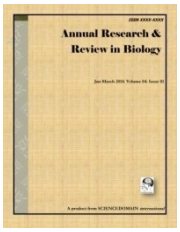

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Teratological Changes Generative Various of Wheat in a Weak Chloride Salinity

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Authors' contributions

This work was carried out in collaboration between all authors. Author NVT performed the statistical analysis and wrote the first draft of the manuscript. Author NAK wrote the protocol, managed the analyses of the study. Author LKM designed the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: We examined the cytological response of generative cells in different varieties and types of wheat under salt stress to understand how the physiological state of plants is related to growth conditions and productivity.
Study Design: This study was conducted in the Laboratory of Cell engineering, Institute of Plant Biology and Biotechnology Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan from 2011-2012.
Methodology: The material for cytological studies served as the young ears are not released from the vagina of the upper leaves of various types of wheat subjected to the stage of stem elongation weak chloride salinity and without saline (control). Between 20 and 25 flowers on each ear were selected for cytological experiments. Samples were prepared and microsporocytes in the anthers were examined for at least 10 fields of view.
Results: In high-salt conditions (0.05% and 0.1% NaCl), viable fertile pollen grains were observed in all wheat species tested but teratological irregularities occurred during flower development and slowed both male and female gametophyte development.
Conclusion: The possibility of the evaluation of the physiological condition of the wheat plants in relation to the conditions of growth and productivity changes by the presence of teratology flowers.

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Keywords: Wheat species; generative sphere; teratology; physiological state.

1. INTRODUCTION

One of the most important factors influencing the formation of the generative cell in plants is exposure to water. According to Skazkin [1], cereals are very sensitive to moisture during the phase in which pollen tubes are exposed, making the flower susceptible to damage under adverse abiotic environmental conditions, such as drought and high soil salt concentrations.

Saini and Aspinall [2,3] examined the effects of water stress during meiotic maturation to determine grain fertility in males and female in wheat generative organs. They showed that if the plants experienced a water deficit during and immediately after meiosis, most anthers were small and shriveled not cracked and pollen showed acytoplasmic pathology. High-temperature stress during meiosis may disrupt pollen cells and reduce the fertility of the female gametophyte. This stress reduced the number of kernels that underwent normal development.

Low light during meiosis was found to cause a reduction in the viability of pollen grains, reducing the number of grains per ear according to Demotes-Mainard et al. [4,5].

Previous studies found that osmotic and salt stress in pollen grains of barley and wheat resulted in plasmolytic processes; prompting the development of salt- and drought-tolerant cereals at the gametic level [6]. Marked cytological abnormalities in the development of male and female gametophytes in wheat under high salt conditions led to changes in growth and productivity [7].

Overall, however, the study of the cytological and physiological responses of reproductive structures resulting from osmotic and salt stress are not well-understood [8]. An understanding of the ecological plasticity and tolerance of reproduction systems are provide important insights into how plants cope with one of the most common stresses experienced in nature. Examining the effects of osmotic and salt stress on plants at different stages of embryonic development would help to determine the most vulnerable stages of reproductive organ formation and to identify the mechanisms of the changes [9,10].

The purpose of this study was to evaluate the response of generative cells from different species and cultivars of wheat to salt stress and measure the effects of high salt on wheat growth and productivity.

2. MATERIALS AND METHODS

Four species of wheat plants, including *Triticum aestivum* L. (sort Saratovskaya-29) (genomes $A^{v}A^{v}$BBDD), *Triticum compactum* L. (genomes $A^{u}A^{u}$BBDD), *Triticum macha* Dek. et. Men. (genomes $A^{u}A^{u}$BBDD) and *Triticum dicoccum* Schuebl. (genomes $A^{u}A^{u}$BB) were grown as winter crops in a field. During the tillering stage, plants were transplanted into pots to apply different salinity conditions and create optimal growing conditions. The soils in the pots were taken from an experimental field plot and were light-loamy with 3% humus content in the upper layer. Between 3 and 5 plants were grown in each pot. Three biological replicates were performed for each experiment.

