Research Article

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Using RAPD markers to predict polyphenol content in aerial parts of *Echinacea purpurea* plants

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Abstract

BACKGROUND: *Echinacea purpurea* (L.) Moench is in increasing demand worldwide owing to its medicinal value, resulting from the combined effects of several phytochemicals. In the present study, the polymerase chain reaction-amplified randomly amplified polymorphic DNA (RAPD) markers generated from 45 pre-selected primers were used to predict the contents of total phenol, caffeoyl phenol and alkamide[8+9] (alkamide 8+ alkamide 9) in aerial parts of 70 *E. purpurea* accessions through stepwise regression analysis. The contents of these phytochemicals were also analyzed chemically.

RESULTS: In the first trial, 16 polymorphic fragments generated by pre-selected RAPD primers showed significant correlations with the examined phytochemical traits in 59 accessions. Phytochemical traits in leaves and florets of another 11 accessions were further analyzed chemically, and the data were compared to the phytochemical data predicted using the regression equations derived from first trial. Statistical analyses revealed significant correlations in total phenol level between predicted and actual values for leaves and florets in these 11 accessions.

CONCLUSION: RAPD markers coupled with stepwise regression analysis can be considered as an initial screening method for identifying *E. purpurea* accessions with high total phenol content in aerial parts of the plants prior to assessing their agronomic performance in the field.

Keywords: alkamide; *Echinacea purpurea*; phytochemical; RAPD; total phenol

INTRODUCTION

Echinacea purpurea (L.) Moench is one of the major medicinal herbs that have continued to gain commercial interest worldwide owing to its antiviral, antibacterial and immunostimulatory activities.[1–3] These activities appear to result from the combined effects of several phytochemicals such as caffeoyl phenol and alkamide.[4,5] Naturally occurring *E. purpurea* plants are reported to be under threat of extinction,[6] and commercial cultivation of *E. purpurea* plants has become an alternative to meet the market demand.[7–9] Thus increasing the levels of dry mass and bioactive compounds has become a central goal for improvement of *E. purpurea* through traditional breeding efforts. However, the conventional method of selection is laborious and time-consuming, which requires an alternative avenue to identify the elite prototype of *E. purpurea* within a stipulated time frame. The recent development of polymerase chain reaction (PCR)-based techniques has played an important role in the management and utilization of plant genetic resources. By means of appropriate statistical methods, several DNA markers such as amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR) and randomly amplified polymorphic DNAs (RAPD) are used to quantify genetic variation among various plant species.[10,11] The high discriminating power of these techniques has also made DNA marker-assisted selection and breeding more feasible.[12–15]

Among the developed molecular techniques, RAPD has been most popular because of its rapidity, simplicity, low cost and absence of any need for prior knowledge of sequence information.[11,16,17] These characters are especially advantageous for the identification of medicinal plants because sequence data are usually difficult to obtain.[18] RAPD markers have been used with success in the study of medicinal plants including *Eleutherococcus senticosus*,[19] *Chamomilla recutita* (L.) Rausch.,[20] *Menispermum dauricum* DC.,[21] *Panax ginseng*[22] and several *Hypericum* species.[23] It has also been used to detect the genetic variation in inter- and intra-specific populations of *E.* purpurea.[24] However, there are no reports of predicting the levels of bioactive phytochemicals in *E. purpurea*

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plants from RAPD markers. The aim of the present study was therefore to assess the possibility of predicting the levels of total phenol, caffeoyl phenol and alkamides, in particular *E. purpurea* lines, from their RAPD markers. The collected data would help us to select and breed a superior *E. purpurea* population with desirable bioactive content.

MATERIALS AND METHODS

Plant materials and DNA extraction

Fifty-nine chemically distinct *E. purpurea* (L.) Moench accessions were collected from a consecutive mass selection program conducted at the experiment farm of the Department of Agronomy, National Chung Hsing University. Total genomic DNA was extracted by using the CTAB (hexadecyltrimethylammonium bromide) procedure described by Doyle and Doyle[25] from freshly harvested leaves ground to a fine powder. DNA concentration was estimated by subjecting samples to 8.5 mg g^{-1} agarose gel electrophoresis and staining with ethidium bromide. Staining intensities of the total DNA were compared visually with a DNA molecular weight marker. DNA yields of 1–20 mg g^{-1} leaf tissues were obtained, and total DNA was diluted with sterile distilled water to give a final concentration of 10 ng μL^{-1}. Additionally, a second set of *E. purpurea* (L.) Moench materials, which consisted of 11 accessions, were also selected for RAPD and chemical analyses.

