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Effects of Different Dam Waters on *Alliumcepa* L. And *Tradescantiapallida* H. Plant Species

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ABSTRACT

Inthisresearch the effect of dam waters which are taken from four different dams around Canakkale-Turkey were used for planting Allium cepa roots and Tradescantia pallida plant species. For comparing the effects of different water sources on these plants, Allium cepaL. root tip test, and Tradescantiapallida stamen-hair mutation (Trad-SHM) test were realized respectively 72 and 96 hours after dam water application. All experiments were carried out under controlled conditions in the laboratory. According to our research results, it seems that different water resources coming from different dams has been decreased the mitotic index values in A. cepa between %8.5-%14.5 72 hours after dam water application when compared with the control group. For the T. pallida pink mutation in stamen hair cells has been increased the pink mutation events (PMEs) between 2.58-5.30 folds 96 hours after dam water application when compared with the control group. These results consistently showed that mutation levels showed differences depending on the dam locations in both plants.

Keywords: *Allium cepa*, *Tradescantiapallida*, pink mutation, mitotic index.

INTRODUCTION

It increases the potential danger in the environmental pollution that the human being constantly creates waste products and rapid industrial development especially in recent years. Biodiversity and human health are under serious threat from pesticides [1]. However, the chemical warfare where the pesticides are used is the most used method in agricultural struggle [2]. The pesticides can have been turned into the mutagenic and carcinogenic agents that show effects as toxic agent vectors on people by vegetation [3]. Many researchers have informed that the pesticides have the mutagenic and carcinogenic effects [4-5].

This threat is more on the vegetation; because it -as primary producers in and ecosystem- takes place in the bottom rungs of the food chain, can also affect other creatures and has economic importance. The plant's transpiration, photosynthesis, enzymeaction, nucleicacid construct, chlorophyll biosynthesis have affected many physiological events such as membrane damage and the disruption of hormonal balance [6]. Genotoxicity assays are used specifically to evaluate the genotoxic potential of environmental and industrial effluent samples. The meristematic mitotic cells of plant roots are appropriate indicator cells in detecting the clastogenicity of environmental pollutants, especially in monitoring water and soil contaminants [5, 6].

Allium cepa L. and *Viciafaba* L. have been used to evaluate chromosomal aberrations [7, 8].There is a large number of short-term bioassays for detecting genetic toxicity. These assays utilize a wide range of organisms and cell types, and measure a variety of different genetic changes. The genetic damage detected represents DNA damage, from point mutations to chromosomal mutations [9]. Some test methods have been developed for certain organisms,

such as the *Salmonella* sp.-Ames test, *Tradescantia* sp.-micronucleus test and stamen hair mutation assay, and *Vicia* sp.-micronucleus test [10].

The Trad-SHM bioassays constitute a unique dual system that can determine the clastogenicity and mutagenicity of various pollutants in water, air and soil [11, 12]. Both assays have been widely utilized in monitoring and assaying for environmental mutagens in the past 20 years [13]. The increased frequency of MCN and the elevated pink mutation rate in *Tradescantia* inflorescence are indicators of clastogenicity and mutagenicity resulting from exposure to environmental pollutants [14].

Aim of this research was to investigate the effects of different dams water resources on plant genomes. Two relatively sensitive and rapid tests, namely *Allium cepa* root tip test and *Tradescantiapallida* stamen-hair mutation test were used in this research to find the genetically damage levels.

MATERIALS AND METHODS

Plant Material and Cultivation

In this research, *A. cepa and T. pallidaplants* were used as a plant source. Thecertified Allium bulbs havebeenprovidedfrom CeylanAgricultural Companies.*Tradescantia* plant cultures were obtained from CanakkaleOnsekiz Mart University, Faculty of Science Department of Biology.

In Vivo Assay

*A. cepa*bulbswere immerged into glass test tubes (2x10 cm) and held for three days in distilled water for germination. Bulbs were germinated under controlled growth chamber (25±2 °C, 16/8 photoperiod) using 3 replicates each of which included 10 onions bulbs.

T. pallida cuttings were sown in plastic pots (50x18x18 cm) containing a mixture of 3:1 soil-peat. Plantlets were grown under controlled growth chamber (25±2 °C, 16/8 photoperiod) and organized with 3 replicates, each of which included 20 plantlets. For reproduction of these plantlets, they were rooted for six months and development was maintained.

Application of Dam Waters

Dam waters collected in to sterilized 5 lt glass sampling bottles from four different dams from different locations of Canakkale-Turkey.Bottles were kept in the dark till application.

Water which is taken from four different dams were applied to Tradescantia plants by spraying to the flowers and irrigated once in a two days for 4 days long. In addition to these four groups, control groups were also prepared by spraying sterile distilled water for each group. In Allium bulbs, dams waters were added directly to the glass test tubes. In the same way, control groups were also prepared by adding sterile distilled water.

Analysis Procedure

Allium cepa Root Tip Test

In *A. cepa* 72hours after dam water application, root tips were taken into the mixture of ethanol/acetic acid (3:1), and hydrolysis was performed with these root tips with 1N HCl for 11 minutes. Following hydrolysis, slides were prepared for each concentration as repetitives, and these slides were observed using an Olympus CX-31 microscope. Dividing and non-dividing cells in each slides belonging to each groups, 1000 mitotic cell were counted triplicate for calculating the mitotic index values.

