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ASPECTS REGARDING THE MITOTIC ACTIVITY IN SOME VARIETIES OF WHEAT (*TRITICUM AESTIVUM*) GROWN IN THE EXPERIMENTAL FIELD AT SCDA CARACAL

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Abstract

Wheat has a great importance as a food product, providing a large part of the carbohydrates and proteins needed by humans and representing more than half of the calories consumed by mankind. The nutritional importance of wheat and its beneficial effects on human health have been emphasized in many studies, both in agricultural and medical fields.
In the current climate conditions and the predicted changes, as a result of the global warming phenomenon, drought resistance is a priority objective of wheat breeding programs. A decrease in soil water potential due to drought decreases the mitotic activity and rate of leaf expansion.
This study presents some results regarding the mitotic activity (MA) in some varieties of wheat (Triticum aestivum) grown in experiences set up at SCDA Caracal (Romania). Water stress caused a decrease of MA in al genotypes and changes in cell wall integrity. The mitotic index (MI) was an efficient index for detecting the mitotic activity deterioration process, which ranged from 9.2 to 17.6%. There was also some alterations of normal mitosis and appearance of some chromosomal and nuclear abnormalities in wheat cells (disorganized cells, chromosome fragmentation, sticky chromosomes, unequal anaphase, ghost nucleus, etc.), with values ranging from 7.15 to 26.08%. These results suggest a reduction of mitotic activity and increase of oxidative stress in wheat under drought conditions.

***Key words**: wheat, drought, leaves, mitotic activity*

INTRODUCTION

Plants are exposed to various environmental stresses during growth and development under natural and agricultural conditions. Among these, drought is one the most severe environmental stresses affecting plant productivity.

Cereals are increasingly exposed to unfavourable growing conditions, from the point of view of climatic factors, especially extreme temperatures and drought. These are major impediments, which limit growth and development, and implicitly, production (Paunescu et al., 2021, 2023). Therefore, the problem of drought resistance and stabilization of cereals productivity to ensure global food security has become a very topical one.

Wheat (*Triticum aestivum* L.) is one of the most significant cereal crops grown in the semi-arid and temperate regions of the world (Rosculete et al., 2021, 2023). Wheat belongs to the *Poaceae* family, which includes the tribe *Triticeae*, the division with the most economically important cereals. The tribe *Triticeae* includes 14 genera, grouped into the subtribes *Triticinae* and *Hordeinae*, and the production of amphiploids and interspecific hybrids suggests that there is genetic or cytoplasmic compatibility between the different genera (Sakamoto, 1973). The most representative wheat species of this tribe are *Triticum aestivum* L. hexaploid (2n = 6x = 42), *Triticum turgidum* L. tetraploid (2n = 4x = 28) and *Triticum monococcum* L. (2n = 2x = 14) (Peng et al. 2011).

Wheat is very important as a food product, providing a large part of the carbohydrates and proteins needed by humans and representing more than half of the calories consumed by mankind. Globally, wheat is contributing to 20% of the caloric and protein intake of human population (Cosgrove, 2021; Ișlicaru et al., 2021).

The forms in which wheat is used in human nutrition are very diverse, the most widespread being bread. On the other hand, the importance of wheat in animal feed is well known and very important due to its richness in crude protein and carbohydrates. But contamination of feed with microorganisms in ruminants can cause multi-contamination, which prompts the immediate call for specific antibiotic treatments (Cola and Cola, 2021). However, the presence of antibiotics is prohibit in milk intended for human consumption (Cola and Cola 2022, 2023).

Drought affects 60% of the wheat production in high-income countries and 30% in least developed countries (Ahmad et al., 2018). All over the world, multi-annual studies have been carried out on the adaptability and stability of cereals (Roșculete et al., 2021).

To keep up with the demand, modern strategies need to be developed to increase wheat yield under this changing environment (Bonciu et al., 2021a,b; Hunter et al., 2017; Verbeke et al., 2022). In this context, modern biotechnology has a significant potential to contribute to food security and sustainable development (De Souza and Bonciu, 2022 a, b).

Wide hybridization is one of the stresses that might trigger reorganization of the parental genomes. Unequal chromosome division at mitosis has also been reported in many wide hybrids (Fu et al., 2013; Gernand et al., 2005; Tang et al., 2012). Wide hybridization between wheat and rye is an important cytogenetic and breeding tool in wheat (Fu et al., 2013).

Cytogenetic activity in wheat can be disturbed by many factors, besides drought: chemicals (Kopytchuk and Sechnyak, 2012; Zhang et al., 2008), age of seeds (Akhter et al., 1992; Ocom Menezes et al., 2014), etc. Thus, mitotic cell division are affected by deterioration induced by artificial aging stress. Mitotic index reduces along prolonged period of aging stress (Ocom Menezes et al., 2014).

Plant chromosomes are excellent biomarkers for mutagenicity studies, and they can be used in the biomonitoring of various stressors (Alkan et al., 2022; Ciğerci et al., 2023).

