2021

Discovering Genes Involved in the Synthesis of Secondary Metabolites From the Seeds of Moringa Oleifera Through Transcriptome Analysis

Vivian A. Panes
Ateneo de Manila University

Ma. Divina Kristi A. Discar
Ateneo de Manila University

Recommended Citation

Panes, V. A., & Discar, M. D. K. A. (2021). Discovering genes involved in the synthesis of secondary metabolites from the seeds of Moringa oleifera through transcriptome analysis. Acta Horticulturae, 1306, 51–58. https://doi.org/10.17660/ActaHortic.2021.1306.7

Discovering genes involved in the synthesis of secondary metabolites from the seeds of *Moringa oleifera* through transcriptome analysis

V.A. Panes[a] and M.D.K.A. Discar

Department of Biology, School of Science and Engineering, Ateneo de Manila University, Loyola Heights, 1108 Quezon City, Philippines.

Abstract

***Moringa oleifera* is a widely used crop that produces seeds with a plethora of benefits encompassing health and nutrition. Secondary metabolite compounds were determined in the seeds of *Moringa oleifera* that possess nutritional and pharmacological benefits. Although various phytochemical researchers reported the presence of secondary metabolites in *M. oleifera* seeds, there is a lack of research on the genes encoding for enzymes that catalyze the synthesis of secondary metabolites in the seeds of *M. oleifera*. In the present study, RNA sequencing was used to analyze the transcriptome of the mature seed embryos of *M. oleifera*. Biological pathway analysis revealed 416 upregulated genes encoding for 11 enzymes involved in the catalytic steps of the phenylpropanoid and flavonoid pathways, and 63 unigenes encoding for 8 enzymes involved in the catalytic steps of the alkaloid pathway. These findings however need further validation using qRT-PCR which is a reliable and robust technique in order to validate the presence and expression of genes encoding for enzymes leading to the synthesis of secondary metabolites in the mature seed embryos of *M. oleifera*.**

Keywords: transcriptome, RNA-sequencing, secondary metabolites, *M. oleifera*

INTRODUCTION

Many studies have reported on the identification of various secondary metabolite compounds in *M. oleifera* leaves, however, there is a lack of information on these compounds in *M. oleifera* seeds, particularly at the transcriptome level. The transcriptome includes the set of transcripts or Ribonucleic acids (RNA) including protein-coding mRNA (mRNA) and non-coding small RNAs (rRNA, tRNA and miRNA). Transcriptomics aims to identify and catalog these transcripts, determine the transcriptional structure of genes, and quantify gene expression levels (Wang et al., 2009). Transcriptomics has been used in establishing secondary metabolic pathways and mining of genes in several economically and nutritionally important seeds in non-model plants such as *Cicer arietinum* or chickpea (Pradhan et al., 2014), *Brassica juncea* or rapeseed (Liu et al., 2013) and *Brassica oleracea* broccoli (Gao et al., 2014). The main objective of this study was to examine the transcriptome profile of the mature seed embryos of *M. oleifera* and to identify genes encoding for enzymes involved in secondary metabolite pathways.

MATERIALS AND METHODS

Plant material for total RNA extraction and RNA sequencing

A total of 30 early mature embryos were collected from 10 trees in Muñoz, Nueva Ecija Philippines. The total RNA extracts from *M. oleifera* mature embryos were used to construct a cDNA library and was subjected to RNA sequencing using the Illumina Hiseq platform through the Ambry Genetics Company in Aliso Viejo, California, USA (Panes et al., 2017). The illumina reads were assembled de novo using the Trinity and SOAP assemblers.

[a]E-mail: vpanes@ateneo.edu

Functional annotation and biological pathway analysis of genes encoding for enzymes for major secondary metabolite pathways of *Moringa oleifera* mature embryo transcriptome

The assembled transcripts were subjected to blastx alignment in NCBI Non-redundant (Nr) database. The FASTA sequences were used as the query sequence and the 'flowering plants (taxid: 3398)' was chosen for 'Organism option'. Transcripts (from Trinity and SOAP-de novo assemblies) were subjected to KEGG classification using KEGG Automatic Annotation Server (KAAS) for KEGG orthology (KO) assignment, enabling KEGG pathway mapping and BRITE mapping (Moriya et al., 2007). Single-directional best hit (SBH) method was used to assign the orthologs using the BLAST program.

