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A metabolomic analysis of medicinal diversity in Huang-qin (*Scutellaria baicalensis* Georgi) genotypes: discovery of novel compounds

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Abstract In vitro manipulation of plant regeneration in the Chinese medicinal species *Scutellaria baicalensis* Georgi (Huang-qin) resulted in 26 chemically distinct germplasm lines. Antioxidant potential, growth rate and concentration of baicalin, baicalein, melatonin, and wogonin were the selective markers used to identify elite lines. Metabolomic analysis of a subset of the most distinct lines revealed that Huang-qin extracts contained over 2,000 compounds including 781 determined to be of putative medicinal importance as determined by a database search, as well as previously unidentified amino-derivatives of baicalin and wogonin. Huang-qin also contained a metabolite with the same net formula as hyperforin, previously thought to be unique to *Hypericum perforatum* L. Together these results provide new insights into the biochemical complexity of an important medicinal species and demonstrate the power of in vitro manipulation in combination with untargeted metabolomic screening for the production of new germplasm.

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Introduction

Huang-qin (*Scutellaria baicalensis* Georgi) is a medicinal species used extensively in Japanese Kampo medicines (Watanabe et al. 2002) and Chinese prescriptions. Huang-qin is used for the treatment of cancers, hepatitis, cirrhosis, jaundice, hepatoma, leukemia, hyperlipemia, arteriosclerosis and inflammatory diseases (Chang et al. 2002). More recently, studies have focussed on the medicinal efficacy of Huang-qin preparations: whole plant preparations were effective in reducing prostate cancer (Hsieh et al. 2002); alcoholic extracts inhibited liver fiberosis (Nan et al. 2002) and delayed apoptosis in neuronal cells (Suk et al. 2003); and individual isolated compounds reduced the symptoms of Type 1 allergic reactions (Lim 2003). Baicalin, baicalein and wogonin—the most commonly isolated medicinal constituents in Huang-qin—were recently shown to inhibit the proliferation of various human hepatoma cell lines (Chang et al. 2002; Okamura et al. 1999). Whole plant preparations are complex mixtures of many thousands of small metabolites and there is an enormous potential for identifying novel compounds in Huang-qin. In 1997, we discovered significant quantities of the human neurohormone and antioxidant, melatonin, in this species (Murch et al. 1997), results recently confirmed by others (Chen et al. 2003). In a complementary human clinical trial, consumption of Huang-qin-containing products was shown to alter diurnal fluctuations in patients' plasma melatonin concentrations (Watanabe et al. 2002). Although there is growing evidence that Huang-qin contains a range of medicinally active phytochemicals from many different chemical classes, many remain unidentified, their phytochemical biosynthesis unknown and the specific details of their mechanisms of action undetermined.

The objective of our research is to develop distinct germplasm lines of Huang-qin with differing phyto-

chemical profiles, and to compare the effects of different growth conditions on the medicinal efficacy and biochemical interdependency of specific compounds. Naturally occurring and chemically induced variations were evaluated for changes in the production of phytochemicals. Individual germplasm lines were clonally propagated and maintained in vitro for more than 4 years to ensure phytochemical profile stability. An assessment was made of the ability of fourier transform ion cyclotron mass spectrometry (FTMS)-based metabolomics technology to profile, identify, and quantitatively compare metabolites specific to the optimised germplasm lines.

Materials and methods

Germplasm

Individual plants of Huang-qin (*S. baicalensis* Georgi) were selected for the development of germplasm lines with naturally occurring or induced variations. De novo shoot organogenesis was induced using the one-step procedure of intact seedling culture previously described (Li et al. 2000). Sterile seeds (450 seeds) were germinated, from which 300 3-week-old seedlings were randomly selected and exposed to a solution of 2.5 μmol l^{-1} ethylnitrosourea (ENU) for 1 h. The untreated (150) and all treated seedlings were then subcultured on a medium that induced regeneration and incubated with a 16 h photoperiod under cool white light (model F40/CW/RS/EW-ll, Philips Canada, Scarborough, Ontario, Canada; 40–60 μmol m^{-2} s^{-1} at shelf) at 24°C. After 28 days shoots were excised and subcultured onto basal medium (Li et al. 2000). Routine maintenance schedules were established based on growth rate. Individual nodes (5 mm) were excised and subcultured on basal medium supplemented with 1 μmol l^{-1} kinetin for shoot growth. Rooting of the nodal cultures was induced by subculture on basal medium supplemented with 1.0 μmol l^{-1} indolebutyric acid (IBA). Although multiple regenerants were developed from each original seedling, a single regenerant was selected and the rest were discarded to maximise diversity between the lines. The selected regenerants were then clonally propagated and maintained over a 4-year period as distinct germplasm lines.

