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Heat stress reduces the accumulation of rosmarinic acid and the total antioxidant capacity in spearmint (*Mentha spicata* L)

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Abstract: Selected high-phenolic lines of spearmint were subjected to a constant 30 °C heat regimen for a period of 4 weeks to determine the effects of heat stress on soluble phenolics, phenols and rosmarinic acid biosynthesis and antioxidant capacity. Heat stress significantly reduced levels of total phenolic acids (71–87%) and soluble phenols (75–87%). This loss was concomitant with a loss of total antioxidant capacity of 21–60% after week 1 and up to 95% by week 4. High-performance liquid chromatography profiling of heat-stressed plants at 270 and 320 nm detected nearly a complete loss of rosmarinic acid in all seven chemotypes. High-temperature drying of non-heat-stressed plants at 80 °C resulted in a similar loss of total antioxidant capacity and rosmarinic acid content an effect not observed in material that was subjected to low-temperature drying first, followed by exposure to high temperature. This suggests that heat stress negatively regulates rosmarinic acid biosynthesis and causes a potential rapid biological breakdown of rosmarinic acid in tissues. 2,2-Diphenyl-1-picrylhydrazyl radical assays of heat-stressed and non-stressed plants clearly show that rosmarinic acid is the major contributor to the antioxidant capacity in spearmint.

Keywords: antioxidants; heat stress; rosmarinic acid; spearmint; phenolics

INTRODUCTION

Plants from the Lamiaceae family have been harvested for traditional spices and essential oils for centuries. Spearmint (*Mentha spicata* L.) is widely used as a source of essential oils for flavouring, and more recently has been used as a valuable source of the potent antioxidant rosmarinic acid for the nutraceutical and cosmetic industries.[1–3] Rosmarinic acid has earned the reputation as a molecule of interest owing to its multiple biological activities against inflammatory lung diseases, autoimmune arthritis, heart disease and suppression of autoimmune rejection in human skin transplant patients as well as its multipurpose activities against reverse transcriptase, integrase and RNase H in HIV infections.[4–8] Therefore interest in cultivating a quantifiable natural source of this potent and versatile antioxidant has become paramount.

The levels of rosmarinic acid and essential oils are largely dependent on two factors, namely genotype and growing conditions. With some success, researchers have developed clones of chemotypes of spearmint that overproduce rosmarinic acid.[9] These chemotypes perform well under controlled laboratory conditions; however, there are limited data on the effects of field environment and product processing on phenolic metabolism. Changing environmental conditions, resulting in an increased occurrence of heat and drought stress and the metabolic effects of heat stress, have become a major focus of plant research, especially on crop and food plants. Studies show an increase in the levels of antioxidants and phenolics in fruits such as strawberries, blueberries and raspberries under heat stress conditions.[10–12] These inflated levels of antioxidants may be a mechanism for the plants to protect plant tissues against reactive oxygen and nitrogen species created by the elevated temperatures.[10] In other crop plants such as wheat the levels of antioxidant enzymes (superoxide dismutase, glutathione reductase, peroxidase) and the antioxidants ascorbic acid and glutathione increase to combat reactive oxygen species.[13] However, there is little information regarding the effects of heat stress on members of the mint family.[14] The growing conditions could have implications on the metabolic activity for spearmints earmarked as a source of high-quality rosmarinic acid and other phenolic antioxidants for commercial use. Owing to the heterozygous nature of seed-produced mint plants, this study makes use of cloned lines of spearmint, individually selected in tissue culture and increased vegetatively to reduce variation.[9] Therefore the effects of heat stress can be attributed directly to biochemical metabolism of the clonal genotype rather than to plant-to-plant variation.

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The present study addresses the effects of constant heat stress on spearmint plants grown in a controlled indoor environment, in terms of the accumulation of rosmarinic acid and other secondary metabolites with respect to the total antioxidant capacity of the plant extracts.

EXPERIMENTAL

Plant material and chemicals

Spearmint seed was sourced from Burpee Seed (Warminster, PA, USA; coded as MSH) and Lake Valley Seed (Arapahoe Boulder, CO, USA; coded as HMS). Spearmint clones were selected in tissue culture for elevated phenolic levels using a combination of proline analogues and *Pseudomonas*[9] and maintained by clonal propagation. These spearmint clones were produced and are owned by the University of Massachusetts, Department of Food Science (Dr K Shetty) and were maintained in a field nursery in 12 m^2 plots per clone at the University of Guelph, Arkell Research Station.

