

through the production of protective foods. Anthocyanins are colourful natural pigments found in berries and in many common fruit and vegetables. Recent evidence indicates that anthocyanins can protect against cardiovascular disease, some cancers and other diseases. We have developed an innovative approach to investigate the relationship between diet and health and to dramatically increase the content of anthocyanins and related phenolic compounds in tomato. Our studies confirm the anti-inflammatory potential of plant-derived phenolic compounds and indicate novel synergistic interactions between specific phytochemicals.

S.25-2

Trace metal metabolism in photosynthetic organisms

H. Küpper

Biology Centre, Academy of Sciences of the Czech Republic, Institute of Plant Molecular Biology, Ceske Budejovice, Czech Republic

Many trace metals such Cu, Fe, Mn, Mo, Ni, Zn are essential micronutrients, and their concentrations in the environment ranges from deficient to toxic levels. This affects primary production, nutritional value and safety of crops. Therefore, one focus of plant research is on the uptake, transport, sequestration, speciation, deficiency, toxicity and detoxification of trace metals. Early studies often used environmentally not relevant conditions, and individual processes often were not mechanistically interconnected, so that causes and consequences of metal effects remained unclear. This talk will focus on recent progress in understanding trace metal metabolism under environmentally relevant conditions. It could be shown, for example, that metal(loid) (As, Cd, Cr, Cu, Ni) concentrations that were previously considered as not having any effect actually have a strong impact on the plants, and with a different sequence of events than observed at very high concentrations. We used a combination of various biophysical and biochemical methods for measurements *in vivo* (e.g. photosynthesis biophysics, formation of reactive oxygen species, metal transport), *in situ* (e.g. quantitative (sub)cellular distribution and speciation of metals, mRNA levels) as well as on isolated proteins (for identification and characterization of metalloproteins). Using a combination of HPLC with ICPMS ("metalloproteomics") on protein extracts from plants that had been treated with heavy metals, changes in target sites of metal binding to proteins from deficient to toxic concentrations could be analyzed. The combination of these techniques showed clearly metal(loid)-induced changes already at very low concentrations. Further, mechanistic interactions between different affected processes could be resolved in some cases. Finally, recent work deals with interactions between plant metal metabolism and pathogens.

S.25-3

Engineered suppression of toxins in crops: possibilities and drawbacks

J. Gressel

Weizmann Institute of Science, Rehovot, Israel

While many natural compounds are beneficial, many more produced by plants and their pathogens are deleterious. Nature is not a signitor to the Geneva Convention on Chemical Warfare, and plants and their pathogens synthesize a wide array of toxins as evolutionary ploys to protect themselves or their food supply from herbivores and competitors. Classical breeding has bred out many toxins, and their protective role has been replaced by more benign pesticides. Genetic engineering can eliminate toxins much

more quickly than breeding and can be very useful in domesticating wild, toxin-bearing crops as well as in dealing with problems that have been intractable to breeding. For example gene editing can be used to delete part of ricin gene in castorbeans, suppressing ricin production, and rendering the protein of this novel oil-seed crop edible to livestock, whereas the wild-type toxic residues after oil removal represent an environmental problem as well as being a tool for bioterrorism. Breeders have only been unsuccessful in breeding grains resistant *Aspergillus* species that produce carcinogenic aflatoxins. Constructs containing multiple RNAi sequences targeting both the fungus and the toxin biosynthesis pathway have been highly successful in suppressing both below regulatory thresholds. Likewise, engineering has been used to suppress the production of soybean allergens that cause diarrhea in babies and render soybean unusable in feed for carnivorous fish. Engineering has the advantage over breeding in that it is far more specific; engineering changes only the target trait without bringing in many other traits as during hybridization. Gene editing that modifies crop genes and the use of crop RNAi against crop genes have been deregulated in certain enlightened countries, because identical results can be obtained by mutation breeding (without the multiple mutations usually present in the latter).

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Evaluation of anti-inflammatory potential and LC-MS/MS analysis of different standards

A. B. Sözmen¹, E. Canbay², E. Yildirim Sozmen², B. Ovez²

¹Izmir Institute of Technology, Izmir, Turkey, ²Ege University, Izmir, Turkey

Standardization of a LC-MS/MS method for phenolic compound content of biomass originated extracts was aimed in this study, *Chlorella miniata* extracts, which were cultivated solely for this study, were used for this purpose. The first step of the study was to compare external standard method, added standard method, and conventional standard method the qualitative identification of phenolic compounds of the extracts were performed by using LC-MS/MS system. The target phenolic compounds that were investigated were Cateic Acid, Kaempferol, Ferulic Acid, Epicatechin, Quercetin, Myricetin, Rutin, 4-Hydroxy Benzoic Acid, Salicylic Acid, Trans-Cinamic Acid, Gentisic Acid, Protocatechuic Acid, Para-Coumaric Acid, Vanilic Acid, Gallic Acid, Syringic Acid, Ethyl Ferulate, CAPE, Catechin, Chlorogenic Acid, Ellagic Acid, Cyanidin, Naringenin, Delphinidin, and β -Carotene. The standards were prepared at 0,1 mg/ml concentration, and standard mix including the standards was prepared using a dilution of 1/20 and internal standard of DMAE Caffeate, which was absent in extracts, with same concentration, which was also added to each algae extract to determine the loss of phenolic compounds due to matrix effect. The standard curves were prepared suitable to internal standard method, response of compound to response of internal standard vs. concentration of compound. Algae samples were diluted by 9/20 with methanol and standard mixture. A linear gradient method had been used for MetOH/Water mobile phase at a flow rate of 0.3 mL/min with Hypersil BDS C18 (250 \times 4.6 mm, 5 μ m) column at 40 °C. The method took 5 minutes, starting with 100% water and a 1 minute wash, and then gradually increasing the amount of MetOH and continued with 100% MetOH for 3 minutes. Our results showed clearly that the external standard method had a higher accuracy and precision in detecting phenolic compound content of the extracts of algae species.