Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Identification and Analysis of Jasmonate Pathway Genes in *Coffea canephora* (Robusta Coffee) by *In Silico* Approach

Kosaraju Bharathi, H. L. Sreenath

Plant Biotechnology Division, Unit of Central Coffee Research Institute, Coffee Board, Mysore, Karnataka, India

Submitted: 22-11-2016 Revised: 02-12-2016 Published: 11-07-2017

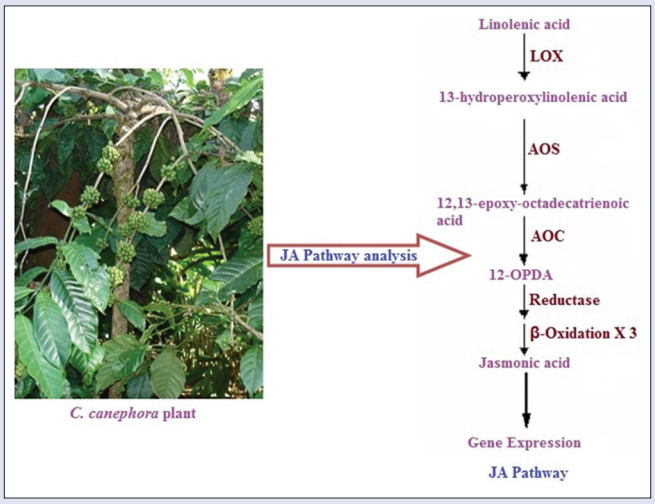
ABSTRACT

Background: *Coffea canephora* is the commonly cultivated coffee species in the world along with *Coffea arabica*. Different pests and pathogens affect the production and quality of the coffee. Jasmonic acid (JA) is a plant hormone which plays an important role in plants growth, development, and defense mechanisms, particularly against insect pests. The key enzymes involved in the production of JA are lipoxygenase, allene oxide synthase, allene oxide cyclase, and 12-oxo-phytodienoic reductase. There is no report on the genes involved in JA pathway in coffee plants. **Objective:** We made an attempt to identify and analyze the genes coding for these enzymes in *C. canephora*. **Materials and Methods:** First, protein sequences of jasmonate pathway genes from model plant *Arabidopsis thaliana* were identified in the National Center for Biotechnology Information (NCBI) database. These protein sequences were used to search the web-based database Coffee Genome Hub to identify homologous protein sequences in *C. canephora* genome using Basic Local Alignment Search Tool (BLAST). **Results:** Homologous protein sequences for key genes were identified in the *C. canephora* genome database. Protein sequences of the top matches were in turn used to search in NCBI database using BLAST tool to confirm the identity of the selected proteins and to identify closely related genes in species. The protein sequences from *C. canephora* database and the top matches in NCBI were aligned, and phylogenetic trees were constructed using MEGA6 software and identified the genetic distance of the respective genes. The study identified the four key genes of JA pathway in *C. canephora*, confirming the conserved nature of the pathway in coffee. The study expected to be useful to further explore the defense mechanisms of coffee plants. **Conclusion:** JA is a plant hormone that plays an important role in plant defense against insect pests. Genes coding for the 4 key enzymes involved in the production of JA viz., LOX, AOS, AOC, and OPR are identified in *C. canephora* (robusta coffee) by bioinformatic approaches confirming the conserved nature of the pathway in coffee. The findings are useful to understand the defense mechanisms of C. canephora and coffee breeding in the long run.

Key words: 12-oxo-phytodienoic reductase, allene oxide cyclase, allene oxide synthase, *Coffea canephora*, jasmonic acid pathway, lipoxygenase

SUMMARY

- JA is a plant hormone that plays an important role in plant defense against insect pests. Genes coding for the 4 key enzymes involved in the production of JA viz., LOX, AOS, AOC and OPR were identified and analyzed in *C. canephora* (robusta coffee) by *in silico* approach. The study has confirmed the conserved nature of JA pathway in coffee; the findings are useful to further explore the defense mechanisms of coffee plants.



