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Investigation of plant hormone level changes in shoot tips of longan (*Dimocarpus longan Lour.*) treated with potassium chlorate by liquid chromatography–electrospray ionization mass spectrometry

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ABSTRACT

The endogenous levels of indole-3-acetic acid (IAA), gibberellins (GAs), abscisic acid (ABA) and cytokinins (CKs) and their changes were investigated in shoot tips of ten longan (*Dimocarpus longan Lour.*) trees for off-season flowering until 60 days after potassium chlorate treatment in comparison with those of ten control (untreated) longan trees. These analytes were extracted and interfering matrices removed with a single mixed-mode solid phase extraction under optimum conditions. The recoveries at three levels of concentration were in the range of 72–112%. The endogenous plant hormones were separated and quantified by liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS). Detection limits based on the signal-to-noise ratio ranged from 10 ng mL^{-1} for gibberellin A4 (GA4) to 200 ng mL^{-1} for IAA. Within the first week after potassium chlorate treatment, dry weight (DW) amounts in the treated longan shoot tips of four gibberellins, namely: gibberellin A1(GA1), gibberellic acid (GA3), gibberellin A19 (GA19) and gibberellin A20 (GA20), were found to increase to approximately 25, 50, 20 and 60 ng g^{-1} respectively, all of which were significantly higher than those of the controls. In contrast, gibberellin A8 (GA8) obtained from the treated longan was found to decrease to approximately 20 ng g^{-1} DW while that of the control increased to around 80 ng g^{-1} DW. Certain CKs which play a role in leaf bud induction, particularly isopentenyl adenine (iP), isopentenyl adenosine (iPR) and dihydrozeatin riboside (DHZR), were found to be present in amounts of approximately 20, 50 and 60 ng g^{-1} DW in the shoot tips of the control longan. The analytical results obtained from the two-month off-season longan flowering period indicate that high GA1, GA3, GA19 and GA20 levels in the longan shoot tips contribute to flower bud induction while high levels of CKs, IAA and ABA in the control longan contribute more to the vegetative development.

1. Introduction

Longan (*Dimocarpus longan Lour.*) is a major economic crop of the northern part of Thailand. Research interests regarding longan have focused on developing ways to enhance the quality and quantity of this fruit. Factors affecting the growth of longan trees in addition to geographical and climatic factors, include light [1–3], water stress [4–6], nutrient or fertilizer [4,7] and certain chemicals [4,8–10]. In recent years, the application of some chemical substances, notably potassium chlorate [8,10], to the soil of longan orchards has been utilized in the northern part of Thailand for off-season longan floral induction. Natural longan usually blooms in the winter when moderately low temperatures stimulate flower bud initiation [8,10], a phenomenon which also commonly occurs with other fruits such as lychees (*Litchi chinensis* Sonn.) [11], mangoes cv. *Kensington Pride.* [9,12], *Phalaenopsis hybrid* (*Minho Princess* × *Phal.* Equestris) [13], and strawberries (*Fraqaria* × *ananassa* Duch.) [14]. To date, most publications on longan have dealt with agricultural aspects such as geographical and climatic factors enhancing fruit yields whereas the effects of chemical compounds associated with the flowering process of longan trees have been less well-documented. It is well known that there are a variety of plant production metabolites which are synthesized in both normal and abnormal plant situations. Some of these compounds are in translocation or storage form and sometimes their amounts are readjusted by differentiation development of growth stage or as a result of some stress reduction. Chemical compounds involved directly in controlling the growth of plants are the plant hormones which can be classified into six major groups, namely: cytokinins, abscisic acid, auxins, gibberellins, ethylene, and other plant hormones such as brassinosteroids, jasmonic acid, and strigolactones [15,16]. Plant hormones are synthesized in shoot or root meristem cells and transported to target areas for plant development including cell division,

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cell enlargement and cellular differentiation depending on the type of plant hormones and their levels. Previous research work has suggested that the levels of plant hormone changes, e.g. of GAs [6,13,17] or CKs [2,17,18], during flowering in certain plants are induced by chemical substance application.

The combined liquid chromatographic and mass spectrometric (LC–MS) technique has been used in chemical research to identify or confirm the chemical components or to measure the levels of specified targets since this technique offers high sensitivity and resolution with a wide range of applications for clinical [19,20], pharmaceutical [19–21], agricultural [22], environmental [21,23,24] and biological samples [19,20,22].

The present research has been aimed at studying the changes in selected plant hormone levels of the IAA, ABA, CKs and GAs groups in shoot tips of longan trees after potassium chlorate treatment for off-season floral induction compared with those of the control longan trees, i.e. those without potassium chlorate treatment, within the same period of time. Analyses were carried out by means of liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS) in both positive and negative ionization modes. The names of the plant hormones investigated in this research are listed in Table 1.

2. Experimental

2.1. Chemicals and reagents

Cytokinins; *trans*-zeatin (t-Z) (purity > 99% for HPLC) was purchased from Fluka (Gillingham, Dorset, UK), benzyladenine (BA) (purity > 98% for HPLC) was purchased from Fluka (Budapest, Hungary), kinetin (K) (purity > 98% for HPLC) was purchased from Fluka (Buchs, Switzerland); trans-zeatin riboside (t-ZR), dihydrozeatin (DHZ), dihydrozeatin riboside (DHZR), zeatin glucosides (Z9G), zeatin riboside-O-glucoside (ZROG), zeatin riboside-5-monophosphate (ZR-MP), isopentenyl adenine (iP) and isopentenyl adenosine (iPR) were products of Olchemin (Olomouc, Czech Republic). Abscisic acid (±ABA) was purchased from A.G. Scientific (San Diego, CA, USA). Indol-3-acetic acid (IAA) (purity > 98% for HPLC) was purchased from Fluka (Buchs, Switzerland). Gibberrellic acid (GA_3) and gibberrellin A4 (GA4) (purity > 90% for HPLC) were purchased from Fluka (Buchs, Switzerland); GA1, 8, 19 and 20 were products of Olchemin (Olomouc, Czech Republic). Alizarin, used as internal standard (I.S.), was purchased from Merck (Darmstadt, Germany). Formic acid, ammonia solution and methanol (Analytical grade) were purchased from Lab Scan (Gliwice, Poland). Methanol and acetic acid (HPLC grade) for mobile phase preparation were purchased from Merck (Darmstadt, Germany). De-ionized water was generated with a Milli-Q water purification system from Millipore (Billeric, MA, USA). SPE C-18 (300 mg) and mixed-mode SPE (C8/SCX, 500 mg) were purchased from Vertical Chromatography (Bangkok, Thailand). Potassium chlorate ($KClO_3$) (Laboratory grade) was locally obtained from Maejo University, Thailand.

