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Phytochemical and molecular analyses of some *Coleus* cultivars cultivated in Egypt

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Abstract

Background: *Coleus* (family *Lamiaceae*) is a popular garden ornamental plant known for its bright-colored foliage. The effective role of different plant growth regulators have been widely reported in plant cultivars (Singh A and Prasad SM Int. J. Sci. Eng. Appl. Sci. 2 (1): 1-10, (2016)). Kinetin has received great interest due to its richness in bioactive phytochemicals including polyphenolic compounds with its antioxidant activity. Molecular markers have been very helpful in enhancing our understanding of genome structure and function. In our study, we investigated the effect of three treatments of kinetin on the phytochemical contents of five *Coleus* cultivars and molecular characterization by ISSR markers.

Results: The studied cultivars of *Coleus* revealed adequate amounts of pigments chlorophyll a (Cha), chlorophyll b (Chb), and total carotenoids (Carot) as well as phytochemical ingredients including total carbohydrates (Carb), total phenolic (TP), total flavonoids (TF), and total tannins (TT). Finger paint cultivar (C₅) revealed the highest values (0.266, 10.853, 11.648, and 6.722 mg/g) of Carb, TP, and TT, respectively, while the highest contents (11.814 and 11.648 mg/g) of TF were identified in Beckwhites gem species (C₂) and finger paint cultivar (C₅), respectively. Inter-simple sequence repeat (ISSR) marker analysis was performed on the samples with 5 primer pairs. These bands were identified as 21 polymorphic and 18 monomorphic ones with 53.8% polymorphism. Ten unique bands were identified in the resulted ISSR profile.

Conclusion: These results suggested that the effect of kinetin treatments stimulates secondary metabolites production. Also, ISSR marker is helpful for possible distinguishing, identifying, characterizing, and selection processing of *Coleus* cultivars.

Keywords: *Coleus*, Cytokinins, Kinetin, Phytochemical, ISSR markers

Introduction

Coleus (family; *Lamiaceae*) is an ornamental plant which can beautify the environment. It is a vegetative pot plant (Singh and Prasad 2016). Cytokinin revealed direct impact on different parameters of photosynthesis in plants including chlorophyll and synthesis and breakdown of chloroplast compounds, enzyme activities, and electron transfer (Pospisilova et al. 2005). The stimulatory effects of cytokinins on biosynthesis process of major secondary metabolite in *Coleus aromaticus* Benth (L), flavonoid

biosynthesis in *Scutellaria alpina* shoot cultures, and cucurbitacin content in *Citrullus colocynthis* were reported (Grzegorzczak-Karolak et al. 2017). Kinetin, one of the most essential members of cytokinins, is promising in such regard (Zhang et al. 2017). The polyphenolic compounds, one of the most important categories of plant secondary metabolites, especially flavonoids, phenolics, and tannins, showed different biological activities including antioxidant and antiviral activities (Mohamed et al. 2016). The chemical contents of bioactive compounds in plants is strongly dependent on the plant species with its different varieties and thanks to the reported stimulatory effects of cytokinins on biosynthesis process of bioactive secondary metabolite in different plants.

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Molecular markers have been widely used in studies on germplasm resource identification, phylogeny of species, plant taxonomy, evolution, and genetic diversity Abdel Khalik and Osman (2017). ISSRs are regions in the genome flanked by microsatellite sequences. Polymerase chain reaction (PCR) amplification of these regions using a single primer yields multiple amplification products that can be used as a dominant multilocus marker system for the study of genetic variation in various organisms (Ng and Tan 2015). ISSR markers are easy to use, cheap, and less demanding compared to other dominant markers, making it an ideal genetic marker for beginners and for plant species whose genetic information is lacking (Li et al. 2007). ISSR markers have been extensively used to determine genetic diversity among *Coleus* sp. (Tripathi 2013), within seven species of the genus *Plectranthus* (*Lamiaceae*) from Saudi Arabia (Abdel Khalik and Osman 2017), in *Radix astragali* (Liu et al. 2016), within pecan plants (Jia et al. 2011), between *A. capillaris* (Shafie et al. 2011), among *Angelica* species (Mei et al. 2015), and in wild Portuguese orchardgrass (Costa 2016). The first exploration of the genetic and chemical diversity of natural populations to identify elite chemotype(s) variability of forskolin content and existing molecular diversity in the wild population of *C. forskohlii* was by Srivastava et al. (2017). This encouraged us to evaluate the effect of kinetin treatments on the five cultivars of *Coleus* with molecular characterization by ISSR markers.