Antioxidant potential

Antioxidant potential of each germplasm line was assessed by measuring the change in absorbance at 520 nm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Takahata et al. 2001). A dilution series was compared to a reaction blank to determine the amount of tissue required for a 50% reduction in oxygen radical production.

Extraction of baicalin, baicalein and wogonin

Analytical methods for quantification of baicalin, baicalein and wogonin were based on previously described methods (Okamura et al. 1999; Lin et al. 1999). Extracts were prepared at low light intensity at room temperature. Frozen shoot tissues from plants grown in vitro (0.25 g fresh weight) were freeze-dried for 24 h (Labconco, Caltec Scientific, Toronto, Ontario, Canada), ground into fine powder and transferred into an amber-coloured 20 ml vial. Bioactive compounds were extracted by sonication for 10 min in 5 ml 70% ethanol (Ultrasonic FS-14 Sonicator, Fisher Scientific, Nepean, Ontario, Canada). Sonicates were then centrifuged at 3,000 rpm for 10 min (Allegra 6/GS-6 Spinchron, Beckman, Palo Alto, Calif.) and 3 ml of the resulting supernatant passed through a 0.45 μm nylon syringe filter (Waters, Mississauga, Ontario, Canada). A 300 μl aliquot of each filtrate was transferred to an amber-colored glass auto-sampler HPLC vial.

Quantification of baicalin, baicalein, and wogonin

Metabolites were separated by HPLC within 15 h of sample preparation (Waters 2695 Alliance HPLC). Baicalin, baicalein and wogonin were separated on a Phenomenex Luna C_{18} column (5.0 μm; 4.6×150 mm) by gradient elution (A: 0.1% phosphoric acid, B: acetonotrile, t=0 min, A/B 90/10, t=14 min, A/B 65/35, t=20 min, A/B 65/35, t=35 min, A/B 45/55, t=40 min, A/B 45/55, t=40.1 min, A/B 90/10) with constant flow at 0.8 ml/min. Metabolites were quantified by comparison to authentic standards using a Waters dual λ absorbance detector at 277 nm. Baicalin, baicalein and wogonin standards were dissolved in 100% methanol (2,000 μg ml^{-1}). Linear standard curves were obtained by plotting concentrations (baicalin: 0.04, 0.15, 1.5, 10, 20, 50, and 100 μg ml^{-1}; baicalein: 0.075, 0.75, 5, 10, 25, and 50 μg ml^{-1}; wogonin: 0.075, 0.5, 1, 2.5, and 5 μg ml^{-1}) as a function of peak area. High linearity (r^2=0.99) was obtained for all calibration curves. The lower limits of detection of baicalin, baicalein and wogonin were 0.01, 0.02, and 0.02 μg ml^{-1}, respectively. To determine percent recovery, duplicate reference samples of *S. baicalensis* were analysed with and without 100 μl of a mixture of baicalin (100 μg ml^{-1}), baicalein (50 μg ml^{-1}), and wogonin (5 μg ml^{-1}). Recovery of baicalin, baicalein and wogonin was above 90, 98, and 92%, respectively.

Analyses of melatonin

Melatonin was quantified in Huang-qin tissue extracts (0.4 mol l^{-1} perchloric acid, 0.05% sodium metabissulfate, 0.1% EDTA) separated on a C_{18} column (NovoPak C_{18} column, 4 μm bead, 3.9×150 mm; Waters) with concurrent electrochemical [Waters 460 electrochemical detector (ECD); 2 nA, 0.85 V] and UV (Waters 484 variable UV detector; 278 nm) detection as described previously (Murch et al. 2000). Analytical methods were validated by comparison to LC-MS/MS identification and RIA quantification procedures described previously (Murch et al. 2000).

Metabolomics analyses

Plant tissues were excised from the in vitro-grown stock plants, flash frozen and stored until FTMS analysis could be carried out. Metabolites were separated into multiple extracts based upon their polarity and acid/base chemistry (Fig. 1). Extraction efficiency was tracked using a series of internal standards; multiple, independent FTMS analyses were then performed on each extract (Aharoni et al. 2002). The resulting data were compiled into a single array for bioinformatics analysis. The assignment of an empirical formula to each low molecular weight molecule present in the extracts, and the clustering of related compounds, was carried out on the basis of accurate mass data (mass accuracies <1 ppm). Putative metabolic transformations of individual compounds within the array could be generated using simple chemical transformations.

