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ORIGINAL ARTICLE

Direct discrimination of different plant populations and study on temperature effects by Fourier transform infrared spectroscopy

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Abstract Fourier transform infrared spectroscopy was used to characterise highland and lowland populations of *Polygonum minus* Huds. grown in different controlled environments. A thermal perturbation technique of two-dimensional correlation infrared spectroscopy (2D-IR) correlation spectra was applied to establish differences between the populations. The absorption peaks at 3,480 cm^{-1} (hydroxyl group), 2,927 cm^{-1} (methyl group), 1,623 cm^{-1} (carbonyl group), and 1,068 cm^{-1} (C–O group) were particularly powerful in separating the populations. These peaks, which indicate the presence of carbohydrate, terpenes, amide and flavonoids were more intense for the highland populations than lowland populations, and increased in environments with a higher temperature. Wavenumbers (1,634, 669 cm^{-1}) and (1,634, 1,555 cm^{-1}) in the 2D-IR correlation spectra provided fingerprint signals to differentiate plants grown at different temperatures. This study demonstrates that IR fingerprinting, which combines mid-IR spectra and 2D-IR correlation spectra, can directly discriminate different populations of *P. minus* and the effects of temperature.

Keywords Fourier transform infrared spectroscopy (FTIR) · Two-dimensional correlation infrared spectroscopy (2D-IR) · Plant populations · Temperature effect · Metabolite screening

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1 Introduction

Polygonum minus Huds. is a herbaceous plant and a member of the Polygonaceae or *Persicaria* family (Bunawan et al. 2011b). This species is widespread in Malaysia and grows in the wild by drains, rivers or lakes in lowland areas but can also be found in highland areas (Yaacob 1987). It has been suggested that *P. minus* leaf is a good source of antioxidant with the amount of total phenolic compounds positively correlated with reducing power (Huda-Faujan et al. 2007). The production of potent antioxidant compounds such as quercetin, kaempferol and cyanidin has been linked to the expression of two recently isolated flavonoid genes in *P. minus*, flavonol synthase and leucianthocyanin dioxygenase (Roslan et al. 2012). Baharum et al. (2010) and Yaacob (1987) reported that *P. minus* chemical compositions mainly comprise of aldehydes and terpenes.

Environmental conditions have an important effect on plant development and metabolism (Dixon and Paiva 1995). Previous studies have demonstrated the chemical composition varied between different plant populations (Vokou et al. 1993; Martz et al. 2009; Said et al. 2011). One factor that contributes to metabolite changes is temperature stress. It can be defined as any drop or rise in temperature that causes reversible or irreversible inactivation of physiological processes or lethal injury in plants (Hayashi 2001). Velikova and Loreto (2005) found that plants capable of emitting greater amounts of isoprenes generally survive better under heat stress. Additionally, changes in sesquiterpenes, for example zingiberene, can be observed from different temperature treatments (Gianfagna et al. 1992).

Fourier transform infrared (FTIR) spectroscopy is an accurate, fast and simple method to determine functional

groups and polar compounds. When a sample is interrogated with an infrared beam, functional groups within the samples absorbed infrared radiation and chemical bonds vibrate in characteristic ways, such as bending or stretching (Allwood et al. 2008). The FTIR wavenumbers are indicative of certain chemical groups (Beekes et al. 2007). FTIR provides fingerprints of organisms where it describes the metabolic status of the cells of the organisms (Oliver et al. 1998). This allows high-throughput screening and classification of biological samples, where it fits the 'omics philosophy' of providing whole-system measurements (Kell 2004). The infrared spectrum is usually divided into three regions; the near- (NIR), mid- (MIR) and far-infrared (FIR). However, only NIR and MIR were widely used in plant studies. Over the past 20 years, the applications of FT-NIR are widespread. For example, Ikeda et al. (2007) and Suzuki et al. (2010) performed the FT-NIR-based fingerprinting to measure the quality of green teas and classified rice-Arabidopsis FOX lines respectively. However, the molecular overtone and combination bands seen in the NIR are typically very broad, leading to complex spectra and it can be difficult to assign specific features to specific chemical components. Thus the use of MIR was important to overcome the limitation. Organic functional group has characteristic and well delineated absorption bands in this spectral region. In addition, because of the metabolites differ from each other by having different combinations of functional groups, their MIR spectra can be used to identify them and characterize their structure (Wilks 2006).

Previously, we reported on the chemical composition of *P. minus* essential oil detected using GC-MS, GC×GC-MS-TOF and GC-FID (Baharum et al. 2010). The aim of this study is to use FTIR as a technique to provide a metabolic fingerprint and to study changes in the chemical composition of *P. minus* from different populations. In this study our main focus is on the freeze-dried leaves and methanol extracts of *P. minus* to analyse the chemical changes due to the effects of temperature treatment. To our knowledge, this is the first attempt to fingerprint the compositions of our tropical herbaceous plant under different temperature treatments using FTIR approach.

2 Materials and methods

2.1 Plant materials

Stem cuttings of *P. minus* were collected from highland populations (altitude >1,000 m): Cameron Highland (CH; 4°29′15.75″N, 101°22′11.02″E), Genting Highland (GH; 3°25′42.18″N, 101°47′21.45″E), Fraser Hill (FH; 3°43′05.24″N, 101°43′57.13″E), and lowland populations (altitude <200 m): Ulu Yam (UY; 3°16′14.63″N, 101°41′11.32″E) and Lenggeng (LG; 2°52′55.40″N, 101°55′39.73″E). Stem cuttings were kept moist in water during transportation back to the laboratory for cultivation. The identity of the plant was confirmed through PCR of *trn*L-*trn*F and nuclear ribosomal ITS sequences (Bunawan et al. 2011a), and by comparing the collected voucher specimens with those of known identity located in the Herbarium of the Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia.