In the output stage, tubes were placed in vessels for introduction of irrigation solutions NaCl at concentrations of 0.05% and 0.1% to create weakly saline conditions. In control conditions the plants were grown without added NaCl. Several ears from each vessel during the formation of the male and female gametophytes were collected for cytogenetic studies. The remaining plant was allowed to reach full maturation and evaluated for crop structure elements such as plant height, ear length of the main ear, the number of ears in the main ear, number of grains in the main spike and grain weight per plant. After harvesting, statistical analysis was performed according to the method of Udolskaya [11]. The arithmetic mean (M) was determined for each feature. The error of the mean values of (m) was calculated using the Moldengauer factor (K constant) with the formula: $m = [a] \times K$, where $K = 1 / (0.79788 \times n \times \sqrt{(n-1)})$. The coefficient of variation was calculated using the formula $C_v = (\sigma \times 100) / M$. The significance of differences between parameters was determined using the *t*-test with the formula (M_1-M_2): $\sqrt{(m_1^2 + m_2^2)}$. Characters * and ** indicate the accuracy of the *t*-test at the 0.05 and 0.01 levels of significance, respectively. The standard error of the mean is also shown. Characters * and ** indicate the accuracy of the *t*-test at the 0.05 and 0.01 levels of significance, respectively.

The young ears which are not released from the vagina of the upper leaves served the material for cytological studies. The young ears was fixed in the morning in freshly prepared Clark's reagent (3:1 96% ethyl alcohol: glacial acetic acid) and stored for 12–24 h. The material was then washed in 96% ethanol for1 h, 80% ethyl alcohol for 1 hour two times, 70% ethyl alcohol for 1 h three times and stored in fresh 70% ethanol.

Staining of flowers and anthers containing microsporocytes was performed in a 2% solution of acetocarmine, prepared using the method described by Pausheva for painting microsporocytes in cereals [12]. Color intensity of flowers and anthers and the number of cells containing normal microsporocytes meiosis and various disorders during meiosis were determined.

For cytological experiments, 20–25 flowers on each spike were viewed and microsporocytes in anthers were counted in at least 10 fields of view.

All staining showing meiosis, as well as flowers, anthers and pollen grains, was examined under a microscope Micros (St. Veit/Glan, Austria), photographed with a Ningbo Yongxin Optics CAM V200 video camera and the Ningbo Yongxin OpticsScopePhoto version 2.4 software (Zhejiang, China) at magnifications of $\times$ 10 (terates) and $\times$ 40 (cells).

3. RESULTS AND DISCUSSION

Wheat flowers contain three stamens and a pistil and bear two feathery stigmas. At the base of the ovary there are two or three small scales, which are lodicula that make up the perianth. During flowering, they swell and push the surrounding flower scales. Changes in the formation of morphological and cytological structures of male and female gametophytes were observed in the control experiment, in which hall flowers developed normally and mature pollen grains were fertile. Under low-salt conditions, although autonomic areas of the plants developed normally, nearly all species showed a number of teratological changes in flower structure and development.

The following shows the process of reduction division (meiosis phases) in all species used in this study (Fig. 1).

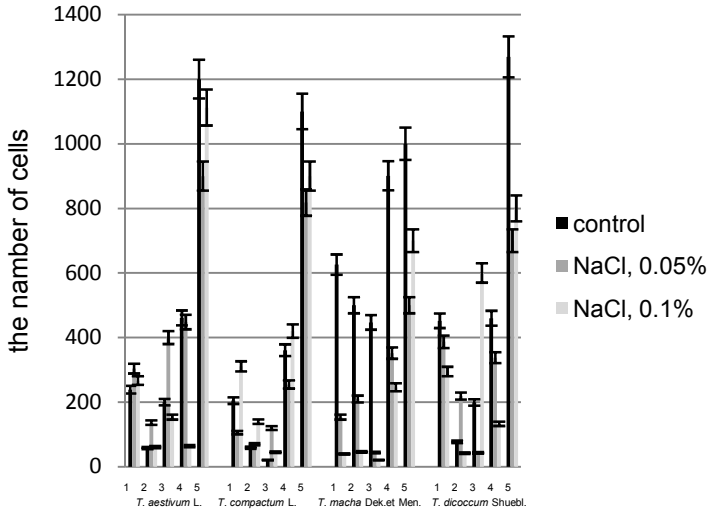


Fig. 1. Characteristics of meiosis in wheat species under low-salt soil conditions
s: 1: Anaphase I; 2: Dyad; 3: Metaphase II; 4: Anaphase II; 5: Tetrads. In this experiment, the number of normal cells was 100% of the total number of observed cells in all phases and all types of wheat. Lag phases of meiosis was time-dependent.