RAPD analysis

PCR DNA amplification was performed using 45 arbitrary oligodeoxynucleotide primers (10-mer), selected on the basis of a preliminary screen of 10 randomly chosen *E. purpurea* accessions (data not presented)). PCRs were performed in a 12.5 µL reaction mixture consisting 100 mmol L^{-1} Tris-HCl (2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride) (pH 8.3), 500 mmol L^{-1} KCl (potassium chloride), 15 mmol L^{-1} $MgCl_2$ (magnesium chloride), 0.1 mg mL^{-1} gelatin, 200 µmol L^{-1} dNTP (deoxyribonucleotide triphosphate), 0.25 U Takara *Taq* polymerase, 0.25 µmol L^{-1} primer and 0.25 ng genomic DNA. Each reaction mixture was assembled in ice to prevent non-specific annealing of primers to DNA template prior to PCR. Amplification was performed in a thermocycler (Thermo Electron PXZ-0.2, Thermo Electron Corporation, Burnham on Crouch, UK) for 39 cycles after initial denaturation at 94 °C for 2 min. Each cycle consisted of denaturation at 94 °C for 45 s, annealing at 35 °C for 45 s, and extension at 72 °C for 2 min. Immediately after the last amplification cycle, the reaction mixture was kept at 72 °C for 5 min and then cooled at 4 °C for 20 min. PCR-amplified DNA fragments were separated by electrophoresis in agarose gel (15 mg g^{-1}) and stained with ethidium bromide and photographed under UV light. All PCR results were tested for reproducibility at least three times. Bands that did not show fidelity were eliminated.

Chemical analyses for total phenol, total caffeoyl derivatives and alkamid^{8+9} (alkamide 8+ alkamide 9)

Total phenolic content was estimated by a colorimetric assay based on procedures described by Taga *et al.*[26] 50 mg of dried ground tissue was extracted using 3 mL of 600 µL mL^{-1} methanol containing 3 µL mL^{-1} HCl for 60 min, and then centrifuged at 18 000 × *g* for 15 min. A 10 µL aliquot of tissue extract was dissolved in 200 µL of 20 µL mL^{-1} Na_2CO_3 (sodium carbonate), and 10 µL of Folin and Ciocalteu's phenol reagent (500 µL mL^{-1}) were added. The mixture was left to stand at room temperature for 30 min. Absorbance measurement was taken at 725 nm using a spectrophotometer, and caffeic acid was used in the construction of the standard curve.

For caffeoyl phenol determination, the tissue extract used for total phenol determination (20 µL) was filtered through a 0.2 µm syringe filter (Minisart RC 15, Sartorius AG, Goettingen, Germany) and then analyzed using high-performance liquid chromatography (HPLC) (Hitachi, Tokyo, Japan) consisting of a pump (L-7100), column oven (655A-52) (35 °C), UV-visible detector (L-4200) (330 nm) and autosampler (L-7200) (Hu and Kitts, 2000). The column used was Mightysil RP-18 GP 5 µm 150 × 4.6 mm (Kanto, Tokyo, Japan). Two different eluents were used: (A) acetonitrile/water 10 : 90 and (B) acetonitrile/water 25 : 75. Various levels of caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid were used in the construction of standard curves. Caffeoyl phenol was the sum of caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid.

For quantification of alkamide^{8+9}, 50 mg of ground dry tissue was extracted with 2.5 mL acetonitrile for 5 min and centrifuged at 18 000 × *g* for 15 min.[27] The supernatant (20 µL) was filtered through a 0.2 µm syringe filter (Minisart RC 15, Sartorius) and then analyzed using HPLC (Hitachi) consisting of pump (L-7100), column oven (655A-52) (35 °C), UV-visible detector (L-7420) (254 nm) and autosampler (L-7200). The column used was a Mightysil RP-18 GP 5 µm, 250 × 4.6 mm (Kanto, Tokyo, Japan). Two different eluents were used: (A) acetonitrile and (B). water. Various levels of alkamide^{8+9} were used in the construction of standard curves.

Statistical analysis

The RAPD dataset and chemical dataset were analyzed with the SAS procedure (SAS system for Windows™ release 6.12, SAS Institute Inc., Cary, NC, USA). The RAPD markers across the 59 accessions were scored as presence (1) or absence (0) of bands for each primer. In all, 369 DNA markers for each of the 59 accessions were recorded with 67 DNA markers showing polymorphic patterns.

The contribution of RAPD markers generated from selected primers to total phenol, caffeoyl phenol and alkamide^{8+9} variations in *E. purpurea* accessions was estimated by using a multiple linear regression analysis with the stepwise option in PROCREG. The analysis was based on the model

$$Y = a + b_1 m_1 + b_2 m_2 + b_3 m_3 + \ldots + b_j m_j + d + e$$

which related the variation in the dependent variable (Y = accession means for a quantitative trait) to a linear function of the set of independent variables m_j. The b_j are the partial regression coefficients that specify the empirical relationships between Y and m_j, d represents the between-accessions residue which is left after regression and e is the error of Y that includes environmental variation. The stepwise selection was performed to keep only those variables that were significant based on their D-statistic ($P < 0.15$). Regression parameters thus estimated were further used to predict the mean value of an additional 11 accessions and the observed and predicted values were compared using Student's t-test and linear regression analysis.