Analysis of expression level of genes encoding for enzymes involved in the synthesis of secondary metabolites in the *M. oleifera* mature seeds

The level of gene expression for secondary metabolites were classified into groups based on their fragment per kilobase per molecule (FPKM) values (Toung et al., 2011): low expression – bottom 25th percentile; medium expression – middle 50th percentile; high expression – top 25th percentile.

RESULTS AND DISCUSSION

Identification of upregulated unigenes encoding for enzymes involved in phenylpropanoid and flavonoid pathways

There are a total of 11 enzymes encoded by 416 upregulated unigenes involved in the phenylpropanoid and flavonoid pathways (Table 1). Phenylpropanoids provide an array of benefits such as defense against pests (Miedes et al., 2014), as structural polymer of plant cell walls (Vogt, 2010), and for pharmacological benefits (Korkina, 2007).

Table 1. Enzymes encoded by the unigenes for the synthesis of phenylpropanoids and flavonoids, the corresponding number and the FPKM values (gene expression levels of *M. oleifera* mature seeds) are also shown.

Enzyme code	Abbreviations	Enzymes	No. of unigenes	FPKM
EC: 4.3.1.24	PAL	Phenylalanine ammonia lyase	19	46.93
EC: 1.11.1.7	E1.11.1.7	Peroxidase	86	702.73
EC: 1.2.1.44	CCR	Cinnamoyl-CoA reductase	1	94.00
EC: 2.3.1.133	HCT	Shikimate O-hydroxycinnamoyl-transferase	13	92.29
EC: 1.14.13.11	CYP73A	trans-cinnamate 4-monooxygenase	5	78.15
EC: 3.2.1.21	E3.2.1.21	beta-glucosidase	250	88.96
EC: 2.1.1.1.104	E21.1.1.104	Caffeoyl-CoA O-methyltransferase	8	395.71
EC: 2.1.1.68	COMT	Caffeic acid 3-O-methyltransferase	9	710.51
EC: 1.14.13.36	CYP98A3	Coumaroylquinate (coumaroylshikimate) 3'monooxygenase	2	41.92
EC: 3.4.16.-2.3.1.91	SCT	Serine carboxypeptidase-like 19	19	294
EC: 1.14.11.9	F3H	Naringenin 3-dioxygenase	4	91.99
Total no. of unigenes			**416**	

Phenylpropanoid biosynthesis initiates from phenylalanine. Through a series of catalytic reactions, various phenylpropanoid-derived metabolites are produced including lignin, flavonoid, coumarin and stilbene (Vogt, 2010) (Figure 1). Flavonoids are generally synthesized through the phenylpropanoid pathway, which provides P-coumaryl CoA, a branching-point metabolite which initiates the central flavonoid biosynthetic pathway.

Phenylalanine ammonia lyase (PAL) is involved in the first and committed step in the

phenylpropanoid pathway (Figure 1) and is therefore involved in the biosynthesis of the polyphenol compounds such as flavonoids, phenylpropanoids, and lignin in plants. There are 19 (Table 1) upregulated unigenes (FPKM 46.93) identified in this study which imply that *M. oleifera* seeds are rich in secondary metabolites, flavonoids and phenylpropanoids. Other abundant (86) (Table 1; Figure 1) unigenes that are upregulated (FPKM 702.73) are those encoding for peroxidase which catalyze the synthesis of guiacyl lignin, syringin lignin, and P-hydroxy-phenyl lignin (Figure 1). Those upregulated unigenes suggest that lignin is accumulated and there is an abundant phenylpropanoid-derived compound in the seeds of *M. oleifera*. Lignin is well-known for its role in structural functions (Vogt, 2010) in plant development and in plant defense (Hematy et al., 2009). Aside from its involvement in plants, there is also a research interest on the potential bioactivities of lignin such as the suppression of apoptosis in neural cells caused by oxidative stress. The catalytic mechanism of plant peroxidase involves the reduction of hydrogen peroxide to water. This function supports various studies on the potential functions of plant peroxidase including soil detoxification and waste water treatment (Gholani-Borujeni et al., 2011), particularly the water purification and detoxification properties of *M. oleifera* seeds (Hamid and Rehman, 2009). There is one upregulated unigene (FPKM 94.00) encoding for the enzyme cinnamoyl-CoA reductase which plays a critical role in lignin formation. Peroxiredoxins (classified as peroxidases) are a ubiquitous family of antioxidant enzymes that control cytokine-induced peroxide levels and thereby mediate signal transduction.