Results

The 300 germplasm lines from the mutagen-exposed cultures were selected on the basis of their morphogenic and growth parameters. Individuals with poor growth or from which there was an accumulation of phytotoxic compounds in the medium were discarded. Several plants exuded substances that significantly lowered the pH of the medium so that it was no longer semi-solid and were

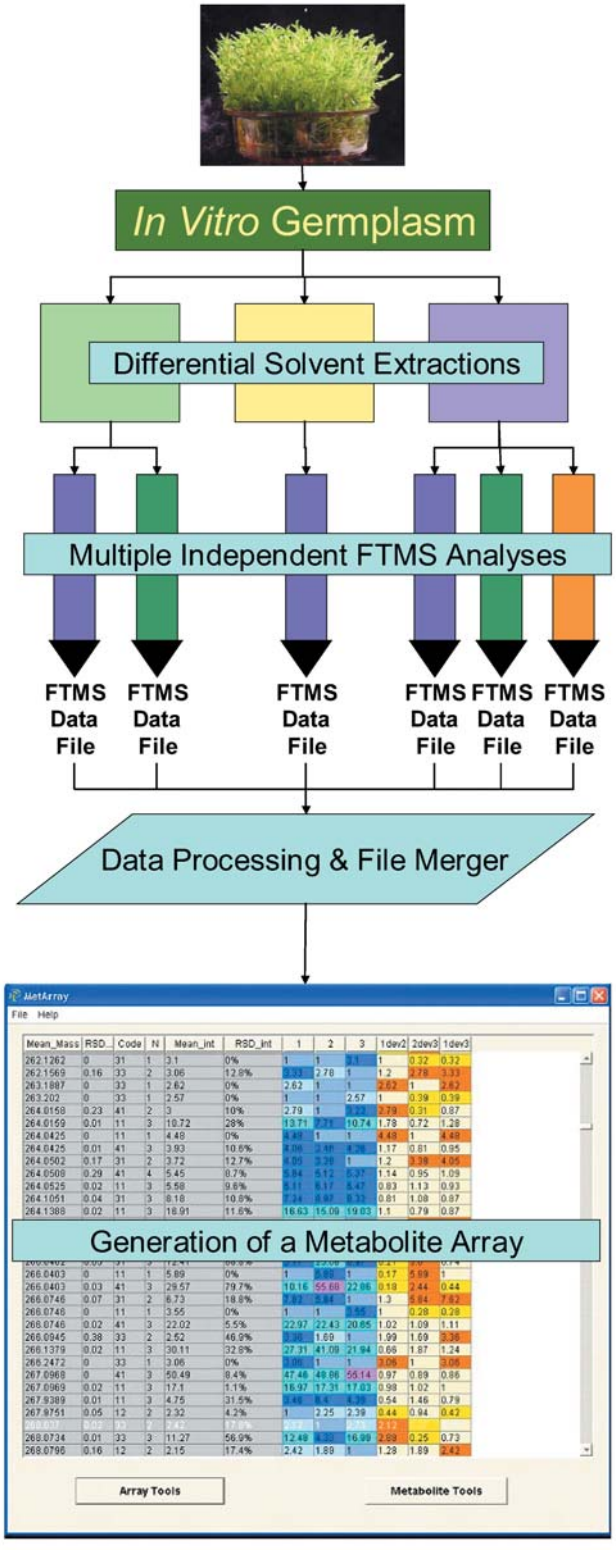

Fig. 1 Schematic representation of differential extractions for metabolomic analyses by Fourier transform ion cyclotron mass spectrometry

discarded. This process likely eliminated the most severe genetic mutations arising from ENU exposure. Thus, those germplasm lines retained may represent both natural and ENU-induced genetic variations. The fastest growing line was 111 with lines 38 and 55 growing at a rate that was not significantly different. For maintenance of the cultures, fast-growing lines (F) were subcultured at 4-week intervals, moderate-growing cultures (M) every 6 weeks, and slow cultures (S) every 8 weeks (Table 1).

There was a greater than 10-fold variability in antioxidant potential ranging between 120 μg and 1,270 μg Huang-qin (Table 1). It is interesting to note that the highest antioxidant potential was observed in naturally occurring germplasm, not that treated with mutagen. Following the antioxidant selection phase, 26 germplasm lines were chosen for further chemical characterisations.

The concentration of baicalin in the lines ranged from 0.417 μg mg^{-1} to 4.519 μg mg^{-1}, dry weight, a 10-fold variation (Table 1). Greater variability was observed in the baicalein and wogonin concentrations of the germplasm lines with a 39-fold difference in baicalein and a 30-fold difference between the highest and lowest concentrations of wogonin (Table 1). By far the greatest variability was observed in the melatonin concentrations of the individual germplasm lines, with a nearly 5,000-fold difference in concentration (Table 1). It is interesting to note that there was no correlation between the antioxidant potential and any of the metabolites of interest. Line 111 was the fastest growing line with moderate antioxidant potential, baicalin, baicalein and very low concentrations of wogonin. Line 31 had a similar antioxidant potential to line 111 but with significantly higher concentrations of all three metabolites of interest. Line 38 had an extremely high antioxidant potential but lower concentrations of baicelin, baicelein and wogonin. Lines 31 and 38 grew at a much slower rate than line 111 and were more susceptible to damage arising from exudation of metabolites that lowered the pH of the culture medium. However, lines 31 and 129, which had the highest concentrations of baicalin and baicalein, were also observed to have significantly lower concentrations of melatonin. In contrast, the highest melatonin concentrations were observed in lines that had significantly less of the other bioactive compounds (Table 1).