RESULTS AND DISCUSSION

One of the crucial objectives in the *E. purpurea* variety improvement program is to identify and select the populations or lines having high phytochemical contents (e.g. total phenol or caffeoyl phenol). However, conventional methods of identifying

phytochemical traits generally require laborious field and laboratory works and are time-consuming. Several molecular markers have been used in marker-assisted selection programs.[14,15] These molecular techniques bypass the reliance on diagnostic morphological and phytochemical traits that take time to collect in a mass selection system. Nevertheless, the success of such selection programs depends on the extent of linkage between molecular markers and the relevant phytochemical traits. RAPD markers are amplified DNA fragments generated by *Taq* DNA polymerase from short and random primers with arbitrary sequence. These markers can reveal single-base changes in the primer target site as well as large deletions or insertions in DNA samples.[16,17] In the present study, RAPD-PCR amplification was performed using 45 arbitrary oligodeoxynucleotide primers (10-mer) (data not presented) selected from a preliminary screen of 10 randomly chosen *E. purpurea* accessions employing 240 RAPD primers (including 180 Operon A–C, E–G, I, J, O and 60 Ubc 1–60) (data not presented). Across all 59 accessions, a total of 369 DNA fragments ranging from 510 to 1700 bp were generated, of which 67 fragments (18%) were polymorphic (Fig. 1). The average number of amplified fragments was 8.2.

Analysis of variance revealed that the variation among accessions was highly significant for all six phytochemical traits (leaf total phenol, floret total phenol, leaf alkamide^{8+9}, floret alkamide^{8+9}, leaf caffeoyl phenol and floret caffeoyl phenol) (data not presented). A summary of these phytochemical traits is presented in Table 1. Phenolic substances extracted from leaves and flowers of *E. purpurea* plants have been used for the treatment of various types of illness.[28] In the present study, the content of total phenol in leaf tissue was generally lower than that in floret (data not presented). These results are in agreement with the previous report of Chen *et al*.[9] The contents of total phenol in leaves and florets ranged from 25.60 (minimum) to 171.43 mg g^{-1} dry weight (DW) (maximum) and from 42.49 (minimum) to 126.62 mg g^{-1} DW (maximum), respectively (Table 1). The contents of total phenol in leaves and florets of 59 accessions averaged 54.42 and 81.68 mg g^{-1} DW, respectively. As with the data in total phenol

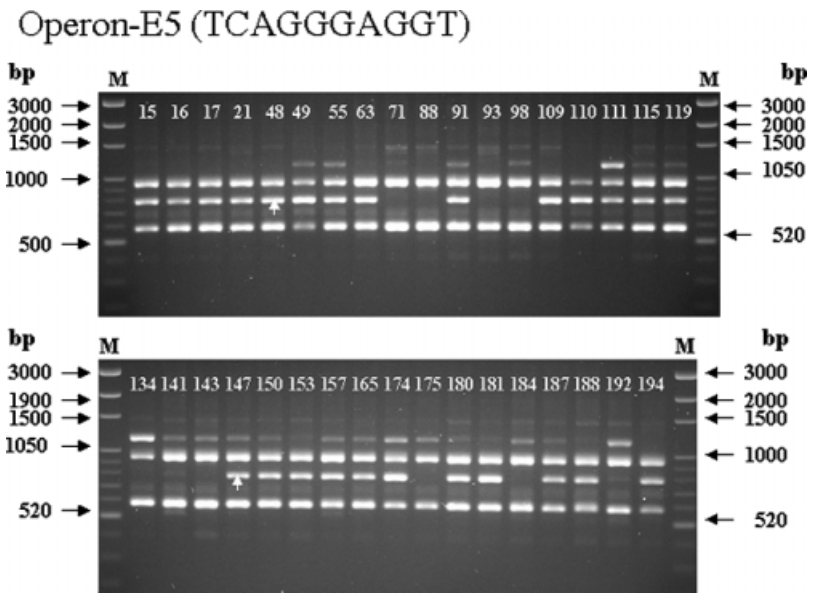

Figure 1. RAPD patterns of 35 accessions of *E. purpurea* plants generated by primer Operon-E5. Arrows indicate the changes in DNA fragments (780 bp) among the tested accessions.

Table 1. Means, maxima, minima and correlation coefficients of total phenol, alkamide^{8+9} and caffeoyl phenol (mg g^{-1} dry weight) in leaf and floret parts of 59 accessions of *E. purpurea*

Character	Leaf total phenol	Floret total phenol	Leaf alkamide^{8+9}	Floret alkamide^{8+9}	Leaf caffeoyl phenol	Floret caffeoyl phenol
Mean ± Standard error	54.42 ± 22.83	81.68 ± 17.60	0.08 ± 0.06	0.57 ± 0.33	23.33 ± 13.39	48.61 ± 15.48
Maximum	171.43	126.62	0.29	1.43	100.58	79.07
Minimum	25.60	42.49	0.00	0.20	0.20	9.11
Correlation coefficient						
Total phenol (leaf)	1.000					
Total phenol (floret)	0.087	1.000				
Alkamide^{8+9} (leaf)	−0.238*	−0.142	1.000			
Alkamide^{8+9} (floret)	0.061	0.073	0.441**	1.000		
Caffeoyl phenol (leaf)	0.865**	0.172	−0.351**	0.042	1.000	
Caffeoyl phenol (floret)	0.161	0.836**	−0.212	0.179	0.311**	1.000

Asterisks indicate significance at * 5% and ** 1% statistical levels, respectively.