Abbreviations used: *C. canephora*: *Coffea canephora*; *C. arabica*: *Coffea arabica*; JA: Jasmonic acid; CGH: Coffee Genome Hub; NCBI: National Centre for Biotechnology Information; BLAST: Basic Local Alignment Search Tool; *A. thaliana*: *Arabidopsis thaliana*; LOX: Lipoxygenase, AOS: Allene oxide synthase; AOC: Allene oxide cyclase; OPR: 12 oxo phytodienoic reductase.

Correspondence:

Ms. Kosaraju Bharathi,
Plant Biotechnology Division,
Unit of Central Coffee Research Institute,
Coffee Board, Manasagangotri,
Mysore - 570 006, Karnataka, India.
E-mail: bharathi.kosaraju@gmail.com

DOI: 10.4103/pm.pm_518_16

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Jasmonic acid (JA) and its derivatives jasmonates (JAs) play a key role in plant metabolic processes and signaling systems when they are in stress and injured from pests.[1-4] JAs, also called as oxylipins, are derived from fatty acid α-linolenic acid through the formation of different intermediates, i.e., 13-hydroperoxy-9,11,15-octadecatrienoic acid by 13-lipoxygenase (LOX), 12-oxo-phytodienoic acid (OPDA) by allene oxide synthase (AOS) and allene oxide cyclase (AOC) in cytoplasm, and finally JA by OPDA reductase (OPR) through 3-oxo-2 (2'[Z]-pentenyl) cyclopentane-1-octanoic acid by β-oxidation in peroxisomes [Figure 1].[4-6] The synthesis and signaling process of JA and JAs what we presently know is from studies on *Arabidopsis thaliana* and *Solanum lycopersicum*.[7-9] Still, in many plant species including coffee, the jasmonate pathway is yet to be studied in detail. These plants have to be studied to know if they possess exactly the same biosynthetic pathway comprising similar enzymes that perform

Cite this article as: Bharathi K, Sreenath HL. Identification and analysis of jasmonate pathway genes in *Coffea canephora* (robusta coffee) by *in silico* approach. Phcog Mag 2017;13:S196-200.

the same biological role in their metabolism as in *Arabidopsis* and *Solanum*.

Coffee is one of the most important agricultural commodities; nearly 124 species are identified and exist around the world.[10] However, only two species are considered commercially important, i.e., *Coffea arabica* L and *Coffea canephora* P, of which *C. arabica* is known for better cup quality compared to *C. canephora*. Between the two species, arabica is more sensitive to pathogens and pests (fungi, nematodes, and insects).[11] In recent years, many coffee growers are shifting to robusta coffee cultivation due to high production costs, labor scarcity, and disease and pest management associated with arabica cultivation.

The objective of the study was to identify key genes of JA biosynthesis pathway in *C. canephora*, which is one of the parents of the tetraploid arabica coffee along with *Coffea eugenioides*. Recently, *C. canephora* genome is sequenced producing a high-quality draft genome of the species.[12] The complete genome sequences of *C. canephora* are available in the web-based database of Coffee Genome Hub (http://coffee-genome.org/). The database served as a very useful resource in this study.

MATERIALS AND METHODS

Databases

GenBank Database of National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov) and database of Coffee Genome Hub (http://coffee-genome.org/) were used to identify the presence of proteins of the key enzymes involved in JA synthesis.

Software

Molecular evolutionary genetic analysis software MEGA6 (http://mega6.software.informer.com/) was used for sequence alignment and construction of phylogenetic trees.

Identification of protein sequence of key genes of *Coffea canephora* involved in jasmonic acid biosynthesis

Protein sequence of different genes involved in the JA synthesis was taken from the model plant *A. thaliana* using NCBI database. Those sequences were used for Basic Local Alignment Search Tool (BLAST) search[13] and the Coffee Genome Hub database to identify the respective gene sequences in *C. canephora*. Among the matches in *C. canephora*, top match for each gene was taken and used for BLAST search against NCBI database. The top matches for *C. canephora* proteins involved in the JA pathway obtained in NCBI BLAST search were used for sequence alignment to obtain phylogeny trees using MEGA6 software.[14]

Construction of phylogenetic trees

Around 14 top matches in NCBI database for each protein involved in the JA pathway and 1 top match from coffee genome database were taken from the BLAST results and transferred the sequence data to MEGA ALIGN tool in MEGA6 software. Using CLUSTALW alignment, the obtained sequences were aligned and by neighbor-joining method with bootstrap value 1000, the phylogenic trees were constructed.[15]