2.2 Plant cultivation

Stem cuttings were propagated in M3 compost within individual seed trays and grown in controlled environment chambers (Conviron Controlled Environments Limited, Canada) with a 12 h day:12 h night cycle, 75 % humidity and 180 μmol m^{-2} s^{-1} light intensity. For temperature experiments three chambers were used, control 25 °C day:18 °C night, lowland condition 28 °C day:20 °C night and highland condition 22 °C day:15 °C night. Each growth chamber had an equal number of plants per population. Plants were watered with reverse osmosis water twice daily. No additional nutrients were supplemented to the soil or water. After 2 weeks, five plants from each population were individually transferred to larger plant pots (15 × 15 × 17 cm^3). Leaf samples were harvested after 6 weeks, immediately frozen in liquid nitrogen and stored at −80 °C. Fully developed leaf samples (leaf node number 3–5) were used in this study, as leaf is directly exposed to environmental changes.

2.3 Sample preparation

2.3.1 Freeze-dried leaf samples

Freeze-dried leaves sample were ground into fine powder. The leaves were first freeze-dried to avoid the interfering absorbance in the spectrum caused by water content. Ground freeze-dried leaves (2 mg) were blended with 200 mg KBr powder. The mixture was then pressed into a tablet using hydraulic pump (Specac, USA).

2.3.2 Methanol extracts

Metabolites were extracted according to Gomez-Romero et al. (2010) with slight modifications. Each sample with weight of 500 mg was freeze-dried and ground to a fine powder. Samples were then extracted using 5 mL of methanol. After standing for 1 h, the extracts were filtered and the procedure was repeated twice. About 500 μL of sample extract were then concentrated under vacuum and dissolved in 100 μL of methanol before analysis.

2.4 Measurement method

FTIR analysis was conducted as described by Xu et al. (2013) with slight modifications using Frontier™ Fourier transform-mid infrared spectroscopy (FT-MIR) equipped with a deuterated triglycine sulfate (DTGS) detector and Spectrum software version 10.3 (Perkin Elmer, USA). For the analysis of methanol extracts by attenuated total reflectance (ATR), 20 µL of sample were placed on the diamond crystal plate for about 20 s to allow the methanol to evaporate. Spectra were then obtained at a resolution of 1 cm^{-1} in the range of 4,000–650 cm^{-1} with accumulation of 32 scans.

For freeze-dried leaf samples, the tablet was placed into holder for transmission IR spectral analysis. Mid infrared (MIR) spectra were recorded from an accumulation of 4 scans in the range of 4,000–450 cm^{-1} with resolution of 1 cm^{-1}. 2D-IR analysis was performed in a similar fashion but the tablet of freeze-dried leaf sample was placed into a holder with a temperature controller (Specac, USA) continuously heated with an increasing rate of 2 °C min^{-1}. Transmission IR spectra were collected at temperature intervals of 10 °C from 40–120 °C to form dynamic FTIR spectra. 2D-IR synchronous correlation spectra were obtained from series of dynamic spectra analysed using a 2D-IR correlation analysis (TD: IR 2D COS) software developed by Tsinghua University, China (Sun et al. 2011).

All analyses were done with three biological replicates, each with three technical replicates. Data sets were baseline-corrected and area-normalised before statistical and multivariate analyses (Kemsley et al. 1994).

2.5 Statistical analysis

Differences between combined data of highland and lowland populations was analysed using a Student's *t* test analysis in SPSS v12.0.1 (Chicago, IL, USA) software. The same method was applied for the comparison between the temperature treatments. Changes with $P < 0.05$ were considered to be significant.

2.6 Multivariate analysis

A table of spectrum wavenumbers (2,000–450/650 cm^{-1}) and absorbance was generated using the Spectrum™ software (Perkin Elmer, USA). All spectrum lists from each sample were collated into a single table in Microsoft Excel 2007 and analysed with SIMCA-P+ v12.0 (Umetrics, Sweden), and the analysis was performed using Pareto scaling (Par). Both a PCA analysis and a partial least square discriminant analysis (PLS-DA) was conducted in order to build a statistical model that optimize the separation between three temperature treatments. The separation of the samples was observed in a scatter plot. Score contribution plot shows the contribution value of each variable in sample separation.

3 Results

3.1 Comparisons between different populations of *P. minus*

In this study, we compared the spectra of *P. minus* leaf samples and methanol extracts obtained from five different populations which have been grown under the same controlled environment (Fig. 1). For comparison between the populations, the spectra of plants grown under baseline temperature were analysed. In general, full FTIR spectra collected from the five different populations of *P. minus* showed broadly similar absorbance patterns. Several prominent peaks in all of the spectra indicate the presence of specific functional groups in common among different plant populations. Both freeze-dried leaf samples and methanol extracts showed high absorbance at the wavenumber region of 3,500–3,000, 3,000–2,800, 1,750–1,500, and 1,200–1,000 cm^{-1}. According to Pavia and Kriz (2001), peak absorbance at 3,500–3,000 cm^{-1} corresponds to hydroxyl absorption. The 3,000–2,800 cm^{-1} absorption peak corresponds to methylene C–H asymmetric stretching vibration. The peak at region 1,750–1,500 cm^{-1} is due to the stretching vibration of carbonyl group. Many C–O groups have peaks at 1,100 cm^{-1} and generally this peak is the vibration peak of C–O in alcohol hydroxyl group (Supplementary Table 1). In the freeze-dried samples, these vibration peaks indicated that *P. minus* plant contains large amount of carbonyl such as decanal and dodecanal, as reported by Yaacob (1987). Previously, the methanol extracts had been analysed using LC-TOF–MS and flavonoids were the major compounds detected (Supplementary Fig. 1). The high absorbance of O–H, C=O and C–O–C groups in methanol extracts suggested that flavones and flavonols could be the dominant compounds.