2.2. Plant materials

Twenty ten-year-old longan trees of the cv. E-Daw, *Dimocarpus longan Lour.*, located in the experimental orchard of Maejo University, Sansai District of Chiang Mai Province in the northern part of Thailand, were the source of plant material with ten trees being taken as the treated source and the remaining ten trees as the control source. The $KClO_3$ application to the surface soil was approximately 500 g per treated longan tree. The shoot tips (2–3 cm) of longan were collected from both the control and the treated sources starting from 4 days before the application of $KClO_3$ into the soil of the treated source until 60 days after the application. Shoot tips were collected at 2–5 day intervals with the first day of the treatment designated as day 0. For the treated source, each sample comprised approximately 100 shoot tips collected randomly and proportionately from the ten treated longan trees at 1.5–3 meters above the soil surface level. The collected shoot tips were immediately kept in liquid nitrogen before being transported to the laboratory where only terminal buds and lateral or auxiliary buds of the shoot tips were freeze-dried and stored at −20 °C prior to the extraction and clean-up steps. As for the control source, each sample was obtained from the ten control longan trees, using the same procedure as above.

2.3. Extraction and clean-up procedure

One gram dry weight (DW) of each longan shoot tip sample was ground to a powder with liquid nitrogen (N_2) and extracted with a cold extraction mixture of 10 ml MeOH:HCO_2H:H_2O (15:4:1, v/v/v) solvent overnight at 4 °C. The extractant was separated by centrifugation (10,000 rpm, 20 min) and then re-extracted for 30 min in an additional 10 ml g^{-1} plant material of the cold extraction mixture. The supernatant liquid was evaporated either to near dryness or until the methanol was removed. The residue was then dissolved in 2 ml of 0.1 M formic acid and applied to mixed-mode SPE preconditioned with 5 ml of methanol followed by 5 ml of 1 M formic acid. The SPE was washed and eluted with 2 ml of 80% MeOH followed by 2 ml of 1 M ammonia in 80% MeOH. All remaining liquid was dried with N_2 gas. Finally, all of the extracts were re-dissolved in 80% MeOH with internal standard and filtered through 4 mm PTFE before determination by liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS).

2.4. Liquid chromatography–electrospray ionization-mass spectrometry conditions

The determinations were performed on a LCMS system consisting of an Agilent series 1100 with a diode array detector (DAD) and an orthogonal spray HP mass spectrometer equipped with an electrospray ionization (ESI) source. The ionization interface was operated in both positive and negative modes. The data acquisition software used was Agilent Technology. The LC separation was carried out by a Zorbax SB C18 column (2.1 mm × 100 mm i.d., 3.5 μm). The elution was performed using solvent A (0.1% acetic acid in methanol) and solvent B (0.1% acetic acid in water) with a linear A/B gradient program of: 0 min: 10% A; 4 min: 30% A; 34 min: 60% A; 40 min: 100% A, and held for 10 min at a flow rate of 0.3 mL min^{-1}. The injector volume selected was 5 μL. The LC–ESI-MS conditions were set as: capillary voltage 4500 V; nebulizer pressure 25 psi, gas temperature 330 °C; nitrogen gas flow rate 10 L min^{-1} and fragmentation 80 V and 70 V for positive and negative modes, respectively. Quantitation was performed by integration of the peak area ratio of the analyte to the internal standard on extracted ion chromatograms (EICs) with the extracted ions of $[M+H]^+$ and $[M-H]^-$ for positive and negative ions respectively. The specific diagnostic ions for each of the plant hormones are listed in Tables 2 and 3.

3. Results and discussion

3.1. Liquid chromatography–electrospay mass spectrometry

Eleven cytokinins (K, BA, iP, iPR, DHZ, DHZR, t-Z, t-ZR, ZR-MP, Z9G and ZROG), auxin (IAA), abscisic acid (ABA) and six gibberellins (GA1, 3, 4, 8, 19 and 20), as listed in Table 1, were studied in this work. Fragmentation of each plant hormone was obtained using ESI-MS with both positive and negative ionization scan modes. The

Table 1
Structures, formulas and masses of plant hormones investigated.

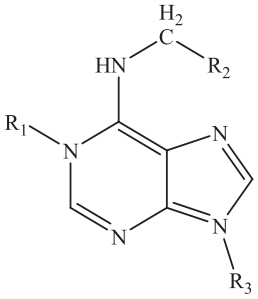
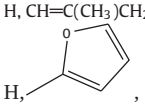
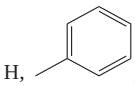
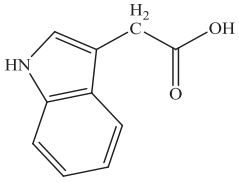
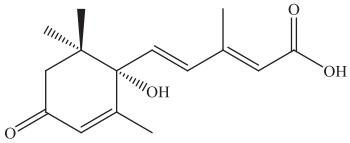
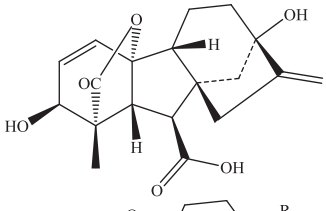
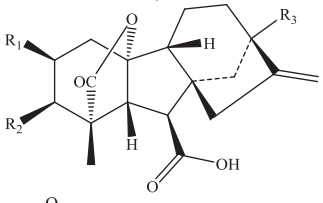
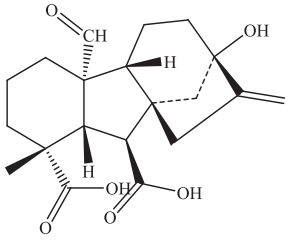
Structure		Formula	Mass	Compound	Abbreviation
	R_1, R_2, R_3	$C_{10}H_{13}N_5O$	219	*trans*-zeatin	t-Z
	H, $CH{=}C(CH_3)CH_2OH$, –	$C_{15}H_{21}N_5O_5$	351	*trans*-zeatin riboside	t-ZR
	H, $CH{=}C(CH_3)CH_2OH$, R	$C_{10}H_{13}N_5$	203	Isopentenyl adenine	iP
	H, $CH{=}C(CH_3)_2$, -	$C_{15}H_{21}N_5O_4$	335	Isopentenyl adenosine	iPR
	H, $CH{=}C(CH_3)_2$, R	$C_{10}H_{15}N_5O$	221	Dihydrozeatin	DHZ
	H, $CH_2CH(CH_3)_2$, -	$C_{15}H_{23}N_5O_5$	353	Dihydrozeatin riboside	DHZR
	H, $CH_2CH(CH_3)_2$, R	$C_{16}H_{23}N_5O_6$	381	Zeatin glucosides	Z9G
	Glu, $CH{=}C(CH_3)CH_2OH$, –	$C_{21}H_{31}N_5O_{10}$	513	Zeatin	ZROG
	Glu, $CH{=}C(CH_3)CH_2OH$, R	$C_{15}H_{22}N_5O_8P$	431	riboside-O-glucoside	ZR-MP
	H, $CH{=}C(CH_3)CH_2OH$, RP	$C_{10}H_9N_5O$	215	Zeatin	K
	 , -, -	$C_{12}H_{11}N_5$	225	riboside-monophosphate	BA
	 , -, -			Kinetin	
	Glu = Glucoside, R = riboside RP = riboside mono-phosphate			Benzyladenine	
		$C_{10}H_8NO_2$	175	Indole-3-ly acetic acid	IAA
		$C_{15}H_{20}O_4$	264	Abscisic acid	ABA
	R_1, R_2, R_3	$C_{19}H_{22}O_6$	346	Gibberellic acid	GA3
	H, OH, OH	$C_{19}H_{24}O_6$	348	Gibberellin A1	GA1
	H, OH, H	$C_{19}H_{24}O_5$	332	Gibberellin A4	GA4
	OH, OH, OH	$C_{19}H_{24}O_7$	364	Gibberellin A8	GA8
	H, H, OH	$C_{19}H_{24}O_5$	332	Gibberellin A20	GA20
		$C_{20}H_{26}O_6$	332	Gibberellin A19	GA19