RESULTS AND DISCUSSION

The main enzymes that play a key role in the biosynthesis of JA are LOX, AOS, AOC, and OPR[1] [Figure 1]. The enzyme LOX is involved in the production of the intermediate compound 13-HPOT by adding oxygen to α-linolenic acid at either C9 or C13 position, with 9S- or 13S-hydroperoxides.[16-18] AOS and AOC are involved in the formation of 12-OPDA through unstable compound, an allene oxide.[19] It is encoded by single gene in *Arabidopsis*,[20,21] two genes in tomato.[22] OPR belongs to a small family of related flavin-dependent oxidoreductases forms the JA through β-oxidation in peroxisome.[4,6,23,24] The proteins of these enzymes were taken from model plant *A. thaliana* in NCBI database. Selected protein sequences of *A. thaliana* were used to identify homologous protein sequences in *C. canephora* genome from Coffee Genome Hub database using BLAST tool. We identified the matching protein sequences related to LOX, AOS, AOC, and OPR enzymes with similarities ranging from 2 to 17 [Table 1].

The same sequences were cross-checked again from coffee genome database with NCBI database and identified as unnamed protein products related to LOX, AOS, AOC, and OPR enzymes in

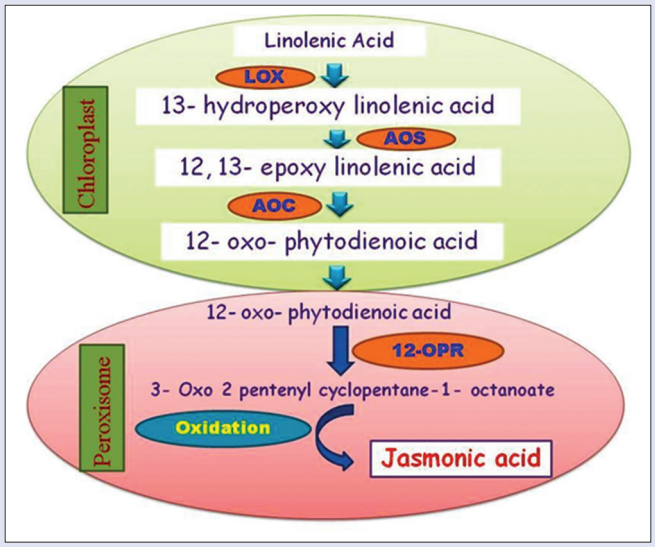


Figure 1: Jasmonic acid is synthesized from fatty acid linolenic acid. Lipoxygenase, allene oxide synthase, allene oxide cyclase are the key enzymes of jasmonate biosynthesis in chloroplast, and they form oxo-phytodienoic acid. Oxo-phytodienoic acid is transported to peroxisome. Reduction of cyclopentanone ring of oxo-phytodienoic acid is catalyzed by peroxisomal oxo-phytodienoic acid reductase enzyme. Three cycles of β-oxidation occur to give finally jasmonic acid

Table 1: *Arabidopsis thaliana* protein sequences of key genes involved in jasmonic acid biosynthesis from National Center for Biotechnology Information used as query and *Coffea canephora* matches obtained in coffee genome hub database

Gene name	NCBI accession numbers of *Arabidopsis thaliana* protein sequence used	Number of matches to *Coffea canephora* proteins in coffee genome hub	Top match in coffee genome hub (locus and *E* value)
LOX	AAF97315.1	11	Cc00 g30760, 0
AOS	NP_199079.1	7	Cc10 g03580, 0
AOC	CAC83763.1	2	Cc07 g09040, 2e-81
OPR	CAB66143.1	17	Cc06 g12110, 0

OPR: OPDA reductase; OPDA: 12-oxo-phytodienoic acids; AOC: Allene oxide cyclase; AOS: Allene oxide synthase; LOX: Lipoxygenase; NCBI: National Center for Biotechnology Information

Table 2: Matches obtained for 12-oxophytodienoate reductase in *Coffea canephora* from coffee genome database. *Arabidopsis thaliana* protein sequence CAB66143.1 was used as query