Despite the shared common features in the spectra, one can observe the distinct intensity of the prominent peaks between different populations. In both the freeze-dried leaf samples and methanol extracts, Genting Highland population showed the most intense of these peaks (Fig. 1a, b). Student's *t* tests were used to determine the significance of the difference between highland and lowland populations. The significant value (*P*-value) was plotted against the wavenumbers of the spectrum. It showed that all of the prominent peaks were significant for freeze-dried leaves (higher in highland population), but not significant for methanol extracts, except in the region of 1,570 cm^{-1} attributed by the aromatic C=C bond (Supplementary Fig. 2).

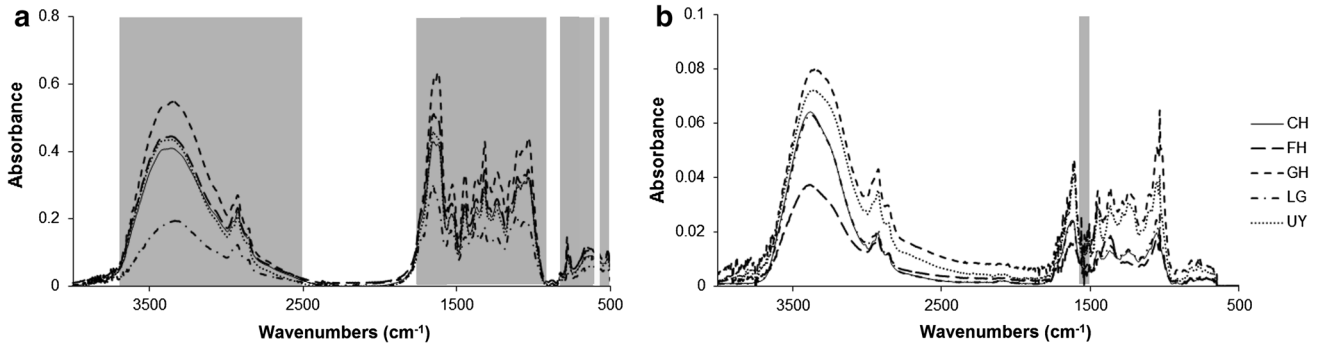

Fig. 1 FTIR absorption spectra of *P. minus* samples from different populations under baseline temperature. **a** Freeze-dried leaves. **b** Methanol extracts. *CH* Cameron highland, *FH* Fraser hill, *GH* Genting highland, *LG* Lenggeng, *UY* Ulu Yam. *CH*, *FH* and *GH* represent samples from highland population while *LG* and *UY* represent samples from lowland populations. *Shaded regions* are significantly ($P < 0.05$) different between highland and lowland populations. The absorbance values are means from 3 biological replicates

Nevertheless, smaller differences between the populations were more difficult to resolve as many spectra overlapped, thus secondary derivative (SD)-IR analysis was applied to enhance the apparent resolution and to amplify small differences in the IR spectra (Fig. 2). The fingerprint region, 900–600 cm^{-1}, was expanded to the range of 2,000–500 cm^{-1} for SD-IR spectra analysis. Overall, the SD-IR spectra from leaf samples (Fig. 2a) were more distinctive among different populations than that of methanol extracts (Fig. 2b).

There were obvious peaks in common for SD-IR of the five samples in the freeze-dried leaves albeit of different relative intensities, including peaks at 1,390, 790 and 690 cm^{-1} (Fig. 2a). Noteworthy is the peak at 1,390 cm^{-1} which was less intense for CH, FH and LG samples compared with that of GH and UY samples while at 790 cm^{-1}, GH sample showed the strongest peak. However, the peak at 690 cm^{-1} showed similar pattern among different samples despite that these three peaks are related to C–H bonds. The 1,500–1,400 cm^{-1} region was unique for each sample with different shapes of the peaks. For the methanol extracts, differences were less apparent, only at the region of 1,800–1,400 cm^{-1} and at 1,034 cm^{-1}, with GH sample being the most distinctive (Fig. 2b).

3.2 Comparisons between different temperature treatments of *P. minus*

To investigate the utilisation of FTIR approach for study on the effects of temperature on plant metabolite changes, plants from different populations were subjected to reciprocal temperature treatments. In this reciprocal transplant experiment, three highland and two lowland populations of *P. minus* as a whole responded to different temperature treatments in a similar way. However, the effects of different temperature treatments were greater in the lowland populations, with more distinct differences in peak intensities than that of highland populations with largely overlapping peaks, especially in the methanol extracts. In freeze-dried leaves, higher temperature treatment resulted in higher intensity of the peak absorbance compared with lower temperature treatment, especially in the lowland plant populations (Fig. 3a, b). Lowland populations showed more significant peaks around the regions of 3,500–2,400 and 1,800–500 cm^{-1}.

For methanol extracts, despite the two control baseline treatments being very similar, the absorbance of putative carbonyl (1,750–1,500 cm^{-1}) and C–O stretching (1,200–1,000 cm^{-1}) changed under both high and low temperature treatments (Fig. 3c, d). Under a higher temperature treatment, the absorbance for hydroxyl group in highland populations was higher than control treatment, but the converse was true for lowland populations. These might reflect the differential responses of highland and lowland plant populations to temperature changes, especially in the flavonoid compositions. These were supported statistically by Student's *t* test plots (Supplementary Fig. 3).

