Accumulation of rishitin in tomato after elicited by silver nitrate

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Accumulation of rishitin as one of a major phytoalexin synthesis in tomato plant was measured in leaves and stems after elicited by 1% of silver nitrate at different plant ages (week nine, ten, eleven, twelve, thirteen, fourteen, fifteen and sixteen). The extraction procedure was using 95% ethanol and followed by isolation of rishitin using Thin Layer Chromatography (TLC). The rishitin band was purple fluorescent color under the ultraviolet light at R_f value of 0.72. No rishitin band was detected in the control plant in both leaves and stems. The quantification of rishitin for each plant part was carried out using spectrophotometer with maximum absorbance of 500 nm. The optical density (OD) value was recorded to evaluate the accumulation of rishitin at different plant ages. The OD values increased gradually from week nine until week sixteen in both leaves and stems. Hence, the rishitin accumulated in tomato plant increases as the plant age increases. The maximum OD value for rishitin in leaves is 0.050 and stem is 0.025 while the minimum mean value of rishitin in leaves is 0.010 and stem is 0.004 which is in week sixteen and week nine respectively. From the data analysis, there was a positive correlation in the accumulation of rishitin in leaves and stems with plant ages.

www.cspp.cn May 29, 2008

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Artemisinin biosynthesis and biotechnology

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Artemisinin is a sesquiterpene lactone from *Artemisia annua* L. that has become one of the most important drugs for malaria therapy, particularly in the form of artemisinin-based combination therapies (ACTs) which are advocated by the WHO in order to reduce the odds of resistance development [1]. Extraction of artemisinin from *A. annua* is still the only commercial source of this antimalarial drug [2]. However, the low artemisinin content of *A. annua*, ranging 0.1-0.8% of the plant dry weight, makes artemisinin a relatively expensive drug, especially for the poor people in developing countries where most of the malaria occurs. One of the most practical ways to reduce the price of artemisinin-based antimalarial drugs is to increase the artemisinin content in the *A. annua* plant [3]. We have established the *Agrobacterium*-mediated transformation system of *Artemisia annua* and transferred a number of key enzyme genes of artemisinin biosynthesis to *A. annua* [3]. The terpenoids profile of transgenic plant as well as the mother plant which is selected high-artemisinin producing *A. annua* were determined by GC and GC-MS, and partial least squares discriminant analysis (PLS-DA) was employed to analyze the GC data. The results provide some useful information to artemisinin biosynthesis and biotechnology [4]. Artemisinin biosynthesis has been studied in some details, but there are still some unclear parts. We have applied retrobiosynthetic NMR approach to study artemisinin biosynthesis and found that the plastid-origin IPP was integrated into the artemisinin molecules. This finding will be very helpful for artemisinin biotechnology.

Acknowledgements: The work was financially supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (KSCX2-SW-329) and 3 projects of Natural Sciences Foundation of China (30470153, 30672623, 60773164).

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www.icspp.cn May 29, 2008

Characterize a Cotton Cytochrome P450 Gene That Related to Gossypol Biosynthesis

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Gossypol and related sesquiterpene phytoalexins of cotton protect the plants against pathogens and insect herbivores. However, the utilization of cotton seeds as excellent source of oil (21%) and high-quality protein (23%) was limited by a high content of gossypol in seeds. We are interested in elucidation of gossypol biosynthesis pathway and have isolated genes for several enzymes, including farnesyl diphosphate synthase (FPS), (+)-delta-cadinene synthase (CAD) and (+)-delta-cadinene-8-hydroxylase (CYP706B1).

To investigate the unknown steps, we selected 13 putative cytochrome P450 monooxygenase ESTs from *Gossypium hirsutum* EST database and analyzed their expression patterns in glanded and glandless cotton cultivars. One of them, named *CQ1*, showed a greatly reduced expression level in the glandless cotton cultivar compared to the glanded cultivar. Northern blot indicated that it was highly expressed in roots, cotyledons and leaves, but the transcripts were also detectable in hypocotyls and sepals. During cotton seed development, its expression started at 15-20 DPA and the expression level was kept high till seed maturation. Moreover, its expression could be strongly and quickly induced by *Verticillium dahliae* elicitor treatment of suspension cell cultures and mechanical wounding of hypocotyls. All these expression patterns were consistent with gossypol accumulation and the expression patterns of other gossypol biosynthesis related genes, such as *(+)-delta-cadinene synthase* (*CAD*), *(+)-delta-cadinene-8-hydroxylase* (*CYP706B1*).

The full length cDNA of *CQ1* was obtained by 5' RACE and 3' RACE. It encodes a protein of 522 amino residues and shares 54% sequence identity with *Glycine max CYP82C1p*. This suggests that CQ1 be a member of CYP82 family. Its 1.6-kb promoter region was obtained by genome walking and the vector *ProCQ::GUS* was constructed and introduced into *Arabidopsis*. GUS staining of transgenic plants showed a similar expression pattern to that revealed by northern blot.

All these results suggest that *CQ1* might be a new cytochrome P450 gene involved in gossypol pathway. The functional characterization of this gene is still underway.

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www.cspp.cn May 29, 2008

Effects of elevated CO_2 levels on the invasive weed *Mikania micrantha*: Induction of β-caryophyllene synthase, changes in volatile sesquiterpenes and allelopathic potential of β-caryophyllene

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Mikania micrantha is one of China's most aggressive invasive weeds. *M. micrantha* secretes volatile sesquiterpenes, which are known to display allelopathic effects on other plants. Elevating CO_2 could enhance allelopathic potential of invasive plant and further accelerate successful invasion. We tested this hypothesis by investigating effects of elevated levels of CO_2 (750 ppm) on *M. micrantha.* Using a suppressive subtractive hybridization (SSH) library approach, 349 expressed sequence tags (ESTs) were sequenced. One of them showed high sequence similarities with a β-caryophyllene synthase gene from *Artemisia annua*. Real-time PCR analysis confirmed that expression of β-caryophyllene synthase in *M. micrantha* leaves was strongly enhanced in response to elevated CO_2. Using gas chromatography-mass spectrometry (GC-MS) and gas chromatography (GC), we analyzed 12 major sesquiterpenes from *M. micrantha*. *M. micrantha* plants exposed to 750 ppm CO_2 showed elevated contents of α-longipinene, α-copaene and β-himachalene, whereas those of α-bergamotene, β-cubebene and δ-cadinene decreased. Contents of β-caryophyllene were transiently increased in *M. micrantha* leaves. Phytotoxicity of β-caryophyllene was studied on *M. micrantha* and three other plants (namely *Raphanus sativus*, *Brassica campestris* and *Lactuca sativa)*. Inhibitory effects caused by β-caryophyllene were dose-dependent and most pronounced in *B. campestris* and *L. sativa*. Phytotoxic effects of β-caryophyllene were stronger at 750 ppm CO_2, especially when tested on *R. sativus*. Taken together, our results suggest that elevated atmospheric CO_2 levels may cause stronger the allelopathic potential of β-caryophyllene, increase the competitiveness of *M. micrantha* and further accelerate successful invasion.

Acknowledgements: This research was financially supported by the National Natural Science Foundation of China (No.30670385) and the Key Program of Ministry of Education of China (No.403037).

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Dynamic of Changes of Diosgenin level in Different Parts of *Dioscorea Zingiberensis* C. H. Wright

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In this paper biennial plants of the *Dioscorea zingiberensis* C. H. Wright were used as research material which is a special Chinese species used for steroid drugs manufacturing. Research of dynamic changes of diosgenin in different parts of *Dioscorea Zingiberensis* C.H.Wright during different periods were carried out by analyzing the evolution of diosgenin levels and immunohistochemical localization of diosgenin in leaf, stem and rhizome. The results indicated that at first the diosgenin level in leaf keep rising but begin to fall after June, the highest level was observed as 0.56%(g/g DW) in early June. The change trend of diosgenin level in rhizome is like it was in the leaf, but the highest level was observed as 2.80%(g/g DW) in early July. The immunohistochemical localization revealed that diosgenin existed in leaf, stem and rhizome and the reaction in the phloem of stem is positive. These results showed that the diosgenin could be synthesized in leaf cells, then transported to rhizome through the phloem of stem and reserved there. Diosgenin level in different parts of rhizome were different from each other during the development of the plant. The highest diosgenin level was observed in the annual part from June to August. From September to October the highest diosgenin level was observed in the biennial part.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (30370152).

www.cspp.cn May 29, 2008

Identification of Key Enzymes Involved in Ginsenoside Biosynthesis of *Panax quinquefolius* L. by Expression Sequence Tag Mining and Functional Expression Assay

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American ginseng (*Panax quinquefolius* L.) is one of the most widely-used traditional Chinese medicines. The major pharmacologically active constituents of American ginseng are dammarene-type ginsenosides. It is generally believed that the first committed step in ginsenoside biosynthesis is the cyclization of 2, 3-oxidosqualene to dammarenediol II catalyzed by dammarenediol synthase[1], followed by hydroxylation via cytochrome P450 (CYP450)[2] and subsequently glycosylation via glycosyltransferase[3]. In contrast to the considerable commercial interest in ginsenosides, little is known about the genes and biochemical pathway of ginsenoside biosynthesis in American ginseng[4].

A cDNA of dammarenediol synthase (DDS) was cloned from roots of American ginseng by homology based PCR. Homology analysis showed high identity of more than 90% between DDSs from *Panax quinquefolius* L. and *Panax ginseng*. Expression sequence tag (EST) mining and functional expression assay were used to identify the CYP450s and glycosyltransferases involved in ginsenoside biosynthesis of American ginseng. Three cDNA libraries were constructed from the root, leaf and flower of 4-year-old American ginseng. Until now, 1248 random clones from the root cDNA library have been sequenced. EST analysis indicates 11 genes, respectively encoding CYP450s, glycosyltransferases and cyclases, are possibly involved in ginsenoside biosynthesis. Four full-length cDNAs of CYP450 candidates and three ones of glycosltrasferase candidates were obtained by RACE (Rapid Amplification of cDNA End) method. Phylogenetic analysis showed that the three novel glycosltrasferases lied within a same clade with UGT71G1, a triterpene glycosyltransferase in *Medicago truncatula*; two of four CYP450 candidates lied within a same clade with CYP450 AB231332 from *Glycine max*, which is a sole triterpene hydroxylase cDNA identified from any plant species until now. These results suggest possible roles for the novel CYP450s and glycosltrasferases in ginsenoside biosynthesis of American ginseng. Among them, two of glycosyltransferases have been expressed successfully in *E.coli* and the product purification is in progress. Our further work will focus on the expression of CYP450 candidates in *Saccharomyces cerevisiae* and identification of CYP450s and glycosyltransferases in the pathway of ginsenoside biosynthesis by enzymatic activity assay.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 30772735) and the 11th Five-year National Key Technologies R&D Program (No. 2006BAI09B02-1). The au-

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thors are grateful to Professor Weiwei Gao for the gift of plant materials of *Panax quinquefolius* L.

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www.cspp.cn May 29, 2008

Introduction of Multiploid to *Artemisia annua L.* through Colchicine Treatment

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In order to obtain the *Artemisia annua L* multiploids, the leaf buds were treated with colchicines. The results indicated that the induction rate of multiploid was 24% when treated with 0.05% colchicines, leading to doubling of chromosome number (2n=4X=36) with a rate of 91%. Apparent phenotypic changes in multiploid plants could be observed. The leaves of multiploids were bottle green and rolling while those of diploids were light green without rolling. In multiploids the stem diameter was increased to 1.5±0.2mm compared with 0.86±0.2mm in diploids. The size of pores was enlarged. The chlorophyll number in guard cells increased 30.2%. The change of artemisinin content was insignificant. As the growth increased 1.25 times in multiploids, it appears that total artemisinin may be increased by planting multiploids for artemisinin production.

Acknowledgements: This study was supported by Key Laboratory of Forest Products and Chemical Engineering.

www.cspp.cn May 29, 2008

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Isolated and Characterization of the Genes Encoding 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase in *Salvia miltiorrhiza* Bunge

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There are increasing data showing that biosynthesis of terpenoids, such as carotenoids, dolichols, ubiquinones, various hormones, is regulated by enzymes distal from HMGR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) [1]. The reaction catalyzed by the HMGR is the first committed step in the mevalonate pathway of isoprenoid biosynthesis [2]. To explain the function of HMGR in the mevalonic acid pathway of *Salvia miltiorrhiza*, two fragments of 458bp and 537bp were amplified from the *Salvia miltiorrhiza* genome using degenerated primers designed in base of the HMGR amino acid consensus sequences. The 458bp sequence had 85% identity after slicing compared to the sequences of *HMGR* in the GenBank from *Salvia miltiorrhiza*. The other 538 sequence with one intron had 77-84% identities with *Catharanthus roseus*, *Picrorhiza kurrooa*, *Gentiana lutea* and *Gossypium barbadense*. With PROSITE analysis (http://us.expasy.org/prosite/), several domain signatures, such as EGF-like domain signature (EGF_1), were found in the two SmHMGR putative protein sequences. These signatures were also founded in others plant HMGR protein. The further experiments such as obtaining the full length of the two genes and analyzing the two enzymes function within *Salvia miltiorrhiza*, which should be beneficial for regulating and accumulating tanshinones through the mevalonic acid pathway.