The metabolomic analysis identified over 2,400 compounds, of which 781 were putatively identified by comparison to a database of medicinal compounds. Metabolite data from the three different Huang-qin genotypes were compared by the molecular masses of each metabolite detected (Figs. 1, 2, 3, 4). The relative concentration of each of the metabolites detected in the series of plant extracts was determined by directly comparing the signal to noise ratio in each case (Figs. 2, 4). Since very accurate mass data were collected (mass accuracies less than 1 ppm), the molecular formulas for most of the detected metabolites could be easily determined (Fig. 2). Armed with this depth of chemical information, putative metabolic transformations of individual compounds within the array could be generated using simple chemical trans-

Table 1 Naturally occurring and induced variation in chemical content of selected germplasm lines of Huang-qin (*Scutellaria baicalensis* Georgi). Values within a column followed by the same letter are not significantly different by Student-Newman-Kuells means separation ($P<0.05$)

Line	Source of variation[h]	Growth rate[I]	Antioxidant potential[j]	Baicalin (μg mg^{-1})	Baicalein (μg mg^{-1})	Wogonin (μg mg^{-1})	Melatonin (nmol g^{-1})
31	N	M	544.9 bc	4.52 a	2.55 b	0.026 bcde	21 c
38	N	F	119.3 c	0.65 fg	0.23 fg	0.002 e	1,660 c
55	N	S	362.9 c	0.94 fg	0.26 fg	0.033 bcd	22,500 b
100	N	S	668 bc	2.10 cdef	0.34 fg	0.035 bc	1,037 c
101	N	S	549.1 bc	1.17 defg	0.39 fg	0.015 cde	261 c
102	N	S	778.6 abc	1.93 cdefg	1.39 de	0.064 a	1,126 c
106	N	M	1,092 ab	3.08 bc	0.49 efg	0.034 bc	215 c
111	N	F	524.4 bc	1.60 cdefg	1.01 defg	0.007 de	9 c
113	N	F	852.9 abc	2.65 c	0.71 efg	0.012 cde	16 c
114	N	S	332.8 c	1.88 cdefg	2.26 bc	0.019 bcde	132 c
115	N	M	770.8 abc	1.89 cdefg	0.64 efg	0.004 e	42 c
116	I	F	1,269.7 a	2.95 bc	0.45 efg	0.044 ab	27 c
117	I	S	301.2 c	0.74 fg	0.19 fg	0.006 de	69 c
120	I	F	336.7 c	2.17 cdef	0.23 fg	0.005 e	31 c
124	I	M	314 c	2.62 cd	1.18 def	0.015 cde	1,258 c
125	I	M	364.5 c	1.67 cdefg	0.95 defg	0.005 e	1,593 c
127	I	M	269.5 c	1.01 defg	0.36 fg	0.019 bcde	7,679 bc
128	I	M	252.9 c	2.06 cdef	1.04 defg	0.012 cde	6,342 bc
129	I	S	187.3 c	3.11 bc	3.13 a	0.028 bcde	81 c
130	I	S	818.6 abc	1.35 defg	0.93 defg	0.028 bcde	44,362 a
132	I	F	397.4 c	1.60 cdefg	0.84 efg	0.004 e	47 c
136	I	S	372.4 c	0.95 fg	0.49 efg	0.006 de	972 c
137	I	S	257.5 c	0.42 g	0.08 g	0.007 de	261 c
138	I	F	525.5 bc	2.99 bc	1.82 cd	0.024 bcde	7,296 bc
141	I	M	332.2 c	2.49 cde	1.01 defg	0.021 bcde	177 c
182	I	F	304.4 c	4.09 ab	1.77 cd	0.028 bcde	597 c

[h] Variation was either naturally occurring (N) or induced by exposure to ethylnitrosourea (I)
[I] Growth rate was determined over more than 50 subcultures and maintenance for a period of approximately 4 years. F fast growing, subculture every 4 weeks. M moderate growth rate, subculture every 6 weeks. S slow growth, subculture every 8 weeks
[j] Antioxidant potential values represent the amount of tissue (μg) required for a 50% reduction in free radical generation therefore a lower value represents a greater detoxification potential

formations (Figs. 2, 3). We used this approach to compare the metabolic transformations of compounds traditionally associated with Huang-qin, viz. baicalin, baicalein and wogonin.

