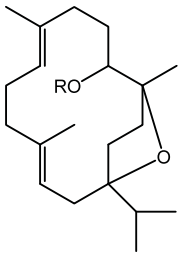
**The molecular characterization of *Boswellia*, and the biosynthesis of incensole acetate – a novel pharmacological agent from an ancient drug**

## Background

The genus *Boswellia* is centered in North-East Africa, where about 75% of the species are endemic. *Boswellia* trees when cut, exude an oily gum resin of great commercial and cultural value. This resin ("frankincense"; "olibanum"; "lebona") is mentioned in numerous ancient texts, and prized by many civilizations. *Boswellia* resin has been used as a major herbal remedy, and in religious and cultural ceremonies for millennia, in Middle East traditions, as well as other traditions. It is mostly used for its putative psychoactivity, and as an anti-inflammatory remedy. It is also an important component of perfumes and toiletries, and is marketed as a food supplement.

Numerous reports attribute the anti-inflammatory and cytotoxic properties of *Boswellia* resin solely to boswellic acid and its derivatives (see Khanna et al., 2007 for a recent review), but up to date no attempts were made to isolate the active components of the resin responsible for its putative psycho-activity.

As the nuclear factor-κB (NF-κB) pathway is central in the inflammatory process, we focused on the inhibitory effect of *Boswellia* resin on this pathway, and isolated two NF-κB inhibitors from the resin, their structures elucidated as incensole acetate (IA) and its non-acetylated form, incensole (IN). Both compounds are macrocyclic diterpenes of the cembranoid group, and are considered to be biomarkers of *Boswellia* species (Hamm et al., 2005). IA inhibited TAK/TAB-mediated IκB kinase activation loop phosphorylation, resulting in the inhibition of cytokine and lipopolysaccharide-mediated NF-κB activation in a specific manner. IA inhibited the synthesis of various cytokines and other inflammatory mediators in macrophage cell lines and primary monocytes. *In vivo*, IA exerted a robust anti-inflammatory effect in a mouse inflamed paw model (Moussaieff et al., 2007).




**Scheme 1. The structure of incensole acetate (IA; R = Ac) and incensole (R = H).**

We examined the effects of IA on the inflammatory process, and on the recovery of neurobehavioral and cognitive functions in a mouse model of closed head injury (CHI). In the brains of post-CHI mice, IA reduced glial activation, inhibited the expression of IL-1β and TNFα mRNAs, and induced apoptosis in macrophages at the area of trauma. Subsequently, hippocampal neurodegeneration was inhibited and a beneficial effect on functional outcome in post CHI-mice was attained (Moussaieff et al., 2008a).

We also examined the effects of *Boswellia* extracts and purified components in several behavioral models, and found that IA elicits an anxiolytic, anti-depressant and sedative effects. Concomitant changes in c-Fos expression in the brain were observed (Moussaieff et al., 2008b). The sedative effects of IA resemble the ones elicited by THC, the active ingredient in marijuana, and when tested in the classical "cannabinoid tetrad" IA exhibits a cannabimimetic profile of action. However, when injected with SR 141716, a specific CB1 receptor inhibitor, the effect of IA on locomotion was abolished, while its effect in the other cannabinoid tests remained with no change (unpublished data). These results imply that IA acts as a partial cannabimimetic compound, probably with relation to its effect on TRP channels. We assayed the compound for its ability to bind to an array of related

receptors, transport proteins and ion channels. IA failed to bind to any of these targets, but robustly activated transient receptor potential vanilloid (TRPV)3 ion channel (Moussaieff et al., 2008b). We repeated the behavioral tests in TRPV3$^{-/-}$ mice and compared them to wild type mice. While significant effects similar to the ones seen in Sabra mice were recorded in wild type C57BL/6 mice, no anxiolytic-like or anti-depressive-like effects were noted in TRPV3$^{-/-}$ mice, suggesting that these effects are mediated via TRPV3 channels. Our results imply that TRPV3 channels in the brain play a role in emotional regulation, suggesting for the first time a role for this channel in the central nervous system (Moussaieff et al., 2008b).