3.2.1 2D-IR correlation analysis

To identify the possible chemical classes that are altered in the three temperature treatments 2D-IR correlation analysis (2D-IR COS) was used. 2D-IR COS provides enhanced resolution of conventional IR spectra through external physical perturbations e.g., temperature heating, which influence the vibration behaviour of complex molecules. Figure 4 shows the representative 2D-IR correlation synchronous spectra of leaf samples from *P. minus* plants under different temperature treatments within the range of 2,000–450 cm^{-1}, generated through the analytical temperature perturbation from 40 to

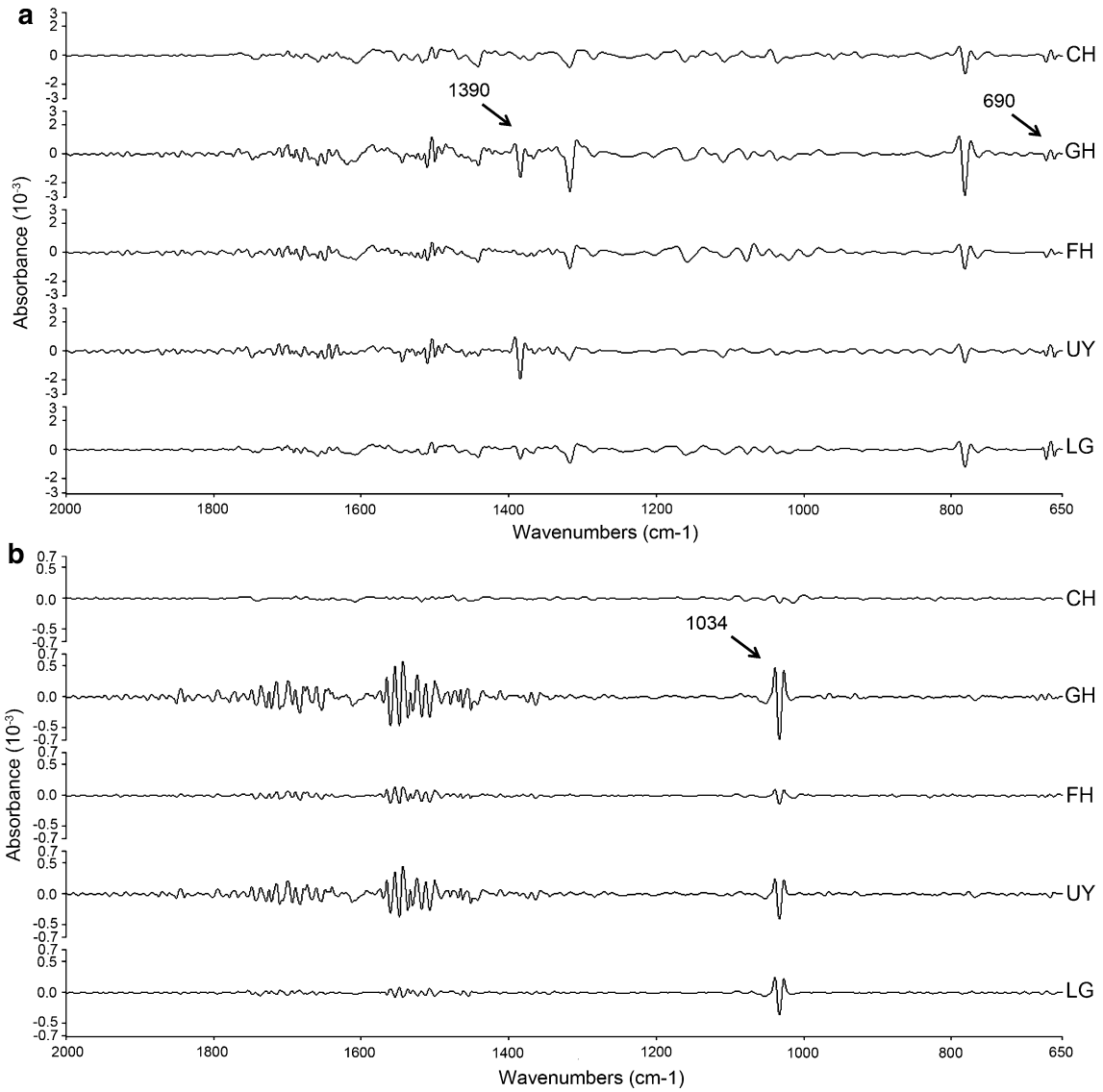

Fig. 2 Secondary derivative IR spectra of *P. minus*: **a** Freeze-dried leaves. **b** Methanol extracts. *CH* Cameron highland, *FH* Fraser hill, *GH* Genting highland, *LG* Lenggeng, *UY* Ulu Yam

120 °C. The contour and stereo-fish-net patterns were different. The autopeaks (square) were apparent near 1,634, 1,203 and 663 cm^{-1} but differ in their relative intensities except for 1,634 cm^{-1}. The most number of crosspeaks (circle) were found at (1,634, 669), (1,634, 1,555) and (1,634, 1,450) cm^{-1}. These crosspeaks can be assigned to alkenyl and aromatic –C–H– bending groups which indicate the present of terpenes and were more pronounced in samples under lower temperature treatment than that of baseline control and higher temperature treatment.

3.2.2 Multivariate analysis

PCA was performed using SIMCA-P+ software based on wavenumbers and absorbance to distinguish samples under different temperature treatments. However, the PCA analysis of the FTIR data was not able to discriminate samples between temperature treatments (result not shown). Thus PLS-DA was applied to optimize the separation between the three classes of treatments. The analysis generated a two component PLS-DA model characterised by a faithful representation of the Y data ($R^2Y = 0.75$) and good cumulative confidence criterion of prediction ($Q^2 = 0.685$). The score plot of PLS-DA model showed the separation of freeze-dried leaf samples under temperature treatments along the first PLS-DA component (PC1) with variation of 44.76 % (Fig. 5). However, this was not the case for methanol extracts with no clear separation on PLS-DA plot (result not shown). This is consistent with the results above showing less apparent differences in the methanol extracts compared with freeze-dried leaf samples.