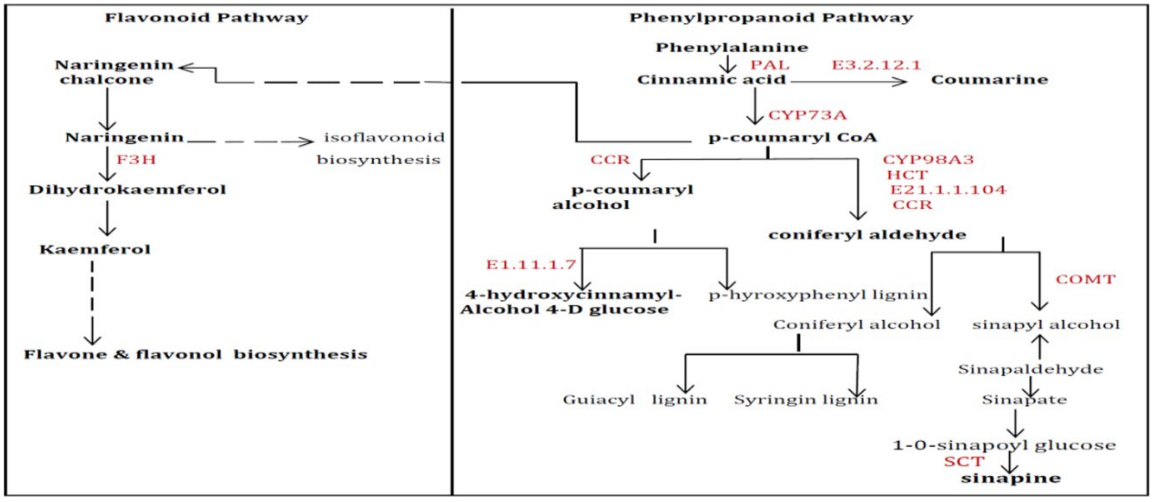

Figure 1. Schematic diagram of the flavonoid and phenylpropanoid pathways modified from KEGG showing the enzymes (represented by enzyme codes) encoded by upregulated unigenes in the mature embryos of *M. oleifera*. PAL (phenylalanine ammonia lyase); E3.2.1.21 (beta-glucosidase); CYP73A (trans-cinnamate 4-monooxygenase); CCR (Cinnamoyl-CoA reductase); CYP98A3 (coumaroylquinate (coumaroylshikimate 3' monooxygenase; HCT (shikimate O-hydroxycinnamoyl-transferase); E21.1.1.104 (caffeoyl-CoA O-methyltransferase); E1.11.1.7 (peroxidase); COMT (caffeic acid 3-O-methyltransferase); SCT (serine carboxypeptidase-like 19); F3H (naringenin 3-dioxygenase).

Peroxiredoxin 6, 1-Cys peroxiredoxin (PRX6) unigenes were identified in the *M. oleifera* mature seeds in this study. The 1-*CysPrx* genes (Stacy et al., 1996) were also identified and expressed in the embryos of *Hordeum vulgare* L. seeds (Aalenf et al., 1994). Expression of *Hv1-CysPrx* in late stage seed development was detected in the embryos of barley grains. Those findings support the present study on the presence of peroxiredoxin 6, 1-Cys peroxiredoxin in the seeds of *M. oleifera*.

Thirteen upregulated unigenes (FPKM 92.29) (Table 1) were identified encoding for shikimate O-hydroxycinnamoyl-transferase (Figure 1) which controls the biosynthesis and

turnover of major plant phenolic compounds such as lignin and chlorogenic acid. There are 5 upregulated unigenes (FPKM 78.15) (Table 1) encoding for trans-cinnamate 4-monooxygenase (Figure 1) that were identified in the *M. oleifera* transcriptome involved in the synthesis of coumaryl. This enzyme participates in phenylalanine metabolism and phenylpropanoid biosynthesis. It belongs to the family of oxidoreductases suggesting an antioxidant activity for *M. oleifera* seeds.

