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Examination of *Trichoderma* strains isolated from the rhizosphere of vegetables for the purposes of developing environment-friendly in field technologies

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Organic farming is becoming nowadays more and more important in the agriculture. Organic farmlands are exposed to dangerous xenobiotics through distinct pollution drift effects such as wind-driven, pesticide-containing dusts and xenobiotic-containing rains. In order to achieve organic farming, there is a need for the development of new techniques which allow the bioremediation of lands previously used in common, intensive agricultural practice. Organic agriculture also faces the problem of pests including the damage caused by plant pathogenic fungi, therefore the implementation of biological control as a possible, environment-friendly solution is also of increasing importance.

Trichoderma strains were isolated from vegetable rhizosphere samples on dichloran-Rose Bengal medium. After purification of genomic DNA, the PCR amplification of the internal transcribed spacer (ITS1-5.8S rDNA-ITS2) region and its sequence analysis were used for the identification of the isolates at the species level. Altogether, 45 *Trichoderma* isolates were identified from the examined samples. The detected *Trichoderma* species were *T. asperellum*, *T. atroviride*, *T. citrinoviride*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. koningiopsis/T. ovalisporum*, *T. longibrachiatum/H. orientalis*, *T. pleuroticola* and *T. virens*.

In vitro antagonism of selected isolates was examined in dual culture tests and the Biocontrol Index (BCI) values were determined for the particular isolates. Certain *T. asperellum*, *T. virens* and *T. atroviride* isolates proved to possess good *in vitro* antagonistic activities against plant pathogenic *Fusarium solani*, *F. oxysporum*, *Phoma cucurbitacearum*, *Alternaria alternata*, *Botrytis cinerea*, *B. pseudocinerea* and *Rhizoctonia solani* strains.

Fungicide susceptibilities were measured by the microdilution method and the Minimum Inhibitory Concentration (MIC) values were recorded. Ten fungicides were tested in the concentration range of 512 µg/ml to 1 µg/ml. Strain *T. asperellum* SZMC 20866 showed resistance to 9 fungicides and was sensitive only to Maneb (MIC: 256 µg/ml). The *T. atroviride* strain SZMC 20781 showed similar fungicide resistance properties to those of *T. asperellum* SZMC 20866. MIC values of *T. harzianum* SZMC 20770 were 256, 512, 32, 64, 512 and 128 µg/ml for Cyproconazole, Fenarimol, Imazalil, Maneb, Penconazole and Thiram, respectively. The strain most sensitive to the tested fungicides was *T. virens* SZMC 20779.

The effect of temperature on growth in a range of 5 – 40 °C was also examined, and the water activity (a_w 0.997 – 0.922) and pH (2.2 – 8.0) dependence determined in the case of the isolated *Trichoderma* strains. Temperature values of 20-30 °C were optimal for the growth of *Trichoderma* strains, while none of the strains were able to grow at 5 °C. The examined strains were able to grow in a wide range of pH from 2.2 to 8.0, the maximal growth was observed under acidic conditions at pH 4.0. The highest tested a_w value (0.997) seemed to be optimal for the growth of all strains. Only limited growth was observed at 0.945 in the case of only three examined strains.

The results of the recent study suggest that the rhizosphere of vegetables may be a rich source of potential biocontrol agents for environment-friendly, organic agricultural production. We identified 3 *Trichoderma* strains which seem to be very promising for the development of microbial products with multiple beneficial effects for the purposes of organic farming.

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Physiological and molecular analysis of salt stress-induced PCD in tomato

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As saline soils and waters are common around the world, salinity is one of the major abiotic stress which largely limits plant growth and productivity. The ability of plants to tolerate salt stress is determined by multiple biochemical pathways; the most important is that the plant facilitates retention and/or acquisition of water, protects chloroplast functions, and maintains ion homeostasis. Severe salinity induces programmed cell death (PCD) in plants takes place in eukaryotic cells of different origin. One typical hallmark of PCD in plants is an increase in the process of protein degradation which is initiated by reactive oxygen species (ROS) and nitric oxid (NO) and involves the action of proteolytic enzymes. ROS and NO generation is one of the earliest response of plant cells under abiotic stresses. Protein degradation is probably the most important degradation process that occurs during PCD. The total protein content of tomato leaf gradually decreased with increasing concentration of NaCl. This decrease in protein content might be due to the increasing activity of cysteine- and serine proteases. For this reason, many of the genes up-regulated during PCD are proteases. The four major classes of proteases: cysteine, serine, aspartic acid and metalloproteases, exist in plant cells. Genes that encode proteases are activated by different ways. Expression of these genes that encode cysteine proteases has been shown to induced by environmental stress such as salinity. We studied different genes, for instance *MCA1*, *CYP*, *CP*, which encode various types of proteases participating in plant PCD. In addition, inhibitors encoding genes (*PI2* and *LTC*)