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www.cspp.cn May 29, 2008

Mining the metaverse - finding and exploiting new metabolic pathways in plants

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Plants produce a huge array of natural products, many of which are specialised metabolites associated with particular species. These secondary metabolites often have important ecological roles, facilitating pollination and seed dispersal and/or providing protection against attack by pests and pathogens. Although the ability of plants to perform *in vivo* combinatorial chemistry by mixing, matching and evolving the genes required for different secondary metabolite biosynthetic pathways is likely to have been critical for survival and diversification of the Plant Kingdom we know very little about the mechanisms underpinning this process. This talk will focus on the function and synthesis of plant natural products and on the origins of metabolic diversity and will draw on our research on triterpene synthesis in crop and model plants.

Acknowledgment: The John Innes Centre is supported by the Biotechnology and Biological Sciences Research Council, UK.

www.cspp.cn May 29, 2008

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Characterization of the key enzymes of phenylpropanoid biosynthetic pathway in *Echinacea angustifolia*

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Currently, extracts and whole plants made from Echinacea sp. comprise one of the largest sectors of the herbal medicine market in North America, as well as in Europe. Caffeinic derivatives from the extracts mainly included echinacoside, cichoric acid and chlorogenic acid and so forth. Researches indicated indicated that caffeinic derivatives from Echinacea sp. have the pharmacological activity of antioxidant and stimulating the immune system. Besides, recent researches also suggested that caffeinic derivatives is neuroprotective[1] and vasorelaxant [2]. Therefore, it has been widely used against infections and for preventing from or treatment for inflammatory. Phenylalanine ammonia lyase(PAL), cinnamate 4-hydroxylase(C4H) and 4-coumarate CoA ligase(4CL), as the three key enzymes, play an important role in the biosynthesis of caffeinic derivatives[3]. Moreover, due to the difficulty of Echinacea sp. seed collection, its low burgeoning rate[4] and its low content of echinacoside, focusing on revealing its molecular mechanism, and finding suitable substitute drug resourses of Echinacea sp. are our main work.

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www.cspp.cn May 29, 2008

Pathway responses in Arabidopsis to infestation of *Bemisia tabaci* B biotype

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Bemisia tabaci (Gennadius) B biotype is a destructive pest all over the world on wide range of host plant species including *Brassicas* with high concentration of glucosinolates. Glucosinolates and their hydrolysis products such as indoles and isothiocyanates can possibly influence several processes related to insect induced resistance and chemical carcinogenesis [1,2]. Therefore, there is a need for more detailed information of plant secondary metabolism after infestation of the whitefly. Proteome differential display of infested and non-infested plants revealed that several proteins changed significantly. GeneChip study reveals that glucosinolates-related pathway is activated in Arabidopsis after infestation by *Bemisia tabaci* (Gennadius) B biotype [1], which can be matched with the results above. Current work is being conducted to determine the glucosinolate pathway involved. Change of glucosinolates in Arabidopsis in responses to whitefly infestation is being analyzed by HPLC-MS/MS.

Acknowledgements: We thank the Proteome Lab at Peking University (Beijing, China) for technical support for proteome differential display analysis. We gratefully acknowledge Dr. Feng-ming Yan for insightful discussions.

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www.cspm.cn May 29, 2008

A new chalcone synthase encoded by an unusual gene with three introns from *Polygonum cuspidatum* Sieb. et Zucc.: the highest bifunctional enzyme activity of chalcone synthase and benzalacetone synthase overexpressed in *Escherichia coli*

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The structure of the chalcone synthase (CHS) gene is conserved in flowering plants. All *CHS* genes studied so far contain a single intron at a conserved site. Since the first *CHS* gene sequence was reported in 1983, none of the enzymes encoded by a multi-intron *CHS* gene had been functionally characterized. In this study, a *CHS* gene containing three introns from *Polygonum cuspidatum* Sieb. et Zucc., a medicinal plant rich in aromatic polyketides, was cloned for the first time. The resulting gene was designated *PcCHS1*. The full-length *PcCHS1* gene is 2424 bp, contains an open reading frame of 1182 bp, 720 bp of promoter region and 209 bp of 3' untranslated region. Interestingly, the coding region of the *PcCHS1* gene is interrupted by three intervening sequences, of 106 bp, 103 bp and 104 bp. DNA gel blot analysis indicated that there are two to four copies of *CHS* genes in the *P. cuspidatum* genome. *PcCHS1* cDNA was amplified with gene-specific primers from rhizomes of *P. cuspidatum* by RT-PCR; it encoded a theoretical protein of 393 amino acid residues with a calculated molecular mass of 43.2 kDa and a pI of 5.8. PcCHS1 showed 57–97% identity at the amino acid level with other type III polyketide synthases (PKSs) of plant origin. *PcCHS1* is highly expressed in the rhizomes and in young leaves, but not in the roots of the plant. *PcCHS1* transcripts in leaves were induced by pathogen infection and wounding. Surprisingly, unlike regular CHS activity, recombinant PcCHS1 expressed in *Escherichia coli* efficiently afforded naringenin as a single product at low-pH; otherwise, it effectively yielded benzalacetone as almost the sole product at high pH. These data suggest that PcCHS1 is a bifunctional enzyme with both CHS and BAS activity. In particular, PcCHS1 exhibited high temperature optima (50 °C) for both the chalcone and benzalacetone formation reactions. In contrast with CHS, which showed broad substrate specificity toward aliphatic CoA esters, PcCHS1 did not accept isobutyryl-CoA, isovaleryl-CoA or acetyl-CoA as a substrate. Moreover, no byproduct of CHS enzyme reactions in vitro such as 4-coumaroyltriacetic acid lactone (CTAL) and bis-noryangonin (BNY) were detected in the enzyme reaction mixture at various pH values. Further, homology modeling predicted that the 43 kDa protein has the same overall fold as *Medicago sativa* CHS. Finally, PcCHS1 is the first detailed, functionally characterized bifunctional CHS that possesses both CHS and BAS activity. Together with recent results [1,2], our study suggests a regulatory mechanism *in vivo* in which the bifunctional CHS with both CHS and BAS activity most likely has a crucial role in the biosynthesis of the C_6-C_4 skeleton of phenylbutanoid derivatives and flavonoids in

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www.cgpp.cn May 29, 2008

several higher plants.

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www.cspp.cn May 29, 2008

Anthocyanin biosynthesis and regulation in sweetpotato

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Anthocyanins are plant secondary metabolites that exhibit antioxidant activities, antimutagenicity and protection for liver damage in medical application. Although many sweetpotato cultivars could accumulate high amount of anthocyanin pigments in their storage roots, little information is available on their biosynthsis and molecular regulation in the different organs of sweet potato as well as their composition of anthocyanins. Using the purple cultivar 'Aymurasaki', which can acummulate high amount of anthocyanins in its young shoots and storage roots, four key genes in the anthocyanin biosynthesis pathway have been cloned from a cDNA library. There are flavanone 3β-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS) and glucosyltransferase (GT). To verify their function in the biosynthesis pathway, the sense and antisense expression constructs were made and sweetpotato transformation has been conducting using *Agrobacterium*-mediated gene delivery. In parallel, *Arabidopsis* plants with or without null mutations in these genes were transformed with corresponding sense constructs in order to analysis the function of these genes and to complement their functions in the mutants. Current result suggested that the four genes isolated from sweetpotato are functional in *Arabidopsis* and could be used for genetic modification of anthocyanin biosynthesis in sweetpotato as well as other plants.

www.cspp.cn May 29, 2008

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Biosynthetic prenylation of flavonoids in plants

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The recent characterization of genes encoding a novel family of aromatic prenyltransferases is challenging discoveries in the biochemistry of natural compounds. This review attempts to summarize current understanding of aromatic prenyltransferases in plants[1,2] and microbes[3], to focus on the biosynthetic prenylation of flavonoids in plants[4] and to outline future opportunities for prenylflavonoids bioengineering.

Aromatic prenyltransferases catalyze an aromatic proton substitution with the prenyl moiety and lead to important classes of biologically active compounds, e.g. phytoalexins such as prenylflavonoids, as well as to essential electron carriers like the ubiquinones and to vitamin E. The presence of isoprenoid chains of varying length and type is a major determinant of the bioactivity of prenylated aromatic compounds which represent new anti-microbial, anti-oxidant, anti-inflammatory, and anti-cancer candidates.

Acknowledgements: This work was supported by The Ministry of Science and Technology of China (2007CB108800).

www.cspp.cn May 29, 2008

Cloning and Functional Analysis of Type III PKS Genes from *Polygonum cuspidatum*

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Stilbene synthase (STS) is a pivotal enzyme in the biosynthesis of resveratrol, using 4-coumaroyl-CoA as a starter, performing three condensations with malonyl-CoA, followed by a aldol type of cyclization reaction to generate resveratrol. Resveratrol has a variety of biologically and pharmacologically important properties. The main biosources of resveratrol are *Vitis vinifera* and *Polygonum cuspidatum* presently. STS gene of *P. cuspidatum* has not been cloned so far. Using degenerate primers generated from the conserved regions of plant type III polyketide synthase (PKS) and young leaf and root cDNA as template, several core fragments were amplified from *P. cuspidatum*, and three full-length genes (PcPKS3, PcPKS2, PcPKS1) were obtained by RACE. Sequence alignment analysis showed that deduced amino acid sequence of PcPKS3 (EU647245) shares 87% identity with stilbene synthase from *Rheum tataricum*, PcPKS2 (EU647246) shares 92% identity with chalcone synthase 2 from *Rheum tataricum*, PcPKS1 (EU647244) shares 93% identity with aloesone synthase from *Rheum tataricum*. Then the recombinant PcPKS3 was expressed in *E. coli*. The purified soluble protein showed a molecular weight of 43-45 kDa on SDS-PAGE. In vitro enzymatic incubation results prove that PcPKS3 performed stibene synthase function. The biochemical functions of the other two genes (PcPKS1, PcPKS2) are under investigation currently.

www.cspp.cn May 29, 2008

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Cloning and Functional Analysis of the Gene Responsible for the Yellow Cotyledon and Reduced Accumulation of Anthocyanins and Proanthocyanidins in *Arabidopsis*

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Flavonoids play important roles in recruitment of pollinators and seed dispersers, protecting plants against various stresses, being signals between plants and microbes and regulating auxin transport [1]. They also have health-promoting properties to animals [2]. Although much is known about the biosynthetic pathway and some universal regulatory proteins such as transcriptional factors with MYB, bHLH or WD40 domains have been identified, how flavonoids accumulation is regulated, particularly in response to environmental cues, remains to be elucidated [3]. Here, we isolated a recessive *Arabidopsis* mutant, *eyc23* (*ems yellow cotyledon 23*), from a collection of mutants created by ethyl methanesulfonate (ems) mutagenesis on Columbia (Col). The *eyc23* mutant showed yellow-green cotyledons and a great reduction of both anthocyanin pigments in the vegetative parts and proanthocyanidins deposition in the seed coat. Purple pigmentation derived from anthocyanins was rarely visible in leaves and stems of *eyc23* plants. The seed coat of *eyc23* was yellow at the ripening stage, and turned to pale-brown color during storage.

To further confirm the observed phenotype, we sowed seeds on half MS solid medium with sucrose concentrations up to 5% in continuous white light to induce anthocyanins synthesis. Consistent with previous report [4], purple pigments were obviously accumulated in the upper hypocotyls, abaxial and marginal regions of cotyledons in the wild type (Col). However, no purple pigments accumulation was observed in *eyc23*. Furthermore, *eyc23* seedlings did not accumulate anthocyanins despite the biochemical complementation by incorporation of naringenin in the inductive medium, indicating that the *EYC23* gene may affect anthocyanins biosynthesis at the downstream of the chalcone isomerase reaction.

To clone *EYC23* gene, the mutant was crossed to L*er* to create a map-based cloning population. First-pass mapping using simple sequence length polymorphisms markers showed that *EYC23* localized between the markers CIW8 and T20O7 on the upper arm of chromosome 5. The physical distance between those two markers is approximately 210kb. Fine-resolution mapping is undergoing.

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www.cspp.cn May 2008

Comparison of melatonin contents in the fruits among three Chinese Cherry *Prunus pscudocerasus* Lindl varieties

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The antioxidant melatonin was recently identified in a variety of edible and herb plants in high concentrations [1]. It was reported that the synthesis and accumulation of melatonin are controlled partially by light and other environmental factors [2, 3]. Cherry fruit has been found to have some curative effects to some chronic diseases, such as gout in China. Sweet cherry (*Prunus avium* L.) and sour cherry (*P. cerasus*) fruits has been proved to contain melatonin which may contribute to the medical function of the fruits. Chinese cherry (*Prunus pscudocerasus* Lindl) originates in China and there are some various local varieties in China. The difference of melatonin concentration in the fruits among Laiyang dwarf cherry、Laoshan red cherry and Wulian yellow cherry was examined by high-performance liquid chromatography with fluorescence detector, and the effect of cultivation conditions on the melatonin concentration in Laiyang dwarf cherry was studied. The results showed that there was an obvious difference of melatonin concentration among these three Chinese cherry varieties, 0.865ng/g、1.053ng/g and 2.458ng/g, respectively, in which the highest melatonin concentration in Wulian yellow cherry was three times than that in Laiyang dwarf cherry. However, no difference was found in the amount of melatonin in Laiyang dwarf cherry fruits between in greenhouse and in the open field. It is suggested that yellow Chinese cherry fruit may contain more melatonin than red ones and cherry fruit not only contains anthocyanins [4] but also other phytochemicals such as melatonin as important antioxidant substances.