The most abundant (250) upregulated unigenes (FPKM 88.96) (Table 1) are those encoding for beta-glucosidase (*bglB*) (Figure 1) which catalyze the synthesis of coumarin from cinnamic acid. Coumarin is a major phenylpropanoid-derived metabolite (Yu et al., 2009). It is used in the pharmaceutical industry as a precursor reagent in the synthesis of a number of synthetic anticoagulant pharmaceuticals, such as warfarin and has a clinical medicinal value as an edema modifier. Eight upregulated unigenes (FPKM 395.71) (Table 1) encoding for caffeoyl CoA O-methyltransferase (Figure 1) were identified. This enzyme methylates the reaction from caffeoyl-CoA to Feruloyl-CoA and plays an essential role in the synthesis of guaiacyl lignin units as well as in the supply of substrates for the synthesis of syringyl lignin units. Derived compounds from the catalytic reactions from P-coumaryl CoA to monolignols through this enzyme such as caffeic acid and ferullic acid were isolated from the seeds of *M. oleifera* and found to have antioxidant activities (Ogbunugafor et al., 2011). Giordano et al. (2016) detected high expression of caffeoyl-CoA-O-methyltransferase genes in the different tissues (petioles, berry skin, pulp and seeds) of *V. vinifera*, one of the top species with the highest protein hits for *M. oleifera* in this study. Unigenes encoding caffeoyl-CoA O-methyltransferase involved in gingerol biosynthesis are upregulated. Products of this reaction lead to the synthesis of gingerol, the major pharmacologically-active component of ginger known for a variety of biological activities including anticancer, anti-inflammation, and anti-oxidation (Wang et al., 2014).

Nine upregulated unigenes (FPKM 710.51) (Table 1) were identified encoding for the enzyme caffeic acid 3-O-methyltransferase (Figure 1) which catalyzes the conversion of caffeic acid to ferulic acid and of 5-hydroxyferulic acid to sinapic acid, an orally bioavailable phytochemical extensively found in spices, citrus and berry fruits, vegetables, cereals, and oilseed crops known to exhibit antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic, neuroprotective, and antibacterial activities. Sinapic acid is a bioactive phenolic acid and has the potential to attenuate various chemically induced toxicities. Piceatannol was also identified in *M. oleifera* mature embryo, a stilbenoid which is a metabolite of resveratrol found in grapes (*Vitis vinifera*) one of the homologous species with the highest protein hits for *M. oleifera* in this study. Piceatannol blocks a viral protein-tyrosine kinase implicated in leukemia, non-Hodgkin's lymphoma and other diseases associated with Epstein-Barr virus (Geahlen and McLaughlin, 1989). There are 2 upregulated unigenes identified (FPKM 41.92) (Table 1) encoding for coumaroylquinate 3’ monooxygenase (Table 1) which belongs to the family of oxidoreductases, acting on paired donors, with O_2 as oxidant and incorporation or reduction of oxygen. This finding confirms that *M. oleifera* seeds contain antioxidants. Nineteen upregulated unigenes (FPKM 294) (Table 1) encode for serine carboxypeptidase-like 19 (SCT) (Figure 1) that catalyzes the synthesis of sinapine expressed in the mature seeds of *Arabidopsis thaliana* (Huang et al., 2008). Sinapine is a derivative of sinapic acid, an alkaloidal amine found in oil seeds of plants in the family *Brassicaceae*. *M. oleifera* is closely related to the cruciferous plants of the family *Brassicaceae* (order *Brassicales*). Four upregulated unigenes (FPKM 91.99) (Table 1) were identified encoding for naringenin 3-dioxygenase (F3H) enzyme (Figure 1) involved in the synthesis of dihydrokaempferol and kaempferol. Kaempferol, derived from quercetin is a flavonol, found in many edible plants and used in traditional medicine (Calderón-Montaño et al., 2011). Numerous studies reported the health potentials of kaempferol including anticancer, antioxidant, antiviral (Calderón-Montaño et al., 2011), antidiabetes and prevention of heart disease (Khalil and Sulaiman, 2010). Flavonol synthase (FLS) catalyzes the reaction from dihydrokaempferol to kaempferol. Lalas and Tsaknis (2002) reported that the seeds of *M. oleifera* contain a type of flavonoid, Myricetin derived from quercetin that had higher antioxidant activity than the commonly used antioxidant BHT and α-tocopherol in the sunflower oil.

