



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2018; 6(5): 1864-1880

© 2018 IJCS

Received: 25-07-2018

Accepted: 30-08-2018

Shubha RDepartment of Genetics and
Plant Breeding, Junagadh
Agricultural University,
Junagadh, Gujarat, India**Rukam S Tomar**Department of Genetics and
Plant Breeding, Junagadh
Agricultural University,
Junagadh, Gujarat, India

Differential gene expression analysis and putative genes identification from three developmental growth stages in fenugreek (*Trigonella foenum-graecum* L.)

Shubha R and Rukam S Tomar

Abstract

Fenugreek (*Trigonella foenum-graecum* L.) popularly known as “Methi” belongs to family fabaceae and subfamily papilionaceae. Gujarat Methi Variety 2 was selected for transcriptome study from the three developmental stages that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS) which were sequenced using Ion S5 genome sequencing machine. Differential gene expression analysis was carried out by using CLC genomic workbench (v10.0.0). In which the data generated from transcriptome sequencing of fenugreek were mapped with the reference genome *Arabidopsis thaliana*, the reference genome with a length of 119,667,750 bp, total number of genes present in the reference genome were 32,833 and 49,477 transcripts. In gene expression analysis from all the three developmental stages, top 10 highly expressed genes and 10 lower regulated genes were selected. In vegetative stage, the highest expression value was 474910 and RPKM was 73070.44 for highly expressed gene, with a gene length of 1223 bp and lowest expression value was 3 and RPKM was 0.291139 for lower regulated gene, with a length of 2833 bp. In reproductive stage, the highest expression value was 233210 and RPKM was 28507.21 for highly expressed gene, with a length of 1223 bp and lowest expression value was 3 and RPKM was 0.268558 for lower regulated gene, with a length of 2332 bp. In maturity stage, the highest expression value was 82652 and RPKM was 6970.07088 for highly expressed gene, with a length of 2334 bp and lowest expression value was 3 and RPKM was 1.104943625 for lower regulated gene, with a length of 468 bp. The sequence data sets were analysed through Blast2GO bioinformatic tool. A total of 445 putative genes were identified. From vegetative stage transcriptome 48 genes were identified, from reproductive stage none of the genes were identified and from maturity stage transcriptome 397 genes were identified.

Keywords: fenugreek, developmental stages, differential gene expression analysis and putative genes identification

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) popularly known as “Methi” belongs to family fabaceae and subfamily papilionaceae. It is an important spice crop largely grown in the northern India during *rabi* season. Fenugreek is used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Cuboid-shaped, yellow- to amber-colored fenugreek seeds are frequently encountered in the cuisines of the Indian subcontinent, used both whole and powdered in the preparation of pickles, vegetable dishes, dal, and spice mixes such as panch phoron and sambar powder. They are often roasted to reduce bitterness and enhance flavour (BBC – 2017) [1].

Fruit is a curved seed-pod, with ten to twenty flat and hard, yellowish-brown seeds. They are angular- rhomboid, oblong or even cubic, and have a deep furrow dividing them into two unequal lobes. Studies reveal that a maximum level of diosgenin [(25R)-5-spirosten- 3h-ol] is found to be in young leaves (20mg/g dry weight) and in mature seeds with the percentage range from 0.28% - 0.92%. Reported that steroidal sapogenins were effective agents for the treatment of hypocholesterolemia, a disorder often associated with diabetes (Raju *et al.*, 2004) [2]. In current study, Gujarat Methi Variety 2 was selected for transcriptome study from the three developmental stages that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS), which were sequenced using Ion S5 genome sequencing machine.

Correspondence

Shubha RDepartment of Genetics and
Plant Breeding, Junagadh
Agricultural University,
Junagadh, Gujarat, India

2. Material and Methods

Present investigation on “Differential gene expression analysis and putative genes identification from three developmental growth stages in fenugreek (*Trigonella foenum-graecum* L.)” was under taken at the Junagadh Agricultural University (JAU), Junagadh during 2015-16 and 2016-17. Laboratory studies on various aspects were carried out at Biotech Cell, Department of Biotechnology, College of Agriculture, Junagadh. Details of the materials and method followed are described here under.

Sample collection

Fenugreek seeds of GMV-2 (Gujarat Methi Variety-2) were sown in plot, under the natural environmental condition, for the collection of the samples from three developmental stages *i.e.*, vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (70-80 DAS) (Figure 3.1). All the tissues were kept in RNA *later* at -20°C till they were used for isolation of RNA.

Transcriptome sequencing and *de novo* assembly construction

Total RNA was isolated by trizol method. mRNA was isolated by Dynabeads[®] mRNA DIRECT[™] Micro Kit of life technology. Whole transcriptome library was prepared by Ion Total RNA-Seq Kit v2 of life technology. Temtube preparation was prepared by Ion S5[™] Temtube OT2 Kit of life technology. Sequencing reagents used from the Ion S5[™] Sequencing 340 Kit of life technology. All the chemicals used in the experiments were of analytical grade from standard manufacturers like Sigma-Aldrich, E-Merck, Hi-media, Qualigenes and SISCO Research Lab. (SRL) etc. In case of fine chemicals, molecular biological grade were used, which were obtained from Bangalore Merck biochem, USA, MWG

biotech Pvt Ltd, Germany, XX-Integrated DNA Technologies, USA.

De novo assembly of the raw data was carried out by using CLC Genomic Workbench (v10.0.1) software from QIAGEN Bioinformatics Tools.

Differential gene expression profile of three stages of growth that were vegetative, reproductive and maturity stages

All assembly read (Contig) was use for deferential expression profile of three stages of growth that are vegetative, reproductive and maturity stages deferential expression profile was carried out using CLC genomic workbench (v10.0.1) Windows, according to the manufacturer's instructions (User manual according respective company) by company.

3. Results and Discussion

Putative/hypothetical genes identification

Putative genes identification is a requirement for gene investigation in the age of transcriptomics. The strategy for discovery of potential ORFs at a large-scale in fenugreek transcriptomes described here will contribute to their annotation and identifies new potential regulators of diverse biological processes in plants and that improve understanding of plant biology. Here analysis revealed that remarkably, 445 putative genes were found from all the developmental stages of fenugreek. 48 putative genes in vegetative stage from 86 contigs, no genes were found in reproductive stage, instead of that GO IDs were found and 397 genes in maturity stage from 57 contigs (Table 3.1).

Putative genes identified in different developmental stages of fenugreek by using Blast 2GO (v4.1) functional annotation tool.

Table 3.1: Vegetative stage putative gene lists

Index	Identifier or Gene location	Gene name	Description
1	Vegetative_(single)_trimmed_contig_2	Vigan.01G480200 PHAVU_003G143800g VHA2 glysoja_005805 GLYMA_13G097900 LR48_vigna627s003100 GLYMA_15G162600 Srha4 GLYMA_09G056300 glysoja_026604 GLYMA_17G061800 glysoja_027574 MTR_4g127710	Ontology term: Integral component of plasma membrane, intracellular membrane bounded organelle, ATP binding, hydrogen-exporting ATPase activity, Phosphorylative mechanism, Metal ion binding, ATP biosynthetic process, Plasma membrane ATPase 4 Ontology ID: GO:0005887, GO:0043231, GO:0005524, GO:0008553, GO:0046872, GO:0006754, GO:0051453, GO:1902600, GO:0006119
2	Vegetative_(single)_trimmed_contig_3	LR48_vigan08g195100 Vigan.06G247800 glysoja_044218 GLYMA_12G020700 MTR_4g076850 GLYMA_11G094300 JCGZ_10564	Ontology term: Cytosol, aminoacyl-tRNA editing activity, isoleucine-tRNA ligase activity, ATP binding-tRNA aminoacylation, regulation of translational fidelity, isoleucine, valine and leucine biosynthetic activity, Isoleucine-tRNA cytoplasmic Ontology ID: GO:0005829, GO:0002161, GO:0004822, GO:0005524, GO:0006428, GO:0006450, GO:0009098, GO:0009099
3	Vegetative_(single)_trimmed_contig_4		Ontology term: Glutamate synthase activity, glutamate bio-synthetic process, oxidation-reduction process, Ontology ID: GO:0015930, GO:0006537, GO:0055114
4	Vegetative_(single)_trimmed_contig_5		Ontology term: Protein binding Ontology ID: GO:0005515
5	Vegetative_(single)_trimmed_contig_6		Ontology term: Oxidoreductase activity, actig on the CH-NH2 group of donors, NAD or NADP as acceptor, iron-sulphur cluster binding, glutamate biosynthetic process, obsolete electron transport Ontology ID: GO:0016639, GO:0051536, GO:0006537,