We discovered that the most abundant metabolite detected was dihydroxyflavone ($C_{15}H_{10}O_4$), which was most abundant in line 111; line 116 had approximately 55% of the amount in line 111 while line 38 had only 13%. These relative concentrations do not correlate with observed antioxidant potentials. A comparison of different common chemical transformations shows that only one (the hydroxylated form, $C_{15}H_{10}O_5$) results in a dramatically different distribution pattern. This metabolite, which corresponds to baicalein, is highest in line 38, lowest in line 116, and at a mid level in line 111. This distribution mirrors the observed antioxidant potentials and the pattern is maintained in three different reactions (hydroxylation, amination and dehydrogenation) among all of the chemical transformations of $C_{15}H_{10}O_5$. In the two other transformations detected, methylation (to give the molecular formula of wogonin) and glucuronidation (to give baicalin) resulted in equal concentrations for all lines and only a small amount in line 116. To carry this exercise further, the transformations of the amino and hydroxyl derivatives were also investigated. Remarkably, all of the detected transformations maintained the new distribution pattern. As we go further and further away from baicalein, the pattern eventually diminishes. If we start with wogonin, we detect two transformations whose distribution pattern mimics the antioxidant properties of the plant extracts. However, these metabolites could have been formed just as easily from the baicalein pathway (Figs. 2, 3, 4).

The data array revealed some surprises, including several compounds that have previously been identified as unique to other species. For example, all three germplasm lines of Huang-qin contained compounds with exactly the same *m/z* value as hyperforin and 8-hydroxyhyperforin, compounds thought to exist only in St John's wort (*Hypericum perforatum* L.). Metabolically related families for these compounds were also generated and these results describe part of a putative pathway for biosynthesis of hyperforin in higher plants (Fig. 5).

Further results included the identification of a range of different phytochemicals of medicinal importance. It is interesting to note that the germplasm line with the highest antioxidant potential contained significantly higher concentrations of compounds consistent with 4-hydroxycinnamoyl ($C_{16}H_{13}NO_5$; MW 299.0796), 7-hydroxy-9-methyl-6-oxo-6H-oxepino[2,3-b][1]benzopyran-5-carboxylic acid ($C_{16}H_{12}O_6$; MW 300.0633), arabutin ($C_{21}H_{22}O_9$; MW 418.1265), umbelliprenin ($C_{16}H_{11}O_5$; MW 418.2362), karatavicinol ($C_{24}H_{34}O_7$; MW 434.2305), Segetalin B ($C_{24}H_{32}N_6O_5$; MW 484.2435), and tantazole A ($C_{24}H_{32}N_6O_2S_4$; MW 564.1477) than any other germplasm line and at least 24 compounds with putative antibiotic activity. In ongoing phytochar-

Common Name	Chemical Transformation	Molecular Formula	Neutral Mass	Signal/Noise 38	111	116	Ratios 38/116	38/111
Dihydroxy Flavone		C15H10O4	254.0578	181.9	1393.5	766.2	0.24	0.13
	-OH	C15H10O3	238.0631	5.7	6.1	4.6	1.25	0.94
	+ NH	C15H11N1O4	269.0687	3.8	10.4	2.0	1.89	0.36
Baicalein	+OH	C15H10O5	270.0527	138.9	71.8	25.0	5.56	1.94
	+H2	C15H12O4	256.0734	2.4	21.3	17.5	0.14	0.11
	+CH2	C16H12O4	268.0734	4.3	17.0	12.5	0.35	0.25
Wogonin		C16H12O5	284.0684	6.0	7.0	8.5	0.70	0.85
	+NH	C16H13N1O5	299.0796	67.6	28.5	1.0	67.57	2.37
	+OH	C16H12O6	300.0633	65.7	28.9	4.4	14.94	2.27
Baicalein		C15H10O5	270.0527	138.9	71.8	25.0	5.56	1.94
Baicalin	+C6H8O6	C21H18O11	446.0846	1.0	1.0	6.4	0.16	1.00
	+NH	C15H11N1O5	285.0638	225.4	30.1	1.0	225.41	7.49
	+OH	C15H10O6	286.0479	149.1	38.0	5.1	29.00	3.93
Wogonin	+CH2	C16H12O5	284.0684	6.0	7.0	8.5	0.70	0.85
	+H2	C15H12O5	272.0684	11.1	4.5	1.0	11.13	2.48
Baicalein(+NH)		C15H11N1O5	285.0638	225.4	30.1	1.0	225.41	7.49
	+H2	C15H13N1O5	287.0796	13.7	2.9	1.0	13.68	4.77
	+CH2	C16H13N1O5	299.0796	67.6	28.5	1.0	67.57	2.37
Baicalein(+OH)		C15H10O6	286.0479	149.1	38.0	5.1	29.00	3.93
	+C6H8O6	C21H18O12	462.0796	31.7	23.8	5.4	5.92	1.33
	+CH2	C16H12O6	300.0633	65.7	28.9	4.4	14.94	2.27
	+OH	C15H10O7	302.0422	6.4	3.1	1.9	3.30	2.07
	+H2	C15H12O6	288.0634	23.4	7.8	1.0	23.38	3.01
Baicalein(+NH+CH2)		C16H13N1O5	299.0796	67.6	28.5	1.0	67.57	2.37
	+OH	C16H13N1O6	315.0739	3.6	1.0	1.0	3.60	3.60
	-H2	C16H11N1O5	297.0638	16.3	4.6	1.0	16.30	3.57
Baicalein(+OH+CH2)		C16H12O6	300.0633	65.7	28.9	4.4	14.94	2.27
	+C6H8O6		476.0954	2.8	2.6	1.0	2.81	1.10
	-H2		302.0791	6.7	1.0	1.0	6.71	6.71
		C16H12O4	268.0734	4.3	17.0	12.5	0.35	0.25
	+ NH	C16H13N1O4	283.0478	9.7	1.0	1.0	9.70	9.70