Table 2
Diagnostic fragments, linear ranges, linear equations, LOD, LOQ and precision values of plant hormones obtained with LC–ESI-MS using positive ionization mode.

Plant hormone		Retention time ($n=5$) (min)	RSD (%)	Linear regression range (ng ml^{-1})	linear regression equation $Y=mX+C$	Correlation coefficient	LOD,[a] ng mL^{-1}	LOQ,[b] ng mL^{-1}
Positive mode	$[M+H]^+$							
t-Z	220 [136]	11.541 ± 0.337	2.92	200–2000	$Y=0.7743X-0.0178$	0.9985	90	320
t-ZR	352 [222]	14.300 ± 0.518	3.62	100–1000	$Y=1.2759X+0.0068$	0.9972	25	100
iP	204 [136]	22.418 ± 0.293	1.30	100–1000	$Y=0.3403X+0.0233$	0.9993	50	120
iPR	336 [204]	26.851 ± 0.561	2.09	100–1000	$Y=0.3440X-0.0258$	0.9993	30	100
DHZ	222 [136]	11.775 ± 0.515	4.37	100–1000	$Y=0.4082X+0.0893$	0.9971	80	250
DHZR	354 [222]	14.874 ± 0.651	4.38	100–1000	$Y=0.2151X+0.0083$	0.9990	45	150
Z9G	382 [220]	10.615 ± 0.586	5.52	100–1000	$Y=0.7109X+0.0321$	0.9988	55	100
ZROG	514 [382]	12.927 ± 0.434	3.36	100–1000	$Y=0.4660X+0.0247$	0.9964	70	140
ZR-MP	432 [222, 136]	9.733 ± 0.512	5.26	100–1000	$Y=0.1503X-0.0047$	0.9982	60	180
K	216 [148]	16.862 ± 0.304	1.80	100–1000	$Y=0.5894X+0.0411$	0.9984	55	150
BA	226	24.162 ± 0.774	3.20	100–1000	$Y=2.8365X+0.0593$	0.9968	50	120

m/z value in the square brackets, [. . .] is the characteristic ion indicating the relevant mass diagnostic fragment of each analyte.
[a] LOD, limit of detection based on 3 times of signal to noise ratio [25].
[b] LOQ, limit of quantitation based on 10 times of signal to noise ratio [25].

Table 3
Diagnostic fragments, linear ranges, linear equations, LOD, LOQ and precision values of plant hormones obtained with LC–ESI-MS using negative ionization mode.

Plant hormone		Retention time ($n=5$) (min)	RSD (%)	Linear regression range (ng ml^{-1})	linear regression equation $Y=mX+C$	Correlation coefficient	LOD,[a] ng mL^{-1}	LOQ,[b] ng mL^{-1}
Negative mode	$[M-H]^-$							
IAA	174 [130]	19.072 ± 0.108	0.56	200–2000	$Y=0.0049X-0.0004$	0.9946	200	600
ABA	263 [153, 219]	27.188 ± 0.716	2.63	100–1000	$Y=0.0325X+0.0043$	0.9992	70	200
GA1	347	16.929 ± 0.708	4.18	100–1000	$Y=0.0351X+0.0013$	0.9977	50	180
GA3	345 [239]	16.702 ± 0.423	2.54	100–1000	$Y=0.0374X+0.0007$	0.9995	30	85
GA4	331 [287]	41.048 ± 1.214	2.96	100–1000	$Y=0.2546X+0.0032$	0.9998	10	20
GA8	363	11.248 ± 0.210	1.87	100–1000	$Y=0.0257X-0.0001$	0.9991	40	120
GA19	361	35.764 ± 0.504	1.41	100–1000	$Y=0.0314X+0.0015$	0.9992	45	150
GA20	331	30.740 ± 0.108	0.35	100–1000	$Y=0.0425X+0.0016$	0.9997	30	75

m/z value in the square brackets, [. . .] is the characteristic ion indicating the relevant mass diagnostic fragment of each analyte.
[a] LOD, limit of detection based on 3 times of signal to noise ratio [25].
[b] LOQ, limit of quantitation based on 10 times of signal to noise ratio [25].

relevant mass diagnostic fragments, in square brackets, are summarized in Tables 2 and 3. Fig. 1 shows overlayed extracted ion chromatograms (EICs) of the plant hormones detected by LC–ESI-MS with positive ionization scan mode while Fig. 2 shows those detected by LC–ESI-MS with negative ionization scan mode. Linearity was evaluated over the range 100–2000 μg L^{-1} and good linear regressions or coefficients were obtained with values above 0.99. The relative standard deviations (RSD %) for each analyte peak area and retention time were well within 10%. Instrumental detection limits or limits of detection (LOD) and limits of quantitation (LOQ) were determined based on the signal-to-noise ratio (S/N), where LOD is 3 times S/N and LOQ is 10 times S/N [25]. LOD and LOQ ranged from 10–200 to 20–600 ng mL^{-1}, respectively, as shown in Tables 2 and 3. These results demonstrate that LC–ESI-MS is quantitatively applicable to plant hormone analysis with both positive and negative ionization modes.