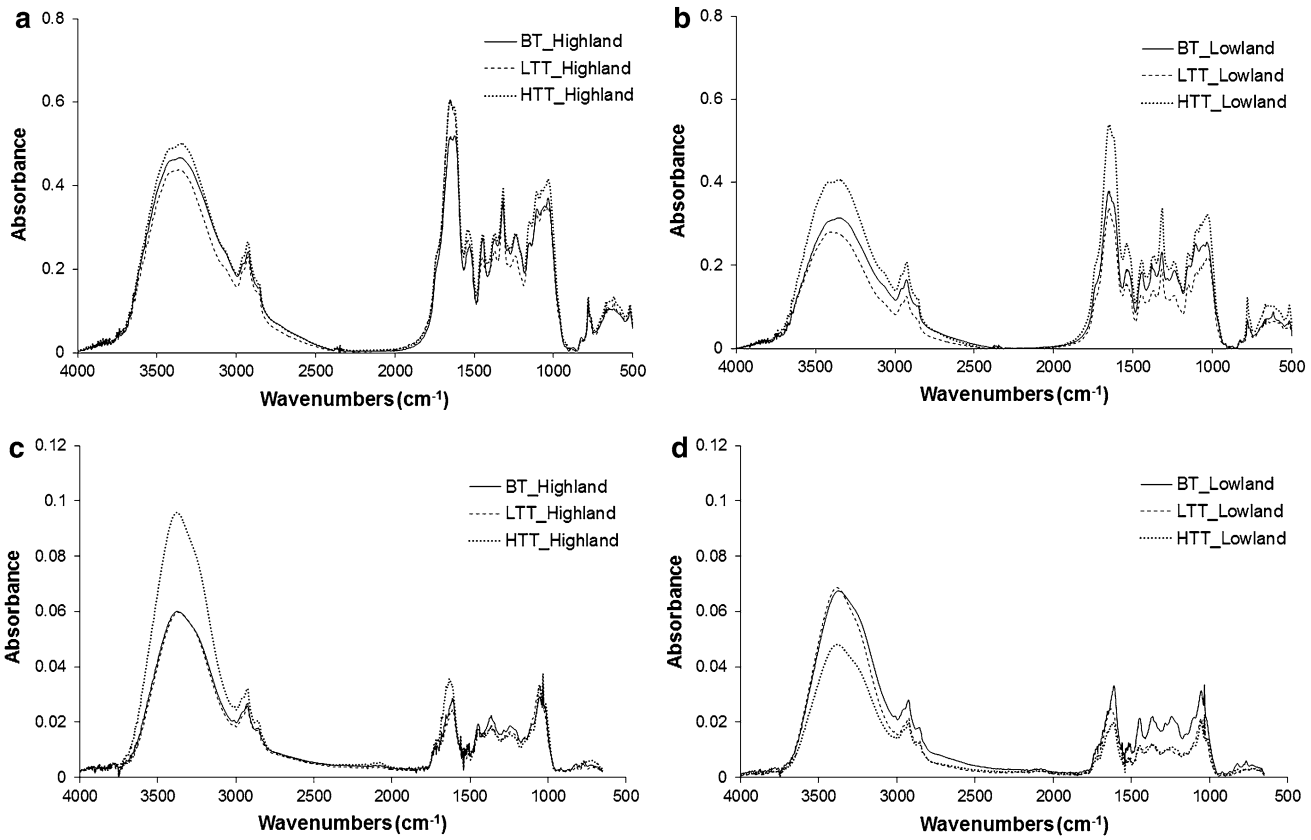

Fig. 3 FTIR spectra of highland and lowland populations of *P. minus* plants grown under different temperature treatments. **a** Freeze-dried leaves of highland populations. **b** Freeze-dried leaves of lowland populations. **c** Methanol extract of highland populations. **d** Methanol extract of lowland populations. *BT* Baseline temperature, *HTT* Higher temperature treatment, *LTT* Lower temperature treatment. The absorbance values are means from three highland and two lowland populations, each with three biological replicates

4 Discussion

4.1 FTIR for plant population study and temperature effect

FTIR is a very efficient technique for screening of plant samples. Samples from different populations can be discriminated based on functional group absorption in a relatively short time. Figure 1 shows typical FTIR spectra of *P. minus* from different populations. The infrared spectra at the region of 3,500–3,000, 3,000–2,800, 1,750–1,500, 1,200–1,000 cm^{-1} for these five populations were similar, differing only in the intensity, which suggest similar major chemical components present in all the samples from different populations. The peaks at the region of 1,750–1,500 and 1,200–1,000 cm^{-1} are sharper in the methanol extracts compared to freeze dried leaves. This indicates a greater relative abundance of phenolic groups especially flavonoid in the methanol extracts than in freeze-dried samples. However, from the statistical analyses, methanol extracts showed no significance difference among the populations compared with freeze-dried leaves (Supplementary Fig. 2). This could be due to the missing compounds in the methanol extracts, which represent only a subset of predominantly phenolic compounds, which might not be responsible for the differences between populations. Freeze-dried leaves are non-extracted sample and contain mixture of all metabolites in the leaves which allowed sample discrimination. Hence it is suggested the use of freeze-dried samples can be better than sample extracts for screening of sample differences.

The absorbance by O–H, C=O and C–O functional groups (3,500–3,000, 1,750–1,500 and 1,200–1,000 cm^{-1} respectively) in freeze-dried leaves are likely indication of carbohydrate and aldehyde compounds (Sun et al. 2011). Stronger absorption peaks at these regions of highland samples suggest a higher of both compounds composition in highland populations. According to Baharum et al. (2010) and Yaacob (1987) aldehyde is present in abundant, predominantly decanal and dodecanal in *P. minus*. This is in accordance with the results from the SPME–GC–MS analysis where decanal and dodecanal were detected to be the most abundant in *P. minus* accounting from 80–90 % of the compounds detected (Supplementary Fig. 4). Another possible chemical group that could be discriminating different plant populations include terpenes, with the presence