All standard phenolics and reagent chemicals were purchased from Sigma-Aldrich (Mississauga, Canada) unless stated otherwise.

Tissue desiccation and solvent extractions

Plant material was dried at various temperatures in order to optimise the levels of rosmarinic acid and total antioxidant capacity. Plant material was dried at three different temperatures (35, 45 and 80 °C) in paper bags for 24–96 h and via lyophilisation at −50 °C. A second protocol involved drying tissue at 35 °C for 96 h and subsequently exposing the dried sample to 80 °C in a high-temperature dryer for another 24 h.

Extracts for chemical and antioxidant assays were prepared using 8–10 mg of dried ground leaf material and microwave extracted in 5 mL of 50% (v/v) ethanol/water (2 × 60 s, 100 W). Extracts were cooled to room temperature, poured into Eppendorf tubes and centrifuged for 2 min at 10 000 × *g* to remove any particulates. Extracts were prepared daily to limit any oxidative degradation.

For high-performance liquid chromatography (HPLC) profiling, 25 mg of ground, dried spearmint was microwave extracted in 2 mL of 50% (v/v) ethanol/water. Extracts were filtered and diluted 1:1 with 55% (v/v) acetonitrile/water prior to injection.

Heat stress experiments on living plants

Seven clonal lines were grown under normal growth room conditions in 15 cm pots for 4 weeks prior to the start of the heat stress experiments. Three plants from each clone were transferred to heat stress conditions using a ConViron growth chamber (Controlled Environments Limited, Winnipeg, Canada) (16 h day, 30/30 °C day/night, 150 lux cm^{-2}), while two were maintained as controls under normal growth room conditions (16 h day, 20/18 °C day/night, 200 lux cm^{-2}). Plants were watered daily to prevent drought stress. Three branches were harvested from each experimental and control plant from each clone every 7 days. The branches were dried at 30 °C in paper bags for 4 days.

Quantification of total soluble phenolics and phenols

Total phenolics were determined using a modified Folin method.[15] Crude extract (125 μL) was transferred to a microfuge tube and reagents were added in the following order: 95% ethanol (125 μL), distilled water (625 μL), 50% Folin reagent (63 μL) and 50 g kg^{-1} K_2CO_3 (125 μL). Samples were mixed gently and placed in complete darkness (45 min) for colour development (blue). Samples were centrifuged (10 000 × *g* for 5 min), an aliquot (250 μL) was transferred to a 96-well micro plate and the absorbance (725 nm) was recorded. *m*-Coumaric acid was used as the standard phenolic, and soluble phenolics were expressed as mg g^{-1} dry weight (DW) basis. All determinations were completed in triplicate and then averaged.

Total soluble phenols were analysed by placing a 15 μL aliquot of crude clarified extract into a 96-well micro titre plate and diluting the sample with 235 μL of 50% (v/v) ethanol/water. The absorbance of the diluted sample solution was recorded at 330 nm and compared against a standard gallic acid solution (standard phenol) ranging in concentration from 0 to 30 μg in an assay volume of 250 μL. All determinations were completed in triplicate and then averaged.

Total antioxidant capacity: DPPH radical-scavenging assay

The nitrogen radical-scavenging activity was measured by monitoring the reduction of the free nitrogen radical 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) in the presence of the spearmint extracts. Briefly, a $DPPH^{\cdot}$ solution (1.0×10^{-4} mol L^{-1} in ethanol) was prepared 24 h prior to each experiment. Sample extracts (5 μL) were added to the wells of a 96-well micro titre plate and the DPPH free radical solution (245 μL) was added to mix with the sample aliquot. Gallic and rosmarinic acids were used as standards. After 30 min, total antioxidant capacity was determined by the observed diminution of absorbance at 515 nm. The radical-scavenging activity was expressed as the % quench of the DPPH radical, calculated by $[(Abs_0 - Abs_{15\,min})/Abs_0] \times 100$. All determinations were completed in triplicate and then averaged.