Acknowledgements: This study was supported financially by NSFC, project 30471200.

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www.cssp.cn May 29, 2008

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Construction and primary analysis of colored Yunnan red pear pericarp SSH cDNA library

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There are many valuable pear (*Pyrus prifolia* Nakai) germplasm resources in Yunnan province of China, and it is noteworthy that several local varieties from Yunnan province possess a heritable characteristic of red pericarp, such as Huoba pear. Because the red pear pericarp is a desirable trait, four pear cultivars with red pericarp, Yunhongli 1, 95-2, 32, and 35, have been developed based on the Huoba pear germplasm. These cultivars were named as Yunnan red pears. It has been known that the colouration of Yunnan red pear pericarp is controlled by endogenous genes and environmental factors. In order to understand the molecular mechanism of red pigment development in Yunnan red pear pericarp, a cDNA library of colored Yunnan red pear pericarps was constructed with the method of suppression subtractive hybridization (SSH). The young fruits of 'Yunhongli 1' pear were bagged with paper, and de-bagged at 20 days before harvest. The pericarps received sunlight for 2, 4, 6, and 8 days respectively, were shaved from the de-bagged fruits, and the pericarps without receiving sunlight were harvested in the same way. The tester cDNA was reverse transcribed from a RNA pool of pericarps received sunlight for 2, 4, 6, and 8 days, and the driver cDNA was synthesized from the RNA of pericarps without receiving sunlight. At last, about 1000 randomly selected positive clones constituted the SSH cDNA library. Agarose gel electrophoresis analysis of PCR products for each clone showed that the inserted cDNA fragments range from 200 to 900 bp in length, and the average length was about 450 bp. Approximately 600 clones were randomly selected and sequenced, then 540 high-quality expressed sequence tags (ESTs) were obtained. Ultimately, the ESTs were assembled into 90 consensus sequences including 37 singletons and 53 contigs. Through gene ontology analysis, 48 consensus sequences were assigned to functional categories. The biggest functional category was metabolism, and the following were secondary metabolism and defense, respectively. It was important that the genes encoding enzymes of anthocyanin biosynthesis constituted the category of secondary metabolism, for example, the genes for chalcone synthase, chalcone flavanone isomerase, dihydroflavonol reductase, flavanone 3 beta-hydroxylase, anthocyanidin synthase, and UDP-glucose:flavonoid 7-O-glucosyltransferase. These genes highly expressed during the coloration of pericarp hint that the pigment in Yunnan red pear pericarp is anthocyanin. In conclusion, the genes involved in anthocyanin biosynthesis of Yunnan red pear pericarp are up-regulated by sunlight, and the accumulation of anthocyanin in pear pericarp donates the red color to fruits of Yunnan red pear. At the same time, the gene encoding WD-40 repeat protein have some copies in our SSH cDNA library, and this suggestes that the WD-40 repeat protein may be the pivotal transcription factor regulating the anthocyanin biosynthesis of Yunnan red pear pericarp.

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www.cspp.cn May 29, 2008

Design of assembled genes cluster for isoflavone biosynthesis

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Isoflavones are important secondary metabolites for plant physiological functions and human being health, produced from *Papilionideae Giseke* of *Leguminosae* and *Euphorbiaceae*, *Ochnaceae*, *Rosaceae*, *Moraceae*, *Celastraceae*, *Amaranthaceae*, *Iridaceae*, etc[1]. Isoflavones biosynthesis are catalyzed by Chalcone synthase (CHS), Chalcone isomerase(CHI) and Isoflavone isomerase(IFS) step by step from coumaroyl-CoA and malonyl-CoA[2,3]. There are two types of mainly CHIs in plants: CHI I in leguminous and nonleguminous plants, isomerizing only naringenin chalcone to naringenin; CHI II only in leguminous plants, having activities to isomerize both naringenin chalcone and isoliquiritigenin to naringenin and liquiritigenin, respectively[4]. For study the isoflavone biosynthesis and isoflavone engineering in *yeast* and *E Coli.*, we cloned all these three key genes: *chs*, *chi* (including *chi I* and *chi II*) and *ifs* from *Glycine max*(soybean)[5,6,7,8,9], then assembled them into cluster genes: *chs-chi I-ifs* and *chi-chi II-ifs*. Finally we designed *pichia* expression vectors pPIC3.5K-rIFS I(*chs-chi II-ifs*) and pPIC3.5K-rIFS II(*chi-chi II-ifs*), *E Coli.* expression vectors pET22b-rIFS I(*chs-chi I-ifs*) and pET22b-rIFS II(*chi-chi II-ifs*)[10,11,12]. Now we are focusing our further research on these vectors expression and optimizing their expression conditions to produce the maximum isoflavones, and test the functions of these assembled genes in other nonisoflavone plants.

www.cspp.cn May 29, 2008

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Effects of phytohormones on carotenoid biosynthesis in tomato fruit

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Carotenoid biosynthesis during tomato fruit ripening is a complex process which is regulated by a number of biotic and abiotic factors[1]. Phytohormones play an important role in regulating carotenoid biosynthesis. Effects of various plant hormones on the content and composition of carotenoids in tomato (*Lycopersicon esculentum*) fruits at different developmental stages have long been studied by exogenous application tests. However, exogenous applications could not reflect the exact cellular influences of these hormones, and contradictory results have been achieved on the influences of hormones on carotenoid biosynthesis[2]. The large populations of well-characterized phytohormone mutants in tomato provide an appropriate system to analyze the relationship between endogenous phytohormone levels and carotenoid biosynthesis in tomato fruit. In this study, a variety of plant hormone mutants and the wild-types (WT), including jasmonate acid (JA)-deficient mutants (*spr2*, *def1*) and the WT Castlemart (CM), absisic acid (ABA)-deficient mutants (*sit* and *flc*) and the WT Rheinlands Ruhm (RR), ethylene (ETH)-deficient mutant *nor* and the WT Ailsa Craig (AC) and ETH-overproduction mutant *epi* and the WT VFN8, were collected and planted. Tomato is a model system to study the fruit development, and the typical ripening stages of tomato fruits are divided into 'mature green' (MG), 'breaker' (BK), 'turning/pink' (T) and 'mature red' (R) [3]. Tomato fruits at above typical developmental stages were sampled for analysis of carotenoid contents (lycopene, β-carotene and lutein). Carotenoid were extracted under dim light and identified by HPLC. The results showed that contents of lycopene and β-carotene were always higher in *sit* and *flc* fruits than in the WT at stages of B, T and R, which indicated that endogenous ABA deficiency increased lycopene and β-carotene contents in tomato fruit. The similar result was also reported by Galpaz et al recently[4]. In *nor* fruits, the lutein content were considerably higher, while lycopene content was significantly lower than that of the wild-type AC at B, T and R stage, which was consistent with the fact that ETH is essential for the biosynthesis of carotenoid, especially the biosynthesis of lycopene during the ripening of tomato fruit[5,6,7]. There were no significant difference in carotenoids content of fruit between mutant *epi* and the wild-type VFN8 and the same results were also reported by Wang et al[8] , which suggested that the overproduction of endogenous ETH had no impact on carotenoid biosynthesis in tomato fruit. The fruit lycopene contents of *spr2* and *def1* were both lower than that of wild-type CM at all three stages of B, T and R , while the opposite effect was observed for β-carotene and lutein contents, which implied that JA promotes lycopene accumulation and inhibits β-carotene and lutein accumulation in tomato fruit. It has been reported that the exogenous applications of MeJA led to a decrease of lycopene content in tomato fruit[9]. The wild-type CM fruits at MG stage were treated with exogenous MeJA at the concentration 0.05–10 µm in our study in order to further investigate the effect of MeJA on lycopene content. The results showed that the lycopene content gradually increased along with the increase of MeJA at the concentration within 0.05-1µm, whereas it decreased by the application of MeJA within 1-10 µm, which indicated that the effects of exogenous applications of MeJA on lycopene content of tomato fruits were varied among different concentration ranges. Further investigations will be carried out to test the effects of JA and ABA on the expression of the key enzymes in carotenoid biosynthesis pathway as well as the storage capacity of carotenoid in tomato fruit.

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www.cspp.cn May 29, 2008

Identification of expressed sequence tags in a petals cDNA library of *Lycoris longituba* and Molecular cloning and analysis of anthocyanin biosynthesis genes expressed in *Lycoris longituba*

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Lycoris longituba is a perennial, bulbiferous, herbaceous plant of the family Amaryllidaceae. It is endemic in China, mainly distributed in the Langya Mountain of Anhui province, and the Baohua Mountain and Xuyi city of Jiangsu province. The three distribution areas are distant about 50 km from one another. *L. longituba* has a unique biological characteristic in that its leaves sprout in spring, die in early summer, and is then followed by flowering. Thus, its vegetative growth and reproduction are discrete, which is rare in angiosperms. The flowers of *L. longituba* have a vigorous and long scope, and are fragrant in smell, exhibiting considerable diversity in both color and floral form. All these indicate that this plant, also called the Chinese tulip, is of a high ornamental value and ideal for ikebana, the Japanese art of flower arrangement. Another value of *L. longituba* is that its bulb has some medicinal potential.

The generation of expressed sequence tags (ESTs) has been proven to be a rapid and efficient approach by which to identify the novel genes and analyze the function of genes. In order to identify and isolate correlative genes of colors, a petals cDNA library of *Lycoris longituba* was constructed, and it was subjected to high-throughout 5'EST sequencing and bioinformatical analysis. We identified 32523 ESTs representing 16474 non-redundant transcripts. BLASTX evaluation indicated that 12960 of the ESTs were homologous to various known genes/proteins in a broad range of organisms, especially gramineae species. The other 16049 ESTs, which showed little if any homology to known sequences, were considered novel. This study provides an overview of gene expression in petals of *Lycoris longituba*, as well as useful information for further investigation color variations in plants' flower.

In order to understand anthocyanin biosynthesis mechanism in Lycoris, the cDNAs encoding flavanone 3-hydroxylase (F3H), flavanone 3'-5'-hydroxylase (F3'5'H),dihydroflavonol reductase (DFR), anthocyanidin synthase (ANS), and flavonoid 3-O-glucosyltransferase (UFGT) were isolated from cDNA library prepared from the petals of *Lycoris longituba*. Deduced amino acid sequences of the cDNAs showed high homology to the sequences from other plants. The mRNAs of anthocyanin biosynthetic genes were detected preferentially in flowers, suggesting that these genes have major roles in determination of petals color.

Acknowledgements: This research was supported by the National 863 Program of China (2002AA241051) and Shanghai KeJiXionNong Foundation (2002).

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www.cspp.cn May 2008

Involvement of tyrosinase in betacyanin biosynthesis induced by dark in C3 halophyte Suaeda salsa seedlings

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As a halophyte, physiological and molecular responses of *Suaeda salsa* to salinity stress have been extensively studied. But nothing is known about the biosynthesis regulation of the red pigments in *S. salsa*. Our previously study showed that the red pigments in *S. salsa* are not anthocyanins but betacyanins. Light suppressed betacyanin accumulation and enhanced their decomposition, and dark at the germination phase is one of the most important factors for the betacyanin accumulation in *S. salsa*. As analog of anthocyanins, betalains are an important class of water-soluble nitrogen-containing pigments, which comprise the red-violet betacyanins and the yellow betaxanthins, and accumulate in most families of the Caryophyllales and some higher fungi.

The role of tyrosinase in the plant secondary metabolism still remains unclear, and the involvement of both hydroxylation and oxidation activities of tyrosinase in betalain biosynthesis has been proposed, but has not yet been shown conclusively. In this paper, seeds of the halophyte *S. salsa* were cultured in the dark for three days and betacyanin accumulation was induced significantly in cotyledons of *S. salsa* seedlings. Accumulated betacyanin in cotyledons of the dark-grown seedlings decomposed with time in light. Along with the betacyanin content declining, the hydroxylation and oxidation activity of tyrosinase extracted from cotyledons of the dark-grown seedlings decreased with time in light. The apparent molecular mass of tyrosinase in cotyledons of *S. salsa* seedlings was about 60 kDa estimated by SDS-PAGE, enzyme activity staining and Western blotting. Furthermore, the tyrosinase only synthesized in the cotyledons of dark-grown *S. salsa* seedlings, and declined with time in light, which was paralleled by the decreases of tyrosinase activity and betacyanin content in light. This result suggests that the specific tyrosinase is positively correlated with betalain biosynthesis, and betacyanin biosynthesis is induced by dark via synthesis of tyrosinase in *S. salsa.*

www.cspp.cn May 2008

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Isolation and Characterization of *SsMYB2* from Coleus, a Novel Transcription Factor Regulate Phenylpropanoid Biosynthesis

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MYB-related transcription factors play wider roles in the regulation of different branches of phenylpropanoid metabolism in general [1]. For the purpose of study on the regulation of phenylpropanoid metabolism, the first MYB R2R3 transcription factor, *SsMYB2* (EF522160 and EF522161 for its cDNA and DNA, respectively) [2], was isolated from Coleus (*Solenostemon scutellarioides*), a famous foliage plant with multicolor and rich in secondary metabolites, using RACE method according to the conserved regions of other function-known MYB R2R3 proteins of plants.