Table 2. Correlation coefficients (*r*) of pre-selected RAPD markers and six phytochemical traits of 59 accessions of *E. purpurea*

Primer code: fragment size (bp)	Phytochemical trait	*r*	Primer code: fragment size (bp)	Phytochemical trait	*r*
A10-900	Leaf alkamide^{8+9}	−0.268*	B4-900	Leaf caffeoyl phenol	0.263*
B4-900	Leaf total phenol	0.264*	E5-780	Floret alkamide^{8+9}	0.284*
E6-1300	Floret alkamide^{8+9}	−0.283*	E6-1000	Floret total phenol	0.261*
E8-870	Floret alkamide^{8+9}	0.369**	E11-780	Floret alkamide^{8+9}	0.266*
E12-850	Leaf alkamide^{8+9}	0.367**	E16-1200	Leaf alkamide^{8+9}	0.268*
G9-600	Floret total phenol	0.325*	I15-1150	Leaf total phenol	−0.268*
O20-1200	Leaf alkamide^{8+9}	0.389**	U49-520	Floret caffeoyl phenol	0.338**
U52-510	Floret total phenol	0.319*	U52-510	Floret caffeoyl phenol	0.282*
U58-510	Floret total phenol	0.319*	U58-510	Floret caffeoyl phenol	0.282*

Asterisks indicate significant at * 1% or ** 5% statistical level, respectively.

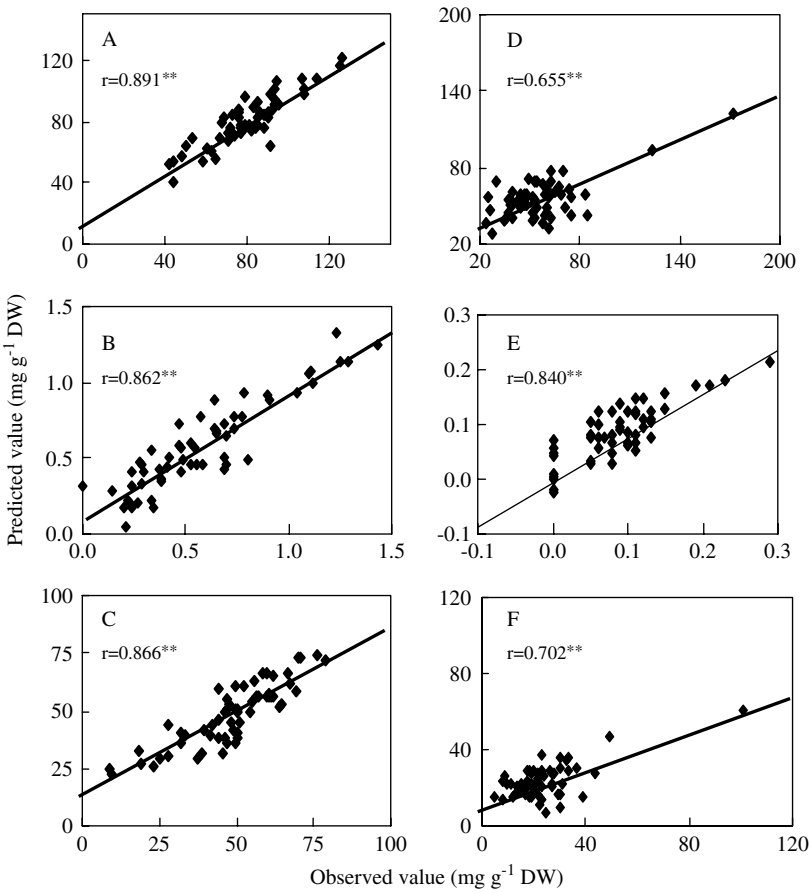

Figure 2. Relationship between observed and predicted values of (A) leaf total phenol, (B) leaf alkamide^{8+9}, (C) leaf caffeoyl phenol, (D) floret total phenol, (E) floret alkamide^{8+9} and (F) floret caffeoyl phenol in 59 accessions, based on the multiple equations generated from phytochemicals and selected RAPD markers of 59 accessions of *E. purpurea* plants.

content, greater variations were also found in the content of caffeoyl phenol in the leaves and florets of *E. purpurea* plant accessions (as indicated by the relatively greater values of standard error). The contents of caffeoyl phenol for the examined 59 accessions averaged 23.33 and 48.61 mg g^{-1} DW in leaves and florets, respectively (Table 1).

Various alkamides have been isolated and identified from *E. purpurea* plant,[29] with dodeca-2*E*,4*E*,8*Z*,10*E*-tetraenoic acid isobutylamide (alkamide 8) and dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (alkamide 9) being predominant.[30] Therefore, only the contents of alkamides^{8+9} were determined in the present study. Alkamides^{8+9} were detectable on leaves and floret portions of the majority of *E. purpurea* accessions (data not presented). The content of alkamides^{8+9} in leaves was lower than that in florets. The levels of alkamides^{8+9} in leaves and florets averaged 0.08 and 0.57 mg g^{-1} dry weight for the tested 59 accessions, respectively (Table 1).

