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Changes in Abscisic Acid and Indole-3-Acetic Acid Concentrations in *Funalia trogii* (Berk.) Bondartsev & Singer and *Phanerochaete chrysosporium* Burds. ME446 Subjected to Salt Stress

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Abstract: Variations in abscisic acid (ABA) and indole-3-acetic acid (IAA) concentrations were studied in *Funalia trogii* (Berk.) Bondartsev & Singer and *Phanerochaete chrysosporium* Burds. ME446 subjected to salt stress. The levels of ABA and IAA were determined by gas chromatography (GC) and UV-VIS spectrophotometry. The levels of hormones vary in the regulation of growth and in response to stress. It was found that ABA concentrations were higher in the salt stressed groups than in the control groups, whereas IAA concentrations were lower. These results show that, in fungi, ABA concentrations are positively and IAA concentrations negatively correlated with salt stress.

Key Words: *Funalia trogii*, *Phanerochaete chrysosporium* ME446, ABA, IAA, salt stress

Tuz Stresine Maruz Bırakılan *Funalia trogii* (Berk.) Bondartsev & Singer ve *Phanerochaete chrysosporium* Burds. ME446'de Absisik Asit ve İndol-3-Asetik Asit Konsantrasyonlarındaki Değişimler

Özet: Tuz stresine maruz bırakılan *Funalia trogii* (Berk.) Bondartsev & Singer ve *Phanerochaete chrysosporium* Burds. ME446'daki absisik asit (ABA) ve indol-3-asetik asit (IAA) konsantrasyonlarındaki değişim incelendi. ABA ve IAA seviyeleri Gaz kromatografisi (GC) ve UV-VIS spektrofotometre kullanılarak belirlendi. Büyümenin regülasyonunda hormon seviyeleri değişmektedir. Deney gruplarında ABA konsantrasyonlarının kontrollerden daha yüksek olduğu belirlendi. Bununla birlikte tuz stresi şartlarında IAA konsantrasyonları kontrollerden daha az bulundu. Bulgularımız funguslarda ABA konsantrasyonlarının tuz stresi ile pozitif, IAA konsantrasyonları ile ise negatif bir korelasyon gösterdiğini ortaya koymaktadır.

Anahtar Sözcükler: *Funalia trogii*, *Phanerochaete chrysosporium* ME446, ABA, IAA, tuz stresi.

Introduction

Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development, although other processes, such as stomatal movement, may also be affected. Plant hormones have also been referred to as phytohormones (Davies, 1995; Iten et al., 1999). Abscisic acid (ABA) regulates seed maturation and maintenance of embryos. It mediates several adaptational responses of the growing plant

towards environmental cues such as desiccation and cold, as well as salt stress, and acts as a negative growth regulator (Iten et al., 1999). Indole-3-acetic acid (IAA) and ABA are synthesized in plants and fungi (Blakesley et al., 1991; Lopez-Carbonell et al., 1994; Ünyayar et al., 1997). Production of ABA under salt stress is well known and fungal growth under salt conditions has been investigated (Adler et al., 1982; Awad and Nair, 1989). However, the effect of salt stress on the level of IAA needs to be investigated. Therefore, the objective of the present work was to determine the relationship of ABA and IAA under salt stress in *Funalia trogii* (Berk.)

Bondartsev & Singer and *Phanerochaete chrysosporium* Burds. ME446.

Materials and Methods

We used strains of *Funalia trogii* and *Phanerochaete chrysosporium* ME446. Fungi were cultivated in a SBM (stock basal mineral) medium containing 0.2 g/L KH_2PO_4 , 0.1 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g/L $\text{NH}_4 \cdot \text{H}_2\text{PO}_4$, 10 g/L glucose. Then 200 mM NaCl was added to the medium (Durusoy et al., 1995) and 1 mL of conidial suspension was inoculated into 100 mL conical flasks, each containing 50 mL of liquid growth medium. The pH levels of the media were adjusted to 4.5. All media were sterilized (15 min, at 15 atm). Culture media: SBM (control) and SBM+NaCl. These culture flasks were incubated at 30°C for 10 days. All treatments were repeated three times.

Purification of ABA and IAA

After incubation, mycelia and culture media were separated by filtration. Mycelium fractions were combined with 100 mL of methanol:chloroform:2N ammonium hydroxide (12:5:3 v/v/v) and then frozen at -60°C after the addition of 1 mg/100 mL butylated hydroxy toluene (BHT).

Extraction, purification and quantitative determination of ABA and IAA in mycelia and culture media were carried out according to Ünyayar et al. (1996).

All experiments were methylated with diazomethane. The methylated ABA and IAA samples were subjected to gas chromatography (GC) and UV-VIS spectrophotometry. GC analysis of me-ABA and me-IAA were carried out on a Hewlett Packard model 6890 Chromatograph equipped a 30 m capillary column (film thickness 0.25 µm) with 5% phenyl methyl siloxane. The carrier gas was nitrogen at a velocity of 62 cm/s. Detector temperature 270°C, flow rate 40 mL/min. Also samples were analyzed at 265 nm with a UV-VIS spectrophotometer (Shimadzu A-160 model).

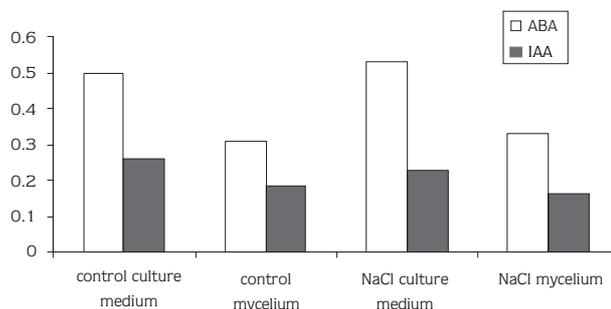
Growth measurements

In order to determine the fresh weight mycelium (FWM), fungus was filtered (No:1 Whatman), weighed and taken off wet tare filter paper.

