis et al. 2001, Fabbro & Körner 2004) organs of plants, as well as anatomical features of plants (Körner et al. 1989, Shi et al. 2004) under the influence of altitude gradient.

A great contribution to the understanding of this problem was made by the study of physiological features of plants in high altitude conditions, in particular, the process of photosynthesis (Körner et al. 1988, 1991) and changes in the chemical composition of plants under the influence of increased background ultra-violet radiation (Rau & Hofmann 1996, Shi et al. 2004, Bernal et al. 2013, Onipchenko et al. 2023) and low temperatures (Rathorea et al. 2018).

Recently, considerable attention has been paid to the study of molecular genetic characteristics of plants as influenced by changes in altitude (Hämälä et al. 2018, Grdiša et al. 2019, Volkova et al. 2021). The effects of polyploidy and apomixis on plant dispersal along an altitudinal gradient are also being actively studied (Schinkel et al. 2016, Rodrigo et al. 2017, Klatt et al. 2018).

Despite such an active and comprehensive study of this problem, the issues of genotoxic effect of high altitude conditions remain insufficiently studied. We aimed to study cytogenetic characteristics of *Plantago major* L. under the influence of conditions related to the altitude gradient. This species was chosen because of its ubiquitous distribution in a wide range of altitudes up to 2000 m above sea level. More than 50 years ago, during the construction of cableways in the Elbrus region, representatives of this species climbed to an altitude of 2700 m above sea level. Now they inhabit the vicinity of the cable car stations on Cheget Mountain (Russia).

MATERIAL AND METHODS

Studies on the influence of highland conditions on the cytogenetic characteristics of plants were carried out in 2013, 2014, 2018 and 2023 on the territory of the Kabardino-

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Balkarian Republic (Russia, Central Caucasus). *Plantago major* was chosen as the object of the study. This is a widespread species that inhabit a wide range of altitudes from the plane (200 m above sea level) to the mountainous (2000 m above sea level) in Kabardino-Balkarian Republic. During the construction of cable cars in the Elbrus region more than 50 years ago, representatives of this species rose to elevation of 2700 m. Now at this altitude they inhabit the vicinity of the cable car on Mount Cheget but not Elbrus. The schematic map of seed collection points is shown in the Figure 1.

Seed collection points for studying the cytogenetic status of *P. major* were located at altitudes of 200 (plain), 600 (low mountains), 2000 and 2700 m (highlands) above sea level. We collected seeds on the plain and in the foothill zone in July, and at altitudes of 2000 m and above in August – early September. Therefore, we provide data on climatic indicators in these periods (Ardavov 2019). The mean temperature in July (1985–2015) at an altitude of 200 m was 22.9°C, precipitation 70 mm; at an altitude of 600 m – 21.4°C, precipitation 81 mm. In August, at an altitude of 2050 m, the mean temperature was 11.7°C, precipitation was 101 mm. For higher altitudes, taking into account that the average annual temperature decreases by 0.1° C for every 100 m of elevation, the average temperature in August at an altitude of 2700 m will be approximately 11°C. Thus, during the period of seed ripening in the mountains, temperatures were lower and precipitation was higher than in the plain and low-mountain zone. Daily temperature fluctuations are of great importance. So, in the low mountains, the average temperature range per day is 8–10°C in summer. In the highlands, the summer daily fluctuations exceed 20°C.

Soil cover of the region varies with altitudinal zonation. Poorly developed alluvial-meadow soils are typical for plain (200 m a.s.l.), dark grey forest soils for lowlands (600 m), and mountain-meadow peaty soils for highlands (from 2000 to 2700 m) (Kerefov & Fiapshev 1968).