Identification of transcripts encoding enzymes involved in alkaloid biosynthesis

Alkaloids are a diverse group of nitrogen-containing compound derived mostly from amino acids (Ziegler and Facchini, 2008). There are a total of 63 unigenes encoding for 8 enzymes involved in the alkaloid pathway (Table 2).

Table 2. Enzymes encoded by upregulated unigenes for the synthesis of alkaloids and the corresponding number of unigenes involved in the alkaloid pathway for *M. oleifera* seed.

Enzyme code	Abbreviations	Enzymes	No. of unigenes	FPKM
EC: 1.4.3.21	AOC3	Primary-amine oxidase	17	364
EC: 2.6.1.1	ASP5	Aspartate aminotransferase (chloroplastic)	2	342.59
EC: 2.6.1.5	TAT	Tyrosine aminotransferase	2	35.13
EC: 4.1.1.25	E4.1.1.25	Tyrosine decarboxylase	6	42.86
EC: 2.6.1.1	GOT1	Aspartate aminotransferase (cytoplasmic)	7	900.53
EC: 2.6.1.1 2.6.1.78 2.6.1.79	PAT	Bifunctional aspartate aminotransferase and glutamate/aspartate-prephenate aminotransferase	4	
EC: 1.1.1.206	TR1	Tropinone reductase I	8	533.04
EC: 2.7.2.4	lysC	Aspartate kinase	17	415.84
Total number of unigenes			**63**	

There are 17 unigenes (Table 2) that are upregulated (FPKM 364) encoding for primary amine oxidase (AOC3) (Figure 2) which catalyze the oxidation of various amines, act as a disulphide-linked homodimer, and catalyze the oxidation of primary amines to aldehydes. A study by Parsons et al. (1995) showed that the biosynthesis from L-Dopa to dihydroxyphenylacetaldehyde is considered toxic to *Aedes aegypti*, similar to the study of Vavricka et al. (2011) that proved that the seeds of *M. oleifera* have larvicidal activity against *A. aegypti.* The primary amine oxidase (AOC3) is involved in the catalytic reaction for the biosynthesis of piperidine (Figure 2) known as a solvent, as a base and as universal building blocks in the synthesis of pharmaceuticals and fine chemicals. Also, piperidine alkaloid in plants were reported to have anti-rheumatism activity and as analgesic (Plunkett and Sainsburry, 1991). Conversely, tropine is a derivative of tropane. Seeds of other species of *Moringa*, *M. peregrina* was reported to contain tropane alkaloids (Rhou-Boroujeni et al., 2016). Through an enzyme tropinone reductase I (TR1, 8 unigenes), 1-methyl pyrrolinium is converted into tropine (Figure 2). Transcripts encoding for primary amine oxidase (AOC3), tropine reductase I (TR1) and aspartate aminotransferase cytoplasmic (GOT1) enzymes are upregulated (Figure 2). This finding suggests that alkaloids are highly expressed in the seeds of *M. oleifera*. This is parallel to the finding of Ijarotimi et al. (2013) that the *M. oleifera* raw seed flour contains alkaloids.

Two unigenes (Table 2) encoding for aspartate aminotransferase chloroplastic (ASP5/ASPB) (Figure 2) that catalyzes the first reaction in the aspartate pathway through phosphorylation of aspartate were determined. The product of the reaction can be used in the biosynthesis of essential amino acids methionine, lysine and threonine (Velasco et al., 2005). Two unigenes (Table 2) encoding tyrosine aminotransferase that catalyze the biosynthesis of 4-hydrophenylpyruvate, a precursor of 4-hydroxyphenylacetaldehyde were identified. Other tyrosine-derived alkaloids identified were dopamine and tyramine. Six unigenes (Table 2) encoding for enzyme tyrosine decarboxylase involved in the conversion of tyrosine to tyramine; and tyrosine from L-Dopa to dopamine were identified. Seventeen upregulated (FPKM 415.84) *lysC* genes encoding for aspartate kinase that catalyzes the phosphorylation of the amino acid aspartate which is the first step in the biosynthesis of three essential amino acids: methionine, lysine, and threonine, known as the “aspartate family” were determined.