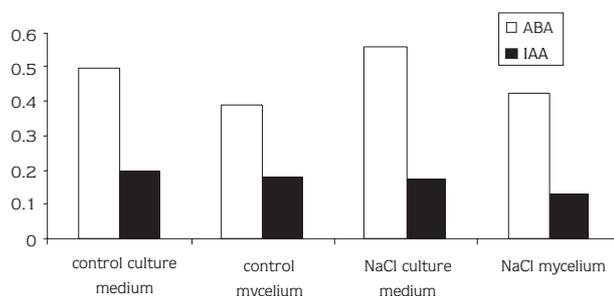
Statistical analysis was done with Statistica 5.0.

Results and Discussion

Changes in ABA and IAA levels related to NaCl stress are shown in Table 1 and Figure 1.



F. trogii



P. chrysosporium ME446

Figure 1. Variations in ABA and IAA contents in the culture media and the mycelia in *F. trogii* and *P. chrysosporium* ME446 under saline conditions.

ABA contents were found to be 0.499 µg/mL in *F. trogii* and 0.496 µg/mL in *P. chrysosporium* ME446, and 0.310 µg/mL in *F. trogii* and 0.390 µg/mL in *P. chrysosporium* ME446 in the control groups (culture medium, mycelium, respectively) (Table 1 and Figure 1). The ABA concentrations in NaCl-treated fungi (0.533 µg/mL and 0.561 µg/mL in culture medium and 0.330 µg/mL and 0.425 µg/mL in mycelium, respectively) were higher than those in the control groups. There were significant differences in ABA levels of NaCl-treated culture media ($P < 0.05$). The IAA concentrations were always lower than those of ABA. IAA contents were found to be 0.259 µg/mL in *F. trogii* (Berkeley) Bondartser & Singer and 0.198 µg/mL in *P. chrysosporium* ME446 Burdsall, and 0.187 µg/mL in *F. trogii* and 0.178 µg/mL in *P. chrysosporium* ME446 in the control groups (culture medium and mycelium, respectively). The IAA

Table 1. ABA and IAA contents in the culture media and the mycelia in *Funalia trogii* (Berkeley) Bondartser & Singer and *Phanerochaete chrysosporium* ME446 under saline conditions. Values are the mean of three replicate culture and error is shown as \pm standard deviation.

	Control		NaCl	
	Culture medium	Mycelium	Culture medium	Mycelium
<i>F. trogii</i>				
ABA content ($\mu\text{g/mL}$)	0.499 \pm 0.065	0.310 \pm 0.004	0.533 \pm 0.006	0.330 \pm 0.029
IAA content ($\mu\text{g/mL}$)	0.259 \pm 0.047	0.187 \pm 0.050	0.227 \pm 0.047	0.166 \pm 0.030
FWM (mg/50 mL)	1190.5 \pm 0.093		1359.9 \pm 0.280	
<i>P. chrysosporium</i> ME446				
ABA content ($\mu\text{g/mL}$)	0.496 \pm 0.072	0.390 \pm 0.049	0.561 \pm 0.008	0.425 \pm 0.047
IAA content ($\mu\text{g/mL}$)	0.198 \pm 0.039	0.178 \pm 0.055	0.172 \pm 0.021	0.128 \pm 0.016
FWM (mg/50 mL)	958 \pm 0.071		852 \pm 0.149	

concentrations in NaCl-treated fungi (0.227 $\mu\text{g/mL}$ and 0.166 $\mu\text{g/mL}$ in *F. trogii* and 0.172 $\mu\text{g/mL}$ and 0.128 $\mu\text{g/mL}$ in *P. chrysosporium* ME446, respectively) were lower than those of ABA. In both of the fungi the ABA concentrations were about three times higher than those of IAA. IAA levels decrease, while the ABA levels increase under stress conditions. Similar results have been reported by Lopez-Carbonell et al. (1996). Hormonal interactions may affect hormonal levels in plant tissues (Lopez-Carbonell et al., 1996; Gadallah, 1996; Hare et al., 1997). Letham et al. (1978) reported that high levels of cytokinins increase the levels of IAA and reduce the levels of ABA. In our study, high levels of ABA may reduce the levels of IAA. The levels of ABA in *P. chrysosporium* ME446 Burdsall were higher than those in *F. trogii*, while the levels of IAA in *F. trogii* were higher than those in *P. chrysosporium* ME446. In contrast, Lopez-Carbonell et al. (1994) showed that both IAA and ABA levels increase

under water stress. Vernieri et al. (1994) reported that ABA accumulated in *Phaseolus vulgaris* L. under stress conditions. Furthermore, Topcuoğlu (1995) demonstrated that the ABA level in *Helianthus annuus* L. cv. Peredovik increased remarkably in 100 mM Na_2SO_4 . In our study, FWM was 1190.5 mg/50 mL in *F. trogii* (Berkeley) Bondartser & Singer and 958 mg/50 mL in *P. chrysosporium* ME446 Burdsall in the control groups. FWM rose in *F. trogii* (1359.9 mg/50mL), while it decreased in *P. chrysosporium* ME446 (852 mg/50mL) in NaCl-treated groups. Fungal growth may be induced in *F. trogii* and reduced in *P. chrysosporium* ME446 Burdsall by stress conditions. The ability of one hormone to affect the level of another is probably of great significance in the regulation of growth and responses to stress (Lopez-Carbonell et al., 1996). Further studies are in progress to relate the results from enzyme activities, hormonal interactions and stress conditions in fungi.

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