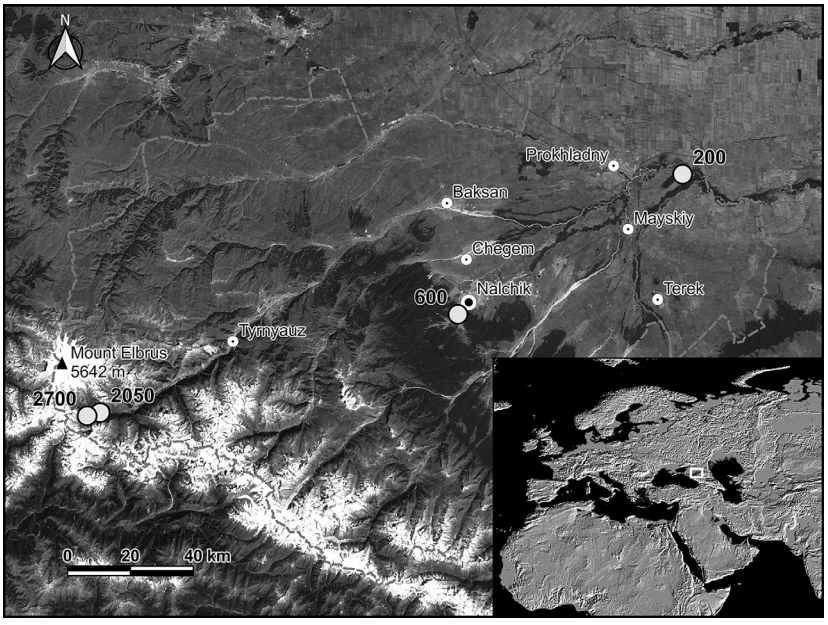

Figure 1 Locations of seed collection points (Kabardino-Balkaria, Russia)

Seed sampling

For each altitude, seeds were collected from at least 50 plants. The sample points were located away from settlements at a distance of at least 200 m from the roads. The collected seeds were dried at room temperature and stored in labeled paper bags. The shelf life of seeds before germination was the same for all altitudes.

Germination of seeds

The seeds were germinated in Petri dishes on wet filter paper at room temperature. If necessary, the filter paper was additionally moistened with tap water. The seeds were germinated until the appearance of roots 5–10 mm long. The seedlings were fixed in a freshly prepared alcohol-acetic (3:1) mixture for at least two days. In this form, the seedlings can be stored in the refrigerator (+4°C) for two to three weeks. If long-term storage was necessary, the fixed seedlings were thoroughly washed with distilled water, dried with filter paper and transferred to 70° ethyl alcohol. They were also stored in the refrigerator at +4°C.

To determine the chromosome numbers, the seedlings were placed in a 0.05 % solution of colchicine for 3 hours, and then fixed.

Staining

The seedlings were stained with 2.5 % orsein (Megske) in 45 % acetic acid. For this purpose, the fixed seedlings were washed with distilled water and placed in a porcelain crucible with 1–2 ml of dye. The dye was used only once. The crucible was then heated on an alcohol burner, bringing to a boil (but not boiling). The procedure was repeated 2–3 times. The seedlings were then left in the dye for 2–3 hours.

Preparation of temporary squashed slides and slides analyses

The stained seedlings were transferred to 45 % acetic acid to lighten the cytoplasm. The seedlings were then placed on a slide in a drop of 45 % acetic acid and the darkly coloured root tip was cut off with a scalpel (or blade). They were covered with a coverslip and two layers of filter paper and crushed with a finger. Quality preparations appear as a uniformly coloured pink stain. These temporary preparations are not to be stored for a long time, so they were analysed immediately.

The slides were evaluated using microscopy at ×1000 bright field magnification. At least 1000 ana/telophase for each height were analysed to determine the frequency of chromosomal aberrations (CA). The remaining phases of mitosis were as many as obtained by analysing 1000 ana/telophase. To determine the mitotic index (MI), 30 seedlings at each height and 1000 cells per seedling were analysed. Micronuclei (MN) frequency was counted in at least

5000 interphase cells for each height. Chromosome number was determined in 50 seedlings for each height.

Statistical analyses

We used the Fisher transform (Urbakh 1963) to determine the reliability of observed differences. The criterion assesses the reliability of differences between the percentages of two samples in which the effect of interest is recorded. Pairwise comparisons of each successive height with the 200 m height were made to determine the reliability of the differences.

Determination of the radiation level

The level of ionising radiation was measured by personal dosimeter-radiometer MKS-03SA. Ultraviolet radiation was measured with a TKA-PKM device designed to determine the energy of light intensity in the UV-C (200 280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) spectra. UV-radiation measurements were carried out on low cloudy or sunny days in a period from 11:00 to 13:00.