As shown in Table 1, six out of 15 correlation coefficients between the six phytochemical traits of 59 accessions were statistically significant. The content of caffeoyl phenol represents

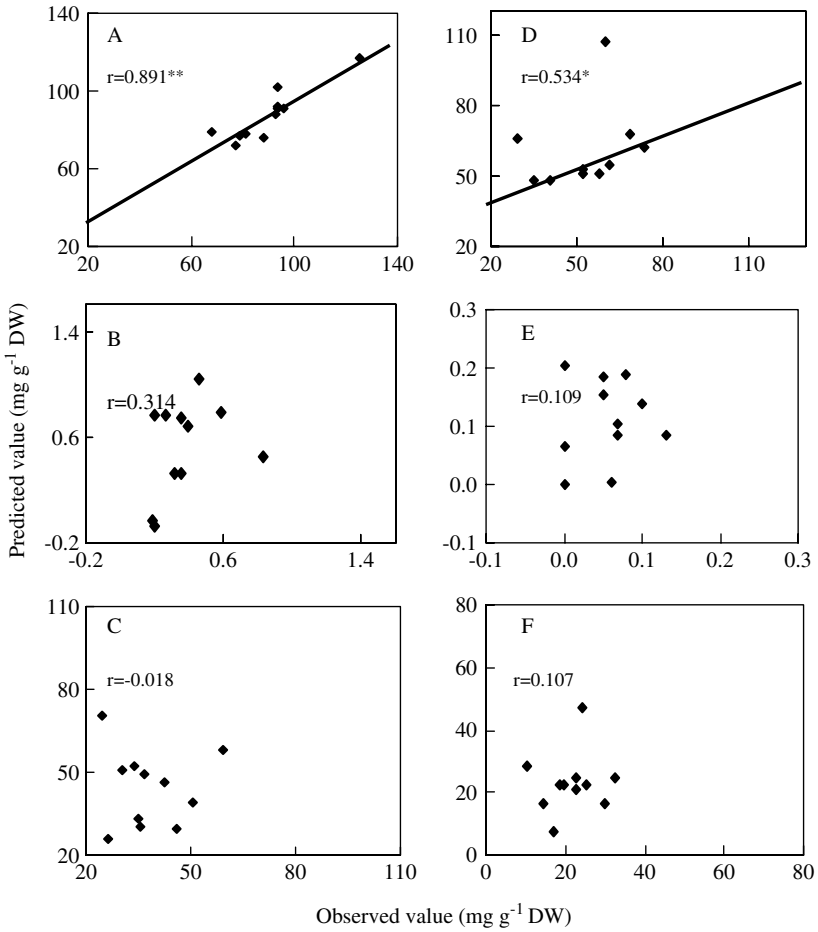

Figure 3. Relationship between observed and predicted values of (A) leaf total phenol, (B) leaf alkamide^{8+9}, (C) leaf caffeoyl phenol, (D) floret total phenol, (E) floret alkamide^{8+9} and (F) floret caffeoyl phenol in 11 accessions, based on the multiple equations generated from phytochemicals and selected RAPD markers of 59 accessions of *E. purpurea* plants.

the greatest portion of phenolic substances in *E. purpurea* plants.[28] Therefore, it is not surprising to find that the contents of caffeoyl phenol were positively correlated with the contents of total phenol in both leaves and florets (Table 1). The levels of caffeoyl phenol in leaves and florets of the plants were also correlated with each other. However, the contents of leaf or floret alkamide^{8+9} were either poorly correlated or negatively correlated with the contents of leaf or floret total phenol (Table 1), suggesting that it is very difficult to breed an *E. purpurea* line possessing both higher total phenol and higher alkamide^{8+9} contents.

The majority of simple correlation coefficients between selected RAPD markers and phytochemical traits had low values (data not presented). As shown in Table 2, only 18 out of 402 calculated coefficient coefficients were significant at 1% or 5% statistical level. Out of these 18 correlation coefficients, in three cases RAPD markers correlated well with phytochemical traits. Marker B4-900bp correlated positively with leaf total phenol and leaf caffeoyl phenol, and markers U52-510bp and U58-510bp correlated positively with both floret total phenol and floret caffeoyl phenol (Table 2). Multiple regression analysis with stepwise option was further used to identify the RAPD markers showing strong association with the assessed six phytochemical traits. The results showed that significant R^2 values (contribution rate) between RAPD markers and phytochemical traits were obtainable for the tested *E. purpurea* accessions, depending on the number of DNA markers used (Table 3). The R^2 values ranged from 0.4293 (leaf total phenol) to 0.8361 (floret alkamide^{8+9}) for the examined phytochemical traits, each with different markers. As shown in Table 3, nine RAPD markers could explain 42.93% of the observed total phenol variation in the leaf part of the 59 examined accessions of *E. purpurea*. For total phenol content in the floret part of *E. purpurea* plants, the estimated R^2 value was much greater than leaf total phenol content, with 15 RAPD markers explaining 79.41% of variation in floret total phenol of 59 *E. purpurea* accessions (Table 4). Likewise, 15, 21, 8 and 16 RAPD markers contributed to the observed leaf alkamide^{8+9} (R^2 = 70.64%), floret alkamide^{8+9} (R^2 = 83.61%), leaf caffeoyl phenol (R^2 = 42.98%) and floret caffeoyl phenol (R^2 = 75.09%) variations, respectively (Tables 3 and 4). These results clearly indicate that a good portion of variation in the assessed phytochemical traits of *E. purpurea* can be statistically estimated by using molecular markers generated through RAPD-PCR, at least in the tested 59 accessions. The plot of the predicted values of the six examined phytochemicals by the DNA data as a function of the actual values of these phytochemicals determined chemically indicated a linear relationship between them (Fig. 2). These results demonstrate that RAPD markers can be used to predict the contents of the six phytochemical traits in the tested 59 *E. purpurea* accessions, depending on the number of DNA markers used.