The full-length cDNA and genomic sequences of *SsMYB2* are 994 bp and 1108 bp, respectively, with a 732bp ORF encoding a polypeptide of 243 amino acids, including a 114bp intron in R3 domain of SsMYB2. NCBI blastp indicated that SsMYB2 shows 75.0%, 64.2% and 58.1% homology with VvMYB4 (ABL61515), AmMYB308 (JQ0960) and AtMYB4 (AF062860), respectively. In N-terminus of SsMYB2, there are two typical conserved R2R3 domains, and a conserved bHLH motif ([D/E]Lx2[R/K]x3Lx6 Lx3R) for interaction with bHLH proteins [3] is found in R3 region. And in non-conserved C-terminal region there are three typically conserved motifs of subgroup 4 [4]: C1 motif (LlsrGIDPxT/SH RxI/l), C2 motif (pdLNLD/ElxiG/S) and Zinc-finger motif ($CX_{1-2}CX_{7-12}CX_{1-2}C$) relating to the special function, in which the C2 motif forms part of the region involved in the repression of transcription[5]. Phylogenetic analysis reveals that SsMYB2 clusters with members of the subgroup 4, in which includes several inhibitors of transcription: AmMYB308 [6], AtMYB4 [5] and FaMYB1 [7]. Semi- quantitative RT-PCR indicated that *SsMYB2* has expression in root, stem, leaf and flower in red cultivar. Overexpression of *SsMYB2* in tobacco plants resulted in decreased expression of C4H and 4CL, and the same phenotype, a dwarfed phenotype and in chlorotic and necrotic lesions on leaves, in transgenic tobacco that had been observed from *AmMYB308* and *AtMYB4* [5,6].

This preliminary characterization of SsMYB2 suggests that this transcription factor may play a key role in regulating phenylpropanoid metabolism in Coleus, possibly acting as a repressor of gene expression.

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Metabolic Engineering of Astaxanthin Production in Arabidopsis by targeting *Chlorella zofingiensis* β-carotene ketolase to Chloroplasts

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Astaxanthin is an important keto-carotenoid pigment, which has been widely used as a feed additive in aquaculture. This pigment contributes to the attractive coloration in bodies of aquatic animals such as salmonids and crustaceans and maintains their normal growth and survival [1]. In addition, astaxanthin was reported to boost immune functions, to reduce oral carcinogenesis and to inhibit the growth of mammary tumors. These properties of astaxanthin are probably related to the strong anti-oxidative activity of astaxanthin over other carotenoids such as β-carotene, zeaxanthin and lutein [2]. Astaxanthin has attracted tremendous commercial interest for medicinal and nutraceutical uses.

The green microalga, *Chlorella zofingiensis* is one of the microbial sources of astaxanthin production owing to the ease of culture and high tolerance to environmental fluctuations. The sequence of a full-length cDNA of *Chlorella zofingiensis* β-carotene ketolase (BKT) gene was cloned and functionally analyzed in *Escherichia coli* in previous study [3]. Here we report the stable transformation of Arabidopsis plants by *Agrobacterium*-mediated method with *C. zofingiensis BKT* cDNA. A 1.7-kb fragment containing the transcriptional promoter and the coding sequence of chloroplast transit peptide of the Arabidopsis 1A Rubisco small subunit gene was used to express and target the *C. zofingiensis* BKT to chloroplasts. Transgenic plants showed slight growth inhibition. Analysis of the pigment synthesis in transgenic plants showed that β-carotene were converted into ketocarotenoids. Several novel pigments were found to accumulate in the leaves of transgenic plants, which were identified as ketolutein, hydroxyl-echeninone, canthaxanthin and astaxanthin.

Acknowledgements: This work was supported by the 863 Program (2007AA02Z102, to Z.-F. Xu) and the Guangdong Natural Science Foundation (05003315, to Y. Li).

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Molecular and enzymatic characterization of a novel benzalacetone synthase encoded by an unusual gene with three introns from *Polygonum cuspidatum* Sieb. et Zucc

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Benzalacetone synthase (BAS) is a novel plant-specific type III polyketide synthase that catalyzes a single decarboxylative condensation of malonyl-CoA to the typical 4-coumaroyl-CoA starter to produce the C_6-C_4 skeleton of phenylbutanoids in higher plants. A cDNA encoding this key enzyme was cloned from rhizomes of *Polygonum cuspidatum* Sieb.et Zucc., a medicinal plant rich in aromatic polyketides. The resulting cDNA was designated *PcBAS1*. It is 1417-bp long and encodes 389 deduced amino acid residues. Surprisingly, the coding region of the *PcBAS1* gene is interrupted by three intervening sequences, of 225bp, 251bp and 89bp. DNA gel blot analysis indicated that there are two to four copies of BAS genes in the *P. cuspidatum* genome. RNA gel blot analysis revealed that *PcBAS1* is highly expressed in the rhizomes and in young leaves, but not in the roots of the plant. *PcBAS1* transcripts in leaves were induced by pathogen infection. Interestingly, the CHS 'gatekeeper' phenylalanines, Phe215 and Phe265 are both lacked in PcBAS1, which are uniquely replaced by Leu and Cys. Unlike *R. palmatum* BAS activity, recombinant PcBAS1 activity expressed in *Escherichia coli* was dependent on pH: at high pH condition (pH 8.5-9.5), the PcBAS1 effectively yielded benzalacetone as a major product along with CTAL, BNY and a little amount of naringenin chalcone; whereas at pH 6.0-8.0, most of the reactions were terminated at the tetraketide stage, leading to formation of CTAL as a major product along with BNY, benzalacetone and a trace amount of naringenin chalcone. In particular, besides 4-coumaroyl-CoA, only the feruloyl-CoA from its derivatives was accepted as starter substrates. In contrast with CHS that showed broad substrate specificity toward aliphatic CoA esters, PcBAS1 did not accept isobutyryl-CoA, isovaleryl-CoA or acetyl-CoA as a substrate. The molecular and enzymatic characterization of PcBAS1 thus contributes to the understanding of the functional diversity and evolution of type III PKSs.

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www.cspp.cn May 2008

Molecular mechanism of flower pigmentation in Japanese gentian plants

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Gentians (*Gentiana triflora* and *G. scabra*) are popular floricultural plants in Japan and widely used as cut or potted flowers. They have unique and brilliant blue-colored flowers, accumulating poly-acylated anthocyanin, so-called 'gentiodelphin', and flavone derivatives in their petals. We have been studying on gentiodelphin biosynthesis, and isolated several biosynthetic structural genes from gentian flowers [1, 2]. Gentiodelphin biosynthetic structural genes can be classified into three groups based on temporal expression profiles during flower development. The first group contains *CHS*, *CHI*, encoding the common enzymes of flavone and anthocyanin biosyntheses; the second belong to *FNSII*, *F3'H*, catalyzing flavone biosynthesis; the third contains *F3H*, *F3'5'H*, *DFR*, *ANS*, *3GT*, *5GT* and *5AT*, involving in anthocyanin biosynthesis. These results revealed that anthocyanin biosynthesis in gentian petals is temporally regulated by the expression levels of the corresponding structural genes.

We isolated two candidate genes as the transcriptional factors regulating anthocyanin biosynthesis in gentian flowers. *GtMYB3* as *R2R3-MYB* homologue and *GtbHLH1* as *bHLH1* homologue exhibited high similarity with petunia *AN2* and *AN1*, respectively. The expression profiles of both genes corresponded well with those of anthocyanin biosynthetic genes and the accumulation pattern of gentiodelphin pigments. Yeast two-hybrid analysis also showed that GtMYB3 interacts with GtbHLH1. Transient expression assay showed that co-expression of *GtMYB3* and *GtbHLH1* could activate the promoter activities of anthocyanin biosynthetic genes. These results strongly supported that GtMYB3 interacts with GtbHLH1 and regulates antchoyanin biosynthesis in gentian flowers.

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www.cspp.cn May 29, 2008

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The Effect of Methyl Jasmonate on Content and Key Enzyme Synthesis of Cyanidin and Partial Signaling Molecule of *Nanguo* Pear

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Nanguo pear is the higher quality variety of *Pyres ussuriensis Maxim*. Its origin lies in Shangduizhuangshi village (Dagushan town, Qianshan district, Anshan city). It has 100-years history. The variety is renowned at home and abroad for its bright color and luster, fine and smooth flesh, sweet and succulency, fragrant and strong flavor. But red fruit ratio of nanguo pear is lower, only 5-10%, especially fade is 100% after rain in maturation period, and recolor slowly. If increasing red fruit ratio, it will bring enormous economic and social benefit.

At present, there is no report on the research of glucoside and the group of acylation for cyanidin synthesis of nanguo pear aroud the world, the key enzyme and metabolic regulation of cyanidin synthesis of pear are not solved yet[6]. The research is based on the signal transduction principle, by applying MeJA on nanguo pear, regulating intracellular signal molecule, improving protein phosphorylation, activating key enzyme of cyanidin synthesis, improving cyanidin synthesis, study the regulating mechanism of MeJA on partial metabolic regulation of cyanidin synthesis of Nanguo pear to establish metabolic regulation technique; by exterior regulation to increase cyanidin content and red fruit ration, all of which will bring enormous economic and social benefit and are urgent tasks for the fruit culture nowadays.

The article studies the possible synthesis mechanism of cyanidin by determining partial signaling molecule and the partial key enzyme of cyanidin of synthesis, Eight-year old trees act as experiment sample, spray them with MeJA during the time of synthesizing cyanin. Result showed that, MeJA improves evidently the content of endogenous ABA, reduces the content of GA_3, increases the ratio of ABA/GA_3, and increases the content of CaM and enzyme activity of Ca^{2+}-ATPase, NADKase. And meanwhile increases the enzyme activity of PAL and CHI, and increases the content of cyanidin.

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The role of *ADC1* gene encoding a carotenoid isomerase in regulation of cyclic electron flow around photosystem I mediated by chloroplastic NADPH dehydrogenase in *Oryza sativa*

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Evidence has been shown that cyclic phosphorylation mediated by chloroplastic NADPH dehydrogenase complex (NDH-1) can keep operation of CO_2 assimilation thereby alleviate photo-oxidative damage under temperature stress in tobacco plant (Wang et al. 2006). However, the regulative mechanism of this pathway is still unclear. In this study, we identified a new mutant called *accumulated damaged chloroplasts 1* (*adc1*), with variegated yellow leaves, in *Oryza sativa* (rice). The *adc1* gene was isolated using map-based cloning approach, and was predicted to encode a carotenoid isomerase. The content of lutein in *adc1* was 70% less than that in the wild type. Damaged chloroplasts with no or less thylakoid membranes accumulated in the mutant leaves. Hydrogen peroxide accumulated under moderate light condition (400 $molm^{-2}s^{-1}$) and more significantly under high light condition (1500 $molm^{-2}s^{-1}$) in the leaves of ADC1.The activity of photosystem II, but not that of photosystem I (PS I) decreased more significantly in the *adc1* mutant than in the wild type under the high light condition. Several photoprotective mechanisms, such as nonphotochemical quenching (NPQ), state 1 - state 2 transitions, especially the cyclic electron transport around PS I were significantly suppressed in the mutant. The activity and amount of NDH-1L with molecular weight around 550 kDa, one of the mediator of the cyclic electron flow in ADC1 were obviously lower than those in the wild type especially under high light condition. The reduced capacity of photoprotective mechanisms were rescued in the complement line. Based on these results, we suggest that except directly dissipating excessive energy, ADC1 is essential for the assemblage of NDH-1L, one of mediators of cyclic electron transport around PS I through its product lutein, thus protecting rice plants from photodamage. The relationship among the photoprotective pathways are discussed.

Acknowledgements: The authors gratefully acknowledge Z.-J. Luo and M.-J. Chen for mutant screening and generation of F2 populations for the mapping, C.-M. Zhang for rice transformation, M.-Y. Zhou for TEM, H. Ma for editing this manuscript. This work was supported by funds from the State Key Basic Research and Development Plan (No. 20007CB108804), the National Key Basic Research Developments Program of the Ministry of Science and Technology, People's Republic of China (2006AA10A102).

www.cspp.cn May 29, 2008

Functional Characterization of a Missense Mutation in CESA4 of Rice (*Oryza sativa* L.)

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Cellulose microfibrils are insoluble cable-like structures that are homogenous polymers of β-1,4-glycan and represent the most abundant component of the cell walls. The synthesis and organization of cellulose polymers largely determines the mechanical characteristics of the wall, as well as the functions in morphogenesis and development of plants [1]. Cellulose microfibrils are synthesized at the plasma membrane by multimeric complexes called rosettes [2]. CESA (cellulose synthase catalytic subunit) proteins are the only known components of the cellulose synthesis complex, which range from 985–1088 amino acids in length and have eight putative transmembrane domains. During the past decade, a large number of mutations that occur in various sites of CESA proteins have been functionally identified and proven to be good materials for verifying the contributions of different domains and work model of CESAs. Here, we reported a missense mutation in *OsCESA4*, which is called *brittle culm11*. This mutant displays the reduced cellulose accumulation and altered distribution of pectin and hemicellulose. The evidence that the mutant cDNA can fully complement the mutant phenotypes accompanying with biochemical data demonstrated that the deficiency in cellulose biosynthesis in *bc11* results from a decreased abundance of CESA4 protein in plasma membrane.

www.cspp.cn May 29, 2008

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A subclade of flavin- monooxygenases involved in biosynthesis of aliphatic glucosinolates

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Glucosinolates (GSLs) are natural metabolites with diverse biological activities and the activity is highly dependent on the modifications of glucosinolate. Here we report the identification of a sub-clade which contains five flavin-monooxygenases (FMO) as *S*-oxygenating enzymes in aliphatic glucosinolate biosynthesis in *Arabidopsis thaliana* which catalyze the conversion of methylthioalkyl (MT) GSLs to methylsulfinylalkyl (MS) GSL. MS GSLs can break down to isothiocyanates such as sulforaphane with potent cancer-preventive properties. The five FMO proteins are designated FMO$_{GS-OX1-5.}$ Biochemical characterization of the recombinant protein and the analysis of GSL content in T-DNA knock-out mutants and overexpression lines show that FMO$_{GS-OX1}$, FMO$_{GS-OX2}$, FMO$_{GS-OX3}$ and FMO$_{GS-OX4}$ can catalyze the conversion from MT GSL to the relative MS GSL independent of chain-length whereas FMO$_{GS-OX5}$ show high substrate specificity for the long chain 8-methylthiooctyl GSL. On the basis of the significant increase of MS especially 4-methylsulfinylbutyl GSL production in the *FMO$_{GS-OX}$s* overexpression lines, the identification of the FMO$_{GS-OX}$ sub-clade will provide a molecular tool for the genetic engineering in production of the cancer-preventive isothiocyanates.