			GO:0055114, GO:0006118
6	Vegetative_(single)_trimmed_contig_7	LR48_vigan09g038100 P25890 P32290 MTR_3g115370 Cat1 PRUPE_ppa004776mg A9PJC6 CAT PHAU_009G011100g Glysoja_014691 GLYMA_04G017500 PCAT1	Ontology term: Glyoxysome, nucleic acid binding, catalyse activity, heme binding, metal ion binding, response to hydrogen peroxide, hydrogen peroxide catabolic process, Oxidation-reduction process, cellular detoxification, tryptophan metabolic process, obsolete peroxidase reaction, catalase Ontology ID: GO:0009514 GO:0003676, GO:0004096, GO:0020037, GO:0046872, GO:0042542, GO:0042744, GO:0055114, GO:0098869, GO:0006568, GO:0006804, GO:0015947
7	Vegetative_(single)_trimmed_contig_8		Ontology term: Protein binding Ontology ID: GO:0005515
8	Vegetative_(single)_trimmed_contig_9		Ontology term: RNA binding, RNA-directed5'-3' RNA polymerase activity, ATP binding, hydrolase activity, acting on acid anhydrides, transcription, DNA-templated, viral RNA genome replication, Ontology ID: GO:0003723, GO:0005524, GO:0005524, GO:0016817, GO:0006351, GO:0039694, GO:0006144, GO:0031379
9	Vegetative_(single)_trimmed_contig_10		Ontology term: Oxidation-reduction process, Transducin family WD-40 repeat Ontology ID: GO:0055114
10	Vegetative_(single)_trimmed_contig_11	GLYMA_10G293500 L484_025582 PHAU_007G006600g JCGZ_14540 Glysoja_013513 Glysoja_032857 PRUPE_ppa001865mg MTR_1g116120 LR48_vigan02g27100	Ontology term: Transketolase activity, pentose-phosphate shunt, carbon utilization, chloplastic Ontology ID: GO:0004802, GO:0006098, GO:0015976
11	Vegetative_(single)_trimmed_contig_12	A0A076KXD2 A0A0F6N0A4 HSP70-1 GSGOC_T00029858001 MIMGU_mgv1a002667mg MTR_7g024580	Ontology term: ATP binding, Heat shock cognate 70kDa Ontology ID: GO:0005524
12	Vegetative_(single)_trimmed_contig_13		Ontology term: Protein and ATP binding, protein metabolic process, Chaperon chloroplatic Ontology ID: GO:0005515, GO:0005524, GO:0019538
13	Vegetative_(single)_trimmed_contig_14		Ontology term: Regulation of transcription from RNA II promoter, regulation of DNA template transcription, elongation, Transcription elongation factor SPT5 homolog 1 Ontology ID: GO:0006357, GO:0032784
14	Vegetative_(single)_trimmed_contig_16		Ontology term: Mammalian STARD2lipid binding START domain, Lipid binding. Ontology ID: GO:0008289
15	Vegetative_(single)_trimmed_contig_17		Ontology term: Auxin response factor 2, Nucleus, DNA binding, regulation of transcription, DNA-templated, response to hormone. Ontology ID: GO:0005634
16	Vegetative_(single)_trimmed_contig_18		Ontology term: RNA binding Ontology ID: GO:0003723
17	Vegetative_(single)_trimmed_contig_19		Ontology term: Iron binding, oxidoreductase activity, acting on paired donors, incorporation or reduction of molecular oxygen, heme binding, oxidation-reduction process, cytochrome p45090b1-like Ontology ID: GO:0005506, GO:0016705, GO:0020037, GO:0055114
18	Vegetative_(single)_trimmed_contig_20		Ontology term: hydolase activity, hydrolysing o-glyccosyl oxidoreductase activity. Acting on paired donors, with incorporation or reduction of molecular oxygen Ontology ID: GO:0005506, GO:0016705, GO:0020037, GO:0055114
19	Vegetative_(single)_trimmed_contig_21		Ontology term: Hydolase activity, hydrolysing O-glyccosyl compounds, carbohydrates binding, carbohydrate metabolic process, endoglucanase 6 Ontology ID: GO:0004553, GO:0005975
20	Vegetative_(single)_trimmed_contig_22		Ontology term: Aldehyde dehydrogenase [NAD(P)+] activity, cellular aldehyde metabolic process, gluconeogenesis, glycolytic process, histidine metabolic process, L-phenylalanine metabolic process, tyrosine metabolic process, aldehyde dehydrogenase family 3 member H1 llike isoform X2

			Ontology ID: GO:0004030, GO:0006081, GO:0055114, GO:0006094, GO:0006096, GO:0006547, GO:0006558, GO:0006570
21	Vegetative_(single)_trimmed_contig_23	TIR-NBS-LRR class	Ontology term: Protein binding, ADP binding, signal transduction, Disease resistance Ontology ID: GO:0005515, GO:0043531, GO:0007165
22	Vegetative_(single)_trimmed_contig_24		Ontology term: COPI vesicle coat, structural molecule activity, binding, intracellular protein transport, vesicle-mediated transport, coatomer subunit beta-1-like Ontology ID: GO:0030126, GO:0005198, GO:0005488, GO:0006886, GO:0016192
23	Vegetative_(single)_trimmed_contig_25		Ontology term: Cytoplasm, ATP binding, kinase activity, phosphotransferase activity, alcohol group as acceptor, metabolic process, Ontology ID: GO:0005737, GO:0005524, GO:0016301, GO:0016773, GO:0008152
24	Vegetative_(single)_trimmed_contig_26		Ontology term: Nucleus, DNA binding Ontology ID: GO:0005634, GO:0003677
25	Vegetative_(single)_trimmed_contig_27		Ontology term: Arginine decarboxylase activity, arginine catabolic process, spermidine biosynthetic proline, proline metabolic process, Ontology ID: GO:0008792, GO:0006527, GO:0008295, GO:0006560
26	Vegetative_(single)_trimmed_contig_28		Ontology term: Integral component of membrane, calcium-transporting ATPase activity, ATP binding, calcium ion transmembrane transport, sarcoplasmic reticulum, Calcium-transporting ATPase plasma membrane-type Ontology ID: GO:0016021, GO:0005388, GO:0005524, GO:0070588, GO:0016529
27	Vegetative_(single)_trimmed_contig_29		Ontology term: Membrane coat, binding, protein transporter activity, intracellular protein transport, vesicle-mediated transport Ontology ID: GO:0030117, GO:0005488, GO:0008565, GO:0006886, GO:0016192
28	Vegetative_(single)_trimmed_contig_30		Ontology term: Translation elongation factor activity, protein binding, ribosome, regulation of translational elongation, Elongation factor Ts Ontology ID: GO:0003746, GO:0005515, GO:0005840, GO:0006448
29	Vegetative_(single)_trimmed_contig_33		Ontology term: Protein binding, LOV domain-containing Ontology ID: GO:0005515
30	Vegetative_(single)_trimmed_contig_34		Ontology term: RISC complex, gene silencing by RNA Ontology ID: GO:0016442, GO:0031047
31	Vegetative_(single)_trimmed_contig_36		Ontology term: RNA binding Ontology ID: GO:0003723
32	Vegetative_(single)_trimmed_contig_37		Ontology term: Zinc ion binding Ontology ID: GO:0008270
33	Vegetative_(single)_trimmed_contig_38		Ontology term: Protein binding Ontology ID: GO:0005515
34	Vegetative_(single)_trimmed_contig_39		Ontology term: Aspartate kinase activity, oxidoreductase activity, amino acid binding, NADP binding, oxidation0-reduction process, glycine meabollic process, L-serine, thronine, lysine metabolic process, phosphorylation Ontology ID: GO:0004072, GO:0016597, GO:0016597, GO:0050661, GO:0055114, GO:0006544, GO:0006563, GO:0006566, GO:0009085, GO:0016310
35	Vegetative_(single)_trimmed_contig_40		Ontology term: Translation initiation factor activity, GTPase activity, GTP binding, ribosome, regulation of translational initiation, translation initiation factor IF-chloroplasmic Ontology ID: GO:0003743, GO:0003924, GO:0005525, GO:0005840, GO:0006446
36	Vegetative_(single)_trimmed_contig_42		Ontology term: catalytic activity, metal ion binding, proteolysis, presequence protease chloroplasmic mitochondrial-like Ontology ID: GO:0003824, GO:0046872, GO:0006508
37	Vegetative_(single)_trimmed_contig_43		Ontology term: Ubiquitin-protein transferase activity, protein binding, protein ubiquitination Ontology ID: GO:0004842, GO:0005515, GO:0016567
38	Vegetative_(single)_trimmed_contig_44		Ontology term: Protein kinase activity, ATP binding, protein phosphorylation, Probable serine thronine-kinase At1g01540 Ontology ID: GO:0004672, GO:0005524, GO:0006468
39	Vegetative_(single)_trimmed_contig_45		Ontology term: Protein binding Ontology ID: GO:0005515
40	Vegetative_(single)_trimmed_contig_46		Ontology term: Membrane, ATP binding, ATPase activity Ontology ID: GO:0016020, GO:0005524, GO:0016887