MATERIALS AND METHODS

For this study, 10 different wheat genotypes were selected, marked with G1, G2...G10.Tip of wheat roots containing the meristem tissue were collected and maintained in the fixative solution (3:1 - ethanol: acetic acid) for 24 hours at room temperature. Also, the samples were stored in 70% ethanol in the fridge, until the preparation of microscope slides.

The wheat meristematic root tips were hydrolyzed in 1 M HCl at for 16 minutes, stained with Feulgen for 30 minutes, and squashed in 45% acetic acid. To determine the mitotic index, 500 cells were counted per control group. All slides were examined at Optika microscope at high power (100X). Chromosomal aberrations were scored according to standard protocols described by Yi et al. (2005). The frequency of chromosomal aberration and micronucleus formation were expressed as the number of cells with chromosomal aberration in 3 replications.

RESULTS AND DISCUSSIONS

The experiments were carried out on a typical argic chernozem soil (non-carbonic), with a well-defined profile and insignificant differences regarding the physical, hydric and chemical properties.

In 2023, the months of August - October were characterized by drought. Temperatures were extremely high for September and October. These were registered against the background of a lack of acute precipitation.

Wheat cytogenetic analysis was carried out through the root-tip smear method. Root cell chromosomes of plants are suitably utilized for research purposes like studying chromosomal abnormalities due to their ease of access, large size, and detectable characteristics.

The mitotic results followed by observing the root-tip cells demonstrated mitotic depression, various types of chromosomal abnormalities, and changes in the MA (Table 1).

In this study, mitotic index (MI) was an efficient index for detecting the mitotic activity deterioration process (Figure 1), which ranged from 9.2 (G5) to 17.6% (G8).

Table 1. The mitotic activity in some wheat genotypes grown in the experimental field at SCDA Caracal

G	TA	CI	CP	CM	CA	CT	MI %
G1	500	439	22	15	13	11	12.2
G2	500	427	29	18	16	10	14.6
G3	500	443	18	14	15	10	11.4
G4	500	426	27	21	15	11	14.8
G5	500	454	16	12	14	4	9.2
G6	500	421	35	10	19	15	15.8
G7	500	422	32	16	18	12	15.6
G8	500	412	43	24	18	3	17.6
G9	500	438	25	16	11	10	12.4
G10	500	441	20	16	17	6	11.8

G=Genotypes; TA=Total number of analysed cells; CI= Number of cells in Interphase; CP= Number of cells in Prophase; CM= Number of cells in Metaphase; CA= Number of cells in Anaphase; CT= Number of cells in Telophase; MI=Mitotic index.

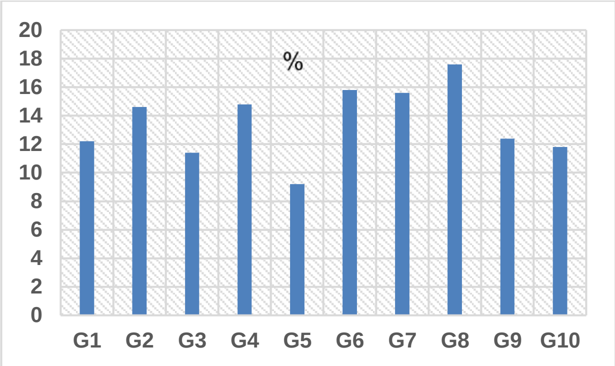

Figure 1. The variation of the mitotic index (MI %) in some wheat genotypes.

The cytological qualities of the wheat meristematic roots were negatively influenced by drought stress through increasing temperatures.

Numerous cytological abnormalities were noted, including disorganized cells, chromosome fragmentation, sticky chromosomes, unequal anaphase, and ghost nuclei (Figure 2). The highest and percentages of chromosomal abnormalities were evidenced in the genotypes G2 (24.1%) and G9 (26.8%). At the same time, the lowest percentage of abnormalities was found in G8 genotype (7.15%) (Figure 3).

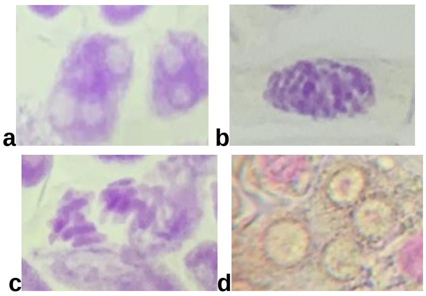

Figure 2. Some cytological abnormalities in wheat genotypes: disorganized cells (a); sticky chromosomes (b); chromosomes fragmentation (c); ghost nuclei (d).