High-performance liquid chromatography (HPLC) profiling of spearmint extracts

Separation of phenolic compounds was achieved using a Gilson Unipoint analytical system (Gilson Corp, Middleton, WI, USA) accessorised with a 4.6 mm × 250 mm C18 ODS-2 reverse phase column (Spherisorb, Supelco, Bellefonte, PA, USA). A gradient solvent system comprising a mixture of acetonitrile (solvent A) and 0.1% (v/v) phosphoric

acid/water (solvent B) was used as follows: 0 min, 25% B; 12 min, 45% B; 15 min, 95% B; 18 min, 95% B for 5 min then re-equilibrated to 25% B. The flow rate was 1 mL min^{-1} and detection was accomplished using a Gilson 118 UV–visible detector set to 320 nm for the detection of free hydroxycinnamic phenolics. The detector was set at 270 nm for the detection of phenols in the extracts. Rosmarinic, caffeic, *m*-coumaric, *p*-coumaric and ferulic acids were identified using retention times with known standards. Quantification of the individual components was accomplished by using the peak area relative to a known standard.

Statistical analyses

To identify significant changes in phenolics, phenols and total antioxidant capacity during the 4 week period that the experimental plants were subjected to 30 °C heat stress, regression analyses were performed. For each clone the initial values and those at the end of 7, 14, 21 and 28 days were plotted. Results of the analysis using the two control plants were then compared with the results from the three experimental plants for each clone. Results were scored as no change, a significant increase or a significant decrease in phenolics, phenols and total antioxidant capacity over the 4 week period. All statistics were done using the program Statistica (StatSoft, Release 5, Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Effect of tissue drying temperature on rosmarinic acid content and total antioxidant capacity

The drying temperature of plant tissues is known to be critical to preserve the antioxidant potential and chemical composition of medicinal plants. Fresh spearmint has a high moisture content, and the preservation of the biochemical and antioxidant properties is largely dependent on the drying process.[16] Different drying regimes were tested to optimise the antioxidant and rosmarinic acid contents in the spearmints prior to extraction.

Drying temperatures of −50 (lyophilisation), 35 and 45 °C for 24–96 h preserved the highest extractable rosmarinic acid in the dried leaves and translated into the greatest total antioxidant capacity in the DPPH assay (Fig 1). The length of the drying time had no effect on the rosmarinic acid content or the total antioxidant capacity. However, increasing the drying temperature to 80 °C resulted in a significant loss of total antioxidant capacity, and rosmarinic acid was nearly undetectable by HPLC in the extracts (Fig 2). The loss of total antioxidant capacity at high temperatures is consistent with the losses observed with high-temperature extractions of other medicinal herbs where chemical degradation of the sample occurs. However, rosmarinic acid is stable to 180 °C, therefore it seemed unlikely that the rosmarinic acid was being broken down via a thermal degradation process. To determine whether the loss of rosmarinic acid was due to chemical or biological degradation, a second drying experiment was set up to dry tissue at 35 °C for 96 h and then expose the same sample to 80 °C for 24 h. After the high-temperature exposure the rosmarinic acid content and the total antioxidant capacity of the extract were unchanged compared with the 35 °C controls. HPLC profiling showed that there were no differences in the chromatograms using detection at 270 or 320 nm. This experiment confirmed the stability of rosmarinic acid (stable to 180 °C) and the absence of any chemical degradation. The results do suggest that some form of biological degradation was occurring in the high-temperature dried material. The metabolic processes in the spearmint are still functional after harvest, and the high-temperature drying potentially could cause a heat stress response in the tissue. Rosmarinic acid is a caffeic acid ester and could be rapidly degraded to provide the plant tissues with an immediate supply of caffeic acid.