Table 3. Intercepts, regression coefficients (*b*) and coefficients of determination (R^2) of derived multiple regression equation to illustrate the relationships between pre-selected RAPD markers and the contents of total phenol, alkamide^{8+9} and caffeoyl phenol in leaves of 59 accessions of *E. purpurea*

Total Phenol		Alkamide $^{8+9}$		Caffeoyl Phenol	
Primer code: fragment size (bp)	*b*	Primer code: fragment size (bp)	*b*	Primer code: fragment size (bp)	*b*
B4-900	28.6597	A6-850	−0.0666	B4-900	13.7620
U19-590	8.2417	A10-900	−0.0177	U19-590	5.6801
U53-580	11.3205	A11-880	−0.0227	U53-580	7.4169
C10-1000	14.3345	U49-900	−0.0227	C10-1000	7.6050
E6-1300	−10.6614	U52-510	−0.0457	E8-870	−11.5644
E8-870	−23.0666	U53-620	0.0336	G8-750	6.2609
E8-600	−13.3117	U53-580	−0.0256	J1-1400	7.3457
J1-1400	11.8187	B13-600	−0.0598	J7-950	5.4358
J7-950	7.8921	C15-800	0.0268		
		E12-850	0.0353		
		E16-1200	0.0466		
		I16-680	0.0266		
		O2-1200	0.0331		
		O2-550	−0.0403		
		O20-1200	0.0425		
Intercept	53.4295		0.0882		7.3913
Probability	0.0006		<0.0001		0.0002
R^2	0.4293		0.7064		0.4298

Table 4. Intercepts, regression coefficients (*b*) and coefficients of determination (R^2) of derived multiple regression equation to illustrate the relationships between pre-selected RAPD markers and the contents of total phenol, alkamide^{8+9} and caffeoyl phenol in florets of 59 accessions of *E. purpurea*

Total Phenol		Alkamide $^{8+9}$		Caffeoyl Phenol	
Primer code: fragment size (bp)	*b*	Primer code: fragment size (bp)	*b*	Primer code: fragment size (bp)	*b*
U53-1700	−7.6858	B5-750	0.2430	A6-850	12.7334
U53-620	−11.2335	U15-890	−0.1796	A10-900	5.7345
U58-510	13.1869	U40-750	−0.3182	A19-1450	−7.4284
C1-1250	9.2849	U49-520	0.1492	B4-900	−11.2243
C1-1200	17.3629	U52-510	−0.1773	U15-890	−7.0767
C1-840	−5.0928	U53-620	0.3333	U41-850	−8.9858
C10-1000	11.5612	U53-580	−0.2151	U49-520	11.0513
E6-1000	17.6664	B14-1250	0.1912	B13-600	8.5779
E8-870	16.9744	C1-1250	−0.1659	B19-1100	7.6350
E12-900	5.4995	C10-1000	−0.2206	E6-1000	10.6693
E16-1150	10.6733	C17-1000	−0.1201	E6-900	19.4500
F7-800	13.0327	E6-1300	−0.2007	E8-870	12.8751
G9-600	25.1896	E6-900	0.1806	E16-1200	−14.7381
I15-1150	13.6268	E8-870	0.4221	I10-1600	−8.7533
J1-1400	5.9412	E11-780	0.1087	O2-1200	−13.7584
		E16-770	−0.1455	O2-550	5.4882
		F7-800	0.3066		
		I10-1600	−0.2127		
		I16-750	0.2090		
		J1-1400	−0.1102		
		O2-550	−0.2420		
Intercept	2.5061		0.5950		30.7884
Probability	<0.0001		<0.0001		<0.0001
R^2	0.7941		0.8361		0.7509

Since the generated stepwise regression equations could effectively describe the relationships between selected RAPD markers and various phytochemical variations for the tested 59 *E. purpurea* accessions, we were curious about whether the derived equations could also be used to predict the contents of phytochemicals in other *E. purpurea* accessions which were excluded from generating the said stepwise regression equation (Tables 3 and 4). Accordingly, a second set of *E. purpurea* materials consisting of 11 accessions was prepared. As shown in Table 5, out of the 66 phytochemical trait and accession combinations for which we made predictions, in 11 cases the actual results assessed chemically differed significantly from the predicted results estimated statistically. The plot of the predicted values of the six phytochemical traits by the DNA data as a function of the actual values that were chemically determined (Fig. 3) indicated a linear relationship in floret total phenol content ($r = 0.891$, $P < 0.01$). A similar result was also obtained for the relationship between leaf total phenolic content using DNA marker as a predictor ($r = 0.534, P < 0.05$). However, the correlation between the observed and predicted values for alkamide^{8+9} and caffeoyl phenol was low (Fig. 3). Only the associations of flora and leaf total phenol levels with selected RAPD markers provide good prediction of the performance of these 11 accessions. Thus it appears that using RAPD markers coupled with stepwise regression analysis can be considered as an initial screening method for identifying *E. purpurea* accessions with high total phenol content in aerial parts of the plants prior to assessing their agronomic performance in the field. The combination of these two techniques should also allow us to estimate indirectly the level of caffeoyl phenol for a given *E. purpurea* accession because both total phenol and caffeoyl phenol traits are closely correlated with each other (Table 2).