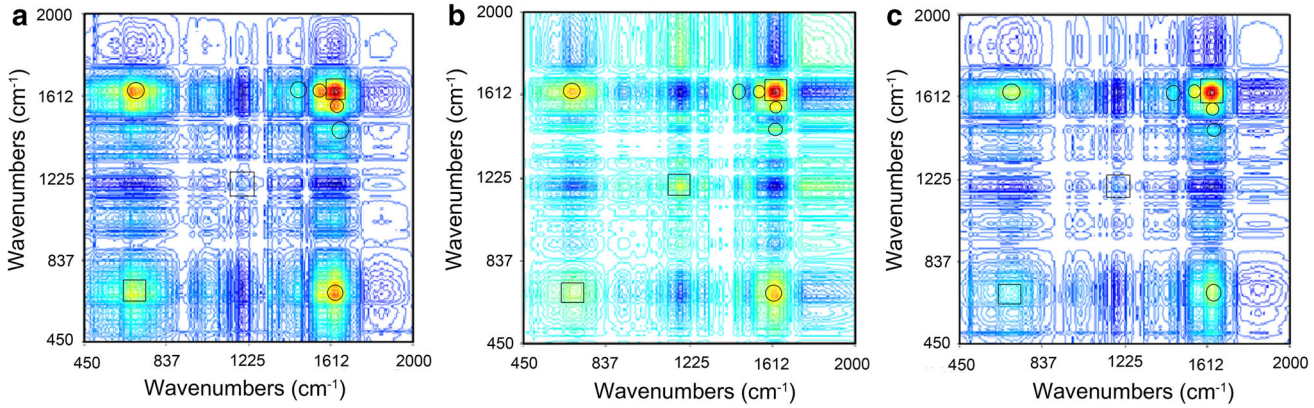

Fig. 4 Contour plot of 2D correlation IR synchronous spectroscopy. 2D spectra from *P. minus* treated with different temperature were analysed in the region 2,000–450 cm^{-1} as described in the method section. **a** Lower growth temperature treatment, **b** Control, **c** Higher growth temperature treatment. *Square boxes* indicate autopeaks. *Circles* indicate crosspeaks. *Red* colour indicated strong peak while *light blue* indicated weak peak (Color figure online)

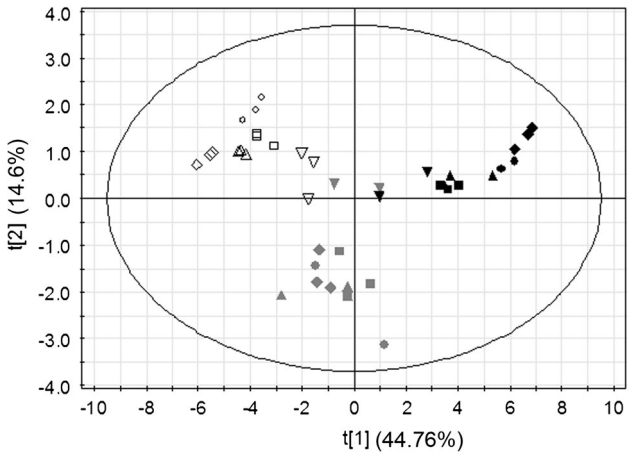

Fig. 5 PLS-DA model obtained from the classification of *P. minus* freeze-dried samples according to the temperature treatments. Separation in the model was based on the compound absorption of *P. minus*. The percentage of the variation of the data explained by each component is provided in the plot. *Box, circle, triangle, diamond* and *inverted triangle* symbols indicated Fraser Hill, Genting Highland, Cameron Highland, Lenggeng and Ulu Yam populations respectively. Baseline, lower and higher temperature treatments are represented respectively by *grey*, *white* and *black* symbols. Each *point* represents one biological sample with three technical replicates

of 3,000–2,800 cm^{-1} absorption peak corresponds to C–H asymmetric stretch, which showed stronger absorbance in highland populations. Further analyses based on SD-IR and 2D-IR will provide a better hint to distinguish which chemical group(s) to play the predominant role in responding to the effects of temperature treatments.

Generally, the SD-IR spectra can enhance the apparent resolution and amplify tiny differences of IR spectrum (Li et al. 2004). For infrared spectra of mixture, adjacent absorption peaks are usually overlapping with each other making the peaks broader. Therefore, SD-IR can be useful to resolve the peaks to reveal weaker spectral features (2,000–500 cm^{-1}), for interpreting the components with low concentration and weak absorption peak (Sun et al. 2011). In freeze-dried leave, the C–O absorbance region which assigned as carbohydrate can be further resolved in the secondary derivative IR. Different peaks suggest different carbohydrate compounds were present in *P. minus*. This is supported by the spectra at the region of 1,500–1,200 cm^{-1} (Fig. 2) corresponding to the absorbance of aromatic group of carbohydrate, which are unique for all five populations. These peaks are separated into individual peaks corresponding to different secondary structures of carbohydrate, indicating that carbohydrate constituents differ among the populations. In methanol extracts, stronger peak absorbance for GH and UY than the other three populations, especially in the region of 1,750–1,400 cm^{-1} indicate higher content of C=O and –C=C– groups, perhaps from the flavonoids.

On the other hand, 2D-IR correlation spectra may indicate possible classes of compound based on different functional groups which vibrate simultaneously (Sun et al. 2011). An autopeaks (peaks along the diagonal axis) show the vibrational self-correlativity of certain functional group at the same wavenumbers. As shown in Fig. 4, the three samples have strong autopeak at 1,634 which indicated the same compounds present in *P. minus* at all growth temperature. The crosspeaks (off-diagonal peaks) indicate the vibrational correlativity between two different functional groups. This provides clues to infer the compound class through the identification of functional groups in common. As the cross peaks around (1,634, 669), (1,634, 1,555) and (1,634, 1,450) wavenumbers can be observed to be more pronounced, we can infer the present of more abundant terpenes in the higher temperature treated samples.