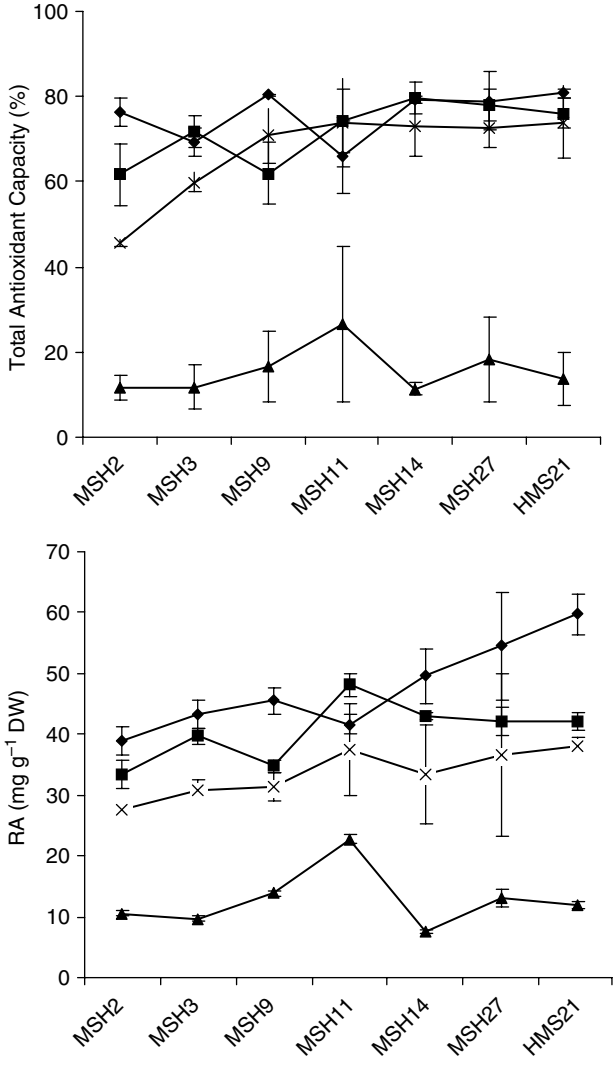

Figure 1. Effects of drying temperature (×, −50 °C; ♦, 35 °C; ■, 45 °C; ▲, 80 °C) on total antioxidant capacity and rosmarinic acid (RA) content of selected spearmint clones. High-temperature drying of plant tissue has a significant negative effect on rosmarinic acid content and total antioxidant capacity.

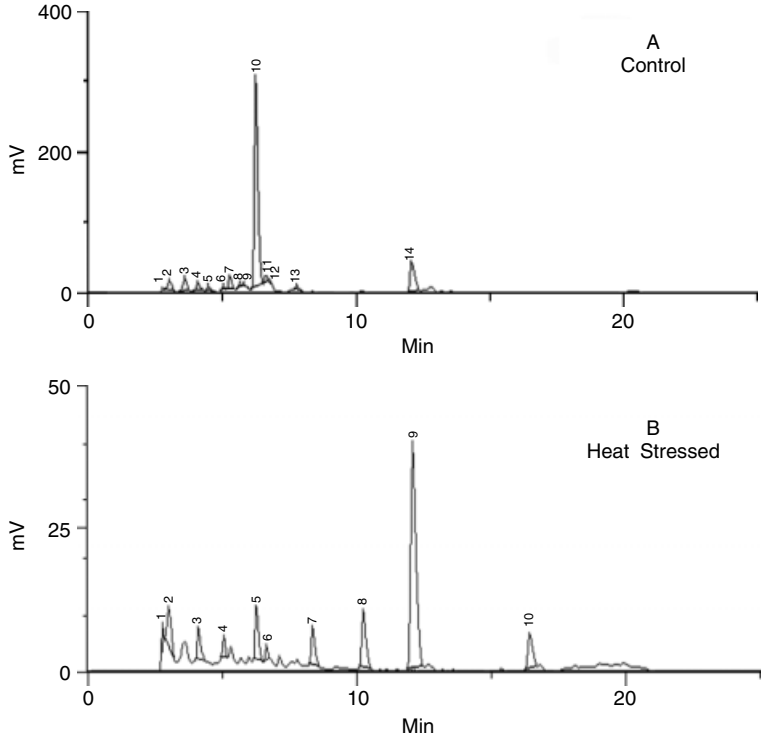

Figure 2. HPLC chromatograms (not to same scale) of MSH2 at 270 nm. (A) Extract of control plants where rosmarinic acid = peak 10 with peak area of 4.41×10^6 units. (B) Magnified (eightfold) chromatogram of heat-stressed plant extract. Rosmarinic acid = peak 5 with peak area reduced to 1.33×10^5 units, or 3.0% of that found in MSH2 controls. Levels of rosmarinic acid for other heat-stressed clonal lines were: MSH3, 2.6%; MSH9, 6.6%; MSH11, 3.0%; MSH14, 10.4%; and HMS 21, 5.3% — a significant reduction compared with the control plants.