Materials and methods

A pot experiment was performed during the two successive seasons, 2016 and 2017, at the green house of the National Research Centre, Dokki, Cairo, Egypt, to study

the effect of kinetin upon growth and chemical constituents of the five cultivars of *Coleus blunei*. For cultivation, the five cultivar's seedlings, uniform in size and shape (2–3 leaves), were kindly supplied from the ornamental and aromatic research station, Ministry of Agriculture. The five cultivars were Color Clouds spicy (C_1), Beckwhites gem (C_2), Solar red (C_3), Colorblaze alligatore tears (C_4), and finger paint (C_5) (Fig. 1). The kinetin treatments and agriculture practices were determined according to Sami et al. (2018).

Extraction of plant materials

Fine air-dried powder (1 g) of each *Coleus* sample was extracted with 10 ml of methanol under shaking at room temperature on an orbital shaker (Heidolph Unimax 2010) for 48 h. Each extract was filtered using filter paper (Whatman No.1). The final volume of each filtrate was adjusted to 10 ml with methanol. A representative plant sample from the three replicates from each treatment was taken after 180 days from planting in which chemical constituents were determined as follows.

Phytochemical analysis

Photosynthetic pigments Cha, Chb, and Carot according to Saric et al. (1967) and total soluble carbohydrate percentage by using colorimetric methods which were described by Smith et al. (1956) were used in the phytochemical analysis. Total phenolic content was determined in methanol extracts by Folin-Ciocalteu reagent according to Singleton and Rossi (1965). The aluminum chloride method was used for the determination of total flavonoid (TF) content in methanol extracts (Zhishen et al. 1999). Total tannin (TT) of each

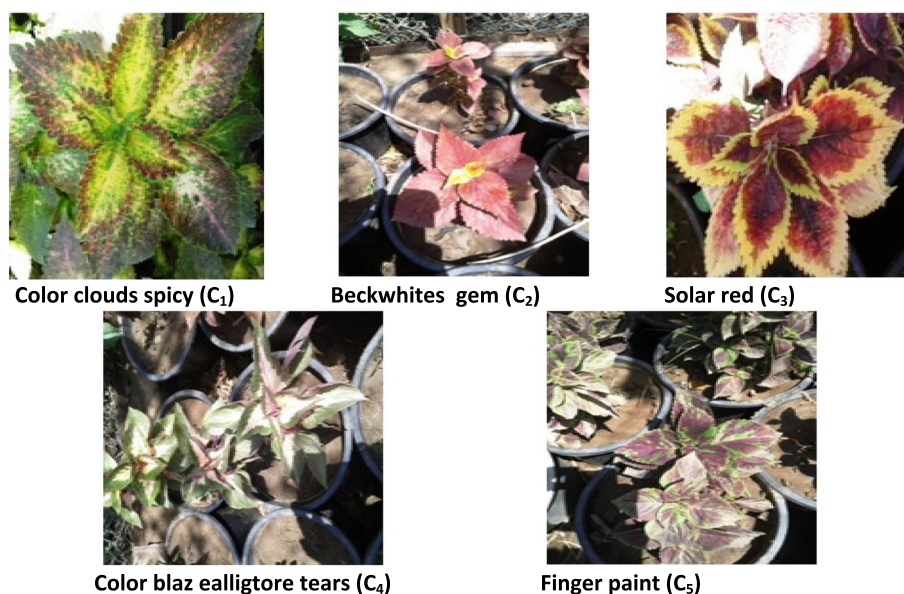


Fig. 1 Morphological description of the five *Coleus* cultivars

extract was measured using the Folin-Ciocalteu reagent assay (Polshettiwar et al. 2007).

Molecular analysis

DNA isolation

Leaves of the five seedlings of *Coleus* were collected and soaked in liquid nitrogen for DNA extraction, and the DNA was extracted by cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990).

Polymerase chain reaction (PCR) procedure

ISSR analyses were performed as described by Adawy et al. (2004) and Hussein et al. (2006). Five ISSR primers were used shown in Table 4. The PCR was performed according to methods described by Shoaib et al. (2016) and Sami et al. (2018).