3.2. Mixed-mode solid phase extraction

Since the amounts of plant hormones in the plant materials investigated were very low and were therefore susceptible to interference by several secondary metabolite compounds present in higher concentrations than the analytes themselves, a purification or clean-up method was required prior to measurement by LC–MS. Modified mixed-mode SPE, consisting of both reverse phase and cation exchange sorbent in single SPE, was employed in this work by adapting the conditions previously reported by Dobrev and Kaminek [26,27]. The optimized mixed-mode SPE was used in the recovery studies performed with blank matrix samples spiked with plant hormone standards at three concentrations. The percentages of recovery at low, medium and high concentration were 72–112, 84–104 and 82–105, respectively, with % RSD in the ranges of 1.96–17.18, 3.26–16.07 and 2.41–14.30, respectively, as listed in Table 4. Replicate extraction ($n=8$) of plant hormone standards at medium concentration yielded good repeatability and reproducibility of the mixed-mode SPE method with 1.95–12.08% RSD and 82–103% recovery. Therefore, mixed-mode SPE was used for the removal of potentially interfering matrices in order to enhance the sensitivity of the LC–ESI-MS system in analyzing for the plant hormones of interest. Knowing the approximate concentrations of the plant hormones of interest in the samples through trial experiments, the final concentrations of the analytes were thus prepared well within the linearity range and at levels higher than the LOQ. The amounts of plant hormones in the shoot tips in g DW were then calculated accordingly.

Floral or leaf induction reflected different physiological plant developments with and without potassium chlorate treatment. After approximately the ninth to twelfth day following potassium chlorate treatment, the treated longan trees were observed to begin their plant development from the stage of flower buds which were later transformed into flowers, anthesis, abscission and longan fruits consecutively with a total of approximately 50 days to reach the fruit induction stage. Longan trees without potassium chlorate treatment generally resulted in growth with leaf induction. Chemical treatment with chlorate is known to activate floral bud induction [8,10] and its effects may be regulated by hormonal changes, as observed in the endogenous IAA, GA and cytokinin transformations after potassium chlorate treatment in this research.

Table 4
Recovery values and relative standard deviations (RSDs) obtained at three concentration levels.

Plant hormone	Low concentration ($n = 3$)[a]			Medium concentration					High concentration ($n = 3$)[a]		
	Spiked ($\mu g\,mL^{-1}$)	Recovery (%)	RSD (%)	Spiked ($\mu g\,mL^{-1}$)	I ($n = 3$)[a]		II ($n = 8$)[b]		Spiked ($\mu g\,mL^{-1}$)	Recovery (%)	RSD (%)
					Recovery (%)	RSD (%)	Recovery (%)	RSD (%)			
Positive mode											
t-Z	0.10	102	13.03	0.20	94	7.39	92	5.08	0.40	96	4.46
t-ZR	0.10	91	7.53	0.20	88	8.12	93	8.00	0.40	92	3.54
iP	0.10	83	14.38	0.20	90	8.75	89	8.01	0.40	99	2.41
iPR	0.10	102	5.44	0.20	91	9.19	88	9.45	0.40	95	6.55
DHZ	0.10	72	10.30	0.20	88	12.86	92	1.95	0.40	89	9.68
DHZR	0.10	99	10.50	0.20	86	3.26	87	9.74	0.40	95	4.73
Z9G	0.10	98	2.95	0.20	98	10.98	84	9.86	0.40	103	9.04
ZROG	0.10	90	5.42	0.20	104	3.38	85	12.08	0.40	102	5.52
ZR-MP	0.10	105	6.08	0.20	96	5.47	91	7.70	0.40	105	10.02
K	0.10	102	8.27	0.20	102	6.90	92	3.80	0.40	96	4.35
BA	0.10	112	6.77	0.20	94	1.25	102	4.16	0.40	103	13.97
Negative mode											
IAA	0.20	nd	–	0.40	84	16.07	82	12.27	0.80	88	6.24
ABA	0.10	108	13.17	0.20	95	10.07	93	11.34	0.40	96	12.38
GA3	0.10	112	1.96	0.20	89	6.54	91	10.22	0.40	82	7.49
GA4	0.10	93	3.21	0.20	92	5.54	96	8.90	0.40	97	11.71
GA1	0.10	95	17.18	0.20	86	3.76	84	8.06	0.40	98	6.75
GA8	0.10	99	11.19	0.20	93	7.47	98	7.79	0.40	91	14.30
GA19	0.10	101	10.38	0.20	90	15.50	93	9.49	0.40	96	6.25
GA20	0.10	110	14.45	0.20	92	12.89	88	11.32	0.40	97	6.42

[a] The results for repeatability and reproducibility for intra-day precision and accuracy at three concentration levels.
[b] The results for repeatability for inter-day precision and accuracy at medium concentrations.