IA is thus a novel pharmacological agent, which may potentially target unaddressed pharmaceutical needs of large segments of the population. The identification of a new prototype drug with beneficial effects in fields of considerable current interest may further open these fields to the discovery of novel drugs for the treatment of diseases that pose unanswered challenges. Moreover, as there is no pharmacological treatment to traumatic brain injury, and current treatments for inflammatory conditions, as well as anxiety and depression conditions are limited by severe adverse effects, such drugs may be of considerable importance. As the synthesis of IA is very difficult, we will try a biosynthetic approach.

**Objectives and methodology**

A. Explore the transcriptome of *Boswellia* species by performing transcriptome analysis of *Boswellia* resin-producing and non-producing tissues. For this aim, we will use a SOLEXA apparatus and the 454 apparatus that is installed in the Weizmann Institute. Only several sequences are currently available from *Boswellia* and we would like to generate a minimum of 30,000 contigs for each tissue.

B. Elucidate the biosynthetic pathway of IA and its derivatives by investigating the composition of IA and related cembrene diterpenoids in *Boswellia* species trough GC-MS conducted on the same tissues employed for transcriptome analyses (see Moussaieff et al., 2007 for a GC-MS finger print).

C. *In-silico* search for specific genes present exclusively in the resin producing tissues and comparison of *Boswellia* transcriptome to *Nicotiana tabacum* transcriptome, a model plant, that is able to synthesize cembrenoids - putative precursors of IA.

D. Genetically engineer the biosynthesis of IA and its derivatives in transgenic *Nicotiana tabacum* and use of these plants as a "factory" for the production of IA which is very difficult to produce synthetically.

E. Use IA and its derivatives as pharmacological tools for the study of TRP channels, their linkage to the endocannabinoid system, and their role in neurodegenerative processes.

For these purposes we intend to:

1. Use the partial cannabimimetic effect of IA, and its robust effect on TRPV3 channel. To this aim we will perform two sets of experiments: 1) Inject IA together with SR141716 – a CB1 specific antagonist to TRPV3 null mice. 2) Inject IA to CB1 null mice, and test the behavioral effects of IA on these mice. This part of the work will be done in collaboration with Professor Raphael Mechoulam from the Hebrew University.
2. Given the neuroprotective activity of IA, a TRPV3 channels activator, and the assumption that TRP channels play an important role in neurodegenerative and neuroprotective processes (Brayden et al., 2008), we intend to use IA as a ligand to TRPV3 channel in the CNS, and examine the possible neuroprotective effects of theis channel. This part of the work will be done in collaboration Professor Esther Shohami from the Hebrew University. We will then screen IA for its effect on an array of other TRP channels. This part of the work will be done in collaboration with Professor Baruch Minke from the Hebrew University and Professor Ardem Patapoutian from the Scripps Institute.

3. We plan to examine whether the neuroprotective effects of IA can inhibit the progress of neurodegenerative diseases. We will first test IA for its effect on the MPTP Parkinson model, and, at a later stage, on Alzheimer animal models. This part of the work will be done in collaboration with Professor Esther Shohami from the Hebrew University.

The proposed research project will take full advantage of the integration of the different competences and skills of the participating researchers and research groups. Dr. Bertea and the Italian research group have a wide expertise in molecular biology and biochemistry of plant secondary metabolism and medicinal plants. The Weizmann research group headed by Asaph Aharoni is focused on plant metabolome, and Arieh Moussaieff – the post doc in charge of this study at the Weizmann Institute side of the collaboration - has a vast experience in the chemistry and the pharmacology of *Boswellia* resin, and has made important contributions to this topic.
Moreover, the collaboration between the two partners will be of paramount importance in terms of technological transfer between the Italian and Israeli research labs and the development of human resources. Students and members of the Italian research group will gain more knowledge in transcriptome analysis and medicinal application of secondary metabolites.

**Currently the laboratory of the Weizmann Institute is equipped with the following analytical instrumentation:**

GC-MS: COMBI PAL autosampler (CTC analytics), a Trace GC Ultra gas chromatograph equipped with a PTV injector, and a DSQ quadrupole mass spectrometer (ThermoElectron Cooperation, Austin, USA).

LC-MS: UPLC_Synapt HDMS™ system (Waters), with the UPLC instrument connected on-line to a PDA detector (Waters, Acquity), and then to the Synapt HDMS detector.