The PCR conditions

The PCR amplification was performed in a 25- μ l reaction volume containing the following: 200 μ l of dNTPs (2.5 mM), 1.5 μ l of Mg Cl₂ (25 mM), 2.5 μ l of 10x buffer, 2.0 μ l of primer (2.5 μ M), 2.0 μ l of template DNA (50 ng/ μ l), 0.3 μ l of Taq polymerase (5 U/ μ l), and 14.7 μ l of sterile dd H₂O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Perkin Elmer Gene Amp PCR System 2400. The reaction was subjected to one cycle at 95 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, then a final cycle of 72 °C for 5 min. PCR products were run at 100 V for 1 h on 1.2% agarose gels which mixed with 1x TBE buffer, and ethidium bromide was added to the melted gel after the temperature became 55 °C. Gels were photographed and scanned with Bio-Rad video densitometer model 620, at a wavelength of 577.

Data analysis

The obtained results were subjected to statistical analysis of variance according to the method described by Snedecor and Cochran (1980), and the combined analysis of the two seasons was calculated according to the method of Steel and Torrie (1980). The similarity matrices of ISSR markers were done using Gel works ID advanced software UVP-England Program. The relationships among cultivars as revealed by dendrograms were done using SPSS for Windows (version 16) program. DICE computer package was used to calculate the pairwise difference matrix and plot the phenogram among cultivars (Yang and Quiros 1993).

Results

Effect of kinetin treatments on the phytochemical contents of *Coleus* cultivars

Chlorophyll pigments Cha, Chb, and Carot ranged from 1.209, 3.654, and 3.733 mg/g in cultivar C₁ to 2.914 mg/g in cultivar C₂ and 12.013 and 10.594 mg/g in C₅,

respectively (Table 1). Cultivar C₅ revealed the highest values (0.266, 10.853, 11.648, and 6.722 mg/g) of Carb, TP, and TT, respectively, as compared to the other cultivars (Table 1). Compared to the other cultivars, the highest contents (11.814 and 11.648 mg/g) of TF were identified in cultivar (C₂) and cultivar C₅, respectively (Table 1).

Different treatments of kinetin showed significant effects ($P \leq 0.05$) on the contents of pigments (Cha, Chb, and Carot), Carb, TP, TF, and TT. One hundred fifty parts per million (K3) treatment gave rise to the highest values (2.221, 9.851, and 9.225 mg/g) of Cha, Chb, and Carot, respectively (Table 2). The highest content of Carb (0.255 mg/g) resulted in 100 ppm (K2) treatment. The highest contents (8.729 and 5.259 mg/g) of TP and TT was associated with 0 ppm (K1) treatment, respectively, while the highest content (9.674 mg/g) of TF resulted in 200 ppm treatment (Table 2).

The results of Table 3 displayed significant effects ($P \leq 0.05$) of different treatments of kinetin on the different cultivars of *Coleus*. K1 and K3 of kinetin gave the highest contents (4.083 and 3.940 mg/g) of Cha with cultivar C₂, respectively, followed by 3.143 mg/g of Cha with cultivar C₅ when treated by K4 of kinetin (Table 3). K3 of kinetin gave rise to the highest values (18.507 and 14.083 mg/g) of Chb with cultivar C₅ and C₂, respectively. The highest value (16.520 mg/g) of Carot resulted of treatment of K3 kinetin with cultivar C₅, followed by 13.523 mg/g of Carot which resulted of treatment of K1 kinetin with cultivar C₂. On the other hand, K2 and K4 of kinetin led to the highest contents (0.282 and 0.277 mg/g) of Carb with cultivars C₃ and C₅, respectively (Table 3). Results pertaining to TP, TF, and TT indicate that treatment of K3 kinetin gave the highest contents (13.633, 15.147, and 8.237 mg/g) of TP, TF, and TT respectively with cultivar C₅.

Molecular characterization using ISSR markers

The amplified fragments of DNA which generated monomorphic bands (MB), polymorphic bands (PB), primer sequences (PS), unique bands (UB), and the percentage of polymorphism ($P\%$) in five *Coleus* cultivars are presented in Table 4 and Fig. 2.

A maximum of 39 DNA bands was scored in the ISSR profiles generated by primers 44B, HB-9, HB-12, HB-14, and HB-15. The size of the amplified bands ranged from about 260 to 1500 base pairs (bp); these bands were identified as 21 polymorphic bands (53.8%) and 18 monomorphic ones. Eleven unique bands were identified in the resulted ISSR-profile generated by primers HB-9, HB-12, HB-14, and HB-15 (Table 4 and Fig. 2).