Triticum aestivum L. in the control variant and high-salt soil, female and male gametophytes developed normally. At 0.05% salinity in the pollen mother cells (MCP) at the stage of diakinesis, metaphase I (MI) was observed in 27 cells at 21 closed bivalents; anaphase I (AI) cells, metaphase II (MII) (Fig. 2a) dyads and anaphase II (AII) dyads had normal tetrads during meiosis. Mature pollen grains were all fertile. When the soil contained 0.1% NaCl, female and male gametophytes developed normally but some of the anthers showed lag phases during meiosis and marked separate, sterile anthers (Fig. 2b). PCR revealed that in diakinesis, 18 MI cells showed 21 closed bivalents, cells of AI, dyads, MII, AII, tetrads, which were normal during meiosis. Mature pollen grains were all fertile.

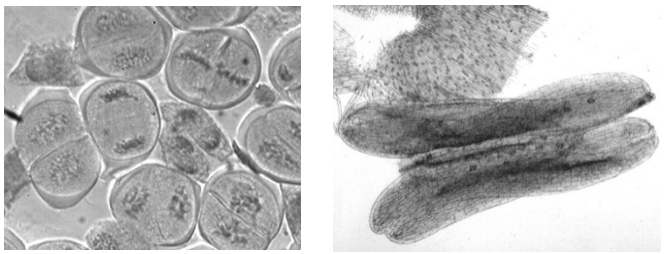
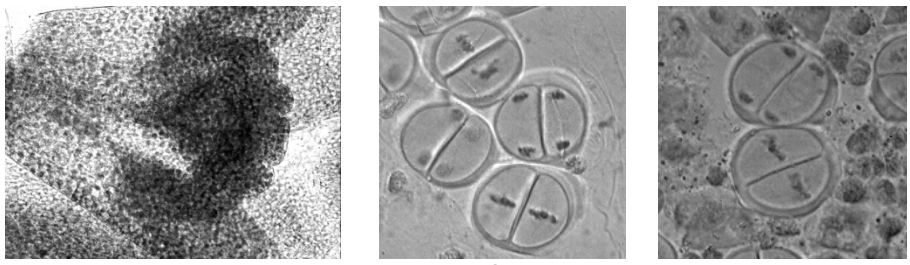


Fig. 2. (a) Normal cell division (dyads, metaphase II) in the plant species *T. aestivum* L. on saline soil 0.05% NaCl and (b) Sterile anther in *T. aestivum* L. flowers in soil containing 0.1% NaCl.

T. compactum L. in the control variant male and female gametophytes were developed normally. But even at a concentration of NaCl 0.05% was observed hypoplasia of both

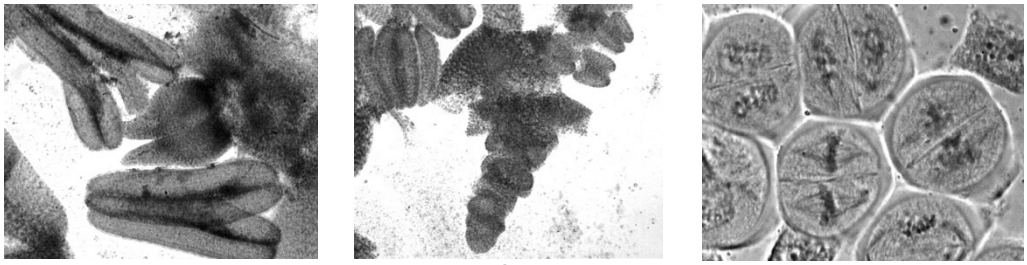
female and male gametophyte. Large flowers were out of the ovary and anthers (Fig. 3a), but where the anthers were developed – were observed during meiosis (Fig. 3b, 3c). In the PCR, in step diakinesis in MI, 12 and 21 cells were observed closed bivalent and 4 cells – by 19-20 bivalent closed and open bivalent 1-2 in a cage, with a NaCl concentration of 0.1% observed many flowers with very small anthers and there, where there was normally developed anthers – the running processes of meiosis. At the stage of MI, 17 cells were monitored by PCR 21 closed bivalents and in 4 cells – 20 closed bivalents and one bivalent open in a cage.



