BARD

FINAL REPORT

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**Involvement of Epicatechin in the Regulation of
Natural Resistance of Avocado Fruit to Postharvest
Pathogens**

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Title: INVOLVEMENT OF EPICATECHIN IN THE REGULATION OF
NATURAL RESISTANCE OF AVOCADO FRUIT AGAINST
POSTHARVEST PATHOGENS

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Abstract

In this project it was found that the activation of the mechanism of resistance in avocado fruits to *Colletotrichum gloeosporioides* depends on the increase of the level of the preformed antifungal diene. This increase is regulated by the synthesis of the flavonoid epicatechin present in the fruit peel. Epicatechin is an inhibitor of the enzyme lipoxygenase whose activity catalyze the breakdown of the antifungal diene. Increase in epicatechin concentration inhibits the breakdown of the antifungal compound and since the compound is continuously synthesized, both combined processes result in the increase of the antifungal level. Biotic and abiotic elicitors affecting the mechanism of resistance, all activate the synthesis of epicatechin. As abiotic elicitors were tested wounding, ethylene and CO₂ treatments. As biotic elicitors were tested challenge inoculation with *C. gloeosporioides*, *Colletotrichum magna* (a non pathogen of avocado) and also non pathogenic strain of *C. magna*. In all the cases activation of the key enzymes of the phenylpropanoic pathway is followed by an enhance in the level of epicatechin and the antifungal diene. In order to determine the level of regulation by the different elicitors of the mechanism, the genes encoding for key enzymes of the phenylpropanoic pathway were cloned and it was found that the different elicitors regulate the expression of those genes at a translational level. Modulation of the mechanism of resistance could also be done by activation of lipoxygenase gene expression. For this purpose lipoxygenase from avocado was cloned and its over-expression, under the effect of methyl jasmonate, determined.

2. Objectives of the original research proposal

1. To determine, using different elicitor factors, the involvement of epicatechin in the regulation of the level of antifungal diene.
2. To follow the expression of genes coding for enzymes involved in the biosynthetic pathway of epicatechin in diene-induced fruits.
3. To clone the gene encoding for polyphenol oxidase and investigate its pattern of expression in diene induced fruits.

Objectives 1 and 2 were mainly studied by the Volcani Center and UC Riverside, while objective 3 was studied by UC Santa Barbara.

In objective 1 several biotic and abiotic elicitors affecting the level of the antifungal diene were studied. Between the biotic elicitors were tested challenge inoculation with *Colletotrichum gloeosporioides*, *C. magna* a pathogen in cucrbits, and a non pathgenic mutant, path-1, of *C. magna*. Between the abiotic elicitors tested, 30% CO₂ treatment, ethylene and wounding.

3. Relevant data, methodology, results and discussion

Effect of biotic elicitors

A non pathogenic mutant strain of *Colletotrichum magna* induces resistance to *C. gloeopsorioides* in avocado fruits

4. Description of cooperation

A very close cooperation between Dr. N.T. Keen and Dr. Prusky has created during several years of work. As it is observed by the several joint publications the team Keen & Prusky has resulted in the best understanding of the mechanism of avocado fruit resistance to postharvest diseases. This collaborations has resulted in 8 ms that have been published in high standard journals and 3 invited reviews in Journals, books and Annual Review books (See published ms and chapters). Beside this activities several invited talks were presented by the team at different invited meetings. About 13 abstract and proceedings on the work were presented (See Abstracts and Proceedings).

The personal collaboration with Dr. Keen and Dr. Prusky have been very successful. Dr. Prusky consider him self very lucky of the possibility of the long term collaboration with Dr. Keen. The joint discussion, and hard working has resulted in the presented results. Several people were directly involved in the project. Dr. Hasna Hamdan worked as a post-doct in Dr. Keen lab. In Keen's lab also Carol Boyd, a permanent staff at UC Riverside, collaborate in several parts of the project. In Dr. Prusky's an undergraduate student Mrs. Ruth Ardi was involved in the project, together with a permanent staff member in the Volcani Mrs. Ilana Kobilier.

5. Evaluation of research achievements

The study of the mechanism of resistance of avocado fruits to postharvest diseases is considered one of the best understood

systems in postharvest pathology. The results obtained up to year 1993 on the regulation of epicatechin inhibition of lipoxygenase has resulted in the possibility of the control of postharvest disease by the use of antioxidants. In this proposal it has for the first time been demonstrated that the regulation of fruit resistance by epicatechin occurs by the induction of the pathway activating several enzymes. The gene encoding for PAL, CHS, F-3-H and LOX have been cloned and we are now in conditions of developing transgenic avocado plants where the gene flavanone-3-hydroxylase could be over expressed. Beside that it has been clearly differentiated the function of lipoxygenase in inducing susceptibility with methyl jasmonate by catalyzing the breakdown of the antifungal diene.

6. Conclusions

We can conclude that the elicitation of the mechanism of resistance in avocado fruits by biotic and abiotic elicitors occurs by the enhancement of the level of epicatechin. The increase in the level of epicatechin occurs by a direct effect of the elicitors on activation of phenyl propanoic pathway. Between the key enzymes that are activated under this conditions are PAL, CHS and F-3-H. This last enzyme seems to have a key function determining the level of epicatechin and the differential expression of this protein in

different cultivars is closely related to the degree of fruit resistance to postharvest decay.