Table 1. Phenolic levels of spearmint tissues grown under constant heat stress[a]

		Phenolics (mg g^{-1} DW)				
Line	Group	Week 0	Week 1	Week 2	Week 3	Week 4
MSH2	Experimental	30.48	21.09	41.03	29.38	19.18
	Control	58.05	83.42	84.76	101.01	122.03
MSH3	Experimental	129.10	94.57	59.90	76.93	39.97
	Control	113.15	175.9	246.00	318.9	229.85
MSH9	Experimental	71.11	32.41	27.08	27.46	12.53
	Control	80.34	67.74	83.47	88.18	59.85
MSH11	Experimental	299.53	117.80	107.70	87.73	62.38
	Control	249.70	295.65	356.65	308.0	212.20
MSH14	Experimental	54.14	33.48	11.01	21.80	19.23
	Control	62.37	94.54	85.22	68.79	71.18
MSH27	Experimental	25.50	13.72	8.49	12.17	11.13
	Control	18.58	14.97	58.76	65.37	84.51
HMS21	Experimental	52.56	33.15	20.17	22.32	10.84
	Control	73.02	116.58	116.19	77.23	80.66

[a] Phenolics were determined determined as the average of three independent samples per plant from three plants from the experimental group and two from the control.

Effect of heat stress on soluble phenols and phenolics in living plants

The levels of phenolics and phenols in the control plants in the 4 weeks prior to heat stress and during the experiment remained high, representative of the levels found in growth room and outdoor field conditions.[17] After plants were moved into the 30 °C growth chamber, a sharp decline was observed in the phenolic levels within 1 week of heat treatment (Table 1). The same trend was also observed for the levels of free phenols found in the spearmint tissue (Table 2). This is the first study to show a reduction in free phenolic and phenol levels, whereas other studies have shown that plants increase phenol and phenolic levels in response to continual elevated temperatures.[10] In that case the authors argued that the increased level of free phenolics in strawberry was the result of an internal defence mechanism to rid plant tissues of dangerous levels of reactive oxygen species formed in the presence of heat stress. Clearly, the observed

Table 2. Soluble phenol levels of spearmint tissues grown under constant heat stress[a]

Line	Group	Phenols (mg g^{-1} DW)				
		Week 0	Week 1	Week 2	Week 3	Week 4
MSH2	Experimental	107.87	52.25	40.04	43.12	28.47
	Control	91.85	152.09	124.55	162.55	208.35
MSH3	Experimental	155.86	121.43	79.10	76.70	51.93
	Control	126.15	191.55	274.65	338.60	244.25
MSH9	Experimental	97.67	51.68	44.04	37.48	19.19
	Control	110.19	109.73	95.72	108.88	73.50
MSH11	Experimental	155.87	121.43	79.13	76.70	51.93
	Control	126.10	191.55	274.70	346.50	244.20
MSH14	Experimental	94.02	69.16	27.60	40.63	30.36
	Control	98.84	146.37	137.30	108.95	125.42
MSH27	Experimental	72.70	46.35	41.47	36.87	27.53
	Control	53.55	98.24	117.25	120.22	155.76
HMS21	Experimental	88.06	56.16	38.87	45.69	27.49
	Control	113.85	172.63	198.14	114.55	125.59

[a] Phenols were determined as the average of three independent samples per plant from three plants from the experimental group and two from the control.