RESULTS

The data on ionising radiation and ultraviolet radiation at the collection points are given in Table 1. As can be seen from the table, the intensity of UV-A radiation (315–400 nm) increased 6.3–10.8 times with increasing altitude; UV-B (280–315 nm) – 6.7–13.0 times and UV-C (200–280 nm) – 4.8–6.7 times.

As for radioactivity, α and β radiation at 2700 m above sea level increased by about 2 times compared to the plain, and γ radiation increased by 1.2 times. Thus, the intensity of all types of radiation increases with increasing altitude.

Cytogenetic characteristics of plants

Cytogenetic studies were carried out using root apical meristem cells of seed progeny of plants at different altitudes.

Mitotic index

Mitotic index characterises the intensity of cell division. The total MI reflects the number of dividing cells at all phases of mitosis. We also determined indices for each phase of mitosis to identify possible changes in their ratio with increasing altitude. The data are presented in Table 2.

As shown in Table 2, there were no significant differences in mitotic index in the root apical meristem cells of the seed progeny of seed progeny of lowland and high altitude plants in 2013 and 2014. In 2018, progeny of lowland (600 m) and highland plants had higher MI than progeny of plain plants. This may be due to the fact that the altitude of 600 m is characterised by more favourable conditions compared to the arid areas of the plain (200 m). In 2018, it was already snowing in the highlands by the time of seed collection. Cold stress may have affected the proliferative activity.

The ratio of mitosis phases in all years did not change with increasing altitude.

Chromosomal aberrations (CA)

Different types of mutations can be registered in dividing cells at different phases of mitosis (Fig. 2). Micronuclei can occur in interphase (Fig. 2b). They are formed from lost chromosomes or chromosome fragments as a result of deletions. Micronuclei can also appear in prophase. This is a rare event. Micronuclei in prophase can form from cells with micronuclei in interphase, but mitosis is usually blocked in such aberrant cells. In metaphase, "lost" chromosomes appear (Fig. 2e). These are chromosomes located outside the metaphase plate. The achromatin strands of the spindle do not attach to them, and after the completion of cell division, micronuclei are formed from them.

Chromosomal aberrations in anaphase and telophase are most diverse. Deletions or lagging of chromosomes may result in fragments of different sizes (Fig. 2d). Subsequently, micronuclei are formed from them. More complex rearrangements involving two or more chromosomes may result in single "bridges" (Fig. 2h) and "double bridges" (Fig. 2i). "Bridges" may also appear in telophase (Fig. 2k).

Table 3 shows the data on the frequency of CA occurrence in cells of the root apical meristem in the progeny of plant seeds from populations located along the altitudinal gradient.

In 2013 and 2018, the total frequency of CA in all phases of mitosis was significantly higher at 2700 m altitude than in the progeny of plants from the lowland zone. Data for 2700 m altitude in 2014 are not available in the table due to early snowfall in the mountains and the end of plant vegetation before full seed maturation. Ambiguous results were obtained for the altitude of 2050 m. In 2013, the mutation level at this altitude was slightly higher than at the plain, but these differences were not statistically significant. In 2014,

Table 1. Radiation and UV radiation levels.

Altitude above sea level (m)	UV, mW/m²			Ionizing radiation		
				1/min•cm²		μSv/hour
	A	B	C	α	β	γ
200	2470–6060	137–420	6–12	33–48	17–21	22
600	12320–18300	114–1097	5–38	43–78	22–24	22
2000	12560–12650	761–774	37–66	52–69	15–28	21
2700	26700–38000	1780–2800	40–58	80–91	35–44	27

Table 2. Mitotic index in cells of the apical root meristem of *P. major*

Altitude above sea level (m)	Prophase	Metaphase	Anaphase + telophase	Mitotic index	P
2013					
200	0.53	0.74	0.81	2.08	
2050	0.80	0.82	0.84	2.43	0.062
2700	0.59	0.89	0.92	2.40	0.062
2014					
200	0.73	0.78	0.80	2.31	
2050	0.75	0.79	0.87	2.41	0.173
2018					
200	0.70	0.81	0.85	2.36	
600	1.08	0.97	1.15	3.20	<0.001
2000	0.99	0.94	0.84	2.77	0.011
2700	0.96	0.80	1.01	2.77	0.011