41	Vegetative_(single)_trimmed_contig_47	Ontology term: Adenylate kinase activity, ATP binding, purine nucleobase process, ATP metabolic process Ontology ID: GO:0004017, GO:0005524, GO:0006144, GO:006034
42	Vegetative_(single)_trimmed_contig_48	Ontology term: Transferase activity, transferring hexosyl groups, metabolic process, Senescence dehydration-associated Ontology ID: GO:0016758, GO:0008152
43	Vegetative_(single)_trimmed_contig_49	Ontology term: Binding, intracellular protein transport, Importin beta-like SAD2 Ontology ID: GO:0005488, GO:0006886
44	Vegetative_(single)_trimmed_contig_50	Ontology term: Nucleic-acid binding, protein binding, Argonaute 4-like Ontology ID: GO:0003676, GO:0005515
45	Vegetative_(single)_trimmed_contig_53	Ontology term: Alpha amylase activity, calcium ion binding, starch metabolic process, sucrose metabolism, Alpha amylase domain Ontology ID: GO:0004556, GO:0005509, GO:0005982, GO:0005985
46	Vegetative_(single)_trimmed_contig_54	Ontology term: Methyl transferase activity Ontology ID: GO:0008168
47	Vegetative_(single)_trimmed_contig_55	Ontology term: Microtubule motor activity, ATP microtubule binding, microtubule based movement, tubulin complex, kinesin FRA1 isoform X1 Ontology ID: GO:0005524, GO:0016301, GO:0050242, GO:0006090, GO:0016310, GO:0015976
48	Vegetative_(single)_trimmed_contig_56	Ontology term: Phosphorelay sensor kinase activity, phosphorelay signal transduction system, nucleoside metabolic process, phosphorylation, protein histidine kinase complex Ontology ID: GO:0000155, GO:0000160, GO:0009116, GO:0016310, GO:0009365
49	Vegetative_(single)_trimmed_contig_57	Ontology term: Methyltransferase activity Ontology ID: GO:0008168
50	Vegetative_(single)_trimmed_contig_58	Ontology term: Membrane, protein binding, zinc ion binding, cellulose synthase activity, cellulose biosynthetic activity, starch metabolic process, sucrose metabolic process, UDP-glucose metabolic process, Cellulose synthase A catalytic subunit 6-like Ontology ID: GO:0016020, GO:0005515, GO:0008270, GO:0016760, GO:0030244, GO:0005982, GO:0005985, GO:0006011
51	Vegetative_(single)_trimmed_contig_59	Ontology term: ATP binding, kinase activity, pyruvate, phosphate dikinase activity, pyruvate metabolic process, phosphorylation, carbon utilization, Phosphate dikinase 2 Ontology ID: GO:0005524, GO:0016301, GO:0050242, GO:0006090, GO:0016310, GO:0015976
52	Vegetative_(single)_trimmed_contig_60	Ontology term: GDP-D-glucose phosphorylase activity Ontology ID: GO:0080048
53	Vegetative_(single)_trimmed_contig_62	Ontology term: Protein binding Ontology ID: GO:0005515
54	Vegetative_(single)_trimmed_contig_63	Ontology term: DNA binding Ontology ID: GO:0003677
55	Vegetative_(single)_trimmed_contig_64	Ontology term: Exocyst, vesicle docking involved in exocytosis, protein transport Ontology ID: GO:0000145, GO:0006904, GO:0015031
56	Vegetative_(single)_trimmed_contig_65	Ontology term: Magnesium ion binding, GTPase activity, GTP binding Ontology ID: GO:0000287, GO:0003924, GO:0005525
57	Vegetative_(single)_trimmed_contig_66	Ontology term: Protein binding, cell redox homeostasis Ontology ID: GO:0005515 GO:0045454
58	Vegetative_(single)_trimmed_contig_67	Ontology term: ADP binding Ontology ID: GO:0043531
59	Vegetative_(single)_trimmed_contig_69	Ontology term: GTP binding Ontology ID: GO:0005525
60	Vegetative_(single)_trimmed_contig_70	Ontology term: Glycylpeptide N-tetradecanoyltransferase activity, N-terminal protein myristoylation, acyl-carrier-protein biosynthetic process Ontology ID: GO:0004379, GO:0006499, GO:0042967
62	Vegetative_(single)_trimmed_contig_72	Ontology term: Membrane, ATP binding, protein targeting, protein import Ontology ID: GO:0016020, GO:0005524, GO:0006605, GO:0017038
63	Vegetative_(single)_trimmed_contig_73	Ontology term: Protein binding Ontology ID: GO:0005515
64	Vegetative_(single)_trimmed_contig_79	Ontology term: ATP binding, Chaperone chloroplastic

			Ontology ID: GO:0005524
65	Vegetative_(single)_trimmed_contig_80		Ontology term: Integral component of membrane, nucleotide binding, metal ion binding Ontology ID: GO:0016021, GO:0000166, GO:0046872
66	Vegetative_(single)_trimmed_contig_82		Ontology term: Oxidoreductase activity, oxidation-reduction process, Dihydrolipoamide dehydrogenase Ontology ID: GO:0016491, GO:0055114
67	Vegetative_(single)_trimmed_contig_83		Ontology term: RNA binding, preotein binding Ontology ID: GO:0003723, GO:0005515
68	Vegetative_(single)_trimmed_contig_84		Ontology term: Ubiquitin-protein transferase activity, protein ubiquitination Ontology ID: GO:0004842, GO:0016567

Table 3.1: Maturity stage putative gene lists

Index	Identifier	Gene name	Description
1	Maturity_(single)_trimmed_contig_1	CISIN_1g004921mg FTSH JCGZ_08123 MTR_3g115110 TCM_034357 PRUPE_ppa002083mg	Ontology term: ATP-dependent zinc metallophosphate chloroplastic, integral component of membrane, chloroplast membrane, metalloproteinase activity, ATP binding, metal ion binding, proteolysis, cell division. Ontology ID: GO:0016021, GO:0031969, GO:0004222, GO:0005524, GO:0046872, GO:0006508, GO:0007049, GO:0051301
2	Maturity_(single)_trimmed_contig_2	Q4F978 W0LLV7 W0LLW2 S5RD17 ORF1 S5RFF1 Q7TD32 G5D8V5 E5KJF1 S5RKI7 E5GB05 S5RCZ6 Q17UV1 A0A0B4VMT5 S5RZU7 A6Q0U7	Ontology term: RNA-dependent RNA polymerase, RNA binding, RNA-directed 5'-3' polymerase activity, ATP binding, methyltransferase activity, acting on acid anhydrides, transcription, RNA-templated, DNA-templated, hydrolase activity, RNA processing, viral RNA genome replication, RNA-directed RNA polymerase complex, mRNA methylation, purine nucleobase metabolic process. Ontology ID: GO:0003723, GO:0003968, GO:0005524, GO:0008174, GO:0016817, GO:0001172, GO:0006351, GO:0006396, GO:0039694, GO:0080009, GO:0006144, GO:0031379
3	Maturity_(single)_trimmed_contig_3	GLYMA_19G065800 Vigan.03G184800 MTR_1g007860 PHAVU_001G075000 g glysoja_043838 glysoja_044510 GLYMA_14G162400 LR48_Vigan07g20470 0	Ontology term: aminoacylate hydrolase, integral component of membrane, hydrolase activity. Ontology ID: GO:0016021, GO:0016787
4	Maturity_(single)_trimmed_contig_5	EUGRSUZ_H04335 GLYMA_20G225000 GLYMA_10G162400 POPTR_0013s03870g C6SXL6 LR48_Vigan10g15330 0 Vigan.05G138700 PHAVU_006G102100 g JCGZ_23500 glysoja_049522	Ontology term: auxin-responsive IAA16-like cellular process, regulation of cellular process Ontology ID: GO:0009987, GO:0050794
5	Maturity_(single)_trimmed_contig_6	U5TZB8 W0LKU8 U5TZC6 U5TZE8 U5TZE5 U5TZC3 D2K9J2 I7DGU6 U5TZF2 U5TZE1 CP S5RZT0 S5RKJ4	Ontology term: coat, viral capsid, structural molecule activity. Ontology ID: GO:0019028, GO:0005198.