UPLC: Waters, Acquity instrument equipped with an Acquity 2996 PDA detector.

GC-FID: Gas chromatograph equipped with a FID detector (ThermoElectron, Focus).

**References:**


Brayden JE, Earley S, Nelson MT, Reading S. (2008) *Clin Exp Pharmacol Physiol.* [Epub ahead of print].

Hamm S, Bleton J, Connan J, Tchapla A (2005) *Phytochemistry* 66:1499-514.

Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB (2007) *Curr Opin Pharmacol* 7:344-51.

Moussaieff, A., Shohami, E., Kashman, Y., Fride, E., Schmitz, M. L., Renner, F., Fiebich, B. L., Munoz, E., Ben-Neriah, Y., and Mechoulam, R. (2007) *Mol Pharmacol.* **72**, 1657-64.

Moussaieff, A., Shein, N. A., Tsenter, J., Grigoriadis, S., Simeonidou, C., Alexandrovich, A., G., Trembovler, V., Ben-Neriah, Y., Schmitz, M. L., Fiebich, B. L., Munoz, E., Mechoulam, R., and Shohami, E. (2008a) *J. Cereb. Blood Flow Metab.* **28**, 1341-52.

Moussaieff A., Fride E., Bregman T., Felder C.C., Shoham S., Kashman Y., Rimmerman N., Huang S.M., Lee H., Caterina M.J., Shohami E., Walker J.M. and Mechoulam R. (2008b) *FASEB J* (Epub ahead of print).

**Budget uses**

The availability of different facilities at the Weizmann Institute of Science virtually eliminates the need of investments in expensive durable equipments, since the laboratory is equipped with most of the top analytical instrumentation. Nevertheless, this multidisciplinary study involves transcriptomics, proteomics and metabolomics, and pharmacological assays, and requires a preliminary investment in a high throughput sequencing followed by consistent amount of investment in consumables (reagents and kits) as well in specialized manpower.

For the high throughput sequencing we will use the SOLEXA apparatus – DYN G.S. Caesarea, Israel. The cost of such sequencing is approximately **10,000€**. The 454 sequencing apparatus, recently installed in the Weizmann Institute may be used to validate the data received from the SOLEXA apparatus, at lower costs, as it is in-house.

The required materials will be mainly consumables (**€ 5,000)**, as state above. A significant part of the budget will be devoted to support Italian students involved in carrying out the project at the Weizmann Institute of Science (**€ 10,000)**.

**Cost estimates:**

| **Description** | **Amount in euros** |
|---|---|
| *Travel Expenses of researchers/students and/or grant holders from Italy to Israel* | **€ 10,000** |
| *Expenses for professional services* | **€ 10,000** |
| *General Expenses (consumables, instrument maintenance, etc.)* | **€ 5,000** |
| **Total budget** | **€ 25,000** |

# CURRICULUM VITAE

## Personal Data


| | |
|---|---|
| *Name:* | Asaph Aharoni |
| *Date and place of birth:* | 25-11-1966, Petach –Tikva, Israel |
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| *Nationality:* | Israeli |
| *Marital status:* | single |

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**Current Position (Since August 2004)**
Senior scientist in the Department of Plant Sciences, The Weizmann Institute of Science, Israel
**Topic of Research Activity:** Genetic regulation of metabolic pathways and its co-ordination with developmental and stress response programs in plant biology

## Education

2002 – 2004 Post-doctorate in Dr. Andy Pereira's group in Plant Research Int., Wageningen, The Netherlands. Topic: gain-of-function mutagenesis tools for the identification of regulators of metabolic pathways in *Arabidopsis*

1996 – 2002: Ph.D. in Plant Sciences, awarded **CUM LAUDE**, Wageningen University, The Netherlands (16 October, 2002).
Research conducted at Plant Research International, Wageningen, The Netherlands.
*Promoter*: Prof. J.N.M. Mol, Department of Genetics, Free University of Amsterdam and Prof. Willem J. Stiekema, Department of Molecular Biology, Wageningen University.
*Co-promoter*: Dr. A. P. O'Connell.
*Thesis*: "Strawberry and Beyond: A Novel and Comprehensive Investigation of Fruit Maturation and Ripening".