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www.cspp.cn May 29, 2008

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Biphenyl and benzophenone metabolism

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Biphenyls and benzophenones are benzoate-derived polyketides. Biphenyls are phytoalexins of Maloideae [1]. This rosaceous subfamily includes economically important fruit trees such as apple and pear. Biphenyls seem to be the precursors of dibenzofurans. Both classes of phytoalexins accumulate in the sapwood of infected Maloideae species [1]. Benzophenones are widespread in Clusiaceae. Polyprenylation leads to the formation of bridged polycyclic compounds with intriguing pharmacological activities. Regioselective intramolecular cyclizations of a simple benzophenone give xanthones [2]. The key enzymes of the biosynthetic pathways are biphenyl synthase (BIS) and benzophenone synthase (BPS) [3, 4]. These type III polyketide synthases (PKSs) use benzoyl-CoA and three malonyl-CoAs to form identical linear tetraketides. While BPS cyclizes the intermediate via intramolecular Claisen condensation, BIS catalyzes intramolecular aldol condensation and decarboxylative elimination of the terminal carboxyl group. The products are 2,4,6-trihydroxybenzophenone and 3,5-dihydroxybiphenyl, respectively. cDNAs encoding BIS and BPS were cloned from cell cultures of *Sorbus aucuparia* (Rosaceae) and *Hypericum androsaemum* (Clusiaceae), respectively [5, 6]. The enzymes share 54% amino acid sequence identity. Expression of BIS and BPS was inducible by elicitor treatment, whereas that of chalcone synthase (CHS) was not. The preferred starter molecule for CHS, 4-coumaroyl-CoA, was no starter substrate for BIS and BPS. In a phylogenetic tree, BIS and BPS grouped together closely within the cluster of functionally diverse PKSs. An individual cluster was formed by CHSs, indicating an ancient duplication of the ancestral gene. Site-directed mutants were generated and rationalized by homology modeling. A single amino acid exchange transformed BPS into phenylpyrone synthase. In the active site cavity of this BPS mutant, the intermediate triketide was redirected into a smaller pocket, resulting in lactonization. When incubated with *ortho*-hydroxybenzoyl-CoA as starter substrate, BIS catalyzed only one decarboxylative condensation with malonyl-CoA and formed 4-hydroxycoumarin.

Acknowledgements: This work was supported by the German Research Foundation (DFG) priority program "Evolution of Metabolic Diversity" (SPP1152).

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Characterization of an *Arabidopsis* T-DNA mutant of the folypolyglutamate synthetase

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Polyglutamylated tetrahydrofolate coenzymes are involved in one-carbon (C1) metabolism [1]. The glutamate residues are attached to the folate molecule via the catalytic activity of folylpolyglutamate synthetase (FPGC) [2]. In *Arabidopsis* there are three isoforms of FPGC, *AtDFB*, *AtDFC*, *AtDFD*. In this study, a T-DNA insertional mutation in the 15th exon of *AtDFC* (At3g10160) was characterized. Morphological observation reveals that the mutant plants displayed one-week delay in bolting and flowering time after sowing 7 weeks comparing with wild type plants. Interestingly the transcripts of some other folate synthesis enzymes, including GTP cyclohydrolase I (GCHI), dihydroneopterin aldolase (DHNA), dihydrofolate reductase (DHFR), were also decreased in the mutant, indicating the existence of the coordinative and interrelated expression within the folate synthesis network. Further characterization on the mutant and changes in folate profiles are under investigation.

Acknowledgements: This research was supported by grant 2007CB108801 to Chunyi Zhang from national basic research program of China.

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Cloning and characterization of glucosyltransferase cDNA from *Rhodiola sachalinensi* cultured cells

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Salidroside has been identified as the most potent ingredient of the Chinese medicine herb, *Rhodiola sachalinensi*. And it was proved to be effect for treating patients suffering tumor, anoxia, microwave, virus, radiation or fatigue by modern pharmacological study[1-5]. However, the resource of wild R. sachalinensis A. Bor has become less and less due to over-exploitation in order to meet the increasing demand[6]. Therefore, in order to reduce the salidroside cost, a effective method of modern biotechnology can be adopted, such as genetic engineering, enzyme engineering or fermentation engineering and so on. In this study, the gene of key enzyme of biosynthesis salidroside was cloned by RT-PCR using a degenerated primer which designed by CODEHOP (COnsensus-DEgenerate Hybrid Oligonucleotide Primers) and RACE-PCR. The key enzyme is glucosyltransferase, which activity was maximized by methyl jasmonate induction in crude protein extract of R. sachalinensis A. Bor cultured cells before total RNA extraction. The cDNA contained an open reading frame encoding 453 amino acids with a calculated molecular mass of 51.2 kDa. The consensus sequence of the plant glucosyltransferases was included in the deduced amino acid sequence. The recombinant glucosyltransferase was expressed and in Escherichia coli and purified by His-Band. Its catalytic activity was examined using tyrosol. The specific activity is 566.8 pkat/mg when the recombinant enzyme catalyzes tyrosol to be salidroside.

Acknowledgements: We thank The Ministry of Agricultrue of the People's Republic of China, for financial support.

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Comparative Analysis of the Chemical Profiles of the Invasive Neophytes *Polygonum cuspidatum* and *Polygonum sachalinensis* by HPLC-DAD-ESI/MS

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Polygonum cuspidatum Sieb. & Zucc (Polygonaceae), a well known traditional Chinese medicine (Hu Zhang in China, Kojo Kon in Japan) has been traditionally used for the treatment of various inflammatory diseases, hepatitis, tumors, and diarrhea. Recently, antiviral activity [1] and bacterial DNA primase inhibiting activity [2] were also reported. But the plant has gained much notoriety in Europe or North America as a pernicious weed (where it is named Mexican Bamboo, Japanese Bamboo or Japanese Knotweed), due to its virtually indestructible growing characteristics [3]. This plant may be confused with giant knotweed (*Polygonum sachalinensis* F. Schmidt ex Maxim), which has a similar growth form, and is also a non-native species with its origins in Asia. Both of these two species are on the black list of invasive alien plants in Switzerland (http://www.cps-skew.ch/english/black list.htm).

It is said that most invasive plants are phytochemically unique in their new habitats, and many of the unique phytochemicals from invasive plants have been reported to have multiple activities, including antiherbivore, antifungal, antimicrobial and alleopathic (phytotoxic) effects, which may provide the plants with several advantages in their new environments [4].

As one part of a research program on the medicinal value of such unique phytochemicals, different crude extracts of *P. cuspidatum* and *P. sachalinensis* were qualitatively analyzed by a validated HPLC-DAD-ESI/MS method: 1) to compare the chromatograms of different species and different organs to show the possibility of alternative medicinal utilization; 2) to compare the chromatographic fingerprints of samples from China and Switzerland to reveal the phytochemical differences between the original and invasive varieties. A total of 36 constituents were identified on-line by comparing the retention times, UV data, mass spectra of detected compounds with those of References compounds or those published in the literature. It was shown that stilbenes and anthraquinones are the main constituents of the root extracts of *P.cuspidatum*, while phenylpropanoid glucosides are the main constituents of the root extracts of *P. sachalinensis*. Thus these two species may not be alternatively utilized for the same therapeutic effect. In contrast, phenylpropanoid glucosides were present as the main constituents for the stems of all these varieties. Comparison of *P. cuspidatum* in Switzerland with the variety growing in China indicated that stilbene glucosides such as piceatannol glucoside and resveratroloside predominate in the invasive variety, as well as proanthocyanidins, flavonol gallates. The significance of these phytochemicals in the robustness of the plant growing in alien areas is worth further consideration.

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Acknowledgements: This work was supported by the Swiss National Science Foundation (grant no. 200020-107775 to Prof. K. Hostettmann). The author also gratefully acknowledges support by the China Scholarship Council.

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www.cspp.cn May 29, 2008

LeGWD – a tomato gene involved in starch phosphorylation and degradation is essential for pollen viability

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Although the sequence of many genes is available, their biological function is largely unknown. The major challenge in the era of genome-wide sequencing research is to find ways to use the vast amount of sequence data to improve our understanding about the biology of organisms.

The most direct approach to determine the functions of the sequenced genes in an organism is to disrupt or generate mutation in the genes and analyze the consequences. In this work we used both forward and reverse genetics strategies in order to isolate and characterize genes involved in primary and secondary metabolism.

Using both forward and reverse strategies, we screened the *Ac/Ds* Micro-Tom tagging population previously produced in Prof. Avi Levy's lab[1,2]. From these screens two new metabolic mutants, the *legwd1::Ds* and the *Orr*^{Ds} were identified. In my talk I will focus on the *legwd1::Ds* mutant. The transposon insertion sites in the *legwd1::Ds* mutant was found to be homologous to the Glucan Water Dikinase (*GWD)* gene from potato (and *SEX1* from Arabidopsis,[3,4]. This protein, was first isolated in potato [3] and was found to function as an overall regulator of starch mobilization by controlling the phosphate content of the starch [3,4,5]. Based on genetic phenotypic and molecular analysis we uncovered a new role for this gene, namely the mutation in *LeGWD* caused male sterility. This sterility is correlated with starch excess in the pollen grains as detected by iodine staining. It was also associated with a reduction in pollen germination. SEM and TEM analyses indicated a mild shrinking of the pollen grains and accumulation of large starch granules inside the plastids. The level of soluble sugars was reduced by 1.8 fold in mutant pollen. Overall, the transfer of the mutant allele through the gametes was only of 0.4% in the male gametes while it was normal through the female gametes. Additional null mutant alleles, obtained through transposon excision, showed the same phenotypes as *legwd::Ds*. Moreover, pollen germination could be restored, and the starch excess phenotype could be abolished by pollen-specific expression of the potato *GWD* homolog (*StGWD*). These results demonstrated, for the first time, that starch phosphorylation and its subsequent breakdown are essential for pollen germination. Pollen sterility has prevented the isolation of a homozygote. To overcome this, we isolated a conditional mutant that is homozygote for *legwd::Ds* but is expressing *StGWD* under a pollen-specific promoter. This mutant is a true knockout of *GWD* in all tissues but the pollen. It will serve in the future as a genetic platform for exploring the role of *GWD* in tomato development and physiology in tissues other than pollen.

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www.cspp.cn May 29, 2008

Metabolomic study of aflatoxin production in *Aspergillus flavus*-inoculated peanut seeds

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Aflatoxin (AFs) is a class of mycotoxins produced mainly by the fungal species *Aspergillus flavus* and *A. parasiticus,* before or during harvesting, or because of improper storage [1]. Four natural present aflatoxins are AFB1, AFB2, AFG1 and AFG2, which are the major contaminates in a wide variety of agricultural products such as peanut, corn, nuts, etc[2]. These compounds are highly toxic and carcinogenic, even at very low concentrations. Therefore, prevention of AF contamination and identification of biomarkers that representing AFs production are very crucial in food safety. Metabolomics has emerged as a valuable technology for comprehensive metabolic profiling and comparison of metabolites in complex biological systems [3]. Metabolomics, which is usually carried out using liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) or NMR technologies, allows us to compare quantitively and qualitatively the differences in metabolites. The results could be futher analyzed statistically using multivariate tools such as principal component analysis (PCA), hierarchical cluster analysis (HCA), discriminate analysis or correlative network analysis.[4]

The aims of this research is to examine the the production of aflatoxins in peanut and studying the relationship between aflatoxins production in different temperature and humidity by means of metabolomic analysis method, which is helpful to choose better storage conditions to prevent the toxin production. Peanuts cotyledons were inoculated by aflatoxigenic and atoxigenic strains of *Aspergillus flavus*, and cultivated at different temperature and humidity. Then, the samples were extracted with appropriate solvents, detected by LC-Q-Tof MS and GC-Tof MS and analyzed using multivariate analysis tools (PCA, PLS, OPLS). Results obtained so far provide several potential markers with known molecular weight. Their molecular formulas and structures have to be identified.