Gene name	*E*	Query cover	Identity percentage	Locus ID
12-OPR 3~OPR3~complete	0	97.44	75.32	Cc06_g12110
Putative 12-OPR 11~OPR11~complete	8e-98	65.22	55.08	Cc10_g09310
12-OPR 2~OPR2~complete	5e-138	95.65	53.99	Cc10_g09320
12-OPR 2~OPR2~complete	2e-139	96.68	52.76	Cc10_g09360
Predicted protein~OPR11~modules	5e-18	18.93	51.35	Cc10_g09300
Putative 12-OPR 11~OPR11~complete	8e-131	95.65	51.32	Cc10_g16500
12-OPR 1~OPR1~fragment	6e-95	71.1	51.25	Cc10_g09330
12-OPR 2~OPR2~complete	8e-134	95.65	51.05	Cc10_g09340
Putative 12-OPR 11~OPR11~complete	5e-110	86.96	50.88	Cc06_g02480
12-OPR 1~OPR1~complete	1e-129	96.68	50.79	Cc10_g09350
12-OPR 2~OPR2~complete	6e-128	95.65	48.35	Cc10_g16490
Putative 12-OPR 11~OPR11~complete	6e-109	91.82	48.06	Cc00_g31410
Putative 12-OPR 11~OPR11~complete	1e-106	122.76	47.82	Cc10_g09290
12-OPR 2~OPR2~fragment	1e-37	35.81	47.14	Cc10_g16510
Putative 12-OPR 11~OPR11~complete	3e-100	134.78	46.97	Cc10_g16520
12-OPR 1~OPR1~complete	2e-103	95.65	44.92	Cc09_g03820
12-OPR 1~OPR1~fragment	5e-35	40.15	43.31	Cc10_g16530

OPR: OPDA reductase; OPDA: 12-oxo-phytodienoic acid

Table 3: Matches obtained for allene oxide synthase in *Coffea canephora* from coffee genome hub database. *Arabidopsis thaliana* protein sequence NP_199079.1was used as query

Gene name	*E*	Query cover	Identity percentage	Locus
AOS, chloroplastic~CYP74A~complete	0	99.42	66.41	Cc10_g03580
Hypothetical protein~CYP74A~missing_functional_completeness	1e-44	22.59	64.10	Cc00_g35230
AOS, chloroplastic~CYP74A~complete	0	89.96	63.81	Cc10_g03570
Hypothetical protein~CYP74A~missing_functional_completeness	2e-42	22.59	62.39	Cc05_g00930
AOS~CYP74A2~complete	1e-172	90.15	52.65	Cc02_g18130
9-divinyl ether synthase~DES~complete	7e-174	89.58	52.55	Cc02_g18120
Putative AOS, chloroplastic~CYP74A~complete	2e-119	86.87	40.66	Cc05_g03650

AOS: Allene oxide synthase

Table 4: Matches obtained for lipoxygenase in *Coffea canephora* genome from coffee genome hub database. *Arabidopsis thaliana* protein sequence AAF97315.1 was used as query

Gene name	*E*	Query cover	Identity percentage	Locus
Linoleate 13S-lipoxygenase 3-1, chloroplastic~LOX3.1~complete	0	87.61	73.41	Cc00_g30760
Linoleate 13S-lipoxygenase 3-1, chloroplastic~LOX3.1~fragment	5e-69	19.96	63.24	Cc05_g01710
Lipoxygenase 6, choloroplastic~LOX6~complete	0	93.42	54.62	Cc11_g16680
Linoleate 13S-lipoxygenase 3-1, chloroplastic~LOX3.1~complete	0	99.56	49.58	Cc02_g13400
Linoleate 13S-lipoxygenase 2-1, chloroplastic~LOX2.1~complete	0	81.69	47.81	Cc01_g04060
Linoleate 13S-lipoxygenase 3-1, chloroplastic~LOX3.1~fragment	2e-20	12.94	45.11	Cc00_g27370
Probable linoleate 9S-lipoxygenase 5~LOX1.5~complete	0	85.96	44.71	Cc02_g33790
Probable linoleate 9S-lipoxygenase 5~LOX1.5~complete	0	85.96	44.71	Cc02_g33800
Probable linoleate 9S-lipoxygenase 5~LOX1.5~complete	0	91.56	44.68	Cc02_g33320
Probable linoleate 9S-lipoxygenase 5~LOX1.5~complete	0	92	44.25	Cc02_g33780
Linoleate 9S-lipoxygenase 5, chloroplastic~LOX5~complete	0	94.74	43.62	Cc03_g03580