		S5S1M2 Q1W2L6 ORF5 S5RZV7 S5RFF8	
6	Maturity_(single)_trimmed_contig_7	PHAVU_008G206200 g GLYMA_14G040800 glysoja_004093 VITISV_037734 MTR_5g088220 GLYMA_02G275400 Vigan.03G054500	Ontology term: UBPI-associated 2A-like, nucleotide binding, nucleic acid binding Ontology ID: GO:0000166, GO:0003676
7	Maturity_(single)_trimmed_contig_8	MTR_2g009080 DHS2 glysoja_005577 I3S9C5 "EUGRSUZ_G00854 EUTSA_v10024959m g PHAVU_006G205200 g LR48_Vigan09g26270 0	Ontology term: phospho-2-dehydro-3-deoxyheptonate aldolase chloroplastic-like, chloroplast thylakoid,3-deoxy-7-phosphoheptulonate synthase activity, protein binding, chorismate biosynthetic process, tryptophan biosynthetic process, tyrosine biosynthetic process, L-phenylalanine biosynthetic process. OntologyID: GO:0009534,GO:0003849,GO:0005515,GO:0009423,GO:0000162,GO:0006571,GO:0009094
8	Maturity_(single)_trimmed_contig_9	B456_001G257000 GLYMA_09G240300 F383_11682 TCM_018390 B456_006G025300 CICLE_v10012084mg MTR_7g009590B7FI K1 PHAVU_008G043300 g PRUPE_ppa007912mg B6VAX6 Vigan.04G053500	Ontology term: V-type proton ATPase subunit d2, proton-transporting V-type ATPase, V0 domain, hydrogen ion transmembrane transporter activity, oxidoreductase activity, ATP hydrolysis coupled proton transport, oxidation-reduction process. OntologyID: GO:0033179, GO:0015078, GO:0016491, GO:0015991, GO:0055114
9	Maturity_(single)_trimmed_contig_10	MTR_2g042330 glysoja_035968 GLYMA_09G070300 I3SVD5 BRADI_4g31310 Vigan.02G299700 EUGRSUZ_F04402 GLYMA_15G178400	Ontology term: aldehyde dehydrogenase family 7 member A1, L-aminoadipate-semialdehyde dehydrogenase activity, betaine-aldehyde dehydrogenase activity, oxidation-reduction process, lysine catabolic process, lysine biosynthetic process, glycine metabolic process, L-serine metabolic process, threonine metabolic process. Ontology ID: GO:0004043, GO:0008802, GO:0055114, GO:0006554, GO:0009085, GO:0006544, GO:0006563, GO:0006566
10	Maturity_(single)_trimmed_contig_11	Vigan.02G312000 glysoja_026613 glysoja_013215 LR48_Vigan04g00370 0 GLYMA_15G163900 PHAVU_009G237900 g MTR_2g036900 GLYMA_09G057800	Ontology term: mRNA binding, pumilio homolog chloroplastic-like Ontology ID: GO:0003729
11	Maturity_(single)_trimmed_contig_13	RCOM_0753130 glysoja_028195 MTR_1g056640 GLYMA_02G294000 TCM_030805 GLYMA_14G020000 A0A089X1Y4	Ontology term: Glucan endo-1,3-beta-glucosidase 12"; "anchored component of plasma membrane, glutathione peroxidase activity, protein histidine kinase activity, polysaccharide binding, glucan endo-1,3-beta-D-glucosidase activity, response to oxidative stress, peptidyl-histidine phosphorylation, oxidation-reduction process, glutathione metabolic process, obsolete peroxidase reaction, protein histidine kinase complex, starch metabolic process, sucrose metabolic process. OntologyID: GO:0046658, GO:0004602, GO:0004673, GO:0030247, GO:0042973, GO:0006979, GO:0018106, GO:0055114, GO:0006749, GO:0006804, GO:0009365, GO:0005982, GO:0005985
12	Maturity_(single)_trimmed_contig_14	GLYMA_01G019200 glysoja_024248 PHAVU_002G139200 g	Ontology term: phosphoenolpyruvate carboxykinase [ATP]-like, cytosol, phosphoenolpyruvate carboxykinase (ATP) activity, ATP binding, kinase activity, gluconeogenesis, phosphorylation, tricarboxylic acid cycle, carbon utilization OntologyID: GO:0005829,GO:0004612,GO:0005524,GO:0016301,GO:0006094,GO:00

			16310,GO:0006099,GO:0015976
13	Maturity_(single)_trimmed_contig_15	U5U2G8 W0LH29 S5RD13 S5S1L1 U5U2G0 U5U2H1 ORF2 S5RKI6 S5RFE7 U5U2J2 S5RZU3	Ontology term: triple gene block 1, ATP binding, Ontology ID: GO:0005524
14	Maturity_(single)_trimmed_contig_16	MTR_3g037230 Vigan.02G253300 glysoja_041632 GLYMA_04G165900 PHAVU_009G185000 g GLYMA_06G196600	Ontology term: zinc C3HC4 type (RING finger), ATP-dependent peptidase activity, zinc ion binding, proteolysis, obsolete ATP-dependent proteolysis Ontology ID: GO:0004176, GO:0008270, GO:0006508, GO:0006510
15	Maturity_(single)_trimmed_contig_17	GLYMA_17G027200 MTR_4g119980 GLYMA_07G246800 glysoja_009826 Vigan.01G521500 glysoja_004381	Ontology term: kinesin KIN-13A isoform X1, kinesin complex, microtubule, microtubule motor activity, ATP binding, microtubule binding, cysteine-type peptidase activity, ATPase activity, proteolysis, microtubule-based movement, tubulin complex Ontology ID: GO:0005871, GO:0005874, GO:0003777, GO:0005524, GO:0008017, GO:0008234, GO:0016887, GO:0006508, GO:0007018, GO:0045298
16	Maturity_(single)_trimmed_contig_18	W0TS91 PHAVU_009G254300 g B456_011G198200 GLYMA_15G143700 LR48_Vigan635s0091 00 GLYMA_09G038600 glysoja_029434 MTR_2g034720 F383_18719	Ontology term: beta-D-xylosidase 1-like"; plant-type cell wall, apoplast, beta-glucosidase activity, xylan 1,4-beta-xylosidase activity, alpha-L-arabinofuranosidase activity, scopolin beta-glucosidase activity, seed coat development, arabinan catabolic process, xylan catabolic process, starch metabolic process, sucrose metabolic process, nucleotide metabolic process. Ontology ID: GO:0009505, GO:0048046, GO:0008422, GO:0009044, GO:0046556, GO:0102483, GO:0010214, GO:0031222, GO:0045493, GO:0005982, GO:0005985, GO:0009117
17	Maturity_(single)_trimmed_contig_19	GLYMA_12G120300 GLYMA_06G285400 MTR_4g079290 ACX3 PHAVU_011G116000 g JCGZ_12221 Vigan.06G121700 Vigan.06G122100 glysoja_033856	Ontology term: Acyl-coenzyme A oxidase peroxisomal-like, peroxisome, fatty-acyl-CoA binding, acyl-CoA dehydrogenase activity, acyl-CoA oxidase activity, electron carrier activity, flavin adenine dinucleotide binding, oxidoreductase activity, acting on the CH-CH group of donors, with a flavin as acceptor, fatty acid beta-oxidation using acyl-CoA dehydrogenase, lipid homeostasis, obsolete electron transport, acyl-CoA metabolic process. Ontology ID: GO:0005777, GO:0000062, GO:0003995, GO:0003997, GO:0009055, GO:0050660, GO:0052890, GO:0033539, GO:0055088, GO:0006118, GO:0006637
18	Maturity_(single)_trimmed_contig_20		Ontology term: F-box At1g47056-like, protein binding Ontology ID: GO:0005515
19	Maturity_(single)_trimmed_contig_21	AnnAh5 I3SGC9 GLYMA_07G106600 Vigan.06G135900 C6TLC9 glysoja_025789 glysoja_017182 GLYMA_12G109800 MTR_4g038440 GLYMA_09G171600	Ontology term: (R,S)-reticuline 7-O-methyltransferase-like, cytosol, calcium ion binding, calcium-dependent phospholipid binding, O-methyltransferase activity, quercetin 3-O-methyltransferase activity, protein dimerization activity, aromatic compound biosynthetic process, methylation, flavonoid biosynthetic process. Ontology ID: GO:0005829, GO:0005509, GO:0005544, GO:0008171, GO:0030755, GO:0046983, GO:0019438, GO:0032259, GO:0009813
20	Maturity_(single)_trimmed_contig_22	VPI GLYMA_13G162800 CISIN_1g004159mg CICLE_v10007524mg RCOM_0003350 VIT_09s0002g07880 CISIN_1g0042302mg A0A0S2IKL7	Ontology term: Energized vacuolar membrane proton pump, integral component of membrane, inorganic diphosphatase activity, hydrogen-translocating pyrophosphatase activity, 2-alkenal reductase [NAD(P)] activity, proton transport, transmembrane transport, oxidation-reduction process, oxidative phosphorylation. Ontology ID: GO:0016021, GO:0004427, GO:0009678, GO:0032440, GO:0015992, GO:0055085, GO:0055114, GO:0006119
21	Maturity_(single)_trimmed_contig_23	Q4F978 W0LLV7 NcLaVgp1 W0LLW2 S5RD17	Ontology term: RNA-dependent RNA polymerase, RNA binding, RNA-directed 5'-3' RNA polymerase activity, ATP binding, mRNA methyltransferase activity, hydrolase activity, acting on acid anhydrides, transcription, RNA-templated, transcription, DNA-templated, RNA processing, viral RNA genome replication, mRNA methylation, purine