1994 - 1996: M.Sc. awarded **CUM LAUDE** at the Hebrew University of Jerusalem, Faculty of Agriculture, Department of Horticulture, Rehovot, Israel (25 June, 1996).
*Supervisor*: Dr. A. Vainstein.
*Thesis*: "Developing Efficient Regeneration and Transformation Methods for Carnation and Gypsophila Plants".

1991 - 1994: B.Sc., Agronomy, The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot, Israel.

## Awards

1. The Weizmann Institute Scientific Council Prize (Levinson prize in Biology), 2008.
2. The European Research Council (ERC) grant for Starting Independent Investigators 2008.
3. The Yigal Alon Fellowship award by the Council for Higher Education in Israel, 2005.
4. The Sir Charles Clore Prize, 2004.
5. Competitive scholarship for the Postdoc position from the Centre for Bio-systems Genomics (CBSG), The Netherlands, 2003.
6. Plant Research International award, November, 2001.
7. Plant Research International award, November, 2000.
8. CPRO-DLO institute award, December 1999.
9. CPRO institute award, September 1998.

## Patents

- Transgenic Plants Having Altered Levels of Aromatic Amino Acids and Metabolic Derivatives. (Submitted December, 2007).
- The strawberry PINH genes and Their Use (Submitted- November, 2004).
- Method for Preventing Dehiscence and Altering Plant Lignification (Submitted- July, 2004).
- The SHINE Clade of Transcription Factors and Their Use (Submitted- June 2004; P215385EP, 04077174.3).
- Terpene Synthase/cyclase and Olefin Synthase and Uses Thereof (Submitted- January 2001; PCT-NL02-00089, 04076757.6-).
- Fruit Flavor Related Genes and Use Thereof (Submitted- June 2000; WO219991202).

## Conference and Workshops Organization

- Organizer of the Ulpana Amos de-Shalit for Life Sciences, a one week course for excellent undergraduate students in Israel, Weizmann Institute, August 2007.
- Chairman of the 6$^{th}$ Congress of The Israel Association for Medicinal Chemistry, Weizmann Institute, March 2007.
- Co-organizer of the MINERVA Workshop on "Metabolism Meets Development", Weizmann Institute, September 2006.
- Organizer of the Ulpana Amos de-Shalit for Life Sciences, a one week course for excellent undergraduate students in Israel, Weizmann Institute, September 2006.