The primer 44B generated 2 monomorphic fragments with molecular sizes 365 and 415 bp. The fragments with molecular sizes 630 bp and 920 bp were scored with cultivars Solar red and finger paint, while the fragment with

Table 1 Different cultivars affect the chemical analysis of *Coleus blunei* (mean of two seasons)

Cultivars	Cha (mg/g, FW)	Chb (mg/g, FW)	Carot (mg/g, DW)	Carb (mg/g, DW)	TP (mg/g, DW)	TF (mg/g, DW)	TT (mg/g, DW)
C1	1.535	5.910	5.494	0.238	6.039	6.871	3.446
C2	2.914	8.398	9.838	0.212	7.108	11.814	4.069
C3	1.209	3.654	3.733	0.224	7.769	6.926	4.501
C4	1.401	5.458	4.901	0.222	7.908	8.648	4.871
C5	2.763	12.013	10.594	0.266	10.853	11.648	6.722
LSD = P ≤ 0.05	0.00519	0.00636	0.01288	0.00062	0.07252	0.22004	0.07884

C₁ Color Clouds spicy, C₂ Beckwhites gem, C₃ Solar red, C₄ Colorblaze alligore tears, C₅ finger paint, Cha chlorophyll a, Chb chlorophyll b, Carot total carotenoids, Carb total carbohydrates, TP total phenolic content, TF total flavonoid content, TT total tannins content

molecular size 485 bp was absent in cultivars C₁ and C₄ only. Two monomorphic fragments with molecular sizes 600 bp and 260 bp were generated by primer HB-9. The fragment with molecular size 1335 bp, was exhibited in cultivars C₁ and C₃ only. Seven unique bands were identified; 3 of the positive markers with molecular sizes 930 bp, 570 bp, and 420 bp had appeared with cultivar C₄ only. While the 4 negative markers with molecular sizes 800 bp, 660 bp, 500 bp, and 340 bp were absent in cultivars C₅, C₁, C₄, and cultivar C₄ respectively. Two positive markers with molecular sizes 1060 bp and 470 bp were identified with cultivars C₂ and C₁ respectively having generated by primer HB-12. Four monomorphic fragments with molecular sizes 1430, 935, 680, and 370 bp were recognized. The fragment with molecular size 820 bp was exhibited in cultivars C₁ and C₂ only, while the fragment with molecular size 715 bp appeared in cultivar C₁ and cultivar C₃ only. The results of primer HB-14 showed that 4 monomorphic fragments with molecular sizes 1500 bp, 1175 bp, 680 bp, and 620 bp had exhibited. The fragment with molecular size 735 bp was present in all cultivars except cultivar C₄ only, so it is a negative marker. While the fragments with molecular sizes 500 bp and 420 bp were absent in cultivars C₄ and C₅ only, at the same time, the fragment was recognized at molecular size 380 bp and was present in cultivar C₄ and cultivar C₅ only. One unique band was identified at about 1120 bp with cultivar C₂ having detected by primer HB-15. Seven monomorphic fragments were recognized with molecular sizes 1470 bp, 970 bp, 725 bp, 685 bp, 600 bp, 460 bp, and 375 bp

The results of ISSR analysis were pooled together to generate the dendrogram (Fig. 3). The five *Coleus* cultivars were divided into two main groups. The first comprises the four cultivars C₁, C₂, C₃, and cultivar C₅ which was subdivided into two subgroups; the first includes cultivars C₁, C₂, and cultivar C₃. The second subgroup had cultivar C₅ only. The first subgroup was divided into two clusters; the first one includes cultivars C₁ and cultivar C₃ and the second cluster had cultivar C₂ only. The second group includes the cultivar C₄ only.

