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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (15): 1957-1960, 2008
ISSN 1028-8880

Structural Changes in Root Tips of Wheat (*Triticum aestivum* L.) in Response to Olive Oil Mill Wastewater

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Abstract: Toxic effects of the wastewater were investigated ultrastructurally in root tips of *Triticum aestivum*. As a result, wall and nuclear degradations, disruptions in all cytoplasmic membranes, irregular nucleus shapes and cellular organization defects were densely detected. Besides, germination ratio, total protein contents, DNA contents and root-shoot growth were found to be decreased significantly when compared to the control group. Results were compared with those of recent studies regarding excessive Na^{+}, Fe^{+2}, P, polyphenols and acidic pH toxicity.

Key words: Olive oil waste water, toxicity, ultrastructure, root, wheat

INTRODUCTION

Waste water from the olive oil industry creates an important environmental problem marked especially in Mediterranean countries where a great number. of plants are involved in the production and refining of olive oil. Various types of treatment have been proposed for detoxification of this wastewater, biological, either aerobic (Benitez *et al*., 1997a; Sidal and Taşkın-Özkale, 2003) or anaerobic (Marquez *et al*., 1998) or chemical, mainly oxidative, either ozonation (Benitez *et al*., 1997b) or photodegradation (Miranda *et al*., 2002). In addition, few studies have been done about toxicity of the wastewater (Filidei *et al*., 2003). Also OMWs contain high ratios of Na, Fe as well as polyphenols and poses a significance with its acidic pH (Sassi *et al*., 2006; Arntzen *et al*., 2008). In present study, we reported that OMWs had direct toxic effects on the nuclear material since it led to formation of multinucleate cells and nucleus fragmentation and several mitotic abnormalities in root tips of *Triticum aestivum*. Besides, germination ratio and total protein contents were found to be decreased significantly when compared to the control group, and natural chromosome numbers varied (Aybeke *et al*., 2000). When checked in the literature, we could not find any study investigating OMWs toxicity on plants, using TEM technique. Therefore, the purpose of this study is to investigate ultrastructurally cytotoxic effects of OMWs on plant root tips.

MATERIALS AND METHODS

Research was conducted in 2008 February in Trakya University, Biology Department. In the study, seeds of *Triticum aestivum* L. cultivar MURAT-1 obtained from Trakya Agricultural Research Institute in 2008 were used. Olive oil wastewater (OMWs) was provided from Olive oil factories in Gömeç, Edremit-Ayvalik (Turkey), where it was directly released without being subject to any detoxifying process. The main characteristics of the OMWs used in this study were pH 4.5-5; polyphenols 3.4±0.3 g L^{-1}, Na^{+} 450 (ppm), Fe^{+2} 42 (ppm), P 500 (ppm), K.O.I. 90-100 (g L^{-1}).

Firstly surface sterilization of caryopsis was carried out. While control group materials were treated with only distilled water, those of test groups were treated with only one of the three concentrations of OMWs, pure 1/1, 1/10 and 1/100 for 3 h (Aybeke *et al*., 2000). Germination assays were carried out in petri dishes incubated at 24°C. Ten root tips from each groups were excised, fixed in 2% glutaraldehyde buffered to pH 7.2 in 0.07 M phosphate buffer for 2 h at 4°C, after washing in buffer solution post fixed in 1% aqueous osmium tetroxide for 2 h at 4°C, dehydration in a graded alcohol series, and embedded in EPON-812. Root tips was sectioned using a Reichert ultramichrotome. Sections were stained with 1% uranyl acetate followed by 2% lead citrate. A Zeiss 9A TEM was used to study the root tip sections.

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The DNA content of 30 early prophase nuclei (4C) was also calculated for the preparation of *Triticum aestivum*. Assuming that the 4C value of *T. aestivum* cv. Chinese Spring was 69.27 pg (Bennett and Smith, 1976), the absorption values for each dose were converted into absolute amounts. The differences in the DNA content were tested by analysis of variance (ANOVA) and comparisons between means were performed with the Tukey test ($p \leq 0.05$) were done on DNA content data. For total protein content was detected by quantifying the amount of nitrogen by selective ammonium electrode and was then calculated as %N ×6.25. After germination, the root lengths measured up to 7 days and their stem lengths were measured up to 14 days (Joaquin *et al*., 2007) and germination rates were calculated. The data were analyzed through one way analysis of variance (ANOVA) to determine the effect of treatments, and least significant difference (LSD) proofs were performed to test the statistical significance of the differences between means of treatments.