Furthermore, the PLS-DA analysis on the freeze-dried showed differences between the highland and lowland populations under different temperature treatments suggests

an overriding effect of temperature over the origin of different plant populations. Biological replicates of each population grouped according to the temperature treatments rather than whether they are of highland or lowland population. This is further supported by similar responses of plants from highland and lowland populations to different temperature treatments.

4.2 Temperature effects on plant chemical compositions

Generally, higher temperature treatment gave higher absorbance for both freeze-dried leaves and methanol extracts. Biosynthesis of secondary metabolites is strongly affected by different abiotic stresses (Naghdi-Badi et al. 2004). Plant leaves are generally more sensitive to changes of environmental factors than other organs, and the differences in leaf traits have been used to classify plants and to establish genetic relationships (Wei et al. 2010). According to Zhang et al. (2008), the differences in chemical composition of leaves were considered a more persuasive metric for studying different populations of plant than the composition of other organs. Infrared spectroscopy could provide an overview of complex components in the plant before further characterisation of specific compounds.

FTIR analysis of samples from plants grown under different temperature treatments indicated that temperature affected plant metabolite production especially carbohydrate, terpene and flavonoid. Analysis of freeze-dried samples from different temperature treatments showed that the signals for carbohydrate and terpene are stronger at elevated temperature treatments. This is in accordance with previous studies which reported the increased of sucrose accumulation under heat stress in potato and tomato (Lafta and Lorenzen 1995; Miguel et al. 2007; Jie et al. 2012). In addition Rosenfeld et al. (2002) also reported an increase in caryophyllene with increasing temperature in carrot root. An increase in temperature changes photosynthesis activity and modified the carbohydrate production. Terpene synthesis increased dramatically at higher temperatures leading to the hypothesis that terpenes may protect plants by deterring herbivores or through plant thermotolerance. The isoprene fumigation increased thermotolerance by 3-10 °C and this was dose-dependent with isoprene concentration (Sharkey and Singsaas 1995; Singsaas et al. 1997).

Most plants suffer from physiological and biochemical damage by being exposed to the temperatures higher or lower than their optimal for growth (Ridley 1967; Lyons 1973). Plants that grow at unfavourable temperatures had been reported with high phenylalanine ammonia lyase activity, which is a key enzyme of phenylpropanoid pathway, leading to the accumulation of flavonoids (Janas et al. 2000). Thermal stress will induce the production of phenolic compounds such as flavonoids and phenylpropanoids, due to a decrease in photosynthesis with the concurrent increase in oxidative stress (Oquist 1983). This is supported by our finding, which showed the higher production of metabolites in higher temperature treatments.

5 Concluding remarks

FTIR is a rapid screening method that can be applied to identify the chemical compositional difference between plant samples. *P. minus* plants from different populations which were subjected to different temperature treatments can be discriminated by IR spectra according to the position and intensity of peaks, based on the tri-step identification+ IR, secondary derivative-IR and 2D-IR correlation spectra. In the temperature treatment experiments, the higher temperature treatment resulted in signs of increasing carbohydrate, terpenes, amide and flavonoids, which were apparent in highland populations of both freeze-dried and flavonoid samples. Hence, the production of desired compounds could potentially be boosted by controlling the temperature conditions of plant cultivation. Terpene and flavonoids are the major compounds in *P. minus*, as it could be assigned as a marker for the temperature changes especially in higher temperature. Furthermore, we can conclude that highland *P. minus* plants generally produced certain secondary metabolites in greater abundance than that of lowland plants.

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References

Allwood, J. W., Ellis, D. I., & Goodacre, R. (2008). Metabolomic technologies and their application to the study of plants and plant–host interactions: A review. *Physiologia Plantarum, 132*, 117–135.

Baharum, S. N., Bunawan, H., Ghani, M. A., Mustapha, W. A. W., & Noor, N. M. (2010). Analysis of the chemical composition of the essential oil of *Polygonum minus* Huds. using two-dimensional gas chromatography-time-of-flight mass spectrometry (GC-TOF MS). *Molecules, 15*, 7006–7015.

Beekes, M., Lasch, P., & Naumann, D. (2007). Analytical applications of Fourier transform-infrared (FT-IR) spectroscopy in microbiology and prion research. *Veterinary Microbiology, 123*, 305–319.

Bunawan, H., Chee, Y. C., Md-Zain, B. M., Baharum, S. N., & Noor, N. M. (2011a). Molecular Systematics of *Polygonum minus* Huds. based on ITS Sequences. *International Journal of Molecular Sciences, 12*, 7626–7634.

Bunawan, H., Talip, N., & Noor, N. M. (2011b). Foliar anatomy and micromorphology of *Polygonum minus* Huds. and their taxonomic implications. *Australian Journal of Crop Science, 5*(2), 123–127.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell, 7*, 1085–1097.

Gianfagna, T. J., Carter, C. D., & Sacalis, J. N. (1992). Temperature and photoperiod influence trichome density and sesquiterpene content of *Lycopersicon hirsutum* f. *hirsutum*. *Plant Physiology, 100*, 1403–1405.

Gomez-Romero, M., Segura-Carretero, A., & Fernandez-Gutierrez, (2010). Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry, 71*, 1848–1864.

Hayashi, H. (2001). *Plant Temperature Stress*. doi:10.1038/npg.els.0001320.

Huda-Faujan, N., Noriham, A., Norrakiah, A. S., & Babji, A. S. (2007). Antioxidative activities of water extracts of some Malaysian herbs. *ASEAN Food Journal, 14*(1), 61–68.

Ikeda, T., Kanaya, S., Kobayashi, A., Yonetani, T., & Fukusaki, E. (2007). Prediction of Japanese green tea ranking by Fourier transform near-infrared reflectance spectroscopy. *Journal of Agricultural and Food Chemistry, 55*, 9908–9912.

Janas, K. M., Cvikrova, M., Palagiewicz, A., & Eder, J. (2000). Alterations in phenylpropanoid content in soybean roots during low temperature acclimation. *Plant Physiology and Biochemistry, 38*, 587–593.