		ORF1 S5RFF1 G5D8V5 S5S1L3 E5KJF1 W6MY60 A0A0K2BN37 S5RKI7 E5GB05 S5RCZ6 A0A0B4VMT5 S5RZU7	nucleobase metabolic process, RNA-directed RNA polymerase complex Ontology ID: GO:0003723, GO:0003968, GO:0005524, GO:0008174, GO:0016817, GO:0001172, GO:0006351, GO:0006396, GO:0039694, GO:0080009, GO:0006144, GO:0031379
22	Maturity_(single)_trimmed_contig_25	Vigan.08G042600 glysoja_020091 TCM_010834 GLYMA_20G126500 GLYMA_10G263800 MTR_1g107555 LR48_Vigan609s003900 PHAVU_007G038200g	Ontology term: multicopper oxidase LPR2-like, viral capsid, copper ion binding, oxidoreductase activity, oxidizing metal ions, oxidation-reduction process Ontology ID: GO:0019028, GO:0005507, GO:0016722, GO:0055114
23	Maturity_(single)_trimmed_contig_26	JCGZ_11398 TUFBI PHAVU_009G173200g VIT_14s0066g00220 TUFA PHAVU_003G205600g POPTR_0001s08770g LR48_Vigan04g067800 GLYMA_05G041900 GLYMA_06G176900	Ontology term: elongation factor chloroplastic, mitochondrion, chloroplast, translation elongation factor activity, GTPase activity, GTP binding, mitochondrial translational elongation, ribosome, regulation of translational elongation Ontology ID: GO:0005739, GO:0009507, GO:0003746, GO:0003924, GO:0005525, GO:0070125, GO:0005840, GO:0006448
24	Maturity_(single)_trimmed_contig_27	A0A0A6ZA38 C0JP19 MTR_5g030430 RCOM_0864470 JCGZ_12669	Ontology term: transcription factor MYC2-like, 2-alkenal reductase [NAD(P)] activity, protein dimerization activity, oxidation-reduction process. Ontology ID: GO:0032440, GO:0046983, GO:0055114
25	Maturity_(single)_trimmed_contig_28	Vigan.09G205300 GLYMA_15G023800 PHAVU_010G139300g MTR_8g016230 glysoja_018073 GLYMA_08G193500 glysoja_022369 GLYMA_07G010400 Bgal	Ontology term: beta-galactosidase 1-like, cell wall, vacuole, beta-galactosidase activity, carbohydrate binding, galactose metabolic process, glycosaminoglycan catabolic process, glycosphingolipid metabolic process, beta-galactosidase complex, glycerolipid metabolic process. Ontology ID: GO:0005618, GO:0005773, GO:0004565, GO:0030246, GO:0006012, GO:0006027, GO:0006687, GO:0009341, GO:0046486
26	Maturity_(single)_trimmed_contig_29	GLYMA_12G228600 GLYMA_13G271200 MTR_2g078060	Ontology term: DJ-1 homolog B, nucleus, mitochondrion, cytosol, transcription coactivator activity, transferase activity, glyoxalase III activity, regulation of transcription from RNA polymerase II promoter, glutamine metabolic process, methylglyoxal catabolic process to D-lactate via S-lactoyl-glutathione, lactate biosynthetic process, cellular response to hydrogen peroxide, transcription factor complex, positive regulation of transcription, DNA-templated. Ontology ID: GO:0005634, GO:0005739, GO:0005829, GO:0003713, GO:0016740, GO:0019172, GO:0006357, GO:0006541, GO:0019243, GO:0019249, GO:0070301, GO:0005667, GO:0045893
27	Maturity_(single)_trimmed_contig_30	glysoja_005735 GLYMA_17G069100 TCM_038525 glysoja_032940 Vigan.01G469700 MTR_4g12963	Ontology term: Eceriferum 3-like, plasma membrane, integral component of membrane, C-4 methylsterol oxidase activity, iron ion binding, fatty acid biosynthetic process, cuticle hydrocarbon biosynthetic process, wax biosynthetic process, alkane biosynthetic process, pollen sperm cell differentiation, oxidation-reduction process, Ontology ID: GO:0005886, GO:0016021, GO:0000254, GO:0005506, GO:0006633, GO:0006723, GO:0010025, GO:0043447, GO:0048235, GO:0055114
28	Maturity_(single)_trimmed_contig_31	C6THS0 LR48_Vigan06g081200 GLYMA_09G208300 TCM_016603 VIT_02s0012g0222	Ontology term: probable xyloglucan endotransglucosylase hydrolase 30, cell wall, integral component of membrane, apoplast, hydrolase activity, hydrolyzing O-glycosyl compounds, xyloglucan: xyloglucosyl transferase activity, xyloglucan metabolic process, cell wall biogenesis, cell wall organization. Ontology ID: GO:0005618, GO:0016021, GO:0048046, GO:0004553,