RESULTS AND DISCUSSION

In the control roots (germinated under absence of OMWs), normal nuclear structures such as, large-homogenous nucleoplasm, regular nuclear envelope and nucleoli were observed (Fig. 1). Regarding test groups, in especially 1/1 concentration, local disintegration of walls,

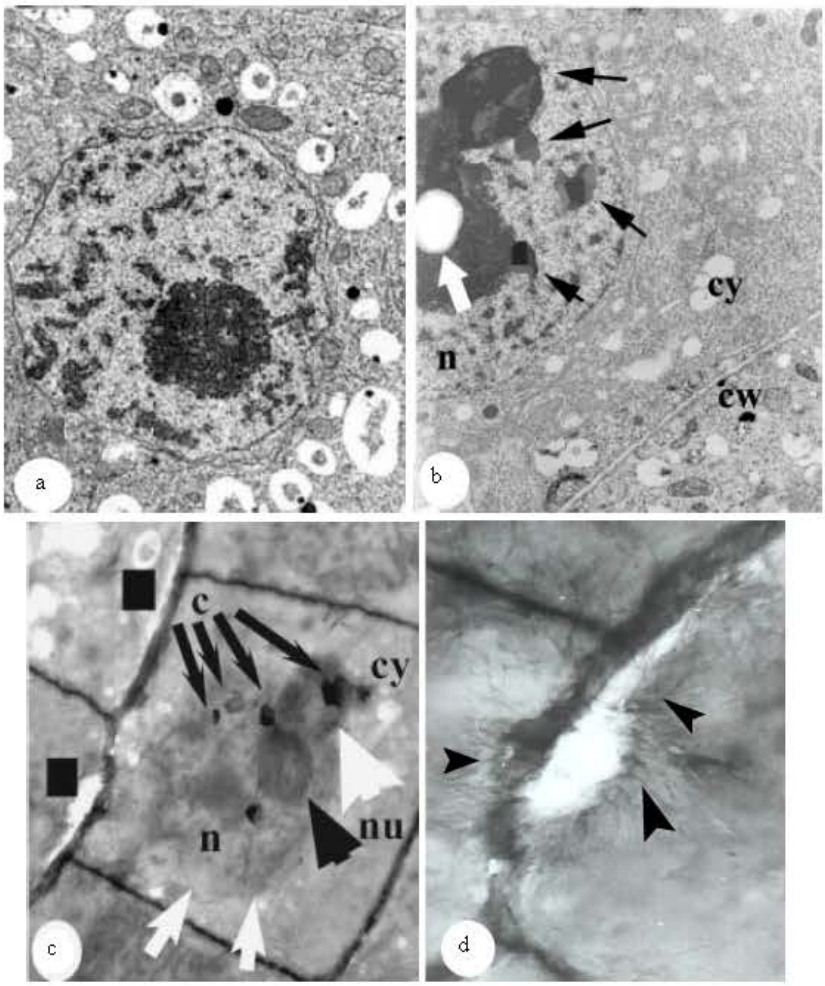

Fig. 1: (a) Comparison of nuclei (n) from control root cell, nucleolus, nuclear envelope and cytoplasm appeared normal, x4200. a-d Test groups with OMWs; (b) in treatments with 1/10 concentrations, big or small crystals (arrows) were in nucleus, and one nuclear vacuole (white arrow) in nucleolus x5300. (c) in pure (1/1) concentration, several nuclear disorganizations: two nucleolus connecting to each other (black/white arrowheads), one of which (white arrowhead) was found in cytoplasm instead of nucleus, crystals (arrows) in nucleus, and cell wall defects (■), x3600, enlarged in (d) (note unidentified fibrous materials (arrowheads), x12000) (n: nucleus, nu: nucleolus, c: crystal, cy: cytoplasm, cw: cell wall)