In conclusion, the present results indicate that the contents of phytochemical traits, including leaf total phenol, floret total phenol, leaf alkamide^{8+9}, floret alkamide^{8+9}, leaf caffeoyl phenol and floret caffeoyl phenol, in aerial parts of *E. purpurea*, vary considerably among the tested accessions. These variations, particularly in the content of total phenol in aerial parts of the plants, were also detectable by using RAPD markers. It appears that using RAPD markers, which are more or less randomly distributed across the genome, coupled with multiple regression analysis with stepwise option, could substantially change and improve the way in which crop biodiversity is used in the future. The combination of these two techniques should allow us to predict what a plant will look like in terms of quantitative phytochemical traits prior to elaborate field trials. If a diverse test array of *E. purpurea* germplasm is scored for important traits requiring specialized assessment conditions, then marker data can provide an efficient means of predicting the value of additional germplasm for such traits, and even identifying suitable material among germplasm *in situ*.

ACKNOWLEDGEMENTS

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Table 5. Observed (O) and predicted (P) contents (mg g^{-1} dry weight) of total phenol, alkamide^{8+9} and caffeoyl phenol in floret and leaf parts of 11 accessions. The predicted values were generated using the multiple regression equations derived from 59 accessions of *E. purpurea*

		Plant code											$LSD_{0.05}$
		142	152	156	160	170	173	182	183	189	197	198	
Leaf total	O	68.50	57.83*	29.31*	35.34	51.90	40.77	61.34	41.35	59.85*	52.45	73.72	7.49
Phenol	P	67.83	50.59*	65.77*	48.43	50.60	48.43	54.52	14.28	107.00*	52.60	61.84	
Floret total	O	92.52	93.25	93.23	95.85	93.55*	77.56	87.90*	81.28	125.20*	67.60*	79.10	23.92
Phenol	P	87.83	91.78	91.33	91.02	101.60*	72.22	76.10*	77.99	117.00*	79.00*	77.39	
Leaf	O	0.07	0.13	0.08*	0.07	0.00	0.10	0.00	0.00*	0.06	0.05*	0.05*	0.39
Alkamide^{8+9}	P	0.08	0.08	0.18*	0.10	0.06	0.14	0.00	0.20*	0.00	0.18*	0.15*	
Floret	O	0.39	0.83	0.20	0.19	0.35	0.59	0.31	0.45*	0.26*	0.35	0.20*	0.09
Alkamide^{8+9}	P	0.68	0.45	−0.07	−0.04	0.74	0.78	0.31	1.04*	0.77*	0.32	0.75*	
Leaf caffeoyl	O	22.69	30.00	10.15*	14.55	19.59	18.35	32.72	17.06	24.00*	25.47	22.73	19.71
Phenol	P	24.45	16.28	28.67*	16.27	22.54	22.53	24.45	7.52	47.13*	22.15	20.70	
Floret caffeoyl	O	36.82	30.44*	26.09	35.71	34.04	50.76	24.80*	42.59	59.18	46.08	34.78	12.56
Phenol	P	49.41	50.80*	25.76	30.28	52.48	38.92	70.40*	46.33	58.10	29.73	32.81	

* Statistically different at 5% significant level.