a b c

Fig. 3. (a) Flower without ovary and anther on 0.05% NaCl soil; tNormal division of cells (metaphase II and anaphase II) in a plant species *T. compactum* L., (b) control and (c) 0.1% NaCl

T. macha Dek. et. Men in the control variant, male and female gametophytes developed normally. At 0.05% and 0.1% NaCl, several anthers displayed slow development INC; – instead of all the stages of meiosis (metaphase, anaphase) marked only the pachytene stage. At 0.1% salt concentration, metaphase I, 10 cells were closed bivalent, 19–20 were closed bivalent and 1–2 cells were open bivalent. In single flowers, marked formation of immature bodies or "clusters" consisting of ovaries, anthers and overgrown tissue were observed (Fig. 4b, 4c).

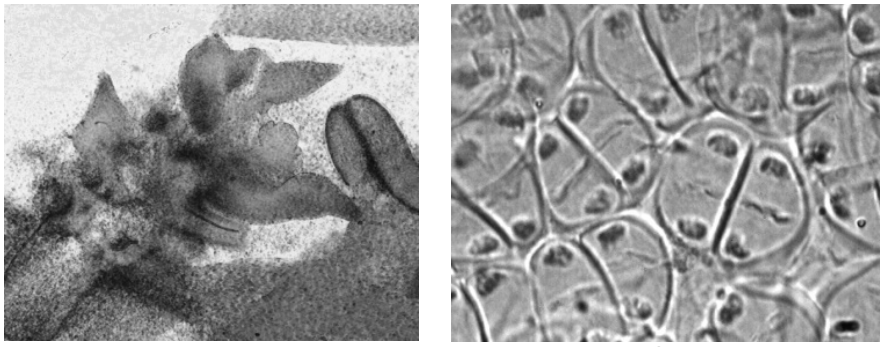


a b c

Fig. 4. Development of flower organs in plants of T. macha Dek. et. Men., (a) normal development control, (b) Development of several flowers per stem blight, combined into "clusters" on saline 0.1% NaCl soil and (c) Division of cells (metaphases II) in the plant species T. macha Dek. et. Men. on 0.1% soil

T. dicoccum Schuebl during normal development of male and female gametophytes in the control variant in 0.05% NaCl in the INC stage MI, 10–14 closed bivalent cells were observed; later, the cells showed anormal course of meiosis. Mature pollen grains were all fertile. Sporadic formation of immature bodies, "clusters" consisting of ovaries, anthers, and

overgrown tissue were observed. Anthers developed normally during meiosis. In 0.1% NaCl soil, in the INC stage MI, 14–16 closed bivalent cells were observed; cell AI, dyads, MII, AII, tetrads were normal during meiosis. Several flowers had very small anthers. However, all mature pollen grains were fertile.



a b

Fig. 5. (a) Teratological abnormalities in development of flowers in *T. dicoccum* Schuebl. on 0.05% NaCl soil and (b) Division of cells (anaphases II) in a plant species *T. dicoccum* Schuebl. on 0.1%NaCl soil.