Discussion

Different five cultivars of *Coleus* revealed adequate amounts of pigments (chlorophyll a, chlorophyll b, and total carotenoids) as well as phytochemical ingredients including total carbohydrates, total phenolic, total flavonoids, and total tannins. These results are in accordance with the former results which discussed the chemical contents of different species of *Coleus* (Zhang et al. 2017). The present results indicated significant variations in the chemical contents of the studied five cultivars of *Coleus* treated by different concentrations of kinetin. The different treatments of kinetin presented general increase in pigment contents in the five studied cultivars of *Coleus*. These results are analogous with the results which indicated that kinetin application improved the contents of leaf chlorophyll and photosynthesis pigments in *Vicia faba*, maize, and tobacco (Ibrahim et al. 2007; Kaya et al. 2010; Rivero et al. 2010). Application of kinetin can increase the concentration of chlorophyll in the

Table 2 Different kinetin treatments affect the chemical analysis of *Coleus blunei* (mean of two seasons)

Treatment	Cha (mg/g, FW)	Chb (mg/g, FW)	Carot (mg/g, DW)	Carb (mg/g, DW)	TP (mg/g, DW)	TF (mg/g, DW)	TT (mg/g, DW)
K1	1.908	4.515	5.851	0.246	8.729	9.223	5.259
K2	1.948	7.200	6.765	0.255	7.168	8.614	4.209
K3	2.221	9.851	9.225	0.215	7.622	9.215	4.573
K4	1.781	6.781	5.807	0.213	8.222	9.674	4.845
LSD = P ≤ 0.05	0.00465	0.00569	0.01152	0.00056	0.06486	0.19681	0.07052

K1 0 ppm, K2 100 ppm, K3 150 ppm, K4 200 ppm, Cha chlorophyll a, Chb chlorophyll b, Carot total carotenoids, Carb total carbohydrates, TP total phenolic content, TF total flavonoid content, TT total tannins content

Table 3 Interaction of different cultivars and kinetin treatments affects the chemical analysis of *Coleus blunei* (mean of two seasons)

Interaction	Cha (mg/g, FW)	Chb (mg/g, FW)	Carot (mg/g, DW)	Carb (mg/g, DW)	TP (mg/g, DW)	TF (mg/g, DW)	TT (mg/g, DW)
C1 + K1	0.782	2.377	2.177	0.232	6.190	5.707	3.633
C1 + K2	1.287	5.033	4.520	0.275	5.397	5.777	2.863
C1 + K3	2.023	7.760	8.670	0.248	6.000	7.633	3.370
C1 + K4	2.047	8.470	6.610	0.197	6.570	8.367	3.917
C2 + K1	4.083	5.073	13.523	0.253	8.713	12.740	4.997
C2 + K2	2.780	9.667	8.720	0.204	6.240	11.813	3.523
C2 + K3	3.940	14.083	12.950	0.195	5.287	10.000	3.053
C2 + K4	0.853	4.770	4.160	0.194	8.190	12.703	4.703
C3 + K1	1.437	3.357	2.960	0.248	7.380	5.593	4.420
C3 + K2	1.150	2.953	4.120	0.282	4.697	4.110	2.727
C3 + K3	1.117	4.327	3.870	0.167	6.523	6.220	4.117
C3 + K4	1.133	3.980	3.983	0.198	12.477	11.780	6.740
C4 + K1	0.893	2.953	2.677	0.229	10.950	11.963	6.807
C4 + K2	1.810	8.127	7.343	0.272	7.000	8.927	4.103
C4 + K3	1.173	4.580	4.117	0.189	6.667	7.073	4.090
C4 + K4	1.727	6.173	5.467	0.198	7.017	6.630	4.483
C5 + K1	2.343	8.817	7.920	0.268	10.413	10.110	6.440
C5 + K2	2.713	10.220	9.120	0.244	12.507	12.443	7.830
C5 + K3	2.853	18.507	16.520	0.275	13.633	15.147	8.237
C5 + K4	3.143	10.510	8.817	0.277	6.857	8.890	4.380
LSD = $P \leq 0.05$	0.01039	0.01273	0.02576	0.00125	0.14495	0.43981	0.15681

C₁ Color Clouds spicy, C₂ Beckwhites gem, C₃ Solar red, C₄ Colorblaze alligore tears, C₅ finger paint, Cha chlorophyll a, Chb chlorophyll b, Carot total carotenoids, Carb total carbohydrates, TP total phenolic content, TF total flavonoid content, TT total tannins content, K1 0 ppm, K2 100 ppm, K3 150 ppm, K4 200 ppm

plants through minimization of electrolytic leakage (Kaya et al. 2010), delaying chlorophyll decomposition by inhibition of chlorophyll activity, and through protection of chlorophyll photo-oxidation by increasing carotenoid concentration (Chernyad 2000). On the other hand, the decrease in chlorophyll and carotenoid content in some species of *Coleus* treated with some concentrations of kinetin might be owing to the degradation of these pigments or decrease in the efficiency of photosynthetic process which causes the low production of these pigments; this is in consensus with results which were