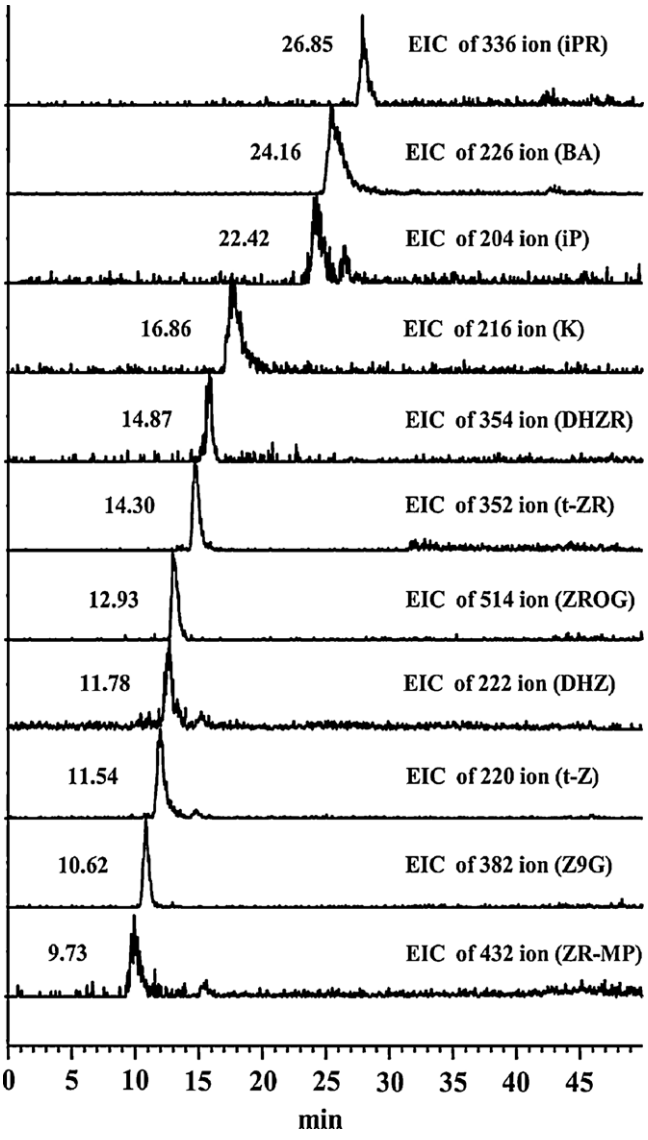


Fig. 1. Overlayed extracted ion chromatograms (EICs) of plant hormones detected by LC–ESI-MS with positive ionization scan mode.

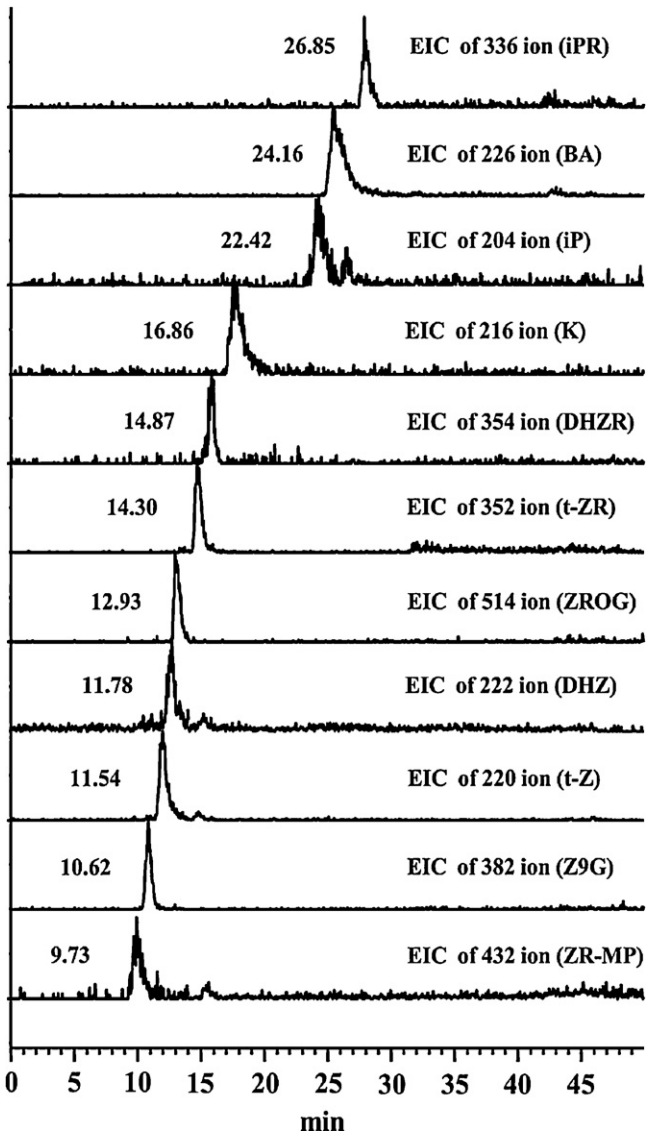


Fig. 2. Overlayed extracted ion chromatograms (EICs) of plant hormones detected by LC–ESI-MS with negative ionization scan mode.

3.3. Analysis for cytokinin level changes

Apical shoot tip samples from both the control (without $KClO_3$ treatment) longan trees and the longan trees treated with $KClO_3$ were extracted and cleaned up using mixed-mode SPE under the optimum conditions previously obtained. Cytokinin bases and their derivatives were simultaneously analyzed by LC–ESI-MS in positive ionization mode. Fig. 3 compares the cytokinin changes in apical shoot tips which developed into leaf buds and flower buds for the control and treated longan trees, respectively. Changes in the concentration levels of cytokinins in the shoot tips of the control trees, particularly those involved in vegetative growth including periclinal division, anticlinal division, and cell enlargement of lateral and terminal buds, such as t-Z, t-ZR, iP, iPR, ZROG, Z9G, ZR-MP and DHZR found to be approximately 15–90, 0–180, 0–30, 0–150, 0–120, 30–120, 2–1000 and 0–100 ng g^{-1} DW respectively, were greater than those in the treated longan trees which were found to be approximately at 20–45, 10–40, 0–40, 0–180, 0–15, 10–60, 5–500 and 0 (undetected) ng g^{-1} DW, respectively, as shown in Fig. 3. However, iP and iPR in the flowers from the treated longan trees during the anthesis period were detected in higher concentrations than those in the shoot tips of the control longan. The first two weeks after treatment with $KClO_3$ is the critical period of endogenous plant hormone change that controls cell differentiation of the leaves or flower buds and influences cell division and cell enlargement during the later stages [28,29]. Fig. 3 indicates that the levels of CKs, such as iP, iPR and DHZR in the shoot tips of the control trees at one week after potassium chlorate treatment were found to be 10, 45 and 30 ng g^{-1} DW, respectively, while those in the treated longan trees could not be detected. The amounts of t-ZR and Z9G of the control trees, found to be approximately 5 and 35 ng g^{-1} DW, respectively, were lower than those of the treated trees which were detected at approximately 30 and 60 ng g^{-1} DW, respectively. Ordinary root or shoot growth and branching developments were in accordance with the combination of the CK and IAA levels [28,30,31].