Increasing the salt concentration in the soil in all species studied except *T. compactum* L. significantly reduced plant height, including *T. aestivum* L. (from 94.5 ± 2.1 cm to 75.8 ± 3.3** cm), at *T. dicoccum* Schuebl. (from 114.2 ± 2.3 cm to 91.5 ± 0.8** cm), and at *T. macha* Dek.et.Men (from 98.0 ± 1.5 cm to 82.5 ± 1.1** cm), and decreased ear length, most significantly for *T. aestivum* L. (from 7.8 ± 0.2 cm to 6.2 ± 0.3** cm). Although a concentration of 0.05% NaCl was used, *T. dicoccum* Schuebl. and *T. macha* Dek. et. Men showed growth stimulation of the ear, while *T. dicoccum* Schuebl showed low stimulation of plant growth. Increasing the salt concentration led to a decrease in the value of these indicators. A decline in the number of spikelets per spike, the number of kernels per ear, and the mass of grains per plant was observed under low-salt stress for all species. The largest decrease in the number of spikelets per spike was observed in *T aestivum* L. (from 13.5 ± 0.4 pcs to 11.7 ± 0.1** pcs), in *T. dicoccum* Schuebl. (from 18.7 ± 0.7 pcs to 14.0 ± 0.7** pcs), and in *T. macha* Dek. et. Men (from 16.8 ± 0.3 pcs to 15.0 ± 0.4* pcs), grain weight per plant in *T. compactum* L. (from 2.5 ± 0.1 g to 1.7 ± 0.1** g) and *T. macha* Dek. et. Men (from 2.0 ± 0.1 g to 1.0 ± 0.1** g). And the greatest decrease in "the number of grains per ear" with increasing NaCl concentration was found in hexaploid spicies *T. aestivum* L. (from 25.7 ± 0.9 pcs to 22.3 ± 0.9* pcs) and *T. compactum* L. (from 28.7 ± 0.8 pcs to 24.0 ± 0.7** pcs). Generally, increasing the salt concentration often led to increased variation of the studied parameters. The *T. compactum* L. species was the most stable in the parameters of growth in stressful conditions and *T. dicoccum* Schuebl. on the parameters of productivity.

The effect of salt concentration on growth parameters and grain productivity of the examined species are shown in Table 1.

Table 1. Characteristics of various species of wheat in low-chloride salinity (0.05% NaCl) of the soil in the pot experiment

The conditions of plant cultivation conditions	Plant height, cm		Length of ear, cm		The number of spikelets on the main spike, pcs		The number of grains in the main spike, pcs		Grain weight of plants, g	
	M ± m	Cv, %	M ± m	Cv, %	M ± m	Cv, %	M ± m	Cv, %	M ± m	Cv, %
***T. aestivum* L. (A^uA^uBBDD)**										
control	94.5±2.1	5.0	7.8±0.2	5.1	13.5±0.4	6.2	25.7±0.9	8.1	1.7±0.0	4.5
NaCl, 0.05%	80.3±2.1*	6.7	7.2±0.2	5.1	13.0±0.4	6.8	24.5±0.8	3.4	1.7±0.0	4.5
NaCl, 0.1%	75.8±3.3**	13.7	6.2±0.3**	15.9	11.7±0.1**	8.9	22.3±0.9*	10.5	1.5±0.1	7.3
***T. compactum* L. (A^uA^uBBDD)**										
control	87.3±2.9	8.5	4.5±0.1	5.1	14.2±0.3	5.3	28.7±0.8	5.3	2.5±0.1	7.5
NaCl, 0.05%	84.3±3.5	8.1	4.3±0.1	5.8	13.8±0.3	5.4	27.7±0.9	7.9	1.8±0.0**	12.3
NaCl, 0.1%	78.7±3.0	8.9	4.2±0.3	14.8	12.8±0.8	13.4	24.0±0.7**	6.9	1.7±0.1**	18.0
***T. dicoccum* Schuebl. (A^uA^uBB)**										
control	114.2±2.3	4.3	7.0±0.2	6.3	18.7±0.7	8.7	25.3±1.1	9.2	1.0±0.0	0
NaCl, 0.05%	117.5±1.4	2.3	7.5±0.3	7.3	14.2±0.7**	10.4	24.0±1.1	9.1	1.0±0.0	0
NaCl, 0.1%	91.5±0.8**	1.8	7.0±0.1	4.5	14.0±0.7**	12.7	24.0±1.1	11.2	1.0±0.0	0
***T. macha* Dek. et. Men. (A^uA^uBBDD)**										
control	98.0±1.5	3.2	6.2±0.3	8.9	16.8±0.3	4.5	23.3±0.6	6.5	2.0±0.1	8.9
NaCl, 005%	91.0±0.7*	1.7	6.8±0.3	14.4	15.5±0.3*	3.5	23.0±0.6	6.2	1.3±0.1**	11.9
NaCl, 0.1%	82.5±1.1**	2.7	6.2±0.2	6.6	15.0±0.4*	4.7	22.7±0.9	8.2	1.0±0.1**	18.9