Table 3. Total antioxidant capacity of heat-stressed dried tissue of spearmint using the DPPH radical-scavenging assay[a]

Line	Group	Total antioxidant capacity (% inhibition of DPPH radical)				
		Week 0	Week 1	Week 2	Week 3	Week 4
MSH2	Experimental	17.96	0.00	1.11	3.21	3.48
	Control	18.27	17.78	22.83	15.84	20.59
MSH3	Experimental	58.15	45.21	33.29	25.97	20.25
	Control	50.51	56.66	70.00	70.11	62.57
MSH9	Experimental	59.21	26.38	26.97	25.09	11.40
	Control	61.27	60.18	62.14	71.02	49.27
MSH11	Experimental	23.95	19.99	16.98	20.29	13.56
	Control	28.90	44.23	39.20	39.81	28.70
MSH14	Experimental	59.30	45.12	14.53	31.42	33.45
	Control	62.97	80.39	70.32	69.93	74.50
MSH27	Experimental	46.41	37.81	22.37	12.44	3.26
	Control	31.94	61.09	59.76	60.50	65.30
HMS21	Experimental	56.30	34.32	8.08	5.06	9.60
	Control	68.79	85.60	74.50	53.61	67.69

[a] Antioxidant levels were determined as the average of three independent samples per plant from three plants from the experimental group and two from the control.

levels of both phenols and phenolics in spearmint do not reflect the same defence response as observed in strawberry plants (Tables 1 and 2). Rosmarinic acid, which accounts for 50–85% of the soluble phenolic compounds in these spearmint clones, was reduced to levels more than two orders of magnitude lower than in non-stressed plants (Fig 2).

The losses of soluble phenols, phenolics and rosmarinic acid can be linked to the sharp decline in the observed total antioxidant capacity of the plants under heat stress. Starting in week 1 of heat treatment, total antioxidant capacity declined significantly in all clones and continued a downward trend during the exposure of the plants to 4 weeks under heat stress (Table 3).

The results of regression analyses are summarised in Table 4. In the case of phenolics, in all comparisons the controls exhibited either no significant change or a significant increase over the 4 week heat period, whereas six of the seven clones showed a decrease in phenolics and one line exhibited no change in the heat-treated plants. Similar results were recorded for phenols. For total antioxidant capacity the controls either did not change or showed a significant increase in all seven cases; however, there was a significant decrease in activity in five of the experimental lines. Two experimental lines showed no change over time. Therefore the decrease seen in phenolics, phenols and concomitant total antioxidant capacity in the 30 °C plants was clearly not reflected in the controls. Statistical analysis of phenolic, phenol and rosmarinic acid levels and total antioxidant capacity clearly shows a positive correlation between phenolic levels and total antioxidant capacity. Therefore heat stress caused a significant change in the metabolic processes of the spearmints.

HPLC profiling of rosmarinic acid in heat stress *vs* non-heat stressed plants

HPLC analyses of spearmint plants grown in a normal, controlled environment revealed that rosmarinic acid was the primary phenolic compound present in the spearmint clones. Rosmarinic acid content ranged from 55 to 85% of the total phenolic profile, with the remainder of the phenolics being *m*-coumaric, *p*-coumaric, caffeic and ferulic acids (Fig 2A). Plants that were grown under constant 30 °C heat stress showed a marked decrease in the phenolic and phenol profiles (Fig 2B; note scale differences in comparison with Fig 2A). Similar profiles were found in all the spearmint clones analysed, resulting in a loss of between 90 and 98% of the rosmarinic acid content found in the 20 °C control plants.

Field-grown spearmints also showed a decrease in rosmarinic acid content during the hottest summer

Table 4. Summary of regression analysis results following change in levels of phenolics, phenols and total antioxidant capacity over a 4 week period[a]

	Phenolics		Phenols		Total antioxidant capacity	
Line	Control	Experimental	Control	Experimental	Control	Experimental
MSH2	NS	NS	NS	NS	NS	Neg. sign.
MSH3	Pos. sign.	Neg. sign.	Pos. sign.	Neg. sign.	NS	Neg. sign.
MSH9	NS	Neg. sign.	NS	Neg. sign.	NS	Neg. sign.
MSH11	NS	Neg. sign.	Pos. sign.	Neg. sign.	NS	NS
MSH14	NS	Neg. sign.	NS	Neg. sign.	NS	NS
MSH27	Pos. sign.	Neg. sign.	Pos. sign.	Neg. sign.	Pos. sign.	Neg. sign.
HMS21	NS	Neg. sign.	NS	Neg. sign.	NS	Neg. sign.
No change or positive change	7	1	7	1	7	2
Negative change	0	6	0	6	0	5