LOX: Lipoxygenase

Table 5: Matches obtained for allene oxide cyclase in *Coffea canephora* from coffee genome hub database. *Arabidopsis thaliana* protein sequence CAC83763.1 was used as query

Gene name	*E*	Query cover	Identity percentage	Locus
AOC 4, chloroplastic~AOC4~complete	2e-81	74.81	64.77	Cc07_g09040
AOC 4, chloroplastic~AOC4~complete	4e-89	100	55.73	Cc11_g10540

AOC: Allene oxide cyclase

C. canephora. The maximum number of similarities obtained in coffee genome database was 17 for OPR [Table 2], 7 for AOS [Table 3] and 11 for LOX [Table 4]. The minimum number of similarities obtained was 2 for AOC [Table 5]. Top one similarity for each enzyme was taken as main source to confirm with NCBI by doing BLAST. Matches in NCBI database for each gene were taken for phylogenetic tree construction. The evolutionary history was inferred using the neighbor-joining method.[25] The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.[26] The evolutionary distances were computed using the Poisson correction method[27] and are in the units of the number of amino acid substitutions per site. The analysis involved

15 amino acid sequences. Evolutionary analyses were conducted in MEGA6.[28] LOX, AOS, AOC, and OPR trees mainly show two broad clusters. Each cluster is further divided into subclusters.

The LOX tree mainly shows two broad clusters. Each cluster is further divided into subclusters. For LOX enzyme protein, one subcluster unnamed protein product from *C. canephora* and 13s-LOX (Cc00_g30760) from coffee genome was showing bootstrap value 100. In addition to this, *Theobroma cacao* and *Gossypium hirsutum* also showed bootstrap value 100 [Figure 2]. In AOS, one subcluster unnamed protein product from *C. canephora* and AOS (Cc10_g03580) from coffee genome was showing bootstrap value 100; in addition to this, *T. cacao*, *G. hirsutum, Glycine max,* and *Lotus japonicus* also showing bootstrap value 100 and *Catharanthus roseus* 99 [Figure 3]. In AOC, one subcluster unnamed protein product from *C. canephora* and AOC 4 (Cc07_g09040) from coffee genome was showing bootstrap value 100. *Nicotiana tabacum* and *Petunia* x *hybrida* were showing 90 and *S. lycopersicum* was showing bootstrap value 100. In addition to this, *T. cacao* and *G. hirsutum* are showing bootstrap value 99 [Figure 4]. OPR tree also mainly shows two broad clusters. Each cluster is further divided into subclusters. In subcluster I, unnamed protein product from *C. canephora* and 12-OPR (Cc06_g12110) which was taken from coffee genome was showing bootstrap value 100. In addition to this, *T. cacao* and *G. hirsutum* also showing bootstrap value 100; *Nicotiana tabacum* and *S. lycopersicum* showing bootstrap value 99 [Figure 5].

The present study confirms the presence of genes involved in the synthesis of JA pathway in *C. canephora,* and further studies are needed to know the complete mechanism of these genes during signal transduction in resistance from pests compared to other coffee species.

CONCLUSION

JA pathway is reported to have mainly LOX, AOS, AOC, and OPR enzymes occurs in chloroplast and peroxisome, but there was no report of the presence of these enzymes in coffee plants. From the above study, we are concluding that the JA synthesis enzymes present in the pest-resistant coffee plant *C. canephora* and identified the close relatives of each gene in the NCBI database related to coffee plant. This study will be useful to understand the resistance mechanism in *C. canephora*, and this study is helpful to increase the resistance in other coffee plants such as *C. arabica* by artificial JA application in their cultivation.