*Characterents * and ** indicate the accuracy of the t-test at the 0.05 and 0.01 levels of significance, respectively.*

Seeds collected from the plants were re-sown in the field site the following year and meiosis was analyzed. Meiosis in the offspring of all studied species and varieties was normal under low salt stress; synapsis in the cells during metaphase I was, as observed for 21−14 closed bivalents, cells at anaphase I, dyad metaphase II, anaphase II, tetrads were normal during meiosis. However, some of the microsporocytes grade *T. aestivum* L. and type *T. compactum* L. and *T. macha* Dek. et Men. in anaphase II showed asynchronous division. Thus, *T. aestivum* L. grade with a salt concentration of 0.1% to 19.5% showed asynchronous cell division. In *T. compactum* L. 21% of cell division was asynchronous in the presence of 0.05% NaCl and 18.5% in the presence of 0.1% NaCl. In *T. macha* Dek. et Men., 22.5% of the cells underwent asynchronous division at 0.1% NaCl. All genotypes showed many flowers with very small anthers but some of anthers showed lag phases during meiosis (Fig. 6).

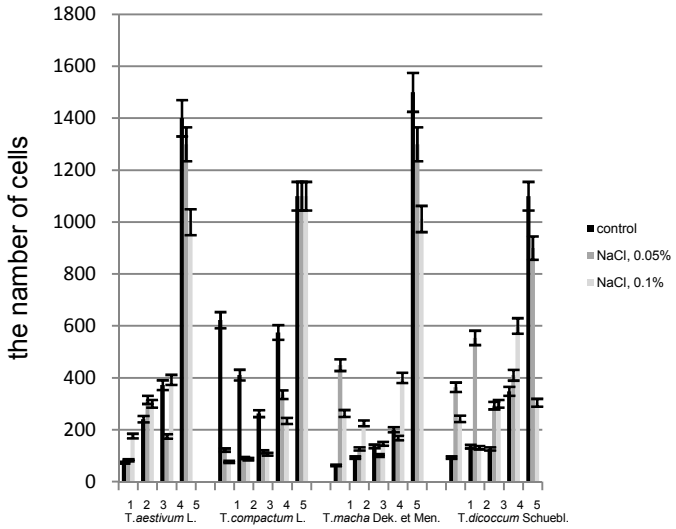


Fig. 6. Characteristics of meiosis in offspring of wheat species subjected to salt stress, one year after salt stress

Note: In this experiment, the number of normal cells was 100% of the total number of observed cells in all phases and all types of wheat except for anaphase II. T. aestivum L.: anaphase II – 85% of normal cell division observed, and 19.5% of the cells – asynchronous; T. compactum L.: 0.05% NaCl at anaphase II – 79.0% in normal dividing cells in asynchronous 21.0%, with 0.1% NaCl – 81.5% of the cells in normal division 18.5% – asynchronous; T. macha Dek. et. Men.: anaphase II – 77.5% of the cells in the normal division of 22.5% – asynchronous. Lag phases of meiosis was time-dependent.

However, for the parental plants and offspring under low-salt conditions, showed the following specific teratological abnormalities in flower development:

a) Underdeveloped and immature ovaries and dense pubescence anthers were established;
b) Stem blight development in plural number of flowers, which were connected to structures resembling "clusters";
c) The absence of some flowers with normal glumes and lemmas, as ovaries and anthers;
d) Separate sterile anther shapes and sizes.