[a] Experimental samples were grown at 30 °C, control samples at 20 °C. NS, no significant change over 4 weeks; Neg. sign., significant decline over 4 weeks; Pos. sign., significant increase over 4 weeks.

months, but not to the same degree as plants grown under continual heat stress.[17] Field-grown plants were subjected to higher temperatures than the 30 °C continual heat stress used in the indoor experiments; however, the heat stress in the field is not continual. Night temperatures were considerably lower than 30 °C, which provided the plants with a recovery period to possibly re-establish normal metabolism. Therefore our results suggest that the significant loss of rosmarinic acid is due to the presence of continual heat stress conditions and that intermittent high temperatures in the field only have a minor effect on rosmarinic acid levels.

The diminution of rosmarinic acid levels during heat stress may be due to two metabolic processes, either a reduction in the biosynthesis of rosmarinic acid or a rapid degradation process. The biosynthetic pathway to rosmarinic acid has been elucidated using *Coleus blumei* cell suspensions.[18] Enzymatically, rosmarinic acid is synthesised from the amino acids L-phenylalanine and L-tyrosine (Fig 3). Rosmarinic acid synthase (EC 2.3.1.140) catalyses the stereospecific union of activated coumaroyl-CoA and (*R*)-3,4-dihydroxyphenyl lactic acid to form the rosmarinic acid precursor 4-coumaroyl-4′-hydroxyphenyllactate. Enzymatic studies suggest that two distinct cytochrome P450 enzymes hydroxylate 4-coumaroyl-4′-hydroxyphenyllactate at the 3 and 3′ positions to form rosmarinic acid.[18] The 3′-hydroxylase has been shown to be sensitive to temperatures above 25 °C in *Coleus*, whereas the 3-hydroxylase is not. The loss of rosmarinic acid accumulation in spearmint grown under continual heat stress at 30 °C may be caused by the failure of the temperature-sensitive 3′-hydroxylase to hydroxylate the 4′-hydroxyphenyllactate ring. If this is the source of diminished rosmarinic acid biosynthesis, we should observe an accumulation of the two precursors to rosmarinic acid, ie 4-coumaroyl-3′,4′-hydroxyphenyllactate and caffeoyl-4′-hydroxyphenyllactate. However, HPLC profiles of the seven genotypes of spearmint used in the heat stress experiments do not show any accumulation of these metabolites (Fig 2B). Therefore heat stress must negatively affect enzyme(s) upstream from the 3′H cytochrome P450 hydroxylation reactions.

Rosmarinic acid synthase (4-coumaroyl-coenzyme A: 4-hydroxyphenyllactic acid 4-coumaroyl transferase) is the next enzyme upstream in rosmarinic acid biosynthesis. Studies using *C. blumei* demonstrated that rosmarinic acid synthase had a temperature optimum at 30 °C using caffeoyl-CoA as substrate and at 40 °C with 4-coumaroyl-CoA as substrate.[18] Therefore this enzyme is heat-stable and should not be affected by heat stress.

Further upstream from rosmarinic acid synthase are tyrosine aminotransferase (EC 2.6.1.5), which converts tyrosine to hydroxyphenylpyruvate (Fig 3), and hydroxyphenylpyruvate reductase (E.C. 1.1.1.237), which converts hydroxyphenylpyruvate to hydroxyphenyllactate.[19] Both these enzymes are also heat-stable, with optimal enzyme activity at temperatures between 30 and 35 °C.[19] Therefore temperature stress would not play a role in changing biological activity for these enzymes.

However, the source of the heat stress inhibition of rosmarinic acid may lie in competing reactions. It is well known that plants catalyse 4-hydroxyphenylpyruvate to homogentisate via hydroxyphenylpyruvate dioxygenase.[20,21] This reaction is very significant in plants, as homogentisate is the precursor to plastoquinones (prenylquinones). Prenylquinones are important scavengers of reactive oxygen species in plant tissues, particularly under stress conditions. Higher demand for prenylquinones during heat stress could lead to increased production of homogenistate, reducing the available 4-hydroxyphenylpyruvate for rosmarinic acid production. This would explain the lack of accumulation of the two rosmarinic precursors 4-coumaroyl-3′4′-hydroxyphenyllactate and caffeoyl-4′-hydroxyphenyllactate and account for the subsequent loss of rosmarinic acid accumulation.