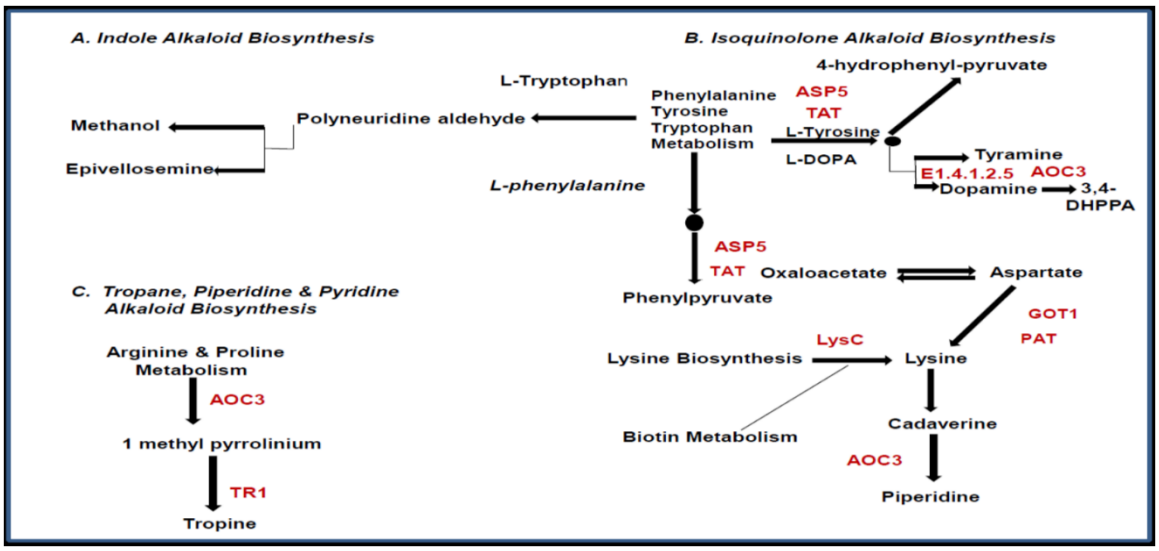

Figure 2. Schematic diagram of the alkaloid pathway modified from KEGG showing the enzymes (in red letters) encoded by upregulated unigenes in the mature embryos of *M. oleifera*. AOC3 (primary-amine oxidase); ASP5 (aspartate aminotransferase (chloroplastic)); TAT (tyrosine aminotransferase); E4.1.1.25 (tyrosine decarboxylase); GOT1 (aspartate aminotransferase (cytoplasmic)); PAT (bifunctional aspartate aminotransferase and glutamate/aspartate-prephenate aminotransferase); TR1 (tropinone reductase I); lysC (aspartate kinase).

CONCLUSIONS AND RECOMMENDATIONS

RNA-sequencing is a high-throughput and cutting edge technology in discovering genes encoding for enzymes and proteins that are vital for the synthesis of secondary metabolites in the mature seeds of *M. oleifera*. The upregulated unigenes in this study are those encoding for enzymes of the phenylpropanoid, flavonoid and alkaloid pathways. These enzymes catalyze the synthesis of secondary metabolites which impart upon *M. oleifera* seeds its medicinal and highly nutritious properties. Although this study was able to identify upregulated genes, encoding for enzymes that catalyze the production of secondary metabolites in *M. oleifera* mature seeds, validation of the expression of these genes is highly recommended to provide evidence that these genes are indeed expressed in the said tissue.

ACKNOWLEGEMENTS

The authors would like to thank Fulbright Philippines Agriculture Advanced Research Fellowship Program for the research funding and opportunity granted to Dr. V.A. Panes and the Department of Science and Technology, Philippines for the research funding granted to M.D.K.A. Discar.

Literature cited

Aalenf, R.B., Opsahl-Ferstad, H.G., Linnestad, C., and Olsen, O.A. (1994). Transcripts encoding an oleosin and a dormancy-related protein are present in both the aleurone layer and the embryo of developing barley (*Hordeum vulgare* L.) seeds. Plant J. *5* (*3*), 385–396 https://doi.org/10.1111/j.1365-313X.1994.00385.x. PubMed

Calderón-Montaño, J.M., Burgos-Morón, E., Pérez-Guerrero, C., and López-Lázaro, M. (2011). A review on the dietary flavonoid kaempferol. Mini Rev. Med. Chem. *11* (*4*), 298–344 https://doi.org/10.2174/138955711795305335. PubMed

Gao, J., Yu, X., Ma, F., and Li, J. (2014). RNA-seq analysis of transcriptome and glucosinolate metabolism in seeds and sprouts of broccoli (*Brassica oleracea* var. *italic*). PLoS One *9* (*2*), e88804 https://doi.org/10.1371/journal.pone.0088804. PubMed

Geahlen, R.L., and McLaughlin, J.L. (1989). Piceatannol (3,4,3′,5′-tetrahydroxy-trans-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. Biochem. Biophys. Res. Commun. *165* (*1*), 241–245 https://doi.org/10.