electron-lucent areas and unidentified fibrous materials seen (Fig. 1c, d). Additionally nuclei are irregular in outline with dense nucleoli and nuclear envelope disappear (Fig. 1). Considering nucleoli, they were distorted and nuclear vacuoles occurred inside (Fig. 1b, c). Similar results were recently reported by Stefanowska *et al.* (2003), showed that cellular degradation occurred because of localisation of numerous phenolic deposits in the nuclei, cytoplasm plasmalemma and the cell wall. In another study concerning Na^+ toxicity, revealed that salt-treatment caused plasmolysis, vesiculation of cellular membranes and degradation of cytoplasm (Mitsuya *et al*., 2003). Additionally under low pH conditions, both plasma membrane disintegrations occurred and lignification reduced cell walls degradability by 25% (Grabber *et al*., 2003). The most striking result is cellular disorganisation, as follows: Crystals were scattered over the nucleus, nucleoli were both contiguous, and were found in the cytoplasm, instead of nucleus (Fig. 1b, c). Lavid *et al.* (2001) also showed typical crystallization in epidermal cells, and consequently noted increased lipid peroxidation, DNA damage, enzyme inactivation and oxidation of protein sulphydryl groups.

According to biochemical and statistical parameters, total protein contents, DNA contents, root - shoot growth and germination ratio were significantly decreased in compared to the control (Table 1, 2). On the basis of these results, *Triticum aestivum* indicates a dose-dependent response. This fits well with the above mentioned structural effects of OMWs on root tip, suggesting that the inhibition of germination and seedling resulted from inhibition of DNA synthesis mechanism and of course protein synthesis process.

Based on present findings and these interpretations, we suggest that toxicity of OMWs is paralleled by toxic effects of each factor (polyphenols, Na^+, Fe^{+2} and acidic pH) contained within. Especially toward 1/1 concentration, it's toxic effects were more destructive. Authors think that with these factors at toxic levels, OMWs effect synergistically all of the cell metabolism and hence disturb seedling, growth and development process of plants. For example, polyphenols are generally regarded to be an environmental pollutants, and their pathologic, mutagenic, genotoxic effects and chromosomal aberrations were reported by Shoji *et al.* (2004) and Sassi *et al.* (2006). In plants of Lamiaceae family, polyphenolic compounds, according to the average number of insects for each case shows mobility, mortality (Regnault-Roger *et al*., 2004). Similarly Na^+ toxicity also caused a decrease in, chlorophyll content and naturally photosynthetic activity; plants suffer from dehydration (Ueda *et al*., 2003). Consequently OMWs causes loses in agricultural production. The water must be used after several chemical purification process producing ethanol, yeast, phenolic compounds, fertilizer and antioxidants (Chtourou *et al*., 2004; Sassi *et al*., 2006). Extractions of these substances will both provide to use and increase economic importance of the waste water.

Table 1: Seed germination, root and shoot length of *Triticum aestivum* treated with OMWs relative to control treated with distilled water

	Root			Shoot			Seed germination (%)		
Parameters	1/1	1/10	1/100	1/1	1/10	1/100	1/1	1/10	1/100
OMWs	0.5*	2.3*	2.5	3.4*	8*	12	65*	78*	88-91*
Control	7	7	7	16	16	16	100	100	100
F-ratio	18,63^A			102,65^A			92,04^A		

Results are means±SD. Means with asterisk are significantly different from each other ($p<0.05$) according to LSD test, A: significant at 0.01 level

Table 2: Effect of OMWs on total DNA and protein content of *T. aestivum* root tip cells

Test groups	Sample size	DNA contents means±SD*	Protein contents
1/1	15	51.43±0.28^A	0.1219±0.011^D
1/10	16	53.09±0.12^A	0.2345±0.006^E
1/100	16	60.89±0.28^B	0.2876±0.0108^F
Control	19	66.24±0.07^C	0.3205±0.0023^F

SD: Standard Deviation, Means with the same letter(s) do not significantly differ in their nuclear DNA content at 0.05 level

CONCLUSION

OMWs showed that the waste water blocked fairly the germination and even disrupted developmental process of wheat because of its' high toxic ingredients. Despite its' damaging effects, it may be possible to use the wastewaters to obtain several useful side products such as ethanol, yeast, phenolic compounds, fertilizer and antioxidants. As solution, the water must be purified before using as irrigation. So, it raises the possibility of both protecting ecological life, and harvesting much more crops, and finally deriving useful side products of the waste water.

ACKNOWLEDGMENT

The authors thank to Prof. Dr. Dürdane KOLANKAYA for providing reference and her valuable comments.

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