Note: P – significance levels of differences compared to the population 200 m. Differences were considered significant at $P \leq 0.05$

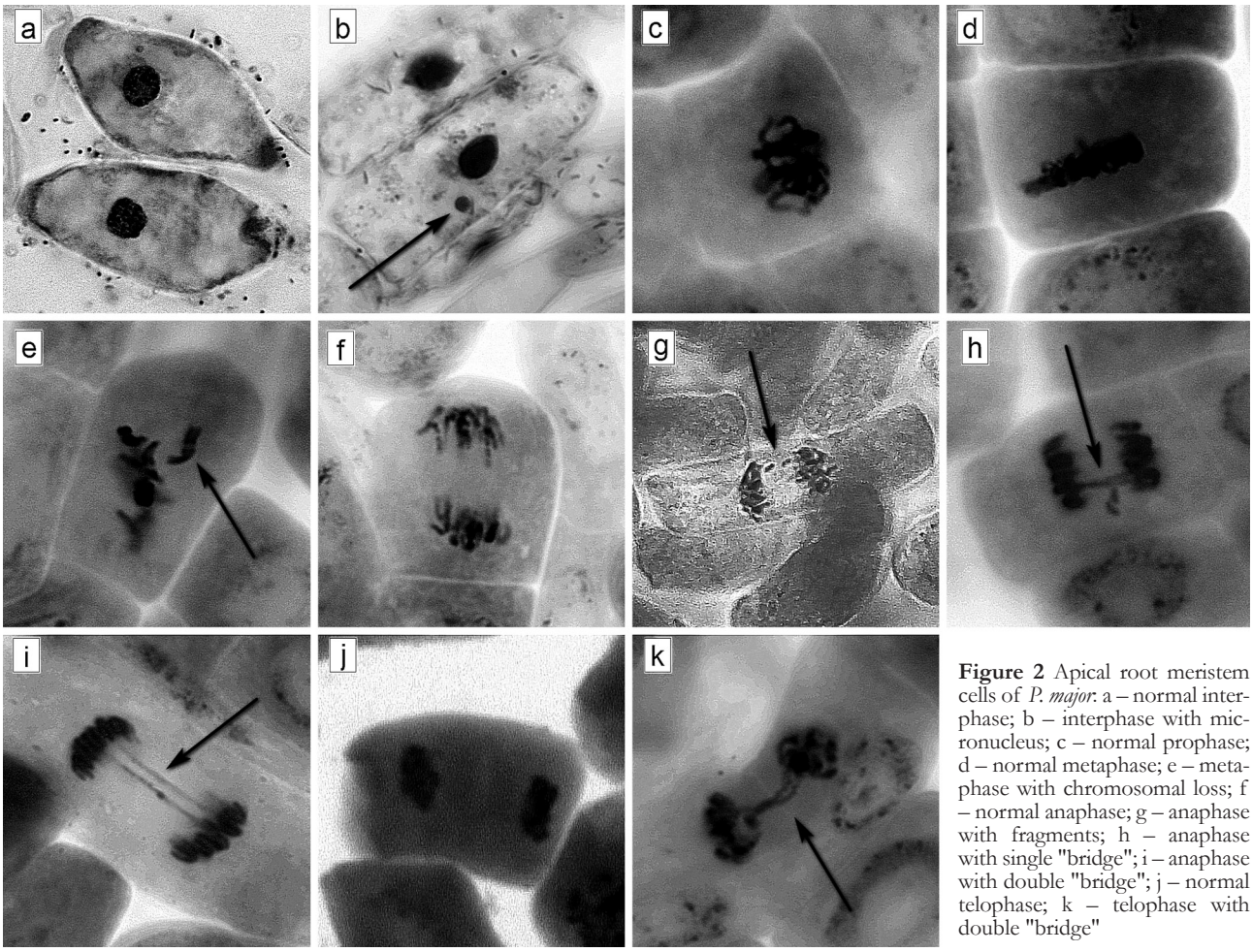

Figure 2 Apical root meristem cells of *P. major*: a – normal interphase; b – interphase with micronucleus; c – normal prophase; d – normal metaphase; e – metaphase with chromosomal loss; f – normal anaphase; g – anaphase with fragments; h – anaphase with single "bridge"; i – anaphase with double "bridge"; j – normal telophase; k – telophase with double "bridge"

Table 3. Frequency of chromosomal aberrations in the root meristem cells *P. major*.