REFERENCES

1 Barrett B, Medicinal properties of *Echinacea*: a critical review. *Phytomedicine* **10**:66–86 (2003).
2 Goel V, Lovin R, Chang C, Slama JV, Barton R, Gahler R, *et al*, A proprietary extract from the Echinacea plant (*Echinacea purpurea*) enhances systemic immune response during a common cold. *Phytother Res* **19**:689–694 (2005).
3 Vimalanthan S, Kang L, Amiguet VT, Liversey J, Arnason T and Hudson J, *Echinacea purpurea* aerial parts contain multiple antiviral compounds. *Pharm Biol* **43**:740–745 (2005).
4 Randolph RK, Gellenbeck K, Stonebrook K, Brovelli E, Qian Y, Bankaitis-Davis D, *et al*, Regulation of human immune gene expression as influenced by a commercial blended Echinacea product: preliminary studies. *Exp Biol Med* **228**:1051–1056 (2003).
5 Matthias A, Banbury L, Stevenson LM, Bone KM, Leach DN and Lehmann RP, Alkylamides from Echinacea modulate induced immune responses in macrophages. *Immunol Invest* **36**:117–130 (2007).
6 Mechanda SM, Baum BR, Johnson DA and Arnason JT, Direct shoot regeneration from leaf segments of mature plants of *Echinacea purpurea* (L.) Moench. *In Vitro Cell Dev Biol – Plant* **39**:505–509 (2003).
7 Seidler-Lozykowska K and Dabrowska J, Yield and polyphenolic acids content in purple coneflower (*Echinacea purpurea* Moench.) at different growth stages. *J Herbs Spices Med Plants* **10**:7–12 (2003).
8 Kreft S, Cichoric acid content and biomass production of *Echinacea purpurea* plants cultivated in Slovenia. *Pharm Biol* **43**:662–665 (2005).
9 Chen CL, Zhang SC and Sung JM, The biomass and caffeoyl phenols productions of *Echinacea purpurea* grown in Taiwan. *Exp Agric* **44**:497–507 (2008).
10 Yonemoto Y, Chowdhury AK, Kato H, Macha MM and Okuda H, Characterization of white sapote (*Casimiroa edulis* Llave & Lex.) germplasm using flora morphology, RAPD and AFLP markers. *Sci Hortic* **112**:366–375 (2007).
11 Behera TK, Singh AK and Staub JE, Comparative analysis of genetic diversity in Indian bitter gourd (*Momordica charantia* L.) using RAPD and ISSR markers for developing crop improvement strategies. *Sci Hortic* **115**:209–217 (2008).
12 Liu Z, Fu T, Tu J and Chen B, Inheritance of seed colour and identification of RAPD and AFLP markers linked to the seed colour gene in rapeseed (*Brassica napus* L.). *Theor Appl Genet* **110**:303–310 (2005).
13 Stendal C, Casler MD and Jung G, Marker-assisted selection for natural detergent fiber in smooth bromegrass. *Crop Sci* **46**:303–311 (2006).
14 Aziz AN and Sauve RJ, Genetic mapping of *Echinacea purpurea* via individual pollen DNA fingerprinting. *Mol Breeding* **21**:227–232 (2008).
15 Agarwal M, Shrivastava N and Padh H, Advances in molecular marker techniques and their applications in plant science. *Plant Cell Rep* **27**:617–631 (2008).
16 Atienzar FA and Jha AN, The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutat Res* **613**:76–102 (2006).
17 Salem HH, Ali BA, Huang T-H, Qin D-N, Wang X-M and Xie Q-D, Use of random amplified polymorphic DNA analysis for economically important food crops. *J Integr Plant Biol* **49**:1670–1680 (2007).
18 Shinde VM, Dhalwal K, Mahadik KR, Joshi KS and Patwardhan BK, RAPD analysis for determination of components in herbal medicine. *Evid Based Complement Alternat Med* **4**:21–23 (2007).
19 Yu CY, Kim SH, Lim JD, Kim MJ and Chung IM, Intraspecific relationship analysis by DNA markers and *in vitro* cytotoxic and antioxidant activity in *Eleutherococcus senticosus*. *Toxicol in Vitro* **17**:229–236 (2003).
20 Wagner C, Friedt W, Marquard RA and Orden F, Molecular analyses on the genetic diversity and inheritance of (−)-α-bisabolol and chamazulene content in tetraploid chamomile (*Chamomilla recutita* (L.) Rausch.). *Plant Sci* **169**:917–927 (2005).
21 Zhou J, Qu F and Nan R, Genetic and alkaloid analysis of *Menispermum dauricum* DC. by RAPD and HPLC. *Phytochem Anal* **18**:509–515 (2007).
22 Yip PY, Chau CF, Mak CY and Kwan HS, DNA methods for identification of Chinese medicinal materials. *Chinese Med* **2**:9–27 (2007).
23 Smelcerovic A, Verma V, Spiteller M, Ahmad SM, Puri SC and Qazi GN, Phytochemical analysis and genetic characterization of six *Hypericum* species from Serbia. *Phytochemistry* **67**:171–177 (2006).
24 Kapteyn J, Goldsbrough PB and Simon JE, Genetic relationships and diversity of commercially relevant *Echinacea* species. *Theor Appl Genet* **105**:369–376 (2002).
25 Doyle JJ and Doyle JL, Isolation of plant DNA from fresh tissue. *Focus* **12**:13–15 (1990).
26 Taga MS, Miller EE and Pratt DE, Chia seeds as a source of natural lipid antioxidants. *J Am Oil Chem Soc* **61**:928–931 (1984).
27 Perry NB, Klink JWV, Burgess EJ and Parmenter GA, Alkamide levels in *Echinacea purpurea*: a rapid analytical method revealing differences among roots, rhizomes, stems, leaves and flowers. *Planta Med* **63**:58–62 (1997).
28 Thygesen L, Thulinn J, Mortensen A, Skibsted LH and Molgaard P, Antioxidant activity of cichoric acid and alkamides from *Echinacea purpurea*, alone and in combination. *Food Chem* **101**:74–81 (2007).
29 Binns SE, Livesey JF, Arnason JT and Baum BR, Phytochemical variation in *Echinacea* from roots and flowerheads of wild and cultivated populations. *J Agric Food Chem* **50**:3673–3687 (2002).
30 Kim H-O, Durance TD, Scaman CH and Kitts DD, Retention of alkamides in dried *Echinacea purpurea*. *J Agric Food Chem* **48**:4187–4192 (2000).