Table 4 Code and sequences of ISSR primers used in molecular characterization of identifying the five *Coleus* cultivars

PN	PS 5'→3'	TB	MB	PB	UB	P%
44B	CTC TCT CTC TCT CTC TGC	5	2	3	-	60
HB-9	GTG TGT GTG TGT GC	10	2	8	7	80
HB-12	CAC CAC CAC GC	8	4	4	2	50
HB-14	CTC CTC CTC GC	8	4	4	1	50
HB-15	GTG GTG GTG GC	8	7	1	1	12.5
Total		39	18	21	11	53.84

PN primer name, PS sequence, TB total band, MB monomorphic band, PB polymorphic band, UB unique band, P% polymorphism percentage

reported earlier (Ouzounidou et al. 2010). The variation in the total carbohydrates in different cultivars of *Coleus* treated with different concentrations of kinetin in the present study might be accompanying with the effectiveness of photosynthetic process. These findings are in agreement with Singh and Prasad (2016); they reported that kinetin treatment increased the amounts of carbohydrate and chlorophyll. The results pertaining to the effects of kinetin treatments on contents of secondary metabolites (total phenolic, total flavonoids, and total tannins) in different cultivars of *Coleus* revealed dissimilarity in the effects of kinetin in *Coleus* cultivars. One hundred fifty parts per million and 200 ppm of kinetin in this study stimulates secondary metabolites production in three cultivars of *Coleus*: Color Clouds spicy (C₁), Solar red (C₃), and finger paint cultivar (C₅). These results are in accordance with Grzegorzcyk-Karolak et al. (2017); they reported the similar stimulatory influence of cytokinins on biosynthesis process of flavonoid in *Scutellaria alpina* shoot cultures. This is analogous to the findings of Govindaraju et al. (2018) which indicated the effectiveness of cytokinin combined elicitor on enhancement of major secondary metabolite biosynthesis with stimulating phenylalanine ammonia lyase (PAL) gene expression in *Coleus aromaticus* Benth (L). The

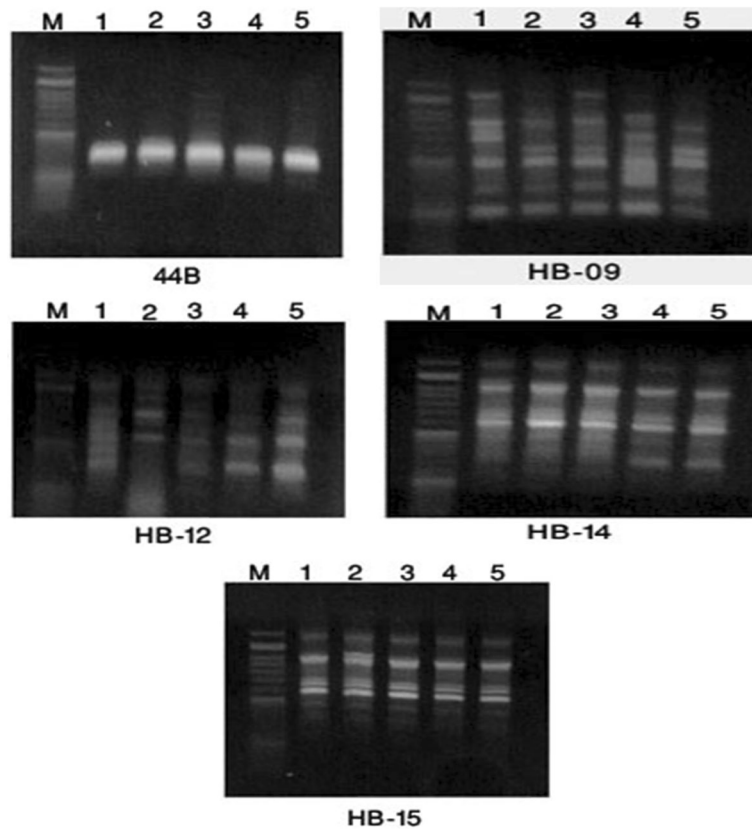


Fig. 2 ISSR profile of five cultivars of *Coleus* generated by 5 primers, M, DNA Marker; 1, Color Clouds spicy, 2, Beckwhites gem; 3, solar red, 4, Colorblaze alligatore tears; 5, finger paint