## Lectures in Congresses and Workshops

1. R*iboswitch-dependent gene regulation and its evolution in the plant kingdom.* 5th Congress of the Federation of the Israel Societies for Experimental Biology (ILANIT), January 2008, **Eilat, Israel**.
2. *Mass spectrometry in Plant Metabolomics*. 21st Israeli Society for Mass Spectrometry Meeting, Weizmann Institute, December 2007, **Rehovot, Israel.**
3. *The Ins and Outs of Tomato Fruit Peel Metabolome.* EUCARPIA Fruit Section Meeting, September 2007, **Zaragoza, Spain**
4. *The Ins and Outs of Tomato Fruit Metabolome.* The 4th Solanaceae Genome Workshop, September 2007, **Jeju Island, South Korea**.
5. *The Ins and Outs of Plant Surface Metabolism.* The 1st Weizmann-Singapore meeting, Weizmann Institute, August 2007, **Rehovot, Israel**.
6. *Using Ultra Performance Liquid Chromatography (UPLC)-QTOF-MS for Metabolome Analysis of Tomato Fruit Peel.* Metabolomics workshop, June 2007, **Wageningen, The Netherlands**
7. *On the Outside of Fleshy Fruit: A Study of Gene Expression and Metabolic Pathways in Peel and Normal mutant Tomato.* Agricultural Biomarkers for Array Technology-COST Workshop, May 2007, **Girona, Spain**
8. *Application of Metabolomics for Improving Fruit and Flowers Quality.* Workshop on Research Directions in Technology and Storage of Agricultural Products, April 2007, **Neve-Ilan (Volcani Institute), Israel**
9. *Gene Expression and Metabolic Pathways in the Arabidopsis and Tomato Fruit Surfaces.* The Otto Warburg Minerva Center Symposium: Comparative Genomics, February 2007, Faculty of Agriculture, **Israel**
10. *Regulation of Plant Surface Metabolism*. MINERVA Workshop on "Metabolism Meets Development", September 2006, **Weizmann Institute, Israel,**
11. *Using Ultra Performance Liquid Chromatography (UPLC)-QTOF-MS for Metabolome Analysis of Tomato Fruit Peel.* SOL 2006 meeting, July 2006, **Madison, USA.**
12. *Using Ultra Performance Liquid Chromatography (UPLC)-QTOF-MS for Metabolome Analysis of Tomato Fruit Peel.* 4th International Plant Metabolomics Conference, April 2006, **Reading, UK.**
13. *Matter of Location: From Fruit Flavour to Attraction of Bodyguard.* Workshop on sensory and aroma, March 2006, Volcani Center, **Israel.**
14. *Using UPLC-TOF-MS and GC-MS for Metabolome Analyses in Plants and Yeast.*ANALYTICA 2006 - The 9th Annual Meeting of the Israel Analytical Chemistry Society, January 2006, **Tel-Aviv**, **Israel.**
15. *Gain and Loss of Fruit Volatile Terpenoids Produced by Wild and Cultivated Strawberry Species.*XVII International Botanical Congress, July 2005, **Vienna, Austria**.
16. *Metabolic Engineering of Terpenoid Biosynthesis in Plants.* The Terpnet 2005 Meeting, April 2005, **Wageningen, The Netherlands.**
17. *Gain and Loss of Fruit Flavor Compounds Produced by Wild and Cultivated Strawberry Species.* American Chemical Society (ACS) meeting, August 2004, **Philadelphia, USA**.
18. *Gain and Loss of Fruit Flavor Compounds Produced by Wild and Cultivated Strawberry Species.* 5th International postharvest Symposium, Workshop-Genomics and Proteomics of Fruit Quality, June 2004, **Verona, Italy**.
19. *Fruit Flavor Formation in Wild and Cultivated Strawberry.* Institute Juan March, Centre for International Meetings on Biology, Workshop on "The Making of a Fruit: From Genes to Molecules to Phenotype". 1-3 March, 2004, **Madrid, Spain.**
20. *From Genomics to Metabolomics – Insight from Activation Tagging Arabidopsis Mutants.* The Society of Genetics in Israel Annual Meeting "Frontiers in Genetics", February 2004, **Tel-Aviv, Israel**.
21. *Altering Plant Terpenoid Metabolism: The Biosafety of Metabolic Changes.* NATO ARW workshop on Genomics for Biosafety in Plant Biotechnology: New Challenges. 15-19 October 2003, **Bansco, Bulgaria**.
22. *Functional Genomics in Strawberry.* COST 836 Workshop, October 2003**, Ancona, Italy.**
23. *Gain of Function Activation Tagged Mutants for Dissecting Metabolic Pathways.* Potential of metabolic profiling in plant science, International workshop, November 2003, **Torino, Italy.**
24. *Non-Targeted Metabolic Profiling Using Fourier Transform ion cyclotron Mass Spectrometry (FTMS).* 7th Int. Congress of Plant Molecular Biology, June 2003, **Barcelona, Spain.**