7. List of publications

Published ms and Chapters

1. Prusky, D., Kobilier, I., Plumbley, R., Fuchs, Y., and Zauberman G. 1993
The effect of CO₂ levels on the symptom expression of *Colletotrichum gloeosporioides* on avocado fruits.
Plant Pathology 42:900-904.
2. Plumbley, R.A., Prusky, D., and Kobilier, I. 1993. The effect of hot-water treatment on the levels of the antifungal diene and quiescence of *Colletotrichum gloeosporioides* in avocado fruits.
Plant Pathology 42:116-120.
3. Prusky, D., and N. T. Keen. 1993
Involvement of preformed antifungal compounds and the resistance of subtropical fruits to fungal decay.
Plant Disease: Feature Article 77:114-119.
4. Prusky, D., Freeman, S., Rodriguez, R.J. and Keen, N.T. 1994.
A non pathogenic mutant strain of *Colletotrichum magna* induces resistance in avocado fruits to *C. gloeosporioides*.
Molecular Plant-Microbe Interactions 7:326-333.
5. Prusky, D., Ohr, H., Grech, N., Campbell, I., Zauberman, Y., Fuchs, Y. 1995.
Evaluation of antioxidant butylated hydroxy anisole and fungicide prochloraz for control of post-harvest anthracnose of avocado fruit during storage.
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6. Wattad, C., Freeman, S., Dinor, A., and Prusky, D., 1995.
A non pathogenic mutant of *Colletotrichum magna* is deficient in extracellular secretion of pectate lyase.
Molecular Plant Microbe Interactions 8:621-626.
7. Prusky, D., Hamdan, H., Ardi, R., and Keen, N.T. 1996.
Induction of biosynthesis of epicatechin in avocado cell suspension treated with enriched CO₂ atmosphere.
Physiological and Molecular Plant Pathology 48:171-178.
8. Prusky, D., Wattad, C., and Kobilier, I. 1996. Effect of ethylene on the activation of quiescent infections of *Colletotrichum gloeosporioides* in avocado fruits.
Molecular Plant Microbe Interactions: In Press.

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In: Novel Approaches to Integrated Pest Management.
Ed. R. Reuveni pp 139-152. Lewis Publishers Inc., Chelsea, Michigan.
2. Prusky, D., 1996.
Pathogen Quiescence in Postharvest Diseases.
Annual Review of Phytopathology 34:413-434
3. Prusky, D. 1996.
Constitutive Barriers and Plant Disease Control. Jack E. Rechcigl Ed. Environmentally Safe Approaches to Crop Disease Control
CRC Press and Lewis Publishers: in Press.

Abstracts and Proceedings

Proceedings

1. Ardi, I., Kobiler, I., and Prusky, D. 1995.
The effect of biological and physical factors on the modulation of resistance of avocado fruit to *Colletotrichum gloeosporioides*.
Proceedings of the 3rd Avocado Symposium. Tel Aviv Israel pp134.
2. Prusky, D., Ohr, H., Grech, N., Campbell, S., Kobiler, I., Zauberman, G. and Fuchs, Y. 1995.
The use of the antioxidant Xedaphen-20 and the fungicide prochloraz for the control of postharvest disease in avocado fruit during storage.
Proceedings of the 3rd Avocado Symposium,. Tel Aviv Israel pp135.
3. Prusky, D., Freeman, S., Rodriguez, R., Keen, N.T., and Kobiler, I. 1995.
Biological control of anthracnose in avocado fruits by non pathogenic strains of *Colletotrichum magna*.
Proceedings of the 3rd Avocado Symposium,. Tel Aviv Israel pp136.
4. Prusky, D. 1996.
New alternatives for the control of postharvest diseases affecting tropical fruits.
5th Siconbiol, Parana, Brasil. 48-58.
5. Prusky, D., Kobiler, I., Ardi, R., and Fishman Y. 1993. Induction of resistance of avocado fruit to *Colletotrichum gloeosporioides* by CO₂ treatment.
Acta Horticultura 343:325-330.

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Expression of lipoxygenase in avocado fruit and its relation to fruit resistance to *Colletotrichum gloeosporioides*.
6th International Congress of Plant Pathology.
Montreal Canada. pp. 229

2. Ardi, R., Kobiler, I., Fishman, Y., Jacoby, B., Prusky, D. 1993.
Activity and expression of key enzymes during induced resistance in avocado against *Colletotrichum gloeosporioides*.
6th Meeting of International Society of Plant Pathology.
Montreal Canada. pp 230.
3. Freeman, S., Rodriguez, R., Keen, N.T., and Prusky, D., 1994.
Induction of resistance in cucurbits and avocado fruits by a nonpathogenic mutant of *Colletotrichum magna*.
Novel Aspects in Host-Pathogen Interactions (Workshop).
Annual meeting of the Israel Phytopathological Society
Phytoparasitica 22:62
4. Wattad, C., Kobiler, D., Dinoor, A. and Prusky, D. 1994.
Pectate lyase is an important factor in pathogenicity of *Colletotrichum gloeosporioides* in subtropical fruits.
4th International Congress of Plant Molecular Biology Amsterdam,
Holland, Abst. 1613.
5. Ardi, R., Kobiler, I., Wattad, C., and Prusky, D. 1996.
Methyl Jasmonate induces susceptibility of avocado fruit to *Colletotrichum gloeosporioides*.
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A-19. Knoxville, TN, USA.
6. Prusky, D., Wattad, C., and Kobiler, I. 1996.
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8th International Symposium on Molecular Plant Microbe Interaction
A-18. Knoxville, TN, USA.
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8. Ardi, R., Kobiler, I., Jacoby, B., and Prusky D. 1996
Effect of ethylene on the mechanism of regulation of resistance of avocado fruit to *Colletotrichum gloeosporioides*. Biology and Biotechnology of the Plant Hormone Ethylene. Hania, Crete, Grece June 1996.