		I3SK34 MTR_5g029100 GLYMA_01G013900 glysoja_024203 PHAVU_002G136200 g Vigan.10G115700	GO:0016762, GO:0010411, GO:0042546, GO:0071555
29	Maturity_(single)_trimmed_contig_32	MTR_4g059680 PHAVU_011G077600 g glysoja_008327 glysoja_021106 GLYMA_11G147900 LR48_Vigan08g15250 0 GLYMA_13G327000 glysoja_008971 Vigan.06G186300	Ontology term: beta-galactosidase 8-like, cell wall, vacuole, beta-galactosidase activity, carbohydrate binding, galactose metabolic process, glycosaminoglycan catabolic process, glycosphingolipid metabolic process, beta-galactosidase complex, glycerolipid metabolic process. Ontology ID: GO:0005618, GO:0005773, GO:0004565, GO:0030246, GO:0006012, GO:0006027, GO:0006687, GO:0009341, GO:0046486
30	Maturity_(single)_trimmed_contig_33	Vigan.05G070200 PHAVU_006G162100 g glysoja_010116 GLYMA_15G097100 MTR_2g020020 GLYMA_13G215800	Ontology term: lipase-like, feruloyl esterase activity, lipid metabolic process. Ontology ID: GO:0030600, GO:0006629
31	Maturity_(single)_trimmed_contig_34	Vigan.01G031800 GLYMA_05G226600 glysoja_036418 PRUPE_ppa004637mg CISIN_1g010895mg GLYMA_08G033300 PHAVU_002G308400 g MTR_8g102460	Ontology term: hexokinase-1, cytosol, glucokinase activity, ATP binding, glucose binding, fructokinase activity, mannokinase activity, cellular glucose homeostasis, glycolytic process, carbohydrate phosphorylation, glucose 6-phosphate metabolic process, starch metabolic process, sucrose metabolic process, galactose metabolic process, gluconeogenesis, streptomycin biosynthetic process, fructose metabolic process, mannose metabolic process. Ontology ID: GO:0005829, GO:0004340, GO:0005524, GO:0005536, GO:0008865, GO:0019158, GO:0001678, GO:0006096, GO:0046835, GO:0051156, GO:0005982, GO:0005985, GO:0006012, GO:0006094, GO:0019872, GO:0006000, GO:0006013
32	Maturity_(single)_trimmed_contig_35	GLYMA_16G175400 GLYMA_09G127700 C6ZJB3 AOA067YB04 AOA024FRX1 GLYMA_09G127200 PHAVU_004G104100 g I1MPE9 glysoja_043178 AdGt-3 GLYMA_16G175300 Vigan.11G168000 GLYMA_16G175200	Ontology term: isoflavone 7-O-glucosyltransferase 1-like intracellular membrane-bounded organelle, quercetin 3-O-glucosyltransferase activity, quercetin 7-O-glucosyltransferase activity, flavonoid biosynthetic process, flavonoid glucuronidation. Ontology ID: GO:0043231, GO:0080043, GO:0080044, GO:0009813, GO:0052696
33	Maturity_(single)_trimmed_contig_36	orf132a T2DPD7 orf114	Ontology term: cell wall-associated hydrolase, mitochondrion, chloroplast. Ontology ID: GO:0005739, GO:0009507
34	Maturity_(single)_trimmed_contig_37	JCGZ_14004 PHAVU_007G215900 g Gm pdis-1b Gm pdis-1a LR48_Vigan02g09970 0 I3SWG3 C6TJT5 glysoja_037686	Ontology term: probable disulfide-isomerase A6, endoplasmic reticulum, protein disulfide isomerase activity, protein folding, response to endoplasmic reticulum stress, cell redox homeostasis. Ontology ID: GO:0005783, GO:0003756, GO:0006457, GO:0034976, GO:0045454
35	Maturity_(single)_trimmed_contig_38	GLYMA_12G192200 C6TJB9 POPTR_0014s15330g VITISV_041925 GLYMA_06G307800 PRUPE_ppa009589mg	Ontology term: usf, cytosol, chloroplast, carboxymethylenebutenolidase activity, 1,4-dichlorobenzene catabolic process. Ontology ID: GO:0005829, GO:0009507, GO:0008806, GO:0019261
36	Maturity_(single)_trimmed_contig_39	MTR_2g006520	Ontology term: T-complex 11, plasmodesma. Ontology ID: GO:0009506
37	Maturity_(single)_trimmed_contig_40	POPTR_0017s07870g LR48_Vigan09g26300 0 MTR_3g069190	Ontology term: nitronate mono-oxygenase, mitochondrion, cytosol, nitronate mono-oxygenase activity, dioxygenase activity, response to symbiotic fungus, response to cadmium ion, oxidation-reduction process,

		GLYMA_08G178100 AT5G64250 PHAVU_006G205400 g	nitrogen compound metabolic process. Ontology ID: GO:0005739, GO:0005829, GO:0018580, GO:0051213, GO:0009610, GO:0046686, GO:0055114, GO:0006807
38	Maturity_(single)_trimmed_contig_41	TCM_004667	Ontology term: blue-light photoreceptor PHR2, lyase activity. Ontology ID: GO:0016829
39	Maturity_(single)_trimmed_contig_42	L484_011957 GLYMA_17G067800 glysoja_005748 Vigan.01G471200L7Y E99 PHAVU_003G150400 g GSCOC_T000413410 01 PRUPE_ppa001301mg	Ontology term: probable alpha, alpha-trehalose-phosphate synthase [UDP-forming] 11, mitochondrion, cytosol, alpha, alpha-trehalose-phosphate synthase (UDP-forming) activity, trehalose-phosphatase activity, trehalose biosynthetic process, dephosphorylation, starch metabolic process, sucrose metabolic process. Ontology ID: GO:0005739, GO:0005829, GO:0003825, GO:0004805, GO:0005992, GO:0016311, GO:0005982, GO:0005985
40	Maturity_(single)_trimmed_contig_43	GLYMA_03G146400 MTR_7g093610 GLYMA_19G149700 LR48_Vigan03g22410 0 RCOM_0446270 TCM_024951 PHAVU_001G144200 g	Ontology term: probable kinase At2g41970, protein tyrosine kinase activity, ATP binding, peptidyl-tyrosine phosphorylation. Ontology ID: GO:0004713, GO:0005524, GO:0018108
41	Maturity_(single)_trimmed_contig_44	GLYMA_12G213400 MTR_2g083210 LR48_Vigan05g14400 0 POPTR_0001s44490g glysoja_001450 PHAVU_005G092500 g GLYMA_13G288300	Ontology term: COBRA 10, integral component of membrane, anchored component of membrane, hydrolase activity, hydrolyzing O-glycosyl compounds, polysaccharide binding, cellulose microfibril organization, cell growth, carbohydrate metabolic process, Ontology ID: GO:0016021, GO:0031225, GO:0004553, GO:0030247, GO:0010215, GO:0016049, GO:0005975
42	Maturity_(single)_trimmed_contig_46	glysoja_041535 PHAVU_009G223000 g glysoja_035373 Vigan.02G295700 JCGZ_26043 GLYMA_09G075400 MTR_2g044580 GLYMA_15G183400 RCOM_1581960	Ontology term: DNAJ ERDJ2A, plasma membrane, integral component of endoplasmic reticulum membrane. Ontology ID: GO:0005886, GO:0030176
43	Maturity_(single)_trimmed_contig_47	Vigan.10G002500 GLYMA_20G001000 PHAVU_003G002000 g LR48_Vigan06g14350 0 glysoja_044851 MTR_1g038840 GLYMA_09G285100 glysoja_024379	Ontology term: 65-kDa microtubule-associated 6-like, microtubule binding, microtubule cytoskeleton organization, cytokinesis, tubulin complex, Ontology ID: GO:0008017, GO:0000226, GO:0000910, GO:0045298
44	Maturity_(single)_trimmed_contig_48	MTR_4g120220 JCGZ_18902 glysoja_009817 PHAVU_003G106700 g GLYMA_17G028000 GLYMA_07G246000 LR48_Vigan01g30420 0	Ontology term: Lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase mitochondrial"; "mitochondrion, zinc ion binding, acetyltransferase activity, dihydrolipoyllysine-residue (2-methylpropanoyl) transferase activity, acyl-carrier-protein biosynthetic process. Ontology ID: GO:0005739, GO:0008270, GO:0016407, GO:0043754, GO:0042967
45	Maturity_(single)_trimmed_contig_49	psbB	Ontology term: photosystem II, chloroplast thylakoid membrane, integral component of membrane, chlorophyll binding, electron transporter, transferring electrons within the cyclic electron transport pathway of photosynthesis activity, photosynthetic electron transport in photosystem II, protein-chromophore linkage, obsolete electron transport Ontology ID: GO:0009523, GO:0009535, GO:0016021, GO:0016168, GO:0045156, GO:0009772, GO:0018298, GO:0006118
46	Maturity_(single)_trimmed_contig_50	MTR_4g075610 C6TNH2 GLYMA_11G097500 PHAVU_002G326900 g glysoja_023504	Ontology term: Plastid movement impaired 1, cell, calcium ion binding, chloroplast relocation. Ontology ID: GO:0005623, GO:0005509, GO:0009902