25. Fruit Flavour Genes: Multiple Pathways and Multiple Substrates for the Corresponding Enzymes. 7th Int. Congress of Plant Molecular Biology, June 2003, **Barcelona, Spain.**
26. *Metabolic Profiling of Activation Tagged Arabidopsis Mutants using QTOF-MS.* Second International Conference on Plant Metabolomics, April 2003, **Potsdam, Germany.**
27. *Strawberry and Beyond: A Novel and Comprehensive Investigation of Fruit Maturation and Ripening.* Netherlands society for plant biotechnology and tissue culture, spring symposium, March, 2003, **Wageningen, The Netherlands.**
28. *Metabolome Activation.* ARANED, February 2003, **Leiden, The Netherlands.**
29. *Profiling Strawberry Fruit Maturation: From Gene Expression to Metabolic Pathways.* The third symposium of plant molecular genetics and breeding (recent advances in plant genomics and molecular breeding), August, 2002, **Seoul National University (Suwon Campus), South Korea**.
30. *Fruit Flavour Genes: From Gene Expression to Metabolic Pathways.* Gordon Research Conference on Postharvest Physiology, August, 2002, **MA, USA**.
31. *Strawberry and Beyond: From Gene Expression to Metabolic Pathways*. SEB annual meeting, fruit development and ripening session, April 2002. **Swansea, Wales**.
32. *Profiling Fruit Maturation: From Gene Expression to Metabolic Pathways*. The first international conference on plant metabolomics, April 2002, **Wageningen, The Netherlands**.
33. *Profiling Fruit Maturation: From Gene Expression to Metabolic Pathways in Strawberry.* Phytochemical society of North America (PSNA) annual meeting, "Phytochemistry in the genomics and post-genomics eras", August 2001, **Oklahoma, USA.**
34. *Function search in plants: expression profiling by DNA microarrays.* Winterschool Bioinformatics, December 2000, **Wageningen, The Netherlands**.
35. *Unraveling metabolic pathways and gene function in strawberry using cDNA microarrays.* IBC's CHIPS to HITS, November 2000, **Philadelphia, USA**.
36. *Unraveling metabolic pathways and gene function in strawberry using cDNA microarrays.* 6th International Congress of Plant Molecular Biology, June 2000, **Quebec, Canada**.
37. *Ripening on Chips.* DNA microarray technology workshop, Cartesian Technologies/GSI-lumonics/TeleChem, April 2000, **Frankfurt, Germany.**
38. *Studying strawberry fruit development and ripening using cDNA microarrays*. Plant Protein Club Workshop "Microarrays", University of York, March 2000, **York, UK**.
39. *Strawberry chips: using DNA microarrays for large scale gene expression analysis in fruits.* 4th International Conference on Postharvest Science, March 2000, **Jerusalem, Israel.**
40. *Strawberry on chips: from gene expression to metabolic pathways.* VIII International Plant and Animal Genome Conference, San Diego, January 2000, **CA, USA.**
41. *Strawberry on chips: gene expression analysis during strawberry development using cDNA microarrays.* The Microarray Meeting: Technology, Application & Analysis. Scottsdale, September 1999, **Arizona, USA.**
42. *Strawberry on chips: from gene expression to metabolic pathways.* Pathway engineering in plants, August 1999, **York, UK.**
43. *Ripening on chips.* Workshop on genomic micro-arrays technology, April 1999, **Amsterdam, The Netherlands.**
44. *Ripening on chips.* Weizmann Institute of Science, March, 1999, **Rehovot, Israel**.
45. *Monitoring the expression of ripening associated strawberry genes using cDNA microarrays.* Lab chips and microarrays for biotechnological applications, January, 1999. **Zurich, Switzerland**.
46. *DNA chip technology as a tool for the isolation of ripening related genes.* SLW Working Party Experimental Plant Sciences, February, 1998, **Lunteren, The Netherlands**.
47. *Isolation and characterization of cytochrome P450 cDNAs from strawberry fruit.* 17th International Congress of Biochemistry and Molecular Biology. Satellite meeting, Cytochrome P450, August, 1997, **San Francisco, USA**.
48. *Isolation and characterization of flavour genes from strawberry.* SLW Working Party Experimental Plant Sciences, April, 1997, **Lunteren, The Netherlands.**