Altitude above sea level (m)	Total dividing cells	Including cells with CA	Persentage, %	P
2013				
200	1735	13	0.75	
2050	3065	37	1.21	0.0625
2700	2275	38	1.67	0.009
2014				
200	2655	14	0.53	
2050	2750	36	1.31	0.0085
2018				
200	2045	16	0.78	
600	1012	9	0.89	0.316
2000	2370	13	0.55	0.103
2700	2417	47	1.94	<0.001

Note: P – significance levels of differences compared to the population 200 m. Differences were considered significant at $P \leq 0.05$.

Table 4. The frequency of chromosomal aberrations in the cells of the root meristem of *P. major*. Data on the phases of mitosis.

Altitude above sea level (m)	Prophase			Metaphase			Anaphase + telophase		
	Total	Incl. cells with CA	%	Total	Incl. cells with CA	%	Total	Incl. cells with CA	%
200	1360	3	0.22	2637	22	0.83	3324	18	0.54
600	172	0	0	393	5	1.27	447	4	0.89
2050	2237	6	0.27	2844	43	1.51	3104	55	1.77*
2700	941	6	0.64	1870	37	1.98*	1875	42	2.24**

Note: * – $P < 0.01$; ** – $P < 0.001$

the frequency of CA in the seed progeny of plants was higher at this altitude than in the plain. And in 2018, the frequency of chromosomal aberrations at this altitude was slightly lower than in the plain, but the differences were not statistically significant.

Further, we present data on the frequency of CA at different phases of mitosis (Table 4) to identify the most sensitive stage of mitotic cell division. The results obtained are similar by year and are not statistically different. Therefore, we present averaged data in Tables 4 and 5.

There are fewer chromosomal aberrations in prophase. We have already noted that this is a rare event because mitosis is usually blocked in aberrant cells. Although the frequency of cells with micronuclei in prophase increases with increasing altitude, these differences are not statistically significant. In metaphase, the frequency of CA is significantly higher. The frequency of aberrant cells with "lost" chromosomes increases with increasing altitude. But these differences become statistically significant only at an altitude of 2700 m. The frequency of cells with chromosomal aberrations increases to the greatest extent in the ana/telophase compared to the plain zone. It becomes statistically significant starting

Table 5. Chromosomal aberrations in the apical root meristem cells of *P. major*. Data on the types of mutations in the ana/telophase.

Altitude above sea level (m)	Total cells in ana/telophase	Including fragments	%	Including single "bridges"	%	Including double "bridges"	%	Total CA	Number of plants with chromosome numbers	
									12 (2*n*)	24 (4*n*)
200	3324	5	0.15	13	0.39	0	0	18	46	4
600	447	1	0.22	3	0.67	0	0	4	50	0
2050	3104	25	0.80	28	0.90	2	0.06	55	41	9
2700	1875	15	0.80	27	1.44	0	0	42	48	2

at an altitude of 2000 m. Thus, the ana/telophase stages appeared to be the most sensitive to the effects of altitudinal conditions.

In Table 5, we present data on the types of mutations in ana/telophase because they are the most diverse. The frequency of cells with fragments increases most with increasing altitude. The occurrence of fragments is due to deletions or lagging chromosomes. To a lesser extent, the frequency of cells with "bridges" increases. The occurrence of this type of chromosomal mutation is usually associated with more complex rearrangements involving two or more chromosomes.