		TCM_024527 PHAVU_011G025600 g GLYMA_05G243200 Vigan.01G008500	
47	Maturity_(single)_trimmed_contig_51	hsp70 AMTR_s00071p00117 740 "MTR_2g005690 VIT_17s0000g03310 Csa_2G122520 RCOM_0759010 JCGZ_00245	Ontology term: Stromal 70 kDa heat shock-related chloroplastic, ATP binding, 2-alkenal reductase [NAD(P)] activity, unfolded protein binding, protein folding, oxidation-reduction process. Ontology ID: GO:0005524, GO:0032440, GO:0051082, GO:0006457, GO:0055114
48	Maturity_(single)_trimmed_contig_52	PHAVU_006G134800 g glysoja_012434 I3SH46 Vigan.05G100200 MTR_2g027800 C6T9Q6 LR48_Vigan09g18910 0 glysoja_015124	Ontology term: PHR1-like 2-like isoform X1, nucleus, DNA binding, regulation of transcription, DNA-templated Ontology ID: GO:0005634, GO:0003677, GO:0006355
49	Maturity_(single)_trimmed_contig_53	Vigan.01G102600 GLYMA_08G124600 glysoja_015544 glysoja_010821 PHAVU_002G247000 g LR48_Vigan01g03510 0 GLYMA_05G166600	Ontology term: YTH domain-containing family 1 isoform X2, RNA binding Ontology ID: GO:0003723
50	Maturity_(single)_trimmed_contig_55	GLYMA_17G258700 LR48_Vigan07g27790 0 NtHsp90er-1 MTR_1g025430 GLYMA_14G219700 glysoja_008600	Ontology term: Endoplasmic homolog, ATP binding, unfolded protein binding, protein folding, response to stress. Ontology ID: GO:0005524, GO:0051082, GO:0006457, GO:0006950
51	Maturity_(single)_trimmed_contig_54	MTR_4g074350 L484_014984 LR48_Vigan08g18680 0 GLYMA_11G103500 DIM PHAVU_011G031700 g glysoja_020397 GLYMA_12G028300 EUGRSUZ_J00851	Ontology term: Delta(24)-sterol reductase, vacuolar membrane, plasma membrane, integral component of membrane, calmodulin binding, oxidoreductase activity, acting on CH-OH group of donors, delta24-sterol reductase activity, flavin adenine dinucleotide binding, lignin metabolic process, uni-dimensional cell growth, plant-type secondary cell wall biogenesis, brassinosteroid biosynthetic process, oxidation-reduction process, Ontology ID: GO:0005774, GO:0005886, GO:0016021, GO:0005516, GO:0016614, GO:0050614, GO:0050660, GO:0009808, GO:0009826, GO:0009834, GO:0016132, GO:0055114
52	Maturity_(single)_trimmed_contig_57	LR48_Vigan11g00240 0 Vigan.09G186600 GLYMA_07G046000 PHAVU_010G123100 gpme1 MTR_8g023310	Ontology term: pectinesterase-like, extracellular region, cell wall, integral component of membrane, pectinesterase activity, aspartyl esterase activity, pectinesterase inhibitor activity, cell wall modification, negative regulation of catalytic activity, pectin catabolic process, starch metabolic process, sucrose metabolic process. Ontology ID: GO:0005576, GO:0005618, GO:0016021, GO:0030599, GO:0045330, GO:0046910, GO:0042545, GO:0043086, GO:0045490, GO:0005982, GO:0005985

Table 3.1: Reproductive stage putative gene lists

Index	Identifier	Description
1	Reproductive_(single)_trimmed_contig_1	Ontology term: Glutamate biosynthesis process, cytoplasm, glutamate synthase activity, ammonia assimilation cycle, oxidation-reduction process Ontology ID: GO:0006537, GO:0005737, GO:0016041, GO:0019676, GO:0055114
2	Reproductive_(single)_trimmed_contig_2	Ontology term: Translation initiation factor activity, translational initiation Ontology ID: GO:0003743, GO:0006413
3	Reproductive_(single)_trimmed_contig_3	Ontology term: Pectin catabolic process, negative regulation of catalytic activity, cell wall, aspartyl esterase activity, integral component of membrane, pectinesterase inhibitor activity, cell wall modification, pectinesterase activity Ontology ID: GO:0045490, GO:0043086, GO:0005618, GO:0045330, GO:0016021, GO:0046910, GO:0042545,

Differential gene expression analysis

Differential gene expression analysis was carried out by using CLC genomic workbench (v10.0.0) in fenugreek genotype GMV- 2 at three developmental stages that were vegetative stage (20-30 DAS), reproductive stage (50-60 DAS) and maturity stage (80-90 DAS). In present study, the data

generated from transcriptome sequencing were mapped to reference genome *Arabidopsis thaliana* with a length of 119,667,750 bp, total number of genes present in the reference genome were 32,833 and 49,477 transcripts (Table 3.2).

Table 3.2: Mapping report of all the three developmental stages of fenugreek with reference genome *Arabidopsis thaliana*.

S. No	Particulars	Vegetative stage		Reproductive stage		Maturity stage	
		Single reads (bp)	% mapped with reference	Single reads (bp)	% mapped with reference	Single reads (bp)	% mapped with reference
1	Counted fragments	5,314,264	22.20	6,689,073	20.90	5,801,433	14.73
2	Unique fragments	4,135,444	17.28	5,601,734	17.50	5,090,342	12.93
3	Non-specifically	1,178,820	4.92	1,087,339	3.40	711,091	1.81
4	Uncounted fragments	18,621,933	77.80	25,315,513	79.10	33,575,263	85.27
5	Total fragments	23,936,197	100.00	32,004,586	100.00	39,376,696	100.00

Mapping report of developmental stages of fenugreek

The total number of reads present in the vegetative stage, were 23,936,197 and longest read was 358 bp and there was no paired reads in a data set. Total counted fragments were 5,314,264 bp and percent mapped with the *Arabidopsis* was 22.20 %, unique fragments were 4,135,444 bp with 17.28 %, non-specifically mapped fragments were 1,178,820 bp with a percent of 4.92, total uncounted fragments were 18,621,933 bp with mapping percentage of 77.80. In reproductive stage, the total number of reads present in the reproductive stage were 32,004,586 bp and longest read was 363 bp and there was no paired reads in a data set. In reproductive stage, total counted fragments were 6,689,073 bp and percent mapped with the *Arabidopsis* was 20.90 %, unique fragments were 5,601,734 bp with 17.50 %, non-specifically mapped fragments were 1,087,339 bp with a percent of 3.40, total uncounted fragments were 1,087,339 bp with mapping percentage of 79.10. In maturity stage, the total number of reads were 39,376,696 bp and longest read was 365 bp and there was no paired reads in a data set. In maturity stage, total counted fragments were 5,801,433 bp and percent mapped with the *Arabidopsis* was 14.73%, unique fragments were 5,090,342 bp with 12.93%, non-specifically mapped fragments were 711,091 bp with a percent of 1.81, total uncounted fragments were 33,575,263 bp with mapping percentage of 85.27.

Differential gene expression analysis in all the three developmental stages of fenugreek

In vegetative stage, top 10 highly expressed genes and 10 lower regulated genes were selected with an expression value of 474910 and RPKM was 73070.44 for highly expressed gene, with a gene length of 1223 bp and expression value of 3 and RPKM was 0.291139 for lower regulated gene, with a length of 2833bp in the vegetative stage. These genes were expressed during, cotyledon, cultured plant cell, juvenile vascular leaf, fruit, guard cell, hypocotyls, leaf mesophyll, petal, plant embryo axis, sepal, shoot axis cortex, shoot axis vascular system, vascular leaf, carpel, cauline leaf, collective leaf structure, flower pedicel, fruit, guard cell, hypocotyls, meristem, leaf apex, leaf lamina base, petal, petiole, plant embryo, pollen, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, four leaves visible stage, mature plant embryo stage, petal differentiation and expansion stage,

plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage and plant sperm cell (Table 3.3). In reproductive stage, top 10 highly expressed genes and 10 lower regulated genes were selected with an expression value of 233210 and RPKM was 28507.21 for highly expressed gene, with a length of 1223 bp and expression value of 3 and RPKM was 0.268558 for lower regulated gene, with a length of 2332 bp in the reproductive stage. These genes were expressed during the mature pollen stage, germinated pollen stage, seedling development stage, cotyledon, cultured plant cell, embryo root, epidermis, fruit, guard cell, hypocotyl, juvenile vascular leaf, leaf mesophyll, petal, pollen, pollen tube cell, root, root endodermis, root tip, root vascular system, sepal, shoot axis cortex, shoot axis epidermis, shoot axis vascular system, vascular bundle, vascular leaf. two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, seed development stage, seedling development stage, vascular leaf senescent stage, collective leaf structure, cotyledon, flower, flower pedicel, guardcell and hypocotyls (Table 3.3).

In maturity stage, top 10 highly expressed genes and 10 lower regulated genes were selected with an expression value of 82652 and RPKM was 6970.07088 for highly expressed gene, with a length of 2334 bp and expression value of 3 and RPKM was 1.104943625 for lower regulated gene, with a length of 468 bp in the maturity stage. These genes were expressed during, carpel, cauline leaf, collective leaf structure, cotyledon, flower, flower pedicel, guard cell, hypocotyl, inflorescence meristem, juvenile vascular leaf, leaf apex, leaf lamina base, petal, petiole, plant embryo, plant sperm cell, pollen, pollen tube cell, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, mature pollen stage, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescent stage, cotyledon, cultured plant cell, guard cell, juvenile vascular leaf and vascular leaf (Table 3.3).