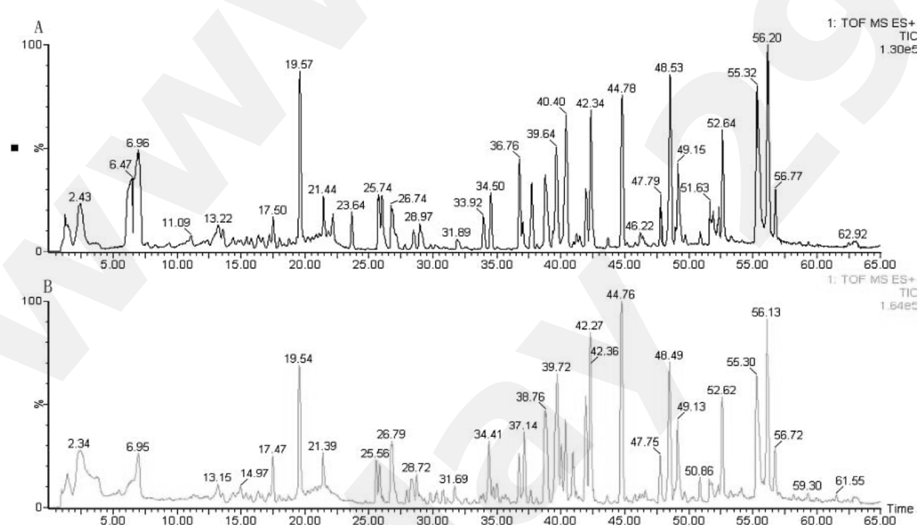

Fig. 1 Total ion chromatogram of crude extract from peanuts cotyledons inoculated by aflatoxigenic (A) and atoxigenic (B) strains of *Aspergillus flavus* respectively and cultivated at 28°C for 4 day.

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Natural variation and QTL analysis of Glucosinolates Concentrations in *Brassica rapa*

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Glucosinolates characterizing the strong flavors and tastes of *Brassica* vegetables, have been found having potential as cancer-prevention agents, crop-protection compounds, and biofumigants in agriculture. We investigated natural variation of glucosinolates in *B. rapa*, which accounts a quarter of total vegetable production in China.

Two sets of *B. rapa* materials were screened for leaf glucosinolates concentrations by using HPLC, one with 96 germplasm accessions belonging to 12 cultivar groups, the other one of variation fixed set with 173 DH lines. The results indicate that the dominant contents of glucosinolates are aliphatic glucosinolates and there are remarkable variations of glucosinolate contents in *B. rapa*. For the germplasm accessions, the most outstanding accession showed a NAP concentration of 40.865 μg g^{-1} DW, which was 1127-fold higher than that of the lowest one. The concentrations of the other two aliphatic glucosinolates varied 5 folds for PRO (0.069-0.344 μg g^{-1} DW) and 151 folds for GBN (0.031-4.693 μg g^{-1} DW). The concentrations of indole glucosinolates, including 4OH, GBC, 4ME, NEO, showed a variation of 7-fold (0.025- 0.173 μg g^{-1} DW), 18-fold (0.089-1.607 μg g^{-1} DW), 20-fold (0.029 -0. 590 μg g^{-1} DW) and 142-fold (0.013-1.850 μg g^{-1} DW), respectively. An 82-fold variation was found for aromatic glucosinolate NAS concentration (0.014 to 1.154 μg g^{-1} DW). More remarkable variations were found for the variation fixed set with the highest NAP concentration of 73.683 μg g^{-1} DW, which was about 2600-fold higher than the lowest one. The concentrations of the other two aliphatic glucosinolates varied 751 folds for PRO (0.071-53.338 μg g^{-1} DW) and 204 folds for GBN (0.066-13.514 μg g^{-1} DW). The concentrations of indole glucosinolates, including 4OH, GBC, 4ME, NEO, showed a variation of 222-fold (0.006- 1.332 μg g^{-1} DW), 291-fold (0.007-2.036 μg g^{-1} DW), 297-fold (0.006 -1.785 μg g^{-1} DW) and 132-fold (0.008-1.057 μg g^{-1} DW), respectively. A 256-fold variation was found for aromatic glucosinolate NAS concentration (0.016 to 4.099 μg g^{-1} DW).

A BC_2S_5 population including 113 lines was constructed by using a rapid cycling line L144 and a Caixin line L58, which showed significant differences in leafy glucosinolates conconcentrations. A genetic linkage map was constracted based on 252 SRAPs and 108 SSRs. Glucosinolates concentrations in leaves were determined for 90 BILs lines grown in green house. A major QTL was detected for NAP and PRO, co-localizing on R3 with explained variance of 82.5% and 36.8%, respectively.

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Promoter cloning and sequence analysis of the alcohol acyltransferase gene (*MdAAT2*) from apple

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Volatile esters are important aromatic components in fermented beverages[1] and many ripened fruits[2,3 ,4]. Alcohol acyltransferases (AATs), members of the BAHD superfamily[5] , are key enzymes in ester biosynthesis, which catalyze the final step in ester formation by linkage of an acyl moiety from acyl-CoA to an appropriate alcohol. Synthesis of esters is most commonly associated with flower development, fruit ripening and alcoholic beverage brewing. However, several recent studies have reported that volatile esters can trigger defense responses and also be induced by herbivore and mechanical damage[6,7,8].Our previous research showed that besides induced by ethylene (ETH), apple alcohol acyltransferase (MdAAT2) was also strongly induced by salicylic acid (SA) and methyl jasmonate (MeJA)[9,10]. SA and MeJA are closely related to the gene expression induced by biotic and abiotic stresses, and also regulate the plant defensive responses mutually with ETH[11]. To investigate the function of apple AAT further, a 1572 bp sequence upstream of MdAAT2 5'UTR was isolated from the genomic DNA of apple using Tail-PCR. Cis-elements and regulation sequences which might respond to ETH and MeJA were found by further analysis of the sequence (PLACE program). These indicated that MdAAT2 might be in response to stresses and locate in the downstream of the signal pathway induced by ETH, SA and MeJA.

Acknowledgements: We thank the staff of Professor Xian-Sheng Zhang's laboratories for providing enthusiastic technical advice and assistance. This work was funded by the National Natural Science Foundation of China (Grant #30700548), the China Postdoctoral Science Foundation (Grant #20070410366), and the Doctoral Program Foundation of Shandong Province of China (Grant #2007BS06001).

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Proteome and glucosinolate changes in Arabidopsis leaves in response to mechanical wounding

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The concentration and composition of certain plant secondary metabolites are known to determine plant responses to herbivores, pathogens and mechanical wounding [1]. Glucosinolates (GSs) are a major group of nitrogen- and sulfur-containing secondary metabolites in *Arabidopsis thaliana* as well as other Brassicaceae plants[2]. In Arabidopsis, mechanical wounding seems to induce the levels of indol-3-ylmethyl GS (I3M) [1]. In this study, we analyzed the changes of glucosinolate levels and the proteome in Arabidopsis leaves in response to mechanical wounding. Rosette leaves were wounded by cutting approximately 40% leaf area across the apical lamina with scissors. Proteins and GSs were extracted from leaves after 0h, 1.5h and 3h after wounding treatment. A total of 12 GSs were detected including 8 aliphatic GSs (3-methylsulphinylpropyl GS, 4-hydroxybutyl GS, 4-methylsulphinylbutyl GS, 5-methyl-sulphinylpentyl GS, 6-methylsulphinylhexyl GS, 8-methylsulphinyloctyl GS, 7-methylthioheptyl GS, and 8-methylthiooctyl GS), and 4 indole GSs (4-hydroxyindol-3-ylmethyl GS, indol-3-ylmethyl GS, 4-methoxyindol-3-ylmethyl GS, and 1-methoxyindol-3-ylmethyl GS). Quantitative analysis using HPLC revealed that the levels of total GSs, aliphatic GSs and indole GSs were all up-regulated after wounding treatment. The highest levels were observed at 3h after treatment. When individual GSs were analyzed, 4-hydroxybutyl GS (4OHB), 4-methylsulphinylbutyl GS (4MSOB), 3-methylsulphinylpropyl GS (3MSOP), 4-methoxyindol-3-ylmethyl GS (4MTI3M) and I3M exhibited predominant changes at 3h after wounding. Proteins from control and 3h wounding-treated samples were analyzed by two-dimensional gel electrophoresis and image analysis. Fifty eight protein spots of on the two-dimensional gels showed significant abundance changes. These proteins were excised, digested with trypsin and identified by LC Q-Trap MS/MS and MALDI TOF-TOF MS/MS. The proteins identified showed significant overlap with those identified to be responsive to MeJA treatment. The results support the notion that wounding and MeJA pathways share common components in plant responses to environmental stresses.

Acknowledgements: This work was supported by funds from the University of Florida and National Natural Science Foundation of China (No. 30528013 and No. 30670325) and a program for New Century Excellent Talents in Chinese Universities (NCET-05-0328).

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www.cspp.cn May 29, 2008

Proteome and glucosinolate changes in leaves of *Arabidopsis thaliana* in response to methyljasmonate

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Glucosinolates (GSs) and their breakdown products exhibit a variety of biological activities[1]. Methyljasmonate (MeJA) is a signaling molecule induced by herbivore attack and wounding to initiate plant defense processes. The levels of GSs in Arabidopsis suspension cells have been characterized. The responses of GSs to MeJA varied greatly with the age of the cells [2]. In Arabidopsis plants, some Indole GSs accumulated in response to MeJA treatment[3]. In this study, we report a detailed analysis of leaf GSs and proteome in response to MeJA. Our results showed that the contents of total GSs, aliphatic GSs and indole GSs, changed at 12h, 24h, 36h and 48h after 200 μM MeJA treatment. The contents of aliphatic GSs and indole GSs reached the peak at 24h and 36h after treatment, respectively. Some aliphatic GSs, such as 3-hydroxylpropyl GSs, 3-methylsulphinylpropyl GSs and 5-methylsulphinylpentyl GSs exhibited significant accumulation in response to MeJA. The contents of indol-3-yl-methyl GS and 4-hydroxyindol-3-ylmethyl GS displayed little change within 12h after the treatment, but increased approximately 42.3% and 65.7% at 24h, and up to 192% and 166% at 48h after treatment, respectively. The levels of 1-methoxyindol-3-ylmethyl GS and 4-methoxyindol-3-yl-methyl GS increased approximately 169% and 4% at 12h after treatment, and then decreased approximately 59.4% and 20.3% at 36h, respectively. Crude proteins from control and MeJA-treated samples were extracted and separated by two-dimensional electrophoresis gels. Comparative gel image analysis has identified many differentially expressed proteins. These proteins were identified by LC Q-Trap MS/MS and MALDI TOF-TOF MS/MS. Most of the MeJA responsive proteins were involved in stress and defense, photosynthesis, energy and protein metabolism. The functional significance of the protein and metabolite changes will be discussed.

Acknowledgements: This work was supported by funds from the University of Florida and National Natural Science Foundation of China (No. 30528013 and No. 30670325) and a program for New Century Excellent Talents in Chinese Universities (NCET-05-0328).

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www.cspp.cn May 29 2008

Variation in E- and Z- guggulsterones of *Commiphora wightii*

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The seasonal and geographical variations in guggulsterone content in oleogum resin have been examined in order to determine the optimum collection time and to identify suitable locations for large scale cultivation of *C. wightii*. The results clearly showed that E-and Z-guggulsterones varied significantly on seasonal basis. Higher guggulsterone concentration was observed during summer (May- July). Over the winter, from November until early spring (March), concentrations of guggulsterones were low, when the plants are in the dormancy stage. A wide variation in guggulsterone contents was also observed over its geographical range in Rajasthan. The southern portion of the state produced lowest guggulsterone yields (< 1.19 %) whiles the central, northern and western regions had higher yields (1.87 to 2.76 %). The guggulsterone yield showed a positive correlation with annual rainfall between 15 and 55 cm and declined in the regions with higher rainfall (> 60 cm). Plants having high guggulsterone content can be utilised for mass propagation and also for genetic improvement.

www.cspp.cn May 29, 2008

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Construction of metabolite database and library in rice

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Plant metabolomics has been established as one of the most important aspects of systems biology and functional genomics [1]. Multi-parallel measurements of the large variety of primary and secondary metabolites need more advanced technology platform, such as GC-TOF/MS, LC-Q-TOF/MS, Obitrap, FT-ICR-MS, NMR[2]. However, identification and classification of the mass spectral components from each single mass spectral profile have not yet been explored and sharing of metabolomic information cross different platform become especially difficult [3~4].

We are trying to establish a metabolite database from the published chemical information that have been generated from different analytic systems, such as MS, NMR, IR, UV. Also, we are constructing a RI(retention time index)-based MS database for rice metabolites. At the same time, we are building rice metabolite library by extraction of metabolic components from different rice tissues at different developmental stages.

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www.cspp.cn May 29, 2008

Discovery of functional genes through analysis of expressed sequence tags (EST) from *Fritillaria cirrhosa* bulbs

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Bulbuls of *Fritillaria cirrhosa* is one of the most widely used traditional Chinese medicines with the function of relieving coughs and eliminating phlegm. Alkaloids were regarded as the effective chemical component. There are currently more than 400 manufactures producing over 200 kinds of Fritillaria preparations which have developed into a large-scale industry worth an estimated US$400 million a year. To investigate the profile of gene expression in bulbs of *Fritillaria cirrhosa* and discover its relative functional genes concerning alkaloid biosynthesis, for the first time, expressed sequence tags (EST) library of *Fritillaria cirrhosa* bulbs has been established. According to BLAST and Gene Ontology analysis, 6 genes (geranyl transtransferase; 3-hydroxy-3-methylglutaryl-CoA reductase; cytochrome P450, family 51, subfamily A (sterol 14-demethylase); etc) are found to be associated with steroids biosynthesis; 1 gene (geranyl transtransferase) has been obtained concerning terpenoid biosynthesis; 1 gene (gibberellin 2-oxidase) connected with diterpenoid biosynthesis, 1 gene (steroid 22-alpha-hydroxylase) with brassinosteroid biosynthesis; 4 genes (aspartate aminotransferase; copper amine oxidase; phenylalanine ammonia-lyase; tropine dehydrogenase) with alkaloid biosynthesis. These results indicate EST is a powerful tool for research on functional genomics of *F. cirrhosa* and it can be applied to the molecular modification of the alkaloid biosynthetic pathway ultimately for improving the quality of *F.cirrhosa* germplasm.