Jie, Z., Xiaodong, J., Tianlai, L., & Zaiqiang, Y. (2012). Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (*Lycopersico esculentum* L.) leaves. *African Journal of Agricultural Research, 7*(3), 487–492.

Kell, D. B. (2004). Metabolomics and systems biology: making sense of the soup. *Current Opinion in Microbiology, 7*, 296–307.

Kemsley, E. K., Belton, P. S., McCann, M. C., Ttofis, S., Wilson, R. H., & Delgadillo, I. (1994). A rapid method for the authentication of vegetable matter using Fourier transform infrared spectroscopy. *Food Control, 5*, 241–243.

Lafta, A. M., & Lorenzen, J. H. (1995). Effect of high temperature on plant growth and carbohydrate metabolism in potato. *Plant Physiology, 109*(2), 637–643.

Li, Y. M., Sun, S. Q., Zhou, Q., et al. (2004). Identification of American ginseng from different regions using FT-IR and two-dimensional correlation IR spectroscopy. *Vibrational Spectroscopy, 36*, 227–232.

Lyons, J. M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology, 24*, 445–466.

Martz, F. O., Peltola, R., Julkunen-Tiitto, R., Fontanay, S., & Stark, S. (2009). Effect of latitude and altitude on the terpenoid and soluble phenolic composition of Juniper (*juniperus communis*) needles and evaluation of their antibacterial activity in the Boreal zone. *Journal of Agricultural and Food Chemistry, 57*, 9575–9584.

Miguel, A. R., Maria, M. R., Rosa, C., Nicolas, C., Juan, M. R., & Luis, R. (2007). Sucrolytic activities in cherry tomato fruits in relation to temperature and solar radiation. *Scientia Horticulturae, 113*, 244–249.

Naghdi-Badi, H., Dastpak, H. A., & Ziai, S. A. (2004). A Review of *Psyllium* Plant (*Plantago ovata* Forsk. and *Plantago psyllium* L.). *Journal of Medicinal Plants, 3*, 1–13.

Oliver, S. G., Winson, M. K., Kell, D. B., & Baganz, F. (1998). Systematic functional analysis of the yeast genome. *Trends in Biotechnology, 16*, 373–378.

Oquist, G. (1983). Effects of low-temperature on photosynthesis. *Plant Cell and Environment, 6*, 281–300.

Pavia, D. L., Lampman, G. M., & Kriz, G. S. (2001). *Introduction to spectroscopy* (3rd ed.). USA: Thomson Learning.

Ridley, H. N. (1967). *The Flora of the Malay Peninsula*. Ashford: L. Reeve & Co.

Rosenfeld, H. J., Aaby, K., & Lea, P. (2002). Influence of temperature and plant density on sensory quality and volatile terpenoids of carrot (*Daucus carota* L.) root. *Journal of the Science of Food and Agriculture, 82*(12), 1384–1390.

Roslan, N. D., Yusop, J. M., Baharum, S. N., et al. (2012). flavonoid biosynthesis genes putatively identified in the aromatic plant *Polygonum minus* via expressed sequences tag (EST) analysis. *International Journal of Molecular Sciences, 13*, 2692–2706.

Said, S. A., Fernandez, C., Greff, S., et al. (2011). Inter-population variability of terpenoid composition in leaves of *Pistacia lentiscus* L. from Algeria: A chemoecological approach. *Molecules, 16*(3), 2646–2657.

Sharkey, T. D., & Singsaas, E. L. (1995). Why plants emit isoprene. *Nature, 374*, 769.

Singsaas, E. L., Lerdau, M., Winter, K., & Sharkey, T. D. (1997). Isoprene increases thermotolerance of isoprene-emitting leaves. *Plant Physiology, 115*, 1413–1420.

Sun, S. Q., Zhou, Q., & Chen, J. B. (2011). *Infrared spectroscopy for complex mixtures: application in food and traditional medicine*. Beijing: Chemical Industry Press.

Suzuki, M., Kusano, M., Takahashi, H., et al. (2010). Rice-Arabidopsis FOX line screening with FT-NIR-based fingerprinting for GC-TOF/MS-based metabolite profiling. *Metabolomics, 6*(1), 137–145. doi:10.1007/s11306-009-0182-2.

Velikova, V., & Loreto, F. (2005). On the relationship between isoprene emission and thermotolerance in *Phragmites australis* leaves exposed to high temperatures and during the recovery from a heat stress. *Plant, Cell and Environment, 28*, 318–327.

Vokou, D., Kokkini, S., & Bessiere, J. M. (1993). Geographic-variation of Greek oregano (*Origanum Vulgare* ssp. *Hirtum*) essential oils. *Biochemical Systematics and Ecology, 21*, 287–295.

Wei, Y. M., Wang, L. H., Cao, F. L., Wei, S. Q., & Liang, Y. D. (2010). Variation and cluster analysis on leaf characters from different provenance sources of *Polygonum multiflorum* Thunb. *Agricultural Science and Technology, 11*, 94–98.

Wilks, P. (2006). NIR versus Mid-IR: How to choose. *Spectroscopy, 21*(4), 43–48.

Xu, C., Wang, Y., Chen, J., et al. (2013). Infrared macro-fingerprint analysis-through-separation for holographic chemical characterization of herbal medicine. *Journal of Pharmaceutical and Biomedical Analysis, 74*, 298–307.

Yaacob, K. B. (1987). Kesom oil: A natural source of aliphatic aldehydes. *Perfumer and Flavorist, 12*, 27–30.

Zhang, Z. X., Liu, P., Kang, H. J., Liao, C. C., Chen, Z. L., & Xu, G. D. (2008). A study of the diversity of different geographical populations of *Emmenopterys henryi* using FTIR based on principal component analysis and cluster analysis. *Spectroscopy Spectral Analysis, 28*(9), 2081–2086.