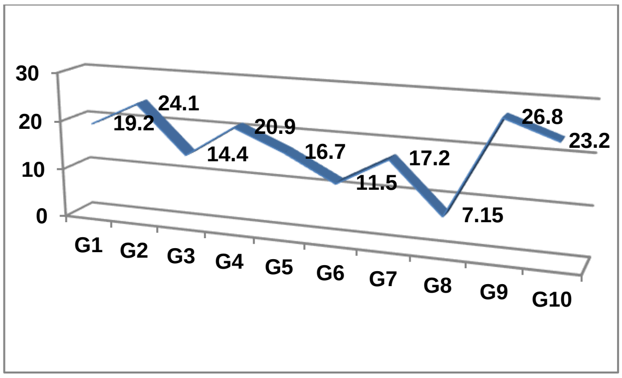

Figure 3. The percentages of chromosomal abnormalities in wheat genotypes in drought condition

Mitotic events determine interphase nuclear morphology (Jevtić et al., 2014). But under the influence of various stress factors, cellular disorganization can occur.

Chromosome stickiness mainly occurs in prophase and metaphase, and it is defined by shortened and thickened chromosomes (Pekol et al., 2015). Chromosome fragmentation is a relevant biomarker of mitotic cell death (Stevens et al., 2007). Also, ghost nucleus may be an indicator of cell death (Mehendiratta et al., 2012).

Drought induces biochemical, physiological, morphological, and genetic responses in plants. As Pekol et al. reported (2015), salt and drought lead to the induction of chromosome aberrations and produced different mitotic abnormalities including chromosomal stickiness, c-mitosis, and micronuclei formation, which indicated their action on the mitotic spindle.

Drought is effectively one of the most destructive natural disasters in terms of loss of life resulting from its consequences such as widespread crop failure, wildfires and water stress. Experts warn that by 2050, droughts may harm approximately three quarters of the world's population (Vogt et al., 2018).

Drought is usually accompanied by heatwaves, with very high temperatures and extremely unfavourable consequences for plants. Heat stress causes great disturbances in plants metabolic processes (Dos Santos et al., 2022). The main factors that limit the obtaining of stable harvests and that have an influence on the growth; development and productivity of plants are climatic factors as well as other factors that represent their consequences, such as osmotic stress. There is a necessity to understand the physiology more in depth and how this physiology is impacted by drought stress (Verbeke et al., 2022).

The response of plants to stress differs depending on the intensity and duration of the stress they are exposed to. Cell membranes are formed by a phospholipid bilayer and proteins. Remarkable metabolic engineering efforts have demonstrated the plasticity of vegetative tissues such as leaves to synthesize and package large amounts of storage lipids, which enable future applications in bioenergy and the engineering of high-value lipophilic compounds (Guzha et al., 2023).

Before anthesis, rewatering restored the xylem and osmotic potential quickly. After anthesis, the osmotic potential was not completely recovered in the stem and flag leaf and remained low for longer. Also, the water flow is prioritized to the flag leaf at the expense of the stem water reserves (Verbeke et al., 2022).

The results reported by Ocom Menezes et al. (2014), have shown that a reduced number of dividing cells were observed in seeds submitted to prolonged period of aging. Chromosomal abnormalities in mitotic cells of aged seeds were detected with the occurrence of micronuclei, chromosome breaks and bridges.

Drought susceptibility index can help to screen more stable genotypes for drought tolerance.

Chromosome fragment formation might be due to chromosomes' stickiness followed by separation failure (Taghvaei et al., 2023). The chromosomes disorganization at this stage may be caused by some disturbances in mitotic spindle. Similar results were reported by Özmen et al. (2022), when drought stress had a reducing effect on the mitotic index and caused chromosomal abnormalities to barley. Also, the G1 and G2 stages increased numerically compared to the control group, while after the PEG+polyamine applications, the G1 stage decreased compared to its control. These results suggest that the application of polyamines at the appropriate time and dose can greatly alleviate the negative effects of drought stress on plant growth and development (Özmen et al., 2022).

Assessment of cytogenetic variability is a highly effective method of identifying the overall impact of environmental factors on the physiological state of plants, and on the unit of heredity (Popova, 2009).

Drought stress causes damages at the cellular, tissue, and organ level by preventing the plants from getting water, while it is seen that the plants have developed various defense mechanisms against the situation (Pinheiro et al. 2004).

According to Ishfaq et al. (2024), drought stress reduced the photosynthetic efficiency, water potential, transpiration rates, stomata conductance, and relative water contents by 18-55% in wheat varieties as compared to control. Oxidative damage is also involved in seed aging; the deleterious role of Reactive Oxygen Species (ROS) in seeds is due to their high reactivity toward biomolecules, including proteins, sugars, lipids and nucleic acids (Ocom Menezes et al., 2014).

One of the main goals of genetic engineering is to produce stable inheritance and expression of drought-tolerant plants carrying single or multiple-desired traits in the following generations.

CONCLUSIONS

Drought conditions during the growing season resulted in a significant decrease in mitotic activity to wheat genotypes.

The cytological qualities of the wheat meristematic roots were negatively influenced by drought stress, through increasing temperatures.

The mitotic results followed by observing the root-tip cells demonstrated mitotic depression and various types of cytological abnormalities, including disorganized cells, chromosome fragmentation, sticky chromosomes, unequal anaphase, and ghost nuclei.

These findings indicate that the number of cells entering mitotic division was reduced and suggest that drought might have mitodepressive impacts on cell division.

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