## Invited seminars

1. Riboswitch-dependent Gene Regulation and its Evolution in the Plant Kingdom. Department of Botany, Hebrew University of Jerusalem, May 2008, **Jerusalem**, **Israel**.
2. *Maintaining the Homeostasis between Primary and Secondary metabolism: A case study from Glucosinolates Biosynthesis.* University of Milano, March 2008, **Milano, Italy**.
3. *Maintaining the Homeostasis between Primary and Secondary metabolism: A case study from Glucosinolates Biosynthesis.* Parco Technologico Padano, March 2008, **Lodi, Italy**.
4. *Gene Expression and Metabolic Pathways in the Arabidopsis and Tomato Fruit Surfaces.* Genetics course seminar series, Faculty of Agriculture, February 2008, **Rehovot, Israel.**
5. *The Ins and Outs of vegetative and reproductive plant organs.* Department of Genetics and Vegetable Crops, ARO, the Volcani Center, January 2008, **Israel.**
6. *The Ins and Outs of vegetative and reproductive plant organs.* Malaga University, October 2007, **Malaga, Spain**
7. *The Ins and Outs of Tomato Fruit Peel Metabolome.* Cordoba University, October 2007, **Cordoba, Spain**
8. *The Ins and Outs of Tomato Fruit Peel Metabolome.* October 2007, **Valencia, Spain**
9. *Regulation of Plant Surface Metabolism.* Max Planck Institute, July 2007, **Koln, Germany**.
10. *Mass-spectrometry based Metabolomics for gene function and metabolic pathway discovery in plants.* The Department of Life Sciences at the Ben-Gurion University, April 2007, **Beer-Sheva, Israel**
11. *Regulation of Plant Surface Metabolism.* The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, February 2007, **Midreshet Ben-Gurion, Israel**
12. *Regulation of Plant Surface Metabolism*. Max Planck Institute of Colloids and Interfaces, November 2006, **Golm, Germany**
13. *The Outer Surface of Plants: Cuticle Related Gene Expression and Metabolic Pathways in Vegetative and Reproductive Organs.* The Danforth Center, October 2006, **St. Louis, Atlanta, USA**
14. *Regulation of Plant Surface Metabolism.* Faculty of Life Sciences, Bar-Ilan University, June 2006, **Israel.**
15. *The Outer Surface of Plants: Cuticle Related Gene Expression and Metabolic Pathways in Vegetative and Reproductive Organs.* KeyGene company, January 2006, **Wageningen, The Netherlands**
16. *Metabolic Engineering of Terpenoid Biosynthesis in Plants.* Department of Plant Biology, University of Turin, April 2005, **Turin, Italy.**
17. *When (and Where) Metabolism Meets Development in Arabidopsis.* Tel-Aviv University, November 2004, **Tel-Aviv**, **Israel.**
18. *When (and Where) Metabolism Meets Development in Arabidopsis.* Department of Genetics and Vegetable Crops, ARO, the Volcani Center, November 2004, **Israel.**
19. *When (and Where) Metabolism Meets Development in Arabidopsis.* Department of Plant Sciences, The Weizmann Institute of Science, February 2004, **Rehovot, Israel.**
20. *From Flavors in Fruit to Resistance in Crops*. Max Planck Institute, November 2003, **Koln, Germany**.
21. *Profiling Strawberry Fruit Maturation: From Gene Expression to Metabolic Pathways*. Technion, April, 2003, **Haifa, Israel.**
22. *New "omics" Tools for Biodiversity Analysis.* The analysis of natural variation within crop and model plants, Int. summer school, Wageningen University, April, 2003, **Wageningen, The Netherlands.**
23. *Strawberry and Beyond: A Novel and Comprehensive Investigation of Fruit Maturation and Ripening.* Faculty of Agriculture, Hebrew University of Jerusalem, February 2003, **Rehovot, Israel.**
24. *Strawberry and Beyond: A Novel and Comprehensive Investigation of Fruit Maturation and Ripening*. February 2003, **Israel.**
25. *Profiling Strawberry Fruit Maturation: From Gene Expression to Metabolic Pathways.* Korean Research Institute of Bioscience and Biotechnology (KRIBB)**,** December, 2002, **Taejon, South Korea**.
26. *Strawberry Fruit Maturation and Ripening: from Gene Expression to Metabolic Pathways.* Curse on microarray technology, Hogeschool Leiden June, 2002, **Leiden, The Netherlands**.
27. *Strawberry Fruit Maturation and Ripening: from Gene Expression to Metabolic Pathways.* INRA, Bordeaux, March 2002, **Bordeaux, France**.