Micronuclei

Micronuclei can appear in interphase cells (Fig. 2b). Micronuclei are formed from "lagging" and "lost" chromosomes, as well as from chromosome fragments resulting from deletions. Since the frequency of such CAs increases significantly with increasing altitude, we hypothesised that the frequency of micronuclei would also increase. The data obtained are summarised in Table 6.

Despite the significant increase in the frequency of aberrant cells with increasing altitude, we did not find a similar increase in the frequency of micronuclei. The frequency of cells with micronuclei in the root apical meristem of seed progeny of plants growing at different altitudes was extremely low and did not vary along the altitude gradient.

Chromosome numbers

We also determined chromosome numbers of plants from populations at different altitudes. The results are summarised in Table 7.

The basic number of plantain is 6 (Shipunov 1998). In all the populations we studied, plants were diploid. Tetraploids were rare and represented by a small number of individuals with approximately equal frequency at different heights. Thus, unlike common dandelion (Reutova et al. 2023), we did not find an increase in ploidy level in *P. major* with increasing altitude.

DISCUSSION

Abiotic and biotic factors change with increasing altitude. Temperature decreases and its daily fluctuations increase. Precipitation increases, the role of wind as an environmental factor increases, soil types change. Oxygen concentration decreases. The intensity of such mutagenic factors as ultraviolet radiation, radioactivity of underlying rocks, cosmic radiation increases. In this connection the study of possible genotoxic effect of all these environmental factors is of great interest. We have studied for the first time a number of cytogenetic parameters in seed progeny of plants from different altitudes.

Table 6. The micronuclei frequency in the apical root meristem cells of *P. major*.

Altitude above sea level (m)	Number of cells	Cells with MN	%	P
2013				
200	5463	3	0.05	
2050	5721	2	0.03	
2700	5154	5	0.10	0.106
2014				
200	5068	0	0	
2050	5054	3	0.06	
2018				
200	5169	1	0.02	
600	5136	1	0.02	
2000	5035	0	0	
2700	5221	3	0.06	0.091

Mitotic index (MI)

It is well known that plant size decreases with increasing altitude. This may be due to the fact that smaller phenotypes are selected for at high altitude, as they are more favourable under growth-limited conditions (Kiełtyk 2021). Smaller plants have lower resource requirements (Herrera 2005), and they are more resistant to the high winds typical of highlands (Takahashi 2014).

We did not detect a decrease in MI in seed progeny of high-altitude plants. Moreover, it was even higher in 2018. This could most likely be due to the fact that seeds from different altitudes were germinated under similar laboratory conditions. This may explain the fact that we did not detect a decrease in the mitotic index. The ratio of mitosis phases also did not change.

Chromosomal aberrations (CA)

The effect of such mutagenic factors as UV radiation and ionising radiation of bedrock increases with increasing altitude. In our study, we found an increase in the intensity of these radiations (Table 1). Thus, α-radiation increased about 3 times, β – 2.6 times and γ – 1.2 times. The intensity of UV radiation also increased with increasing altitude (Table 1). In this regard, it was logical to assume that the level of mutations would also change.

The frequency of CA increased with increasing altitude (Tables 3–5), but these differences became statistically significant only in the highlands at altitudes above 2000 m. The altitude of 2700 m is not typical for *P. major*, it inhabits only in the vicinity of the cable car station on Cheget

Mountain. In other areas of Kabardino-Balkaria we did not meet representatives of this species at such altitudes. At an altitude of 2000 m, the frequency of CA was significantly higher than on the plain only in 2014. This altitude is a common habitat of *P. major*, and it is widely represented in disturbed areas.

As for the phases of mitosis, the most sensitive was ana/telophase. The frequency of aberrant cells at this stage of cell division increased significantly. Even at an altitude of 2000 m, the differences in the frequency of cells with CA were statistically significant compared to the plain zone. In metaphase, this increase becomes significant only at an altitude of 2700. This is due to the fact that in ana/telophase it is possible to register such types of mutations that are not visualised in other phases of mitosis. These are "bridges" and small fragments resulting from deletions. In ana/telophase, these small fragments are clearly visible because they are located between diverging chromosomes. In metaphase, they are located in any part of the mitotic plate and can be missed because of their small size. "Bridges" are formed mainly by dicentrics, which are standardly located in the metaphase plate and are not visible at this stage.