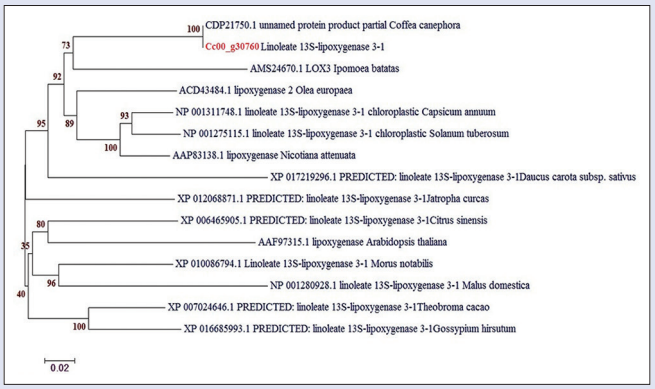


Figure 2: Phylogenetic tree for lipoxygenase enzyme protein

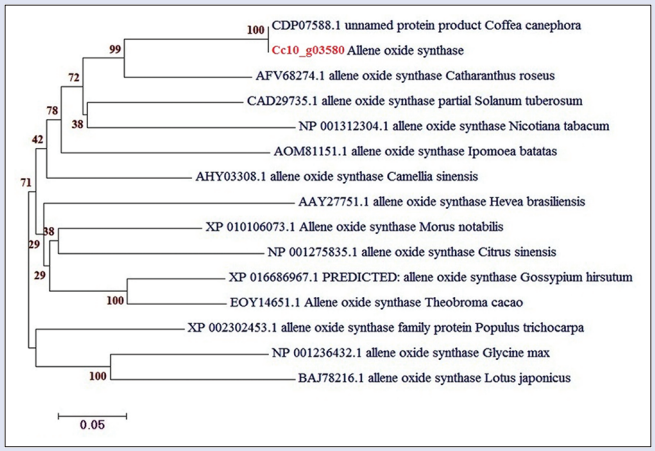


Figure 3: Phylogenetic tree for allene oxide synthase enzyme protein

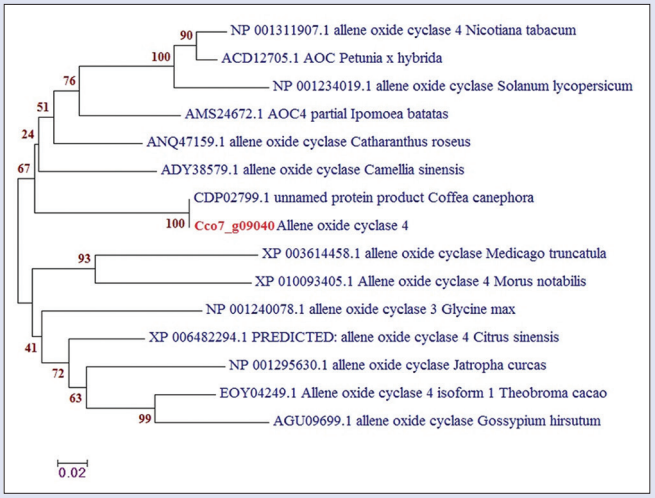


Figure 4: Phylogenetic tree for allene oxide cyclase enzyme protein

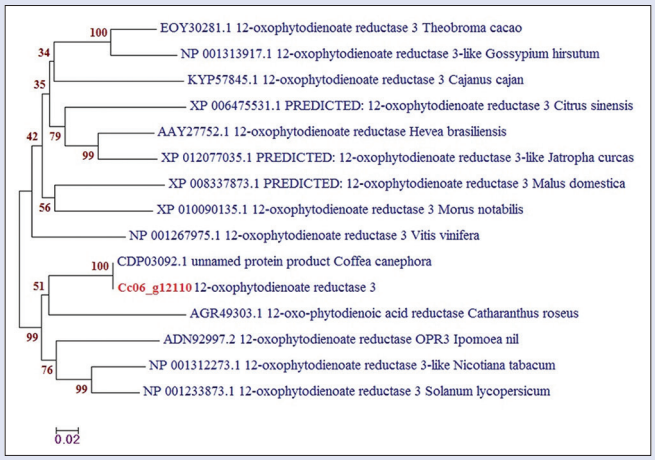


Figure 5: Phylogenetic tree for the oxo-phytodienoic acid reductase enzyme protein