Fig. 2 Metabolite analysis of dihydroxyflavone and baicalin, baicalein, wogonin and related compounds in Huang-qin (*Scutellaria baicalensis*). Colours in the array indicate the signal to noise ratio and the magnitude of the difference between germplasm lines. *Red* Increase, *S/N* ratio >100, line ratio >2.5; *green* near unity, *S/N* ratio >10, line ratio 0.5–2.5; *yellow* decrease, *S/N* ratio <0.5, line ratio <2.5

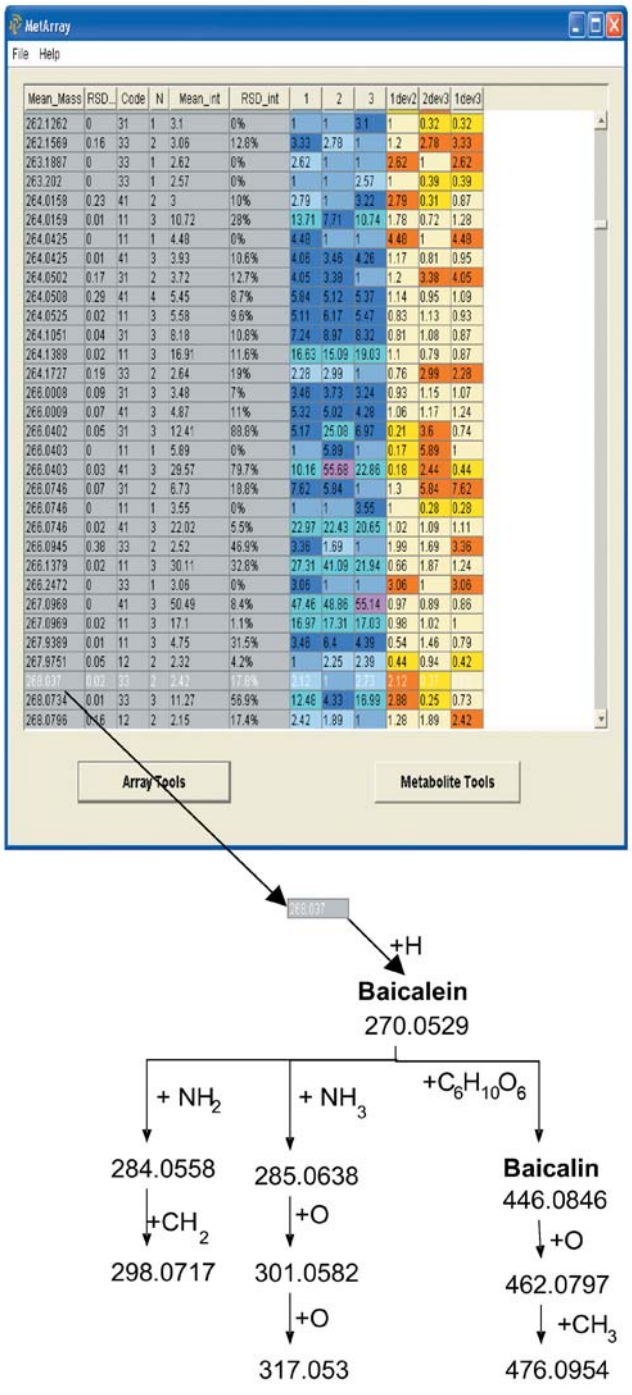

Fig. 3 Pathway analysis of flavone metabolites identified in the extracts of Huang-qin (*S. baicalensis*)

acterisation studies, this germplasm line has demonstrated an enhanced ability to survive insect, bacterial and fungal infestations. In all lines of Huang-qin, large quantities of annojahnin ($C_{37}H_{66}O_5$; MW 590.491), moreollin carboxylic acid ($C_{34}H_{40}O_9$; MW 592.2683), and pyrophaeophorbide a ($C_{53}H_{72}N_4O_3$; MW 812.5614) were found among the compounds with medicinal potential.