and BAX inhibitor-1 homolog gene (*BI1*) were also tested. According to our results in addition to the cysteine proteases their inhibitors also have fundamental role in the regulation of protein degradation. It is very important to see the connection between the processes of salt induced PCD and different stress hormones from which one of the most important is abscisic acid (ABA) which might have a role in this regulatory pathway. ABA is commonly recognized as naturally occurring plant hormone. ABA plays a key role in many developmental processes, from the promotion of seed desiccation tolerance to the synthesis of storage proteins and organ senescence. In addition, ABA acts as an endogenous messenger in the regulation of plant-water status and regulates some aspects of the plant's physiological responses to environmental stresses, such as osmotic stress-induced stomatal closure and salt, drought and cold tolerance. Our first results show that ABA might induce protease activity during PCD.

To gain a better understanding of the salinity stress responses at physiological and molecular level in cultivated tomato we carried out a comparative physiological analysis. Tomato has a medium tolerance to salinity and it can acclimate to high salinity at morphological and physiological level. In addition to the wild-type, an ABA deficient-tomato mutant, *flacca* was studied, too. Plants were treated with sublethal and lethal concentrations of NaCl. The growth of this plant is not inhibited by medium NaCl stress but it is affected by strong one. The salt stress-induced changes in ROS content and in the gene expression level were shown at the beginning of the treatment. Protein content and protease activity were also studied as a function of time. There was a nice correlation between decreased protein content and increased protease activity in the first 24 hours. Finally, we suggest that cysteine proteases might participate in salt-induced PCD in tomato as a function of time depending on intensity of the stress.

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Nuclear function for the actin binding cytoskeletal protein, moesin

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The most dynamic component of the cytoskeleton in every eukaryotic cell is the microfilament network of linear polymers of actin subunits. Extensive research in the past decade has significantly broadened our view about the role actin plays in the life of the cell and added novel aspects to actin research. The discovery of the existence of nuclear actin became evident only recently. Nuclear activities, including transcriptional activation in the case of all three RNA polymerases, export of certain mRNAs and proteins, chromatin remodeling, and nuclear assembly after mitosis, all depend on actin.

Moesin, the well-known cytoplasmic actin binding protein is the only member of the evolutionary conserved mammalian ezrin-radixin-moesin (ERM) protein family in *Drosophila melanogaster*. ERM proteins are responsible for the organization of the cortical actin network and anchor membrane proteins to it. They all have an N-terminal FERM domain, which is a general protein binding domain, a mid-domain which is a flexible hinge region and a C-terminal actin-binding domain. Our laboratory demonstrated previously that moesin is present in the interphase nucleus but the biological significance of this localisation remained unknown.

We are studying currently the exact localisation and function of moesin in the interphase nucleus. Our experiments showed that moesin accumulates as a ring at the nuclear envelope; it is present in the nucleoplasm, in some chromosome regions and occasionally in the nucleolus. We found that the quantity of moesin in the nucleus increases upon heat stress, which suggests a function for moesin in the nucleus and that its transportation into the nucleus is an active process.

To further analyse the chromosomal localisation of moesin, we performed immunostaining experiments on larval polytene chromosomes. Moesin was detected in the euchromatic bands moreover, it also showed colocalisation with the active form of RNA Polymerase II, and the intensity of the accumulation of the two proteins on the chromosomes was identical. Moesin staining was found especially strong in the chromosome puffs which are special euchromatic regions of extremely active transcription sites in the polytene chromosomes. The transcription on a transgene regulated by an inducible promoter resulted in the formation of an extra moesin band in the corresponding chromosome region suggesting that moesin is required for transcription rather than the formation of the puff structure. This idea was confirmed by the finding that the disassembly of the RNA polymerase complex caused by the drug triptolide, resulted in the detachment of moesin from the chromosomes.

We have also performed a preliminary screen to identify the proteins that are responsible for the nuclear transport of moesin. Our results both with cultured cells and in the live animal revealed that the Nup98 protein is involved in the nuclear export of moesin.

In summary, our results demonstrate that besides its cytoplasmic functions, moesin also plays important roles in the nucleus. We have shown that moesin is actively transported to the nucleus where it participates in the process of RNA transcription.

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