PAL stimulates the transformation of L-phenylalanine in phenylpropanoid pathway to trans-cinnamic acid which is the initiator of different polyphenolic compounds (Dixon and Paiva 1995). In this concern, Kostenyuk et al. (2002) stated that the phenolic production was improved because of overexpression of PAL in citrus transgenic roots. Consistently, Takshak and Agrawal (2018) reported that overexpression of cinnamyl alcohol dehydrogenase (CAD), PAL, dihydroflavonol reductase (DFR), 4-coumarate CoA ligase (4CL), and chalcone isomerase (CHI) was associated with amelioration of the concentrations of flavonoids, tannins, anthocyanins, and lignin in *Coleus forskohlii* Briq. under ultraviolet-B radiation.

As for the ISSR analysis, similar results were obtained by many authors. Tripathi et al. (2013) used 10 ISSR primers to assess the morphological and molecular genetic diversity in 18 *Coleus forskohlii* cultivars, which produced 80 fragments and showed 68.75% polymorphism. Twelve germplasm from different populations were assessed for molecular fingerprinting by ISSR marker and densitometric quantification of forskolin in *Coleus forskohlii* Briq. A positive correlation between molecular and chemical fingerprints reflects the feasibility of the ISSR markers in analyzing genome information related to forskolin biosynthesis from varied phylogeography (Srivastava 2017). Liu et al. (2016) assessed 25 ISSR primers which were selected to amplify 95 *Radix*

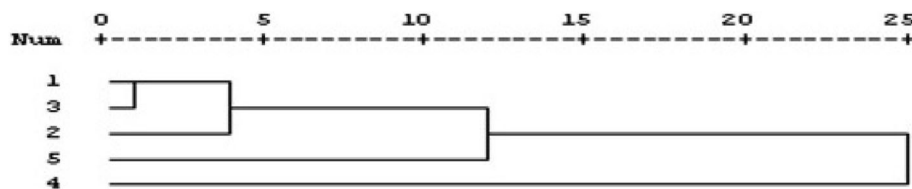


Fig. 3 Dendrogram illustrating genetic distance between the five cultivars of *Coleus* based on ISSR data

Astragali. Jia et al. (2011) selected 10 ISSR primers to analyze the genetic diversity in pecan cultivars. Shafie et al. (2011) used ISSR markers for comparative analysis of genetic diversity in wormwood capillary (*Artemisia capillaris*). Twenty ISSR primers were tested for the amplification in *Angelica sinensis* (Oliv.) by Mei et al. (2015). The genetic diversity and phylogenetic analyses of seven species of *Plectranthus* (Lamiaceae) from Saudi Arabia were carried out by using 5 ISSR primers and generated 137 polymorphic amplified fragments, which pointed a relatively high level of genetic variation in *Plectranthus* (Abdel Khalik and Osman 2017).

Conclusion

The effects of kinetin treatments (150 ppm and 200 ppm) stimulate secondary metabolite production in three cultivars of *Coleus*: Color Clouds spicy (C_1), Solar red (C_3), and finger paint species (C_5). ISSR analyses are important and are potent molecular tools for the study of genetic diversity and authentication of *Coleus* cultivars. Our study signifies the utility of metabolic and molecular fingerprints for identification of elite accessions and provides a lead to the industry for commercial exploitability of *Coleus* cultivars including its location in specific commercial cultivation.

Abbreviations

4CL: CoA ligase; bp: Base pairs; CAD: Cinnamyl alcohol dehydrogenase; Carb: Total carbohydrates; Carot: Total carotenoids; Cha: Chlorophyll a; Chb: Chlorophyll b; CHI: Chalcone isomerase; DFR: Dihydroflavonol reductase; ISSR: Inter-simple sequence repeats; MB: Monomorphic bands; P%: Percentage of polymorphism; PAL: Phenylalanine ammonia lyase; PB: Polymorphic bands; PCR: Polymerase chain reaction; PS: Primer sequence; TB: Total bands; TF: Total flavonoids; TP: Total phenolic; TT: Total tannins; UB: Unique bands

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Authors' contributions

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All data generated or analyzed during this study are included in this manuscript.

Ethics approval and consent to participate

The manuscript does not contain studies involving human participants, human or animal data, and animal or human tissue.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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