Acknowledgements: The authors are grateful for the support by the project ''Study on establishing expressed sequence tags (EST) library and cloning 3-Hydroxy-3- Methylglutaryl-Coenzyme A Reductase in *Fritillaria cirrhosa*'' (No. 30772735), funded by National Natural Science Foundation of China (NSFC)

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HPLC-DAD-ESI-MS Analysis of the Rosemary Metabolites

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Rosemary (*Rosmarinus officinalis L.*) is used as natural antioxidant and possesses many biological activities which are attributed to its secondary metabolites such as polyphenolic acids, flavonoids, phenolic diterpenens and essential oil. In this work, we systematically characterized 30 secondary metabolites of the rosemary extracts from water, 50% CH_3OH and $CHCl_3$-CH_3OH(v/v, 3/1) using HPLC-DAD-ESI-MS. The water extracts were dominated by polyphenolic acids such as vanillic acid, syringic acid, *p*-coumaric acid and caffeic acid, and flavonoids including diosmin, genioside, hesperidin, luteolin 3'-O-β-D-glucuronide and 6-methoxylutelin-7-glycoside. In contrast, the aqueous methanol extracts contained, apart from the above, polyphenolic acids such as rosmarinic acid, sagerinic acid and salvianolic acid B, and flavonoids including 6-methoxylutelin-7-glycoside, luteolin 3'-O-(4''-O-acetyl)-β-D-glucuronide, luteolin 3'-O-(3''-O-acetyl)-β-D-glucuronide, 6-hydroxyluteolin-7-glycoside, criocitrin, and cirimaritin together with some terpenens such as rosmanol, epiisorosmanol, epirosmanol, carnosic acid and carvacrol-2-O-β-glucopyranosyl-β-glucopyranoside. The chloroform-methanol extracts contained mainly phenolic diterpenens such as rosmadial, rosmanol, epirosmanol, epiisorosmanol, epiisorosmanol methyl ether, carnosic acid, methylcarnosate, carnosol and 12-methyoxy carnosic acid. The results showed that HPLC-DAD-ESI-MS is a powerful tool for identification of plant secondary metabolites and the selection of appropriate solvents is crucial for such studies. chloroform-methanol is an excellent solvent system for selective extraction of phenolic diterpenes and that methanol-water is a better solvent for extraction of polyphenolic acids and flavonoids than water. Among all the metabolites detected, *p*-coumaric acid, syringic acid, carvacrol 2-O-β-glucopyranosyl-β-glucopyranoside, genioside, salvianolic acid B and quinic acid were found in rosemary for the first time.

Acknowledgements: Financial supports from NSFC and the Chinese Academy of Sciences (20575074, 06S138) and technical assistance from Dai Hui and Liu Hongbing are acknowledged.

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Metabonomic Studies of *Salvia miltiorrhiza* Bunge by NMR

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The metabolite composition of the aqueous methanol extracts of the dry roots of *Salvia miltiorrhiza* Bunge (SMB), a phytomedicine used for treatments of cardiovascular diseases, were characterized using ^{1}H NMR spectroscopy coupled with multivariate data analysis. The results showed that the SMB metabolites were dominated by amino acids, sugars and salvianolic acid B. The PCA scores plot illustrated clear clustering of SMB extracts from three different drying methods. OPLSDA results showed that compared with freeze-drying, air-drying led to significant level increase for proline, glutamine, malate and salvianolic acid B but decrease for sugars. Compared with air-drying, sun-drying induced significant level increase for proline, glucose and sucrose but decline for glutamine, malate, salvianolic acid B. Compared with air-drying, sun-drying caused significant elevation of glucose and sucrose and decline of proline, glutamine, malate, salvianolic acid B. This suggests that prolonged drought stress (e.g. air-drying) causes increase levels of proline, glutamine, malate, salvianolic acid and decrease levels of glucose and sucrose; more severe and rapid drought stress (e.g. sun-drying) induces the opposite results. The results also indicate that the NMR-based metabonomics technology is a powerful tool for composition-based quality control of traditional Chinese medicines and probably for plant metabolic response to stresses.

Acknowledgment: NSFC and The Chinese Academy of Sciences are acknowledged for financial supports (20575074, 06S138). We thank Chaoni Xiao for some experimental assistance.

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www.cspp.cn May 29, 2008

Modular co-evolution of metabolic networks

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Recent studies suggest that the architecture of biological networks exhibit high level of modularity, and to some extent, topological modules of networks overlap with known functional modules. However, how the modular topology of the molecular network affects the evolution of its member proteins remains unclear.

In this study, we try to find to which extent the identified topological modules of metabolic networks co-evolve. We explore this question by analyzing the metabolic network of H. sapiens reconstructed from the KEGG database [1, 2, 3]. We first break up the metabolic network into modules by the simulated annealing algorithm proposed in [4] and study the linkage pattern between modules. Then we investigate the evidence for co-evolution of modules by analyzing the phylogenetic profiles, evolutionary ages and evolutionary rates of enzyme genes within modules. To mine the inherent relations between structure, function and evolution of metabolic networks, the features from H. sapiens network were then compared with those of the randomized counterparts, here, topological null model and biological null model, respectively. Network decomposition shows that the metabolic network is organized in a highly modular core-periphery way, in which the core modules are tightly linked together and perform basic metabolism functions, whereas the periphery modules only interact with few modules and accomplish relatively independent and specialized functions. Moreover, over half of the modules exhibit co-evolutionary feature and belong to specific evolutionary ages. Peripheral modules tend to evolve more cohesively and faster than core modules do.

Our results suggest that the core-periphery modularity organization reflects the functional and evolutionary requirements of metabolic systems. The correlation between functional, evolutionary and topological modularity indicates that the evolutionary history and functional requirements of metabolic systems have been imprinted in the architecture of metabolic networks. Such systems level analysis could demonstrate how the evolution of genes may be placed in a genome-scale network context, giving a novel perspective on molecular evolution.

Acknowledgements: This work was supported in part by grants from Ministry of Science and Technology China (2006AA02Z317, 2004CB720103, 2003CB715901, 2006AA02312), National High Technology Research and Development Program of China (2006AA020805), National Natural Science Foundation of China (30500107, 30670953, 30670574), International Cooperation Project of Science and Technology Commission of Shanghai Municipality (06RS07109), and Grant from Science and Technology Commission of Shanghai Municipality (04DZ19850, 06PJ14072, 04DZ14005).

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www.cspp.cn May 29, 2008

Plant Secondary Metabolites Research Management Platform

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We present PSMP (Plant Secondary Metabolites Platform), a platform supporting data sharing and project progress tracking system under control of a Word-Wide Web (WWW) user interface and making extensive use of database to track and support users. Considering the complexity and diversity of Plant Secondary Metabolites research, we developed this platform for the collaboration in multi-unit development projects, researchers from different institutions or with different academic backgrounds can form research groups unrestricted to complete various research projects. Each group members are free to share research findings and research data. In addition, People in a group can also be noticed the project's latest progresses through E-mail system. Another major function of the platform is the project progress tracking. The project progress tracking system deploy and supervise the overall research on three different levels such as project, module and task, with the help of the underneath database, from the group leaders to common researchers of a certain project can manage the their work well. Moreover, the record of problem solving can offer invaluable clues for dealing with new related affairs.

www.cspp.cn May 29, 2008

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Changes in the composition and distribution pattern of pigments in petunia flowers caused by a decrease in Rubisco protein content via gene silence

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Genetic engineering for photosynthesis in higher plants was conducted in our Lab., the *petunia* with magenta flower was transformed with a plant expression vector containing tomato rbcS-3C promoter. When the transgenic petunia plants were grown on soil in a green house or on a field, a few lines (5%) exhibited yellow or white color in their young leaves, we examined the mechanism for such phenotype alteration.

SDS-page analysis showed that Rubisco protein was decreased in the leaves with yellow color (moderate) or white color (severe) as compared with that in green (normal) leaves. RNA expression level of *rbcS* was also declined in these leaves while that of Cab was not affected. This result indicated that *rbcS* gene was silenced in the leaves. The decrease in Rubisco protein content in the transgenic *petunia* reduced the chlorophyll content in leaves. This result is in correspondence with those described by Hudson et al [1] and Matt et al [2]. The changes in the composition and distribution pattern of the pigments in flowers were also observed. The shoots with moderate reduce in Rubisco protein produced flowers with thicker color and some of them with red stripes on flower petals (type I flower). The shoots with severe reduce in Rubisco protein produced flowers with thicker color and purple stripes or spots on flower petals (type II flower). The total anthocyanin was largely increased in Type I and TyepII flowers. A novel anthocyanin as a major component appeared in type I flowers and two novel major components presented in type II flowers. RT-PCR analysis showed that expression levels of *F3'H*, *F3'5'H*, *DFR* were increased in type II flowers. Recently, some studies reported that sucrose specifically activated the expressions of several genes coding for flavonoid and anthocyanin biosynthetic enzymes in plants [3, 4]. We thus investigated the changes in sugar contents in the transgenic *petunia* leaves and petals. The results showed that the glucose content was decreased significantly in severe leaves as compare with normal leaves while sucrose and starch contents were increased markedly in type I flowers and Type II flowers as compare with normal flowers.

Acknowledgements: This work was supported in part by the foundation from Yunnan province and Kunming University of Science and Technology for training adult and young leaders of Science and technology in Yunnan and by the foundation (30670163) from National Natural Science Foundation of China.

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Design of flower color and pattern by genetic engineering

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www.cspp.cn May 29, 2008

Flower color is one of the important breeding targets for floricultural plants. Transgenic approach has been applied to many plant species as a method to alter flower color and commercial transgenic carnations are available now. Here, we present new approaches to modify flower color and pattern using model plant tobacco and practical plant gentian. Gentian is a major floricultural plant in Japan and its original flower color is blue derived from delphinidin-type anthocyanins.

1) Comparison of promoters used for RNAi induction

We surveyed the effects of three different promoters on flower color modification in tobacco plant [1]. The results clearly showed that promoters are important factors for modification of flower color and pattern by RNAi. We also demonstrated that a *rol*C promoter from *Agrobacterium rhizogenes* can be used in gentian [2]. Gentian with magenta, white to faint blue and bicolor flowers were sucessfully produced.

2) Regulation of multiple genes by chimeric RNAi construct

We produced red-flowered tobacco by using a single construct controlling three flavonoid biosynthetic genes, *F3'H*, *FLS*, *DFR*, at the same time [3]. Transgenic tobacco plants accumulated pelargonidin derivatives in addition to original cyanidin derivatives. Flavonoid biosynthetic pathway is complexly-linked, therfore it is important to control each flux to accumulate the desired pigments.

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Improvement of Chinese kale by metabolic engineering of glucosinolate

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Chinese kale (*Brassica alboglabra* Bailey) is a well-known traditional Chinese vegetable with high nutritional value[1]. It is a rich source of glucosinolates, a sulfur- and nitrogen-containing secondary metabolites[2,3,4]. HPLC analysis of glucosinolates showed that some varieties of Chinese kale contain a high level of glucoraphanin, a glucosinolate precursor of sulforaphane, which is the most potent, naturally occurring, anticarcinogenic phytochemical identified thus far, it inhibits the development of cancer by specific modification of carcinogenic metabolism, induces the programmed cell death in human cancer cells, and therefore plays an important role in chemoprevention of cancer[2,3,5]. As the most abundant glucosinolate in Chinese kale is gluconapin (3-butenyl glucosinolate), which is much higher than glucoraphanin in all Chinese kale varieties tested [2,3]. The conversion of glucoraphanin to gluconapin was catalysed by AOP2, a tandem 2-oxoglutarate-dependent dioxygenases[4,6]. Studies on *Arabidopsis* indicated that manipulation of glucosinolate composition and content by metabolic engineering was possible[7]. In our study, the *AOP2* gene was cloned in Chinese kale by the homologic sequence in *Arabidopsis*, and an antisense gene fragment of 495 bp including an important domain was amplified from the *AOP2* gene. The fragment was introduced into pCAMBIA2301 to generate pCAMBIA-JAOP2 vector which contained CaMV35S promoter. By using the callus from cotyledon-petiole as the gene transformation acceptor£¬an anti-gene *AOP2* was transferred into plant via *Agrobacterium tumefaciens*. Total number of 120 transgenic plants were gained. PCR analysis, Southern blot and GUS activity assays indicated that anti- *AOP2* was integrated into chromosome of transgenic plants. HPLC analysis of glucosinolate showed that the level of glucoraphanin in T1 progeny was threefold higher than that of CK. The expression of *AOP2* gene and alkenyl modification of glucoraphanin was significantly inhibited in transgenic plants, while no significant changes in visual quality and contents of main nutrition components were found between the transgenic plants and the control. This is the first report of crop improvement by metabolic engineering of glucosinolate.