1016/0006-291X(89)91060-7. PubMed

Gholani-Borujeni, F., Mahvi, A.H., Naseri, S., Faramarzi, M.A., Nabizadeh, R., and Alimohammadi, M. (2011). Application of immobilized horseradish peroxidase for removal and detoxification of azodye from aqueous solution. Res. J. Chem. Environ. *15*, 217–222 https://doi.org/10.1186%2F2193–1801–2-341291X(89)91060–7.

Giordano, D., Provenzano, S., Ferrandino, A., Vitali, M., Pagliarani, C., Roman, F., Cardinale, F., Castellarin, S.D., and Schubert, A. (2016). Characterization of a multifunctional caffeoyl-CoA O-methyltransferase activated in grape berries upon drought stress. Plant Physiol. Biochem. *101*, 23–32 https://doi.org/10.1016/j.plaphy.2016.01.015. PubMed

Hamid, M., and Rehman, K. (2009). Potential application of peroxidases. Food Chem. *115* (*4*), 1177–1186 https://doi.org/10.1016/j.foodchem.2009.02.035.

Hematy, K., Cherk, C., and Somerville, S. (2009). An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. *161* (*2*), 105–121 https://doi.org/10.1111/j.1095-8339.2009.00996.x.

Huang, J., Rozwadowski, K., Bhinu, V.S., Schäfer, U., and Hannoufa, A. (2008). Manipulation of sinapine, choline and betaine accumulation in *Arabidopsis* seed: towards improving the nutritional value of the meal and enhancing the seedling performance under environmental stresses in oilseed crops. Plant Physiol. Biochem. *46* (*7*), 647–654 https://doi.org/10.1016/j.plaphy.2008.04.014. PubMed

Ijarotimi, O.S., Adeoti, O.A., and Ariyo, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented *Moringa oleifera* seed flour. Food Sci. Nutr. *1* (*6*), 452–463 https://doi.org/10.1002/fsn3.70. PubMed

Khalil, M.I., and Sulaiman, S.A. (2010). The potential role of honey and its polyphenols in preventing heart diseases: a review. Afr. J. Tradit. Complement. Altern. Med. *7* (*4*), 315–321 https://doi.org/10.4314/ajtcam.v7i4.56693. PubMed

Korkina, L.G. (2007). Phenylpropanoids as naturally occurring antioxidants: from plant defense to human health. Cell. Mol. Biol. *53* (*1*), 15–25 https://doi.org/10.1170/T772. PubMed

Lalas, S., and Tsaknis, J. (2002). Characterization of *Moringa oleifera* seed oil variety "Periyakulam 1". J. Food Compos. Anal. *15* (*1*), 65–77 https://doi.org/10.1006/jfca.2001.1042.

Liu, X., Lu, Y., Yuan, Y., Liu, S., Guan, C., Chen, S., and Liu, Z. (2013). De novo transcriptome of *Brassica juncea* seed coat and identification of genes for the biosynthesis of flavonoids. PLoS One *8* (*8*), e71110 https://doi.org/10.1371/journal.pone.0071110. PubMed

Miedes, E., Vanholme, R., Boerjan, W., and Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. Front. Plant Sci. *5*, 358 https://doi.org/10.3389/fpls.2014.00358. PubMed

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A.C., and Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction servers. Nucleic Acids Res. *35* (*Web server issue*), w182-w185. doi:10.1093/nar/gkm321.

Ogbunugafor, H.A., Eneh, F.U., Ozumba, A.N., Igwo-Ezikpe, M.N., Okpuzor, J., Igwilo, I.O., Adenekan, S.O., and Onyekwelu, O.A. (2011). Physicohemical antioxidant properties of *Moringa oleifera* seed oil. Pak. J. Nutr. *10* (*5*), 409–414 https://doi.org/10.3923/pjn.2011.409.414.