Discussion

The significance of this study lies in both the approach and the data collected. The wide range of selectable phytochemical variants of Huang-qin clearly illustrates the potential for selection of specific lines. Our ability to select distinct genotypes on the basis of growth rate (slow, medium, fast) and morphology resulted in selection of 26 distinct, stable lines with 7- to 10-fold variation in antioxidant potential and marker compounds. This observation established a correlation between morphological and medicinal potential and may also serve as a model for other species. Individual lines maintained in vitro over a 4-year period by clonal propagation remained morphologically and chemically stable, providing consistent plant material. In a broader sense, these individual clonally propagated lines mimic inbred lines and can be used for reconstitution of "designer hybrids" with specific combinations of different bioactives by conventional breeding or somatic hybridisation. Further, the selectable variation in biochemical profiles of individual germplasm lines provides an interesting new tool for evaluation of the role of specific phytochemicals. The individual lines with significantly low and high levels of specific metabolites provide the first research approach for evaluation of the concept of synergy. For example, individual lines with high antioxidant properties may be especially useful in

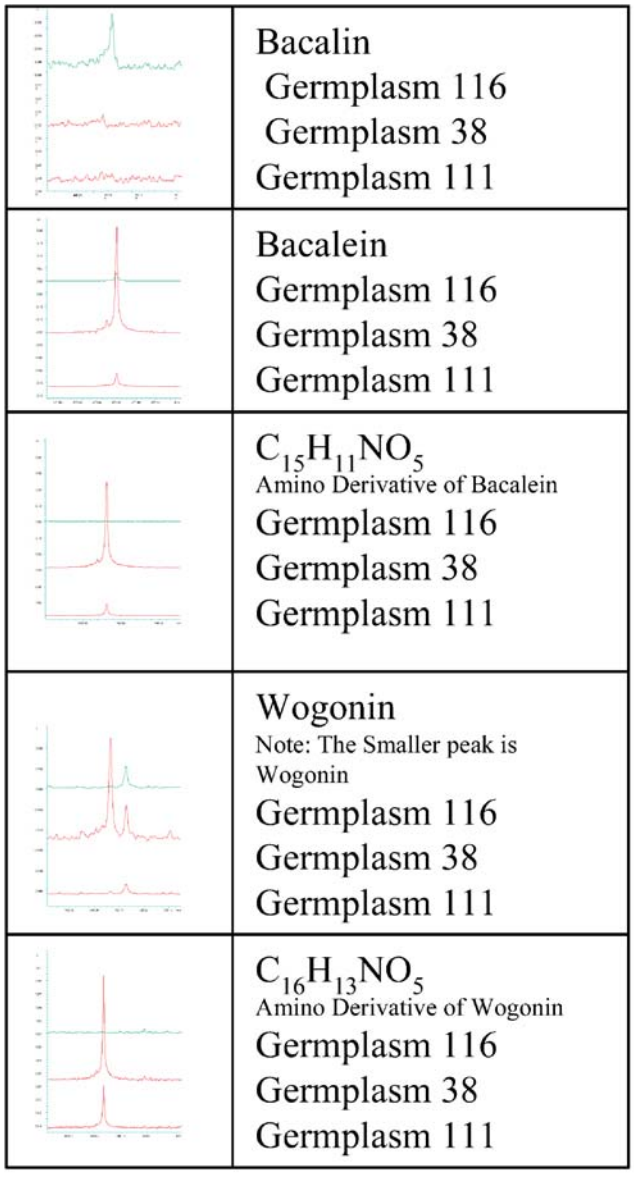

Fig. 4 Detailed metabolite analysis for the medicinal compounds in three germplasm lines of Huang-qin (*S. baicalensis*) including amino-derivatives of baicalein and wogonin

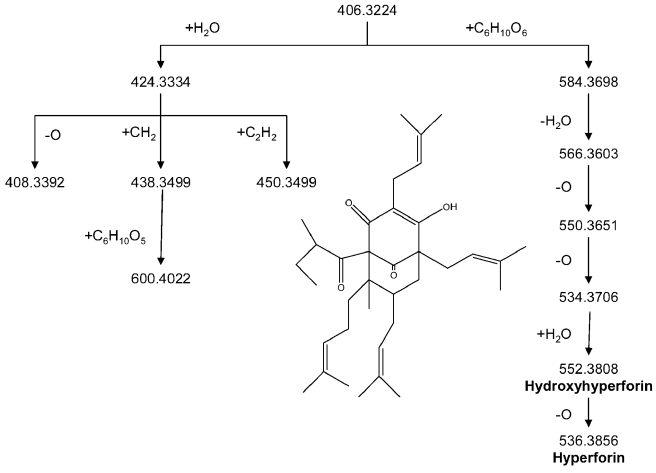

Fig. 5 Structure of hyperforin and the family of compounds with identical *m/z* values as hyperforin, 8-hydroxyhyperforin and related metabolites in *S. baicalensis*

specific preparations. Huang-qin extracts have previously been shown to provide protection against H_2O_2 oxidation in neuronal HT-22 cells (Choi et al. 2002). Despite the opportunity to do so, there has been very limited research to develop elite clones and hybrid varieties of medicinal plants.