Tradescantiapallida H. stamen hair mutation test (pink mutation)

Young inflorescences were selected for each application groups consisting of approximately 1200 stamen hair cells belonging to 12 flowers. One flower is containing around 6 stamens, each stamen containing around 25 stamen hairs, each stamen hairs containing around 20 stamen hair cells which had been immersed in a mixture of 1:1 glycerol: water for each replicate in each different group. The stamen hairs were aligned in the manner usually used for dissection needles, and then examined with a binocular microscope which is connected to the PC screen.

The aim of this observation was to determine the pink mutations and count their number in each stamen hair cells. As a result of the genotoxic substances applied, mutations were observed as pink in stamen hairs [13]. For each groups with replicate totally 3600 stamen hair cells has been counted. Olympus SZ51 microscope was used to obtain clear images of the slides (40x magnification) to determine mutant stamen hair cells.

RESULTS AND DISCUSSION

Allium cepa mitotic index results

According to our research results, it seems that different water resources coming from different dams has been decreased the mitotic index values in *A. cepa* between %8.5-%14.5 72 hours after dam water application when compared with the control group (Fig. 1).

Alliumcepa L.	**72 hrs. (%MI)**
Control	9.24 ± 2.63
Umurbey Dam	8.45 ± 1.40
Bakacak Dam	8.35 ± 1.48
Bayramiç Dam	7.90 ± 1.56
Atikhisar Dam	7.95 ± 1.47

Figure 1 - Mitotic index results in *A. cepa* 72 hours after dam water application. (MI:mitotic index)

***Tradescantiapallida* H. stamen hair mutation test**

According to our research results, it seems that different water resources coming from different dams has been increased the pink mutation events (PMEs) in *T. pallida* between 2.58-5.30 fold 96 hours after dam water application when compared with the control group **(Fig. 2).**

***Tradescantiapallida* H.**	**96 hrs. (PME in 3600 SHC)**
Control	60 (0.017)
Umurbey Dam	215 (0.059)
Bakacak Dam	325 (0.090)
Bayramiç Dam	258 (0.072)
Atikhisar Dam	158 (0.044)

Figure 2- Pink mutation events results in *T. pallida* 96 hours after dam water application.

(PME:pink mutation event, SHC:stamen hair cells)

Somatic mutations that take part in the stamen hairs were seen as pink mutation events (PMEs). Mutation frequencies increased depending on the different water resources.

There are many researches about the degree of cytotoxicity and genotoxicity of different water sources which under pressure of environmental problems. Recent years model plant tests like Allium root tip test and Tradescantia stamen hair mutation tests (pink mutation event PME) mostly using for determining the degree of genotoxicity.

Athanásio et al. (2014) have been investigate the genotoxic, mutagenic, and cytotoxic potential of surface waters in urban streams using *A.cepa* and analyzes the applicability of this assay for environmental monitoring. Physicochemical evaluation were indicated that all samples had various degrees of environmental impact. All samples increased the frequency of chromosome aberrations [15]

Gomes et al., (2014) have been analyzed the cytotoxic and genotoxic potential of the Guandu River's (Brasil) waters, through the use of the *A. cepa* test system. The analyses of 5000 cells per treatment showed that all the points studied had some degree of cytotoxicity and/or genotoxicity [16].

Olorunfemi et al. (2014) have been studied on the analysis of drinking water because of it's importance as contaminated water jeopardizes both the physical and social health of all people. They were analyzed for physicochemical parameters and subjected to cyto-genotoxic evaluation using the *A. cepa* assay. Their results showed that most of the parameters of the lake water in both seasons exceeded World Health Organisation (WHO) permissible limits. At the end of the research they found significantly decreasing in mitotic index and they found chromosomal aberrations like bridges, fragments, sticky chromosomes [17].

Kwasniewska et al. (2013) have been used Tradescantia-micronucleus (Trad-MCN) test for the genotoxicity of water samples from two natural water reservoirs in Poland. Research results suggested the Tradescantia 4430 was a more sensitive bioindicator of genotoxicity than *C. capillaris* hairy roots [18].

When compared with the results of the other researches, most of the researchers used Allium and Tradescantia for evaluate the genotoxicity in plants. Our results showing that different water resources coming from different dams has been changed mitotic index and pink mutation levels depending to the locality and exposure time.

CONCLUSION

In conclusion, it seems that different water resources coming from different dams has been decreased the mitotic index values in *A. cepa* between %8.5-%14.5 72 hours after dam water application when compared with the control group. For the *T. pallida* pink mutation in stamen hair cells has been increased the pink mutation events (PMEs) between 2.58-5.30 folds 96 hours after dam water application when compared with the control group. These results consistently showed that mutation levels showed differences depending on the dam locations in both plants. In addition, location of dam which is affected from different chemicals and harmful substances from agricultural fields, household waste and environmental pollution factors as can also be effective factors on water resource quality. Therefore, it is necessary to protect the water reservoir from this kind of pollutant for public health. According to our research, if we provide moresafewaterresourceswhich is not pollutedfromenvironmantalfactorspublichealthwill be betterdaybyday.

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