The concentrations of cytokinin and auxin in the root and shoot apices are essential factors in cellular differentiation since high CK/IAA ratios control leaf induction or vegetative growth while low CK/IAA ratios and high GAs affect leaf inhibition and induce floral bud or reproductive growth [30]. These findings suggest that the different concentrations of CKs observed indicate their influence in

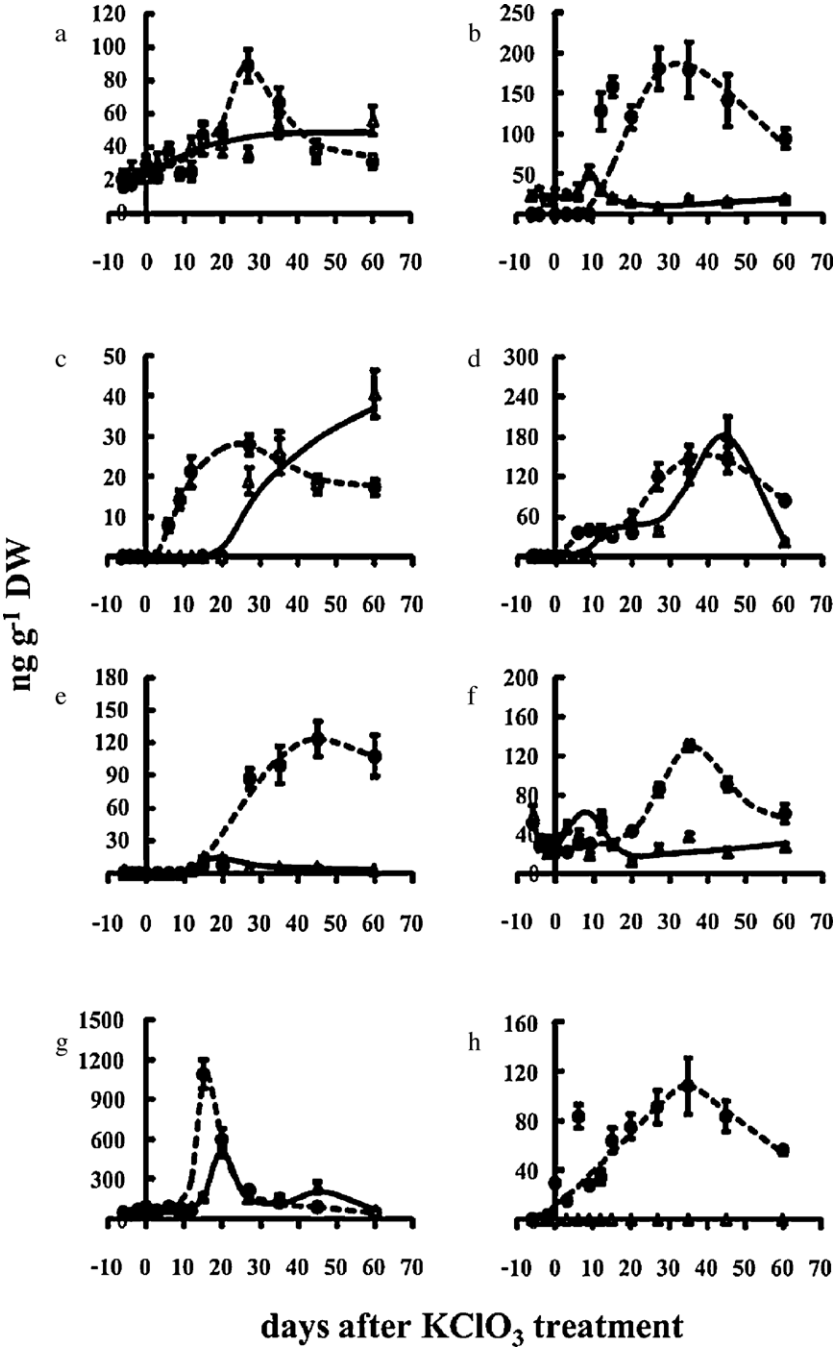


Fig. 3. Endogenous cytokinin level changes in: (a) t-Z, (b) t-ZR, (c) iP, (d) iPR, (e) ZROG, (f) Z9G, (g) ZR-MP, and (h) DHZR in shoot tips of longan trees with (▲) and without (●) $KClO_3$ treatment from the period before floral bud induction until anthesis.

transformation of general bud into leaf induction. Although it has previously been suggested that leaf induction could be controlled by high endogenous free bases of CKs such as Z, iP and DHZ [18], data for DHZ is not presented here in this paper due to its levels in the extracted samples being below the detection limit. The relatively high concentrations of DHZR, iP and iPR in the first week resulted in the shoot tips developing into apical leaves. In contrast, unusually low levels of these CKs would result in the shoot tips developing into apical flowers [18]. Significantly, kinetin and benzyladenine cytokinins were not detected or their concentrations were below the LOD. The function of plant hormones in different development processes such as root or shoot growth [11] and branching or leaf senescence [32] is generally based on plant physiological observations. However, in this work, an analytical approach has been adopted from which Fig. 3(c) and (d) indicates the role of some CKs such as iP and iPR in cell enlargement for both vegetative and reproductive growth.

3.4. Analysis for GA level changes

Although CKs affect cell and plant growth, gibberellin is the most prominent hormone for controlling plant development. In this work, GAs have influenced flower bud initiation. The results of GA monitoring demonstrate that the levels of GAs have an effect on floral induction of the longan trees treated with potassium chlorate, as shown in Fig. 4. The symbol ○ with dashed lines in Fig. 4 represents GA changes in longan trees without potassium chlorate treatment and represents a natural plant development