Acknowledgement

The authors thank the authorities of Plant Biotechnology Division, Unit of Central Coffee Research Institute, Coffee Board, Mysore, for providing the necessary support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Farmer EE, Ryan CA. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 1992;4:129-34.
2. Creelman RA, Mullet JE. Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 1997;48:355-81.
3. Kramell R, Atzorn R, Schneider G, Miersch O, Bruckner C, Schmidt J, *et al.* Occurrence and identification of jasmonic acid and its amino-acid conjugates induced by osmotic-stress in barley leaf tissue. J Plant Growth Regul 1995;14:29-36.
4. Browse J. Jasmonate passes muster: A receptor and targets for the defense hormone. Annu Rev Plant Biol 2009;60:183-205.
5. Vick BA, Zimmerman DC. Biosynthesis of jasmonic acid by several plant species. Plant Physiol 1984;75:458-61.
6. Rosahl S, Feussner I. Oxylipins. In: Murphy DJ, editor. Plant Lipids: Biology, Utilization and Manipulation. Oxford and Boca Raton: Blackwell Publishing Ltd./CRC Press; 2005. p. 329-54.
7. Blechert S, Bockelmann C, Brummer O, Fusslein M, Gundlach H, Haider G, *et al.* Structural separation of biological activities of jasmonates and related compounds. J Chem Soc Perkin Trans 1997;23:3549-59.
8. Narvaez-Vasquez J, Florin-Christensen J, Ryan CA. Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. Plant Cell 1999;11:2249-60.
9. Turner JG, Ellis C, Devoto A. The jasmonate signal pathway. Plant Cell 2002;14 Suppl 1:S153-64.
10. Davis AP, Maurin O, Chester M, Mvungu EF, Fay MF. Phylogenetic relationship in *Coffea* (*Rubiaceae*) inferred from sequence data and morphology. Proc Int Sci Colloq Coffee 2006;21:868-75.
11. De Castro, Renato D, Marraccini P. Cytology, biochemistry and molecular changes during coffee fruit development. Braz J Plant Physiol 2006;18:175-199.
12. Denoeud F, Carretero-Paulet L, Dereeper A, Droc G, Guyot R, Pietrella M, *et al.* The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science 2014;345:1181-4.
13. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, *et al.* BLAST: Architecture and applications. BMC Bioinformatics 2009;10:421.
14. Kumar S, Tamura K, Nei M. MEGA: Molecular evolutionary genetics analysis software for microcomputers. Comput Appl Biosci 1994;10:189-91.
15. Higgins DG, Sharp PM. CLUSTAL: A package for performing multiple sequence alignment on a microcomputer. Gene 1988;73:237-44.
16. Heitz T, Bergey DR, Ryan CA. A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. Plant Physiol 1997;114:1085-93.
17. Feussner I, Wasternack C. The lipoxygenase pathway. Annu Rev Plant Biol 2002;53:275-97.
18. Howe GA, Schilmiller AL. Oxylipin metabolism in response to stress. Curr Opin Plant Biol 2002;5:230-6.
19. Song WC, Funk CD, Brash AR. Molecular cloning of an allene oxide synthase: A cytochrome P450 specialized for the metabolism of fatty acid hydroperoxides. Proc Natl Acad Sci U S A 1993;90:8519-23.
20. Laudert D, Weiler EW. Allene oxide synthase: A major control point in *Arabidopsis thaliana* octadecanoid signalling. Plant J 1998;15:675-84.
21. Sivasankar S, Sheldrick B, Rothstein SJ. Expression of allene oxide synthase determines defense gene activation in tomato. Plant Physiol 2000;122:1335-42.
22. Ziegler J, Stenzel I, Hause B, Maucher H, Hamberg M, Grimm R, *et al.* Molecular cloning of allene oxide cyclase. The enzyme establishing the stereochemistry of octadecanoids and jasmonates. J Biol Chem 2000;275:19132-8.
23. Delker C, Stenzel I, Hause B, Miersch O, Feussner I, Wasternack C. Jasmonate biosynthesis *in Arabidopsis thaliana* – Enzymes, products, regulation. Plant Biol (Stuttg) 2006;8:297-306.
24. Liechti R, Farmer EE. Jasmonate biochemical pathway. Sci STKE 2006;322:1-3.
25. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4:406-25.
26. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 1985;39:783-91.
27. Zuckerkandl E, Pauling L. Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ, editors. Evolving Genes and Proteins. New York: Academic Press; 1965. p. 97-166.
28. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30:2725-9.