Our former work has identified *IQD1,* the first regulator gene involved in glucosinolate metabolism in *Arabidopsis*. Overexpression of *IQD1* leads to a signicant increase in glucoraphanin content and resistance against herbivory such as generalist-chewing green peach aphid and phloem-feeding lepidopteran[8]. The overexpression vector 35S: IQD1 was constructed and transfered into Chinese kale by *Agrobacterium tumefaciens*, Ninety six transgenic plants were gained. The molecular and genetic analysis of transgenic plants and the role of *IQD1* in Chinese kale remain to be further investigated.

Acknowledgements: This study was supported by National Natural Science Foundation of China (30320974), Fok Ying Tong Education Foundation (104034), and NCET-05-0516.

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www.cspp.cn May 29, 2008

Manipulation of artemisinin content in *Artemisia annua* L. by engineering artemisinin biosynthetic pathway

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Artemisinin is an effective anti-malarial drug isolated from *Artemisia annua* L. Due to the low content of artemisinin in *A. annua*, great efforts have been endeavored to improve artemisinin production but not so successful.

In this study, we tried to use genetic engineering to increase artemisinin content in *A. annua* by overexpressing genes encoding the rate-limiting enzymes of artemisinin biosynthesis pathway individually or in combination, including *HMGR*, *FPS*, *ADS* and *CYP71AV1*, and by suppressing the expression of *SQS*, a key enzyme of sterol pathway—a competitive pathway of artemisinin through hairpin-RNA mediated RNAi technique. More than one hundred independent transgenic plants were obtained through *Agrobacterium tumefaciens*-mediated transformation, which were confirmed by PCR and Southern blot analyses. RT-PCR analysis demonstrated that the expression of *SQS* was depressed in hpRNAi transgenic plants, and the expression of transferred rate-limiting enzyme genes was enhanced in the overexpressing transgenic plants. HPLC-ELSD analysis showed that artemisinin contents in some transgenic plants were significantly increased, with the highest reaching up to 2.8% dry weight. This study demonstrates that genetic engineering strategy is an effective means to increase artemisinin content in plants.

Acknowledgements: This work was funded by Shanghai Science and Technology Committee.

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www.cspp.cn May 29, 2008

Transgene research for artemisinin accumution in *Artemisia annua* L

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Artemisinin, a sesquiterpene lactone isolate from *Artemisia annua*, is an effective drug against *Plasmodium* species involved in malaria. The relative low yield of artemisinin in *A. annua* limits wide application of the drug. In recent years, genes (cDNAs) encoding enzymes involved in artemisinin biosynthesis have been investigated. Amorpha-4,11-diene synthase (ADS), a sesquiterpene synthase, converts farnesyl diphosphate (FDP) to amorpha-4,11-diene, a key precursor of artemisinin biosynthesis. A cytochrome P450 mono-oxygenase gene, *CYP71AV1* was isolated from the glandular trichomes. It performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid.

We are interested in sesquiterpene biosynthesis in *Artemisia annua* and cotton. For *Artemisia*, our laboratory has cloned and characterized two monoterpene synthases ((3R)-linalool synthase and β-pinene synthase) and a sesquiterpene synthase (β-caryophyllene synthase). We investigated the spatial expression pattern of *ADS* gene and the transcript level of *ADS* in different *Artemisia annua* ecotypes. Because amorpha-4,11-diene is a key precursor for artemisinin biosynthesis, we transformed the *ADS* gene to *Artemisia annua* ecotype QT, which has a high yield of artemisinin. Several transgenic lines were obtained via *Agrobacterium*-transformation. The *ADS* transcript level of some transgenic lines was elevated according to RT-PCR and Northern blot. RT-PCR also showed that the transgene had no effect on the upstream genes of cytosolic isoprenoid pathway, including those of *HMGR* and *FPS*, but the downstream gene *CYP71AV1* was up-regulated. These lines produced about 1.8 times of artemisinin and 4 times of artemisinic acid compared to the wide-type.

Acknowledgements: This work was supported by the National Naturl Science Foundation of China (30630008), the National HighTech Program of China (2007AA021501).

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www.cspp.cn May 29, 2008

Anti-tumor effects and mechanism of polysaccharides from sweet potato

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In this paper, a study on anti-tumor effects and mechanism of Polysaccharides from sweet potato (PSP) has been made. Experiments in vivo and in vitro show that PSP inhibit tumor cells fissiparity directly .The K562 Mammary Carcinoma cells and Hca-f solid tumor cells with the treatment of PSP have been greatly inhibited. The inhibition rate reach to 75% (P<0.01) while PSP does little harm to the natural cells (FC, flbroblast cell). In the mean time,immune defence system in vivo is activated.Compared with the control,lymphocyte transformation rate increase noticeably as well as thymus index and spleen index (P<0.01). It indicate that PSP can activate immune defence reaction in vivo.The anti-tumorm echanism of PSP is : PSP bind specifically to tumor cell receptor and this kind of binding could affect intracellular physiological function of the tumor. Then immune defense reaction is activated to defence tumor cells.

Acknowledgements: Thanks to Laboratory of Instrumentation and Analytical Chemistry, Dalian Institute of Chemical Physics, Chinese Academy of Sciences; Department of Immuno,Dalian Medical University; Bio-engineering institute of Liaoning Normal University.

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www.cspp.cn May 29, 2008

Chemical Composition and Biological Activities of Essential Oil from the Rhizomes of *Iris bulleyana* in Yunnan Province, Southwest of China

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The genus *Iris* belongs to the family Iridacea comprising over 220 species, 58 of which are found in China[1]. *Iris* species have an immense medicinal importance that are used in the treatment of cancer, inflammation, bacterial and viral infections[2,3,4]. *Iris bulleyana* distributes mainly in Yunnan Province, southwest of china, has a long history of use in folk medicine. Traditional healers in Southwest of China use it to eliminate stasis and resolve swelling. Hydrodistillation was used to extract the essential oil from the rhizomes of *Iris bulleyana*. The essential oil yield obtained was 0.23%. By using GC–MS analysis, Aristolone, Cuparene, β-Gurjunene, δ-Amorphene, α-Muurolene, α-Cadinol, Camphor, γ-elemene, τ-Cadinol, 1-Hydroxy-1,7-dimethyl-4-isopropyl -2,7-cyclodecadiene, Linalool, γ-Muurolene, β-Pinene, Spathulenol, Limonene, Camphene, α-Gurjunene, α-Himachalene, Trans ocimene, α-Bulnesene, α-Pinene, β-Elemene, Alloaromadendrene, a-Terpineol, linalool oxide, Germacrene D, α-Longipinene, Citral, α-elemene, (-)-4-Terpineol, 2,6-Octadien-1-ol, 3,7-dimethyl- were identified. The hole plate diffusion method was used for antibacterial testing. The essential oil exhibited antibacterial activity against *Acetobacter calcoacetica*, *Bacillus subtillis*, *Clostridium sporogenes*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella typhii*, *Staphylococcus aureus* and *Yersinia enterocolitica*. The mycelium growth inhibition method was used for the antifungal testing. The oil exhibited antifungal activity against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium notatum*. By using the β-carotene, acetone and linoleic acid method for the antioxidant testing, the essential oil showed antioxidant activity threshold of 14.7mm mean zone of color retention.

This study indicated that the essential oil frim *Iris bulleyana* has potential as antibacterial, antifungal and antioxidant agent and may be useful in the pharmaceutical and cosmetics industries. More research is needed to ascertain this.

Acknowledgements: This research work was supported by the Yunnan Provincial Natural Science Foundation (Project no.2007C001M). The authors are grateful to Prof. S.B. Ma for identification of plant material.

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www.cspp.cn May 2008

Glucosinolate analysis and Quinone reductase bioassay of Chinese kale and breedings for functional variety

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Chinese kale (*Brassica alboglabra* Bailey) is an original Chinese vegetable with high nutrient value; it is also a rich source of phytochemical components such as glucosinolate, a kind of sulfur- and nitrogen-containing secondary metabolites with diverse biological functions. In present study, the composition and content of GSs were determined in a collection of 27 cultivars of Chinese kale by HPLC-MS. Twelve kinds of GSs were identified, including eight aliphatic GSs (sinigrin, glucolberin, progoitrin, glucoerucin, glucoraphanin, gluconapin, glucoalyssin, gluconapolelferin), three indole GSs (glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin) and one aromatic GS (gluconasturtiin). The most abundant individual GS in each cultivar was gluconapin, ranging from 27. 89% to 69.01%. Other GSs, such as glucoraphanin, progoitrin, sinigrin and glucobrassicin were relatively abundant in different cultivars. Sulforaphane, the degradation produce of glucoraphanin is the most potent, naturally occurring, anticarcinogenic phytochemical identified thus far, it inhibits the development of cancer by specific modification of carcinogenic metabolism, induces the programmed cell death in human cancer cells, and therefore plays an important role in chemoprevention of cancer. The glucosinolate analysis showed that glucosinolate composition and content differed among different cultivars, the total GS content ranging from 0.6826 to 17.5613 ìmol g-1 dw. Aliphatic GSs are primary, ranging from 55.45% to 91.21%, the glucoraphanin content of edible part differed among different cultivars, and 4 cultivars (JL-01, JL-10, JL-15, and JL-23) with significantly high level of glucoraphanin in edible parts were identified. The glucosinolate content of sprout was significantly higher than other stages, while the lowest glucosinolate content was observed at the stage of cotyledon. The extremely rich of glucoraphanin in sprout of Chinese kale indicated the potential role of Chinese kale sprout in anticancer functional food. Chinese kale contained relatively less indole glucosinolates compare to aliphatic ones, the indole glucosinolate content of Chinese kale shifted among different cultivars and at different stages, but the biological function of indole glucosinolate at different stages in Chinese kale remained to be further investigated. The direct assay of the activity of quinone reductase in murine Hepa 1c1c7 cells provides a rapid and reliable indicator of the ability of vegetable extracts to induce enzymes that detoxify carcinogens [1], and hence of putative anticarcinogenic activity via a blocking mechanism [2]. The QR assay system has also been used to detect inducers of anticarcinogenic enzymes in human diet, assess the potential of extracts from cruciferous vegetables, and chemopreventive glucosinolates [3-5]. The QR assay was further modified and simplified in our test. The phosphate buffer (5 mM K_2HPO_4–KH_2PO_4, 1 mM EDTA, pH 7.6) was used in extraction instead of common triple solvent extracts, 0.4% Nonidet P-40 was substituted for 0.8% digitonin during cell lysis, and the incubation time of QR reaction mixtures in QR assay was shortened to 5 - 10 minutes instead of 15 minutes. The QR induction potency and activity of edible part of 27 Chinese kale cultivars were analyzed, and the results showed that both the QR induction potency and activity of edible parts were significantly higher in JL-01, JL-10, JL-15, and JL-23 than other cultivars, which was coincided with the significantly high content of glucoraphanin in the same cultivars. These cultivars with higher level of glucoraphanin and QR inducing activity will be used for breeding of varieties with enhanced anticarcinogenic activity

Acknowledgements: This study was supported by National Natural Science Foundation of China (30320974),

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Fok Ying Tong Education Foundation (104034), and NCET-05-0516.

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www.cspp.cn May 29, 2008

The effect of AHLs on the synthesis and release of volatile compounds in bloom-forming cyanobacteria *Microcystis aegurinosa*

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Cyanobacterial blooms are widely acclaimed to occur in eutrophic lakes and reservoirs worldwide. *Microcystis aegurinosa* is the most widespread and frequently encountered bloom-forming organism. It is known to destroy the pristine quality of water and cause many problems such as occurrence of musty odors, fish kill, deterioration of recreational worth and toxins releasing [1, 2]. Chemical-mediated interactions among aquatic organisms have attracted the attention of researchers worldwide and are used in dealing with the cynaobacterial blooms. In such interactions, organisms secrete chemical substances into the surrounding medium. And these chemicals may serve as a form of communication among the biological componets of ecosystems. In this study, signals of AHLs (N-acyl-homoserine lactone) were selected to be investigated whether it has effect on the interactions among cyaobacteria and bacteria. AHLs are quorum sensing signals of Gram-negative bacteria, which regulate many physiological processes including secondary metabolite production, swimming and swarming motility, conjugal plasmid transfer, antibiotic resistance, biofilm maturation and virulence [3, 4].

The volatile compounds were prepared with simultaneous distillation and extraction equipment (SDE). They were isolated and identified by capillary GC/MS method. Volatile compounds of *M. aeruginosa* in different growth period (exponential growth period and stationary growth period) were analyzed and the effect of additional AHLs substances on the change of volatile compounds was studied. 70 compounds were identified from the cells in exponential growth period, while only 45 and 29 compounds were identified from the cells in stationary growth period and the cells with additional AHLs respectively. Chemicals including a benzene cycle and ester compound were greatly increased in cells of stationary growth phage and cells dealing with the AHLs. These increased chemicals have potential allepathetic functions.

The results showed that AHLs have effect on the synthesis and release of volatile compounds in *M. aegurinosa*. Additional AHLS in exponential growth period resulted in the increasing synthesis of allepathetic compounds, which also detected in cells of stationary growth period.

Acknowledgements: This research work was supported by National Key Project for Basic Research (2002CB412306) and the Yunnan Provincial Natural Science Foundation (Project No. 2007C001M).

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www.cgpp.cn May 29, 2008