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Identification and characterization of up-regulated genes in the halophyte *Limoniastrum monopetalum* (L.) Boiss grown under crude oil pollution

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Abstract Differential display method was applied to transcripts extracted from leaves of *Limoniastrum monopetalum* to identify genes that are differentially expressed in response to crude oil pollution. The results showed that 201 bands with different molecular sizes were differentially expressed in polluted plants. Ten cDNA bands were considered to be consistently over-expressed under crude oil stress and selected for sequencing. Comparative analysis of these cDNA sequences allowed us to classify them into six categories: (1) enzymes increase its activity under petroleum stress and were a good marker of petroleum stress (e.g. xanthine dehydrogenase, metallothionein type 2, and arginine decarboxylase), (2) nitrogen metabolism (e.g. glutamine synthetase and amidophosphoribosyltransferase), (3) drought genes (e.g. CPRD2), (4) salinity stress (e.g. retrotransposon protein), (5) plant growth (e.g. aminocyclopropane-1-carboxylic acid and ribulose-1,5-bisphosphate carboxylase oxygenase), and (6) transport related genes like proton-dependent oligopeptide transport (POT) family protein. Coincidently with the differential display results, the amount of the total protein differed significantly between unpolluted and polluted plants ($T = 3.687$, $P < 0.006$). The electrophoretic patterns (SDS–PAGE) for water soluble proteins revealed that 11 peptides with

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different molecular masses disappeared and eight different peptides were synthesized in polluted plants. The results of up-regulated genes identified in this study may explain the way that *L. monopetalum* populations established on the crude oil polluted soil and vigorous vegetative growth of adult plants.

1. Introduction

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Soil contamination with hydrocarbons causes extensive damage of the local system, since accumulation of pollutants in plant tissue may cause death or mutations [2]. The effect of oil in the soil on vascular plants has been shown to be quite different. Previous observations indicated that crude oil at a certain concentration can stimulate plant growth [1,9,18]. This has been attributed to the presence of substances such as naphthenic acids [7]. It was reported that low level of oil pollution could be easily degraded by natural rehabilitation in soils, increase organic matter in soil and improve the fertility, physical, and chemical properties of the soil. Oil at both moderate and high levels is quite toxic to all higher plants [5]. Shortly after surface application of oil, most plants lose their leaves, show losses in root viability, and die. Initial recovery and revegetation have generally been from woody plants with protected buds or from vegetative regrowth from surrounding non-oiled soils [23,38,39,60]. The toxicity of petroleum hydrocarbon at higher concentrations has been linked to displacement of nutrients and nutrient linkage [3]; reduction in available phosphorus and total nitrogen [8] and interference with soil chemotoxis by crude oil [49], ending with growth retardation [58].

Plants are involved, both directly and indirectly, in the degradation of petroleum hydrocarbons into products (e.g. alcohols, acids, carbon dioxide, and water) that are generally less toxic and less persistent in the environment than the parent compounds [20]. Soil enzymatic activity which can be determined quite promptly and precisely is a reliable indicator reflecting the current biological state of the soil [63]. Accordingly, the indirect role that plants play in the degradation of petroleum hydrocarbons involves the release of enzymes from plants. These enzymes are capable of transforming organic contaminants by catalyzing chemical reactions in soil. Schnoor et al. [51] identified plant enzymes as the relevant agents in the transformation of contaminants mixed with sediment and soil. Isolated enzyme systems included dehalogenase, nitroreductase, peroxidase, laccase, and nitrilase. For example, Aryl hydrocarbon hydroxylase is an enzyme found in mammals, fish, fungi, and vascular plant roots exposed to oil or related hydrocarbons [22,38,45,46].

The molecular knowledge of uptake, biochemistry of PAH stress responses in plant transformation and storage of toxic metals and their derivatives in plants have led to promise biotechnological applications [11,17]. The first goal in phytoremediation is to find a plant species which is resistant to or tolerates a particular contaminant with a view to maximizing its potential for phytoremediation. Resistant plants are usually located growing on soils with underlying metal ores or on the boundary of polluted sites. The potential use of phytoremediation at a site contaminated with hydrocarbons was investigated. Plant growth was found to vary depending upon the species. Presence of some species led to greater total petroleum hydrocarbon (TPH) disappearance than with other species or in unvegetated soil.

Limoniastrum monopetalum (Plumbaginaceae) is a dwarf shrub of whitish-gray aspect inhabiting arid or saline, often coastal, environments [32]. In Egypt it is widely distributed along the Western Mediterranean costal land [13]. Former to contamination with crude oil, the natural population of *L. monopetalum* was associated with *Arthrocnemum macrostachyum*, *Zygophylum album* and *Zygophylum suaedauinosa*. After contamination most of the species were eliminated except *L. monopetalum*. Previous study of *L. monopetalum* populations revealed that the morphological characters between polluted and unpolluted populations were significantly variable. Plants growing in the polluted sites were physically larger than that of the unpolluted sites. The study concluded that RAPD profiles in *L. monopetalum* of unpolluted and polluted plants varied in band intensity, disappearance of bands, and appearance of new PCR products and the difference were correlated to sources of pollution [21]. However, the precise mechanism of the plant response to the toxicity of crude oil is not clear. It varies according to the degree of habitat disturbance and differs between different plants and among populations of the same species inhabiting different habitats. A better understanding of the plant mechanisms growing in oil-contaminated soil is necessary. This information would assist in the development of phytoremediation as a viable alternative to mechanical and chemical approaches in remediation of oil-contaminated soils.

In this study, the differential display technique [34,35] was applied to transcripts extracted from unpolluted and polluted leaves of *L. monopetalum* plants. RNA fingerprinting using arbitrarily primed PCR (RAP) [61,62] allows the semiquantitative simultaneous comparison of the abundance of several hundred randomly sampled RNAs. The goal of this study was to identify and analyze up-regulated RNAs of *L. monopetalum* growing in soil containing crude oil. For more confirmation, on the translation level, the total protein was analyzed. The present work aims to contribute to basic knowledge on the mechanisms of tolerance of crude oil pollution in *L. monopetalum*.

2. Plant sample collection from unpolluted and polluted sites

The study sites are located 120 km west of Alexandria in the Egyptian Western desert. Three populations collected from sites affected by crude oil leakage (Western Desert Petroleum Company (WEBCO)). The oil content in the soil ranges