28. *Strawberry Fruit Maturation and Ripening: from Gene Expression to Metabolic Pathways.* INRA, Toulouse, March 2002, **Toulouse, France.**
29. *Strawberry Fruit Maturation and Ripening: From Gene expression to Metabolic Pathways.* University of Michigan, department of biology, August 2001, **Ann-Arbor, USA.**
30. *Functional Genomics in Plants.* University of Milano, July 2001, lecture for students in a course by Dr. L. Colombo, **Milano, Italy**.
31. Strawberry fruit maturation and ripening: from gene expression to metabolic pathways. The Volcani Center, June 2001, **Israel.**
32. *DNA microarray technology and its applications.* Free University of Amsterdam, May 2001, **Amsterdam, The Netherlands**.
33. *Unraveling metabolic pathways and gene function in strawberry using cDNA microarrays.* Novartis (Syngenta), November 2000, **North Carolina, USA**.
34. *From DNA sequence to metabolic pathways.* Fermenich, July 2000, **Geneva, Switzerland**.
35. *Unraveling metabolic pathways and gene function in strawberry using cDNA microarrays.* University of Amsterdam, BioCentrum, July 2000, **Amsterdam, The Netherlands.**
36. *Ripening on chips.* Zeneca Wheat Improvement Centre, March 2000, **Norwich, UK**.
37. *Ripening on chips.* University of California Davis, January 2000, **CA, USA.**
38. *Ripening on chips.* Experimental Plant Science seminar. **Wageningen University, The Netherlands**. June 1999.
39. *Ripening on chips.* Weizmann Institute of Science, March, 1999, **Rehovot, Israel**.
40. *Strawberry genes and chips.* Department of Horticulture, Faculty of Agriculture, April, 1998, **Rehovot, Israel**.

# CURRICULUM VITAE

## List of publications during the last 5 years

1. Faye M. Rosin, **Asaph Aharoni**, Elma M. J. Salentijn, Jan G. Schaart, Marjan J. Boone, David J Hannapel (2003). Expression Patterns of a putative homolog of AGAMOUS, STAG1, from Strawberry. *Plant Science* 165: 959-968.
2. Elma M.J. Salentijn, **Asaph Aharoni**, Jan G. Schaart, Marjan J. Boone, Frans A. Krens (2003). Differential Gene Expression Analysis of Strawberry Cultivars that Differ in Fruit-firmness. *Physiologia Plantarum* 118: 571-578.
3. **Asaph Aharoni**, Ashok P. Giri, Stephan Deuerlein, Frans Griepink, Francel W.A. Verstappen, Harrie A. Verhoeven, Maarten A. Jongsma, Wilfried Schwab and Harro J. Bouwmeester (2003). Terpenoid Metabolism in Wild-Type and Transgenic *Arabidopsis thaliana* Plants. *The Plant Cell* 15(12):2866-2884.
4. Jules Beekwilder, Mayte Alvarez-Huerta, Evert Neef, Francel W.A. Verstappen, Harro J.-Bouwmeester and **Asaph Aharoni** (2004). Functional Characterization of Enzymes Forming Volatile Esters from Strawberry and Banana. *Plant Physiology* 135(4): 1865-1878.
5. **Asaph Aharoni**, Shital Dixit, Reinhard Jetter, Eveline Thoenes, Gert Van Arkel, and Andy Pereira (2004). The SHINE Clade of AP2 Domain Transcription Factors Activate Wax Biosynthesis, Alter Cuticle Properties and Confer Drought Tolerance when Overexpressed in Arabidopsis (2004). *The Plant Cell*, 16(9):2463-2480.
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**Curriculum vitae of the Italian Applicant**

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Cinzia Margherita Bertea is Assistant Professor of Plant Physiology at the Plant Biology Department of the University of Turin. Born on May 2nd 1970, she graduated in Biological Sciences at the University of Turin in 1994 and obtained the PhD Degree in Biochemical Sciences from the University of Turin in 2000. During the PhD studies (1998-1999) she moved to Washington State University in Pullman (USA) and worked on heterologous expression of cytochrome P450 hydroxylases involved in monoterpene biosynthesis in the genus *Mentha*, under the supervision of Prof. Rodney Croteau. In 2001 she got a post-doctoral Marie Curie Fellowship and moved to Dr. Harro J. Bouwmeester lab at Plant Research International of Wageningen (The Netherlands) where she studied the biosynthesis of artemisinin in *Artemisia annua*. Major fields of research cover physiology, biochemistry and molecular biology of plant responses to abiotic (UV-radiation, mechanical damage) and biotic (pathogen and herbivore attacks) stress. She is also interested in molecular biology and biochemistry of secondary metabolites with particular regard to terpenoids and phenolic compounds in medicinal plants.

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