Hierarchical structural and functional organization of living organisms involves a tiered system of responses to external stimuli. The fact that the cellular level of organization may be treated as system failure due to stress at the organismal level can sometimes receive different assessment [13]. The use of different methods for screening stresstolerance forms increases validity of the results. Thus, maximum performance can be achieved by selection during assessments of a germinating grain in a cell culture or gametophyte selection [14].

Stress factors can lead to cell death. However, meristematic tissue eliminates the most-affected elements and recovery is possible at the expense of the full repopulation of cells. An intracellular recovery system also helps prevent death, but often in repair errors that lead to increased genetic abnormalities in the genome and can confer adverse effects to the following generations. A comprehensive approach to address the long-term effects of exposure to stress creates a holistic view of these changes [15].

Exposure to stress affects the development of generative cells [16]. However, few studies have been performed to examine cytological characterization and the influence of abiotic stresses on the teratological aspects of generative cells formation in flowers.

Morphogenesis is important in cases of abnormal growth and development. This mechanism can often solve particularly difficult problems associated with the emergence and development of a trait. Therefore, abnormal growth and its role in morphogenesis should be further examined because understanding morphogenesis and trait development allows selection at various levels of the growth process. Particularly teratoma generative cells are important. Moreover, structural changes may occur in the entire flower whole or in parts of the flower [17].

Reproductive cells, as well as vegetative cells, are eventually restored after radiation stress. However, large doses of irradiation affect seed progeny for several years; these subsequent generations show reduced viability characterized by a high teratological effect [15].

Nearly all forms of terates show uniformity in flower structure. Teratological flowers are easily observed and allow for the study of taxonomy [17].

Such teratological changes in the development of male and female gametophytes were observed in the preparation of double-haploid barley *in vitro*. For hybrid forms, the embryos were repeatedly passaged in fresh medium [18]. Flowering glumes showed numerous immature ovaries and anthers, which resulted in sterile pollen grains, on the flowering glumes and lateral and central flowers with "clusters" of immature anthers and ovaries, and at flowering glumes side flowers develop additional scales with numerous immature anthers and ovaries, flowered, and glumes sometimes had a long lanceolate, sharply differing in size from the normal oval-shaped scales of barley. However, meiosis in all the studied plants, including the original forms and hybrids involving dihaploids, proceeded normally; metaphase, anaphase, and tetrads occurred, and pollen grains in bulk are fertile. Previous reports have described a similar external appearance of teratomas Salix lapponum and Salix alba showing non-specific morphological changes under the influence of technogenic [19].

Thus, our results confirm the non-specificity of teratological changes in generative cells of cereal plants due to various adverse factors.

Analysis of meiosis in different species of wheat plants under low-salt conditions showed that plants and their offspring were significantly affected by the stress conditions. – In most

cases, all stages of meiosis were completed in all examined species of wheat pollen. However, the experiment revealed that the number of grains per spike in different wheat species under low-salt conditions was significantly reduced. This phenomenon has a different physiological cause, which plays an important role in the observed chloride salinity teratological reproductive disorders and deceleration of the male and female gametophyte development, which may lead to insufficient fertilization, apparently normal pollen grains, the formation of defective grains, and incomplete formation of grain in the ear. Consequently, even low-salt significantly affects the transition from vegetative to reproductive growth of grasses, which is directly related to grain productivity.

Importantly, long-term effects of salts, which were expressed during flower development in the spikelets, were observed in the next generation. Additional genetic studies should be conducted to examine the mechanism of these effects.

The terates in different species of wheat shown in the next generation allow us to make the assumption that the general laws of the effects of stress and that the responses of plant organisms to stress represent genetically fixed mechanisms that emerged in the early stages of evolution.

Thus, detection of teratological changes in flowers can be used to estimate the physiological state of plants in relation to growth and productivity.

4. CONCLUSION

Low soil salt slowed the development of male and female gametophytes, and various teratological changes in the formation of reproductive organs caused lower productivity, including fewer grains per ear of wheat. Developmental disorders affecting generative organs in wheat under salt stress are genetically determined. By detecting the presence of teratological changes in flowers, it is possible to estimate the physiological state of plants in relation to growth and productivity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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