Of all types of mutations, the frequency of fragments increases the most in ana/telophase. This is the simplest type of mutation, associated with deletions (in the case of small fragments) or abnormalities in the mitotic spindle (in the case of lagging chromosomes). The frequency of complex rearrangements leading to the appearance of 'bridges' increases to a lesser extent.

It should be emphasised that we determined the frequency of CAs in the root apical meristem not in plants living at different altitudes, but in their seed progeny. This is a standard technique used to detect environmental mutagens (Reutova 2018). In such studies, seedlings with roots 5–10 mm long are used. This means that it is not the first mitosis. Seeds were germinated under the same conditions for all heights, no additional exposure was carried out. Consequently, the increased level of mutations is a consequence of the impact of extreme conditions of high altitude on parental organisms.

Micronuclei

Since the frequency of CAs leading to micronuclei in interphase cells increases along the height gradient (Tables 3–5), we expected that the frequency of MJA would increase accordingly. But we did not find this to be the case (Table 6). Micronuclei were extremely rare. Although their frequency increased with height, these differences were statistically insignificant. Consequently, aberrant cells are successfully eliminated.

Polyploidy

Many studies (Parisod & Besnard 2007, Beest et al. 2012, Schinkel et al. 2016) have been devoted to the polyploidy and the success of autopolyploids in plant colonisation of new habitats, including high-altitude habitats. We also found a significant increase in ploidy levels (up to 9*n* and 12*n* at 2700 and 3050 m a.s.l.) in *Taraxacum officinale* Wigg along an altitude gradient (Reutova et al. 2023, 2024). We conducted these studies in the same areas. Dandelion reached the altitude of 3050 m above sea level, while *P. major* reached only 2700 m. Currently, dandelion is actively dispersing along hiking trails at 2700 m altitude due to a significant increase in the number of tourists. In addition, dandelion has settled in the vicinity of the cable car station on Mount Elbrus at the altitude of 3000 m, while plantain has not. That is, the common dandelion was more successful in settling new territories.

Polyploidy is always associated with problems of seed reproduction. But this problem is easily solved by switching to asexual reproduction by apomixis. Dandelion is a facultative apomictic plant. Apomixis is useful for expansion because mating partners and pollinators are not required. Polyploidy offers advantages in adapting to unstable environmental conditions. Switching to asexual reproduction and polyploidisation can lead to increased tolerance to colder and more extreme conditions, which in turn provides the ability to overcome altitudinal barriers and migrate to higher altitudes (Kirchheimer et al. 2018). The combination of endogenous selection for apomictic seed formation and exogenous selection for cold-adapted polyploid genotypes has resulted in the predominance of apomictic polyploids of *Ranunculus kuepferi* at high altitudes in the Alps, where diploids have no advantage in terms of adaptability. (Kirchheimer et al. 2016, 2018).

For *P. major*, we did not find data in the literature on its ability to undergo asexual reproduction by apomixis, although there are reports of polyploid plants (Shipunov 1998). It can be assumed that due to the inability of this species to switch to asexual reproduction, autopolyploids lose to diploid individuals, despite all the advantages of polyploidy in extremely unstable conditions. This is due to seed reproduction, as polyploids have problems with meiosis due to polyvalent formation. We found polyploid plants in the populations from the studied altitudes, but these were single individuals (Table 7).

CONCLUSION

Under the influence of high altitude conditions, the frequency of chromosomal aberrations increased in the seed progeny of plants from 2700 m above sea level, indicating the presence of a genetic effect. No similar increase in the frequency of micronuclei was observed in interphase cells. No changes in cell proliferation (mitotic index) and the ratio of mitotic phases according to the altitudinal gradient were observed.

The two species *P. major* and *T. officinale*, inhabiting the same disturbed areas of cable car stations in the Elbrus Mountains, have completely different ploidy levels. In *T. officinale* ploidy increases along the altitude gradient, while in *P. major* plants are mostly diploid at all altitudes. Polyploid representatives of *T. officinale*, which is a facultative apomictic, are more successful in dispersal at high altitudes compared to *P. major*.

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