Panes, V.A., Kitazumi, A. , Butler, M., Baoas, R., and De los Reyes, B.G. (2017). Analysis of the oil biosynthesis transcripts of the *Moringa oleifera* Lam. mature seed embryos using RNA sequencing. Acta Hortic. *1158*, 55–62 https://doi.org/10.17660/ActaHortic.2017.1158.7.

Parsons, M.R., Convery, M.A., Wilmot, C.M., Yadav, K.D., Blakeley, V., Corner, A.S., Phillips, S.E., McPherson, M.J., and Knowles, P.F. (1995). Crystal structure of a quinoenzyme: copper amine oxidase of *Escherichia coli* at 2 A resolution. Structure *3* (*11*), 1171–1184 https://doi.org/10.1016/S0969-2126(01)00253-2. PubMed

Plunkett, O., and Sainsburry, M. (1991). Pyridine and piperidine alkaloid. In Second Supplements to 2nd Edition of Rodde's *Chemistry of Carbon Compounds*, Vol. 4, 2nd edn (Elsevier Science B.V.), p.365–421. https://doi.org/10.1016/B978-044453347-0.50194-4.

Pradhan, S., Bandhiwal, N., Shah, N., Kant, C., Gaur, R., and Bhatia, S. (2014). Global transcriptome analysis of developing chickpea (*Cicer arietinum* L.) seeds. Front. Plant Sci. *5* (*698*), 1–14 https://doi.org/10.3389/fpls.2014.00698. PubMed

Rhou-Boroujeni, H., Heidarian, E., Rhoui-Boroujeni, H., and Rafiecan-kopaei, M. (2016). Phytochemical constituents of *Moringa peregrina* seeds. Pharma Chem. *8*, 80–86.

Stacy, R.A., Munthe, E., Steinum, T., Sharma, B., and Aalen, R.B. (1996). A peroxiredoxin antioxidant is encoded by a dormancy-related gene, Per1, expressed during late development in the aleurone and embryo of barley grains. Plant Mol. Biol. *31* (*6*), 1205–1216 https://doi.org/10.1007/BF00040837. PubMed

Toung, J.M., Morley, M., Li, M., and Cheung, V.G. (2011). RNA-sequence analysis of human B-cells. Genome Res. *21* (*6*), 991–998 https://doi.org/10.1101/gr.116335.110. PubMed

Vavricka, C., Han, Q., Huang, Y., Erickson, S.M., Harick, K., Christensen, B.M., and Li,, J. (2011). From L Dopa to dihydroxyphenylacetaldehyde: a toxic biochemical pathways plays a vital physiological function in insects. PLos ONE *6* (*1*), e16124 https://doi.org/10.13.71/journal.pone0016124.

Velasco, I., Arévalo-Rodríguez, M., Marina, P., and Calderón, I.L. (2005). A new mutation in the yeast aspartate kinase induces threonine accumulation in a temperature-regulated way. Yeast *22* (*2*), 99–110 https://doi.org/10.1002/yea.1197. PubMed

Vogt, T. (2010). Phenylpropanoid biosynthesis. Mol. Plant *3* (*1*), 2–20 https://doi.org/10.1093/mp/ssp106. PubMed

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. *10* (*1*), 57–63 https://doi.org/10.1038/nrg2484. PubMed

Wang, S., Zhang, C., and Yang, Y. (2014). Biological properties of 6-gingerol: a brief review. Natural Product Communications *9*, 1027–1030 https://doi.org/10.1177%2F1934578X1400900736.

Yu, E.S., Min, H.J., Lee, K., Lee, M.S., Nam, J.W., Seo, E.K., Hog, J.H., and Hwang, E.S. (2009). Anti-inflammatory activity of p-coumaryl alcohol-ɣ-O-methyl ether is mediated through modulation of interferon-ɣ production in Th cells. Brit. J. Pharm. *156*, 1107–1114 https://doi.org/10.1111/j.1476–5381.2009.00114.x.

Ziegler, J., and Facchini, P.J. (2008). Alkaloid biosynthesis: metabolism and trafficking. Ann. Rev. Plant Biol. *59,* 735–769 https://doi.org/10.1146/annurev.arplant.59.032607.092730.