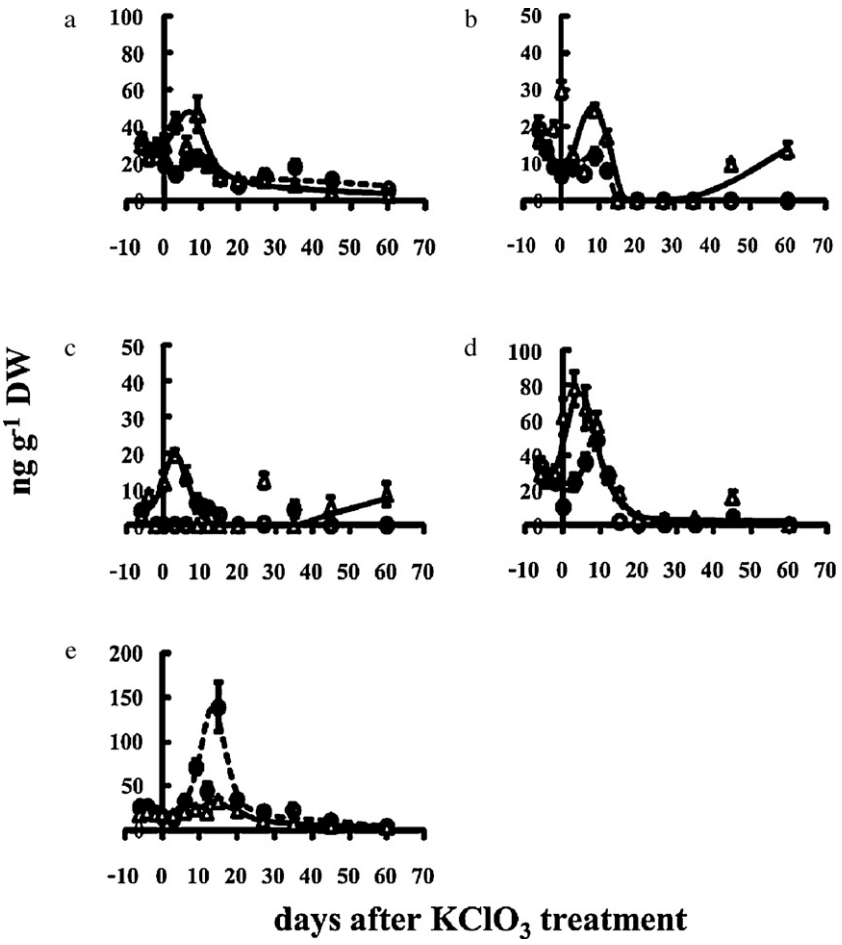


Fig. 4. Endogenous gibberellin level changes in: (a) GA3, (b) GA1, (c) GA19, (d) GA20, and (e) GA8 in shoot tips of longan trees with (▲) and without (●) $KClO_3$ treatment from the period before floral bud induction until anthesis.

process involving shoot branching at both terminal and auxiliary leaf buds and vegetative growth. The amounts of GA1, GA3, GA8, GA19 and GA20 found in the shoot tips of longan trees for normal growth were in the ranges of 0–20, 5–30, 5–140, 0–10 and 0–50 ng g^{-1} DW, respectively, while those in the treated trees were 0–30, 5–45, 5–35, 0–20 and 0–80 ng g^{-1} DW, respectively. Significant GA changes in GA1 (25 ng g^{-1} DW), GA3 (50 ng g^{-1} DW), GA19 (20 ng g^{-1} DW), and GA20 (60 ng g^{-1} DW) of the treated trees were observed after a week of $KClO_3$ treatment and were higher than those of the controls. However, the results for the GA8 levels indicated an opposite change as the concentrations obtained with shoot tips of the treated trees at 20 ng g^{-1} DW were found to be lower than those of the control trees at 80 ng g^{-1} DW. Considering the GA-biosynthetic pathway GA19 → GA20 → GA1 → GA8 [17,33,34], it appears that GA8 is an inactive GA in terms of flower bud initiation [17]. Thus, the control trees yielded relatively higher GA8 levels than the treated trees. As for GA4, its concentrations were so low that they could hardly be detected which could be explained in terms of the GA-biosynthetic pathway [17,33,34] in which GA4 is transformed into an inactive form resulting in such low concentrations Because the plant hormones that influence plant growth are in the free base forms such t-Z, iP, DHZ and GA3, and their levels are very low, analyses for both the free base and conjugated forms would be useful for establishing the relationship between the plant hormones and the plant growth stage. For example, the higher levels of iP, iPR, DHZR (Fig. 3) in the vegetative growth stage or the GA19 and GA20 levels (Fig. 4) could indicate their association with the GA3 level [12,13,33].

Flower bud induction of longan treated with $KClO_3$ was found to be prominent within the first 1–2 weeks after $KClO_3$ treatment during which certain giberrelins, particularly GA1, GA3, GA19 and GA20, were found to increase to higher levels than those of the controls (Fig. 4). There have been reports revealing the role of gibberellins in various developmental processes in mangoes [12], Phalaenopsis hybrida [13] and Macadamia integrifolia [17] but no report to date has given detailed accounts of gibberellins or other plant hormones in longan treated with $KClO_3$. However, one can deduce from the data of this work that certain gibberellins could influence longan flower bud induction in early stages (1–3 weeks) but these gibberellins might have transformed into other forms of gibberellins at later stages as transformations are common in the GA-biosynthetic pathway [33,34].

3.5. Analysis for IAA and ABA level changes

Different IAA contents were obtained between the vegetative and reproductive developments. IAA contents in the apical and lateral buds of the control trees at 1–2 weeks after day 0 were found to be in the range of 25–35 ng g^{-1} DW which were higher than those of the treated trees which were found in the range 5–20 ng g^{-1} DW. In combination with the high CKs contents, IAA could have promoted leaf bud induction in the control trees and leaf development, as shown in Fig. 5(a). Fig. 5(b) shows the ABA concentration levels in apical shoot tips of the trees before treatment until fruit formation. Although the role of ABA in floral bud initiation has not yet been established, the ABA levels obtained for the samples from

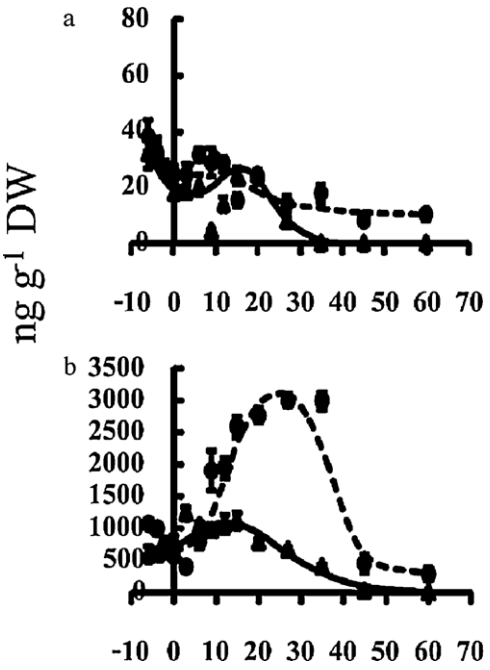


Fig. 5. Endogenous plant hormone level changes in: (a) IAA, and (b) ABA in shoot tips of longan trees with (▲) and without (●) $KClO_3$ treatment from the period before floral bud induction until anthesis.

the control trees of 400–3000 ng g^{-1} DW are clearly higher than 0–1200 ng g^{-1} DW from the treated trees. The ABA functions in plant development are control stomatal closure, dormancy, abscission, seed germination and growth, flowers and fruit senescence [29].