It is interesting to note that the fastest growing Huang-qin germplasm contained the lowest concentration of melatonin, while the highest concentrations of melatonin were found in two of the slow-growing lines. Melatonin is a mammalian neurohormone primarily involved in circadian rhythms with activity as a signal of dark period, and an effective free radical scavenger and antioxidant (Reiter et al. 1997; Reiter 1998). Melatonin was first reported in Huang-qin and other medicinal plants used in the treatment of neurological disorders in 1997 (Murch et al. 1997). The current results support earlier suggestions that increased melatonin may be an indication of a genetic restriction on growth rate in a nutrient-rich environment or a protection against environmental stresses (Murch and Saxena 2002).

Metabolomics is a term that has been coined for the comprehensive, unbiased, high volume mass spectroscopic analysis of the whole profile of metabolites in a complex system such as a plant cell (Hall et al. 2002). The technologies of metabolomics complement genomics data arrays as a great number of genes have been sequenced but have not been assigned putative functions (Fiehn 2001). To date the principle applications of the technology have been to generate comparisons between transgenic plants of the same species for a clear representation of the effects of transformation or for comparisons of plant parental and F1 generations in breeding schemes. Often such profiles are restricted to certain compound classes or pathways of interest (Hall et al. 2002).

Our results show that there is a great potential for the use of this technology in identifying and understanding possible interactions of unique plant secondary metabolites. For instance, metabolite analyses offered an explanation for the different antioxidant potentials of Huang-qin lines. It does appear that the dihydroxyflavone metabolite is a potential flavone sink and that alterations in flavone biosynthesis and equilibrium are responsible for the modified antioxidant capabilities of Huang-qin lines. Since most mutations result in loss of function rather than gain, it can be hypothesised that the mutations in line 38 resulted in the inhibition of the dehydroxylation of trihydroxyflavone to dihydroxyflavone. This inhibition leads to an accumulation of trihydroxyflavone followed by a cascading metabolic effect that normally would not occur. Line 116 is a little more difficult in that the concentrations of the dihydroxyflavone are roughly half of

those in line 111, although there is no increase in trihydroxyflavone levels.

Huang-qin extracts contained a wide range of medicinal compounds from diverse classes of phytochemicals. Over 780 compounds matched metabolites in the medicinal database. In addition, the data array revealed some surprises, including several compounds that have previously been identified as unique to other species. For example, all three germplasm lines of Huang-qin contained compounds consistent with hyperforin and 8-hydroxyhyperforin, previously thought to exist only in St John's wort (*H. perforatum* L.). Metabolically related families for these compounds were also generated and these results describe part of a putative pathway for biosynthesis of hyperforin in higher plants (Fig. 5). In addition, differences in the identity and quantity of metabolites were found between germplasm lines, thereby providing chemical evidence for the diverse mammalian physiological responses to whole plant medicinal preparations.

This is the first report of hyperforin and related compounds in a Huang-qin extract. Hyperforin is a selective serotonin reuptake inhibitor previously thought to be characteristic of St John's wort. More importantly, hyperforin is also known to adversely affect metabolism of several drugs (Wentworth et al. 2000), including the immunosuppressant cyclosporine (with the attendant danger of transplant rejection), reduction in the level of an anti-HIV protease inhibitor, indinavir, and the active ingredient of oral contraceptives. Thus, products that may contain a single example of an entire class of unknown chemicals pose serious health concerns. Together, these results provide further evidence of the chemical complexity of whole plant preparations and emphasise the need for further metabolomic analyses to diagnose aspects of medicinal efficacy.

In conclusion, the data generated in this study clearly demonstrate the diversity between individuals within a medicinal plant species potentially resulting in wide variations in medicinal dose in whole plant preparations. This study also provides the first example of the use of a metabolomic approach for generating a more complete chemical profile of Huang-qin, and possibly other medicinal plants, with an emphasis on the discovery of commonalities and differences between medicinal species. Additionally, families of compounds derived from plant secondary metabolites can be used as the foundation for future investigations of pathways of biosynthesis of these complex molecules. Such analyses will increase our understanding of the complex nature of medicinal species and provide new perspectives for understanding human responses to whole plant treatments.

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