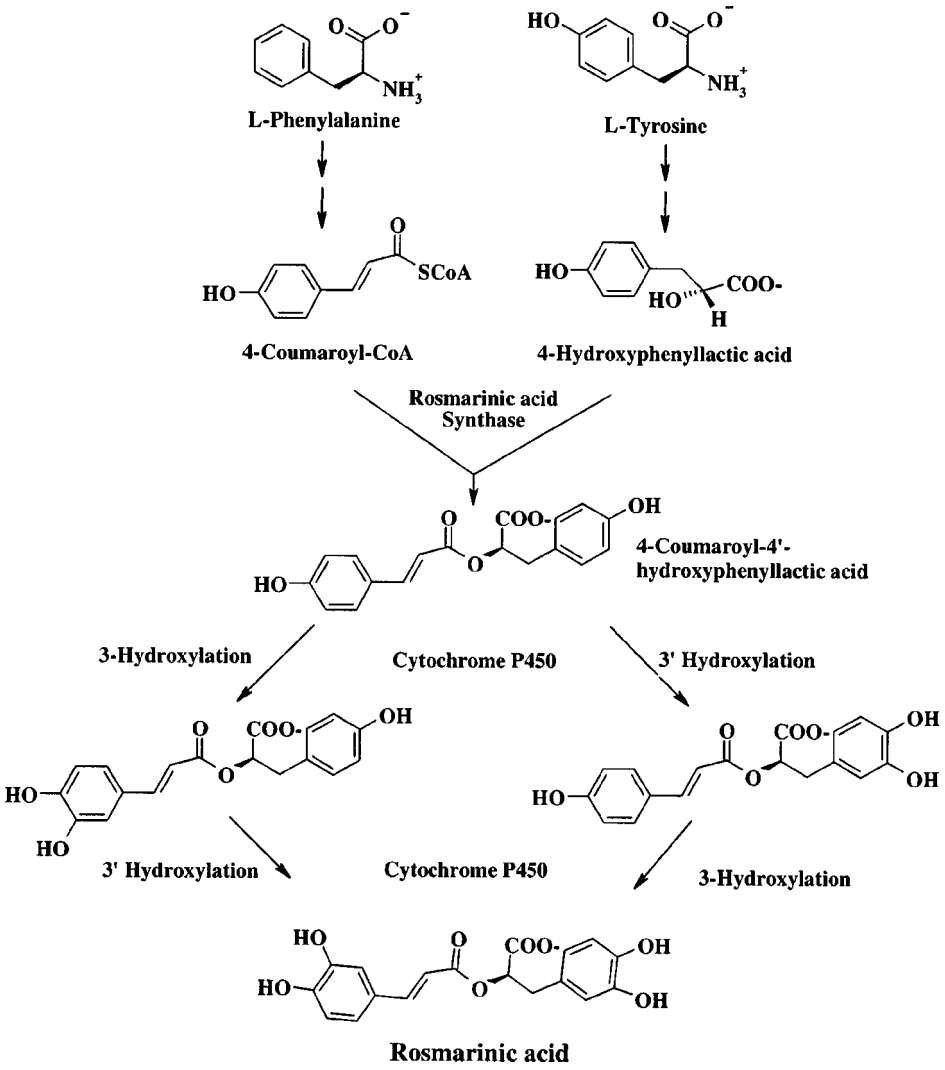

Figure 3. Biosynthetic pathway for rosmarinic acid from amino acids L-phenylalanine and L-tyrosine. Heat stress did not increase the levels of coumaric, cinnamic or hydroxyphenylpyruvate precursors that would suggest a block in rosmarinic acid biosynthesis.

In conclusion, the results clearly demonstrate that heat stress is deleterious to rosmarinic acid accumulation and counterproductive for capturing high levels of rosmarinic acid from harvested spearmint tissues. The results indicate that spearmint requires moderate-temperature conditions for optimum rosmarinic acid accumulation, and the growing of spearmint for large-scale production of rosmarinic acid would not be favourable in subtropical and tropical regions.

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