4. Conclusions

This work has demonstrated the ability of LC–ESI-MS to be used after a single mixed-mode solid phase extraction for determining plant hormone level changes in longan trees treated with $KClO_3$ compared with untreated controls. The findings of this work have indicated that the effect of $KClO_3$ can induce apical buds of longan trees to flower within 21 days after treatment for off-season flowering. Although the mechanism of $KClO_3$ in the control of flowering is not yet established, the analytical data obtained here supports the view that $KClO_3$ could induce changes in endogenous plant hormone (IAA, GAs, ABA and CKs) levels in the shoot tips of longan trees. Determination of these analytes was achieved using the LC–ESI-MS technique. Important hormonal changes involving the floral bud transformation were prominent within the first 1–2 weeks after $KClO_3$ treatment. Flower bud induction has been found to occur with $KClO_3$ treatment resulting in increased levels of certain plant hormones, notably GA1, GA3, GA19 and GA20, and decreased levels of other plant hormones, including GA8, IAA and CKs, as compared with those from untreated samples.

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References

[1] A. Mouradov, F. Cremer, G. Coupland, The Plant Cell (2002) 111–130.
[2] S.T. Chang, W.S. Chen, C.Y. Hsu, H.C. Yu, B.S. Du, K.L. Huang, Plant Physiol. Biochem. 37 (1999) 679–684.
[3] G. Bernier, C. Perilleux, Plant Biotech. J. 3 (2005) 3–16.
[4] F. Ramirez, T.L. Davenport, Sci. Hort. 126 (2010) 65–72.
[5] T.H. Li, S.H. Li, Agric. Sci. China 6 (2007) 58–67.
[6] Y. Koshita, T. Takahara, Sci. Hort. 99 (2004) 301–307.
[7] I. Kostenyuk, B.J. Oh, I.S. So, Plant Cell Rep. 19 (1999) 1–5.
[8] P. Manochai, P. Sruamsiri, W. Wiriya-alongkorn, D. Naphrom, M. Hegele, K.F. Bangerth, Sci. Hort. 104 (2005) 379–390.
[9] S.J. Blaidie, V.J. Kulkarni, W.J. Muller, Sci. Hort. 101 (2004) 51–68.
[10] P. Potchanasin, K. Sringarm, P. Sruamsiri, K.F. Bangerth, Sci. Hort. 122 (2009) 288–294.
[11] T.J. O'Hare, C.G.N. Turnbull, Sci. Hort. 102 (2004) 257–266.
[12] T.L. Davenport, D.W. Pearce, S.B. Rood, J. Plant Growth Regul. 20 (2001) 308–315.
[13] W.R. Su, W.S. Chen, M. Koshioka, L.N. Mander, L.S. Hung, W.H. Chen, Y.M. Fu, K.L. Huang, Plant Physiol. Biochem. 39 (2001) 45–50.
[14] G. Paroussi, D.G. Voyiatzis, E. Paroussis, P.D. Drogoudi, Sci. Hort. 96 (2002) 103–113.
[15] V. Gomez-Roldan, S. Fermas, P.B. Brewer, V. Puech-Page, E.A. Dun, J. Pillot, F. Letisse, R. Matusova, S. Danoun, J. Portais, H. Bouwmeester, G. Be'card, C.A. Beveridge, C. Rameau, S.F. Rochange, Nature 455 (2008) 189–194.
[16] M. Umehara, A. Hanada, S. Yoshida, K. Akiyama, T. Arite, N.T. Kamiya, H. Magome, Y. Kamiya, K. Shirasu, K. Yoneyama, J. Kyozuka, S. Yamaguchi, Nature 455 (2008) 195–201.
[17] A.T. Fletcher, J.C. Mander, J. Plant Growth Regul. 26 (2007) 351–361.
[18] W. Dewitte, A. Chiapetta, A. Azmi, E. Witters, M. Strand, J. Rembur, M. Noin, D. Chriqui, H.V. Onckelen, Plant Physiol. 119 (1999) 111–121.
[19] K. Mitrowska, A. Posyniak, J. Zmudzki, Talanta 81 (2010) 1273–1280.
[20] C.Y. Chen, M.R. Lee, F.C. Cheng, G.J. Wu, Talanta 72 (2007) 1217–1222.
[21] R. Varga, I. Somogyvari, Z. Eke, K. Torkos, Talanta 83 (2011) 1447–1454.
[22] K.H. Lu, C.Y. Chen, M.R. Lee, Talanta 72 (2007) 1082–1087.
[23] S. Wangkarn, P. Soisungnoen, M. Rayanakorn, K. Grudpan, Talanta 67 (2005) 686–695.
[24] J. Parshintsev, T. Hyötyläinen, K. Hartonen, M. Kulmala, M.L. Riekkola, Talanta 80 (2010) 1170–1176.
[25] J.C. Miller, J.N. Miller, Statistics and Chemometrics for Analytical Chemistry, fifth ed., Pearson Education, Harlow, 2005, 109-122.
[26] P.I. Dobrev, M. Kaminek, J. Chromatogr. A 950 (2002) 21–29.
[27] K. Hoyerova, A. Gaudinova, J. Malbeck, P.I. Dobrev, T. Kocabek, B. Solcova, A. Travnickova, M. Kaminek, Phytochemistry 67 (2006) 1151–1159.
[28] J. Deikman, M. Ulrich, Planta 195 (1995) 440–449.
[29] D.W.S. Mok, M.C. Mok, Annu. Rev. Plant Physiol. Plant Mol. Biol. 52 (2001) 89–118.
[30] E. Shani, O. Yanai, N. Ori, Curr. Opin. Plant Biol. 9 (2006) 484–489.
[31] S.P. Chatfield, P. Stirnberg, B.G. Forde, O. Leyser, Plant J. 24 (2000) 159–169.
[32] M.E. Ghanem, A. Albacete, C.M. Andujar, M. Acosta, R.R. Aranda, I.C. Dodd, S. Lutts, F.P. Alfocea, J. Exp. Botany 59 (2008) 3039–3050.
[33] P. Hedden, W.M. Proebsting, Plant Physiol. 119 (1999) 365–370.
[34] P. Hedden, A.L. Phillips, Trends Plant Sci. 5 (2000) 523–530.