Table 3.3: Differential gene expression analysis in all the three developmental stages of fenugreek

Index	Gene name	Expression value	RPKM	Gene ID	Gene length	Unique gene reads	Expressed during
Top 10 up-regulated genes in vegetative stage							
1	LHCB1.2_1	474910	73070.44	AT1G29910	1223	83631	Cotyledon, cultured plant cell, juvenile vascular leaf, fruit, guard cell, hypocotyls, leaf mesophyll, petal, plant embryo axis, sepal, shoot axis cortex, shoot axis vascular system, vascular leaf, carpel, cauline leaf, collective leaf structure, flower pedicel, fruit, guard cell, hypocotyls, meristem, leaf apex, leaf lamina base, petal, petiole, plant embryo, pollen, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, four leaves visible stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage and plant sperm cell.
2	LHCB1.3	292913	44811.6	AT1G29930	1230	52079	
3	LHCA3	251468	29119.66	AT1G61520	1698	251454	
4	AT2G05100	117024	15661.97	AT2G05100	1491	111576	
5	AT2G34420	98021	17650.61	AT2G34420	1045	0	
6	RCA	89431	8009.749	AT2G39730	2947	89450	
7	PSBO1	63193	7885.414	AT5G66570	1890	62870	
8	PSAD2	59794	11769.46	AT1G03130	956	59695	
9	LHCB3	57421	8699.735	AT5G54270	1404	57226	
10	RBCS-1A	56273	8350.985	AT1G67090	1510	53152	
Top 10 lower regulated genes in vegetative stage							
11	AT5G63230	3	1.219262	AT5G63230	463	3	Mature pollen stage, germinated pollen stage, seedling development stage, cotyledon, cultured plant cell, embryo root, epidermis, fruit, guard cell, hypocotyl, juvenile vascular leaf, leaf mesophyll, petal, pollen, pollen tube cell, root, root endodermis, root tip, root vascular system, sepal, shoot axis cortex, shoot axis epidermis, shoot axis vascular, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, seed development stage, seedling development stage, vascular leaf senescent stage, collective leaf, structure, cotyledon, flower, flower pedicel, guardcell and hypocotyl,
12	FLS2_2	3	0.409071	AT5G63580	1899	9	
13	AT5G65005	3	0.692661	AT5G65005	815	1	
14	AT5G65040	3	0.705648	AT5G65040	945	4	
15	AT5G65207	3	1.089804	AT5G65207	518	2	
16	AT5G66595	3	1.443781	AT5G66595	2304	11	
17	AT5G66970	3	1.069164	AT5G66970	681	2	
18	AT5G67080	3	0.422544	AT5G67080	1336	3	
19	AT5G67170	3	0.370906	AT5G67170	2330	7	
20	AT5G67610	3	0.291139	AT5G67610	2833	11	
Top 10 up-regulated genes in reproductive stage							
21	LHCB1.2_1	233210	28507.21	AT1G29910	1223	46691	Carpel, cauline leaf, collective leaf structure, cotyledon, flower, flower pedicel, guard cell, hypocotyl, inflorescence meristem, juvenile vascular leaf, leaf apex, leaf lamina base, petal, petiole, plant embryo, plant sperm cell, pollen, pollen tube cell, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, mature pollen stage, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescent stage, cotyledon, cultured plant cell, guard cell, juvenile vascular leaf and vascular leaf.
22	LHCB1.3	190531	23157.66	AT1G29930	1230	38986	
23	RBCS-1A	147817	17427.66	AT1G67090	1510	138822	
24	RCA	112746	8022.489	AT2G39730	2947	112779	
25	LHCA3	94263	8672.054	AT1G61520	1698	94257	
26	AT2G05100	91161	9692.991	AT2G05100	1491	89004	
27	A3_4	82489	5304.044	AT5G60390	2425	75954	
28	AT2G34420	74382	10641.08	AT2G34420	1045	0	
29	PSAD2	53619	8384.842	AT1G03130	956	53562	
30	UBQ14_2	49392	3612.516	AT4G05320	2332	36116	
Top 10 lower regulated genes in reproductive stage							
31	AT5G61050	3	0.590899	AT5G61050	1134	6	Carpel, cauline leaf, collective leaf structure, cotyledon, flower, flower pedicel, guard cell, hypocotyl, inflorescence meristem, juvenile vascular leaf, leaf apex, leaf lamina base, petal, petiole, plant embryo, plant sperm cell, pollen, pollen tube cell, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, mature pollen stage, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescent stage, cotyledon, cultured plant cell, guard cell, juvenile vascular leaf and vascular leaf.
32	AT5G62080	3	0.64162	AT5G62080	1014	6	
33	AT5G62340	3	0.480185	AT5G62340	934	2	
34	AT5G63250	3	0.779987	AT5G63250	575	3	
35	AT5G63820	3	0.661494	AT5G63820	1318	5	
36	AT5G64450	3	0.821415	AT5G64450	813	5	
37	AT5G65610	3	0.816926	AT5G65610	1587	38	
38	AT5G09615	3	1.057766	AT5G09615	584	2	
39	ERF122	3	0.74131	AT5G67000	605	3	
40	AT5G67160	3	0.268558	AT5G67160	1670	3	
Top 10 up-regulated genes (highly expressed) in maturity stage							
41	UBQ14_2	82652	6970.07088	AT4G05320	2332	68958	Carpel, cauline leaf, collective leaf structure, cotyledon, flower, flower pedicel, guard cell, hypocotyl, inflorescence meristem, juvenile vascular leaf, leaf apex, leaf lamina base, petal, petiole, plant embryo, plant sperm cell, pollen, pollen tube cell, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, mature pollen stage, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescent stage, cotyledon, cultured plant cell, guard cell, juvenile vascular leaf and vascular leaf.
42	PIP1-2	78883	8514.187731	AT2G45960	1902	67861	
43	UBQ14_1	69187	7285.184238	AT4G02890	1637	55152	
44	UBQ11	46832	5629.350277	AT4G05050	1434	34738	
45	PIP1-5	44739	5807.014581	AT4G23400	1828	42237	
46	ACT11	40000	3856.179095	AT3G12110	2310	37435	
47	LHCB1.2_1	37273	5253.304942	AT1G29910	1223	8965	
48	PIP1-3	37090	4127.338937	AT1G01620	1879	29878	
49	RPL40B_1	30412	6781.569343	AT2G36170	1280	25718	
50	UBQ3_1	27379	2542.753899	AT5G03240	1856	24751	
Top 10 lower regulated genes (less expressed) in maturity stage							
51	ATMG00540	3	1.401391915	ATMG00540	369	0	Carpel, cauline leaf, collective leaf structure, cotyledon, flower, flower pedicel, guard cell, hypocotyl, inflorescence meristem, juvenile vascular leaf, leaf apex, leaf lamina base, petal, petiole, plant embryo, plant sperm cell, pollen, pollen tube cell, root, seed, sepal, shoot apex, shoot system, stamen, stem, vascular leaf, mature pollen stage, two leaves visible stage, four leaves visible stage, six leaves visible stage, eight leaves visible stage, ten leaves visible stage, twelve leaves visible stage, germinated pollen stage, flowering stage, mature plant embryo stage, petal differentiation and expansion stage, plant embryo bilateral stage, plant embryo cotyledonary stage, plant embryo globular stage, vascular leaf senescent stage, cotyledon, cultured plant cell, guard cell, juvenile vascular leaf and vascular leaf.
52	ATMG00720	3	1.596029681	ATMG00720	324	2	
53	ATMG00970	3	1.460772928	ATMG00970	354	1	
54	ATMG01020	3	1.119293542	ATMG01020	462	0	
55	ATMG01230	3	1.180624695	ATMG01230	438	3	
56	ATMG01250	3	1.401391915	ATMG01250	369	3	
57	RPS16	3	2.154640069	ATCG00050	1105	6	
58	RPL20	3	1.460772928	ATCG00660	354	3	
59	RPS7-A_2	3	1.104943625	ATCG01240	468	0	
60	RPL23-A_2	3	1.833736229	ATCG01300	282	0	

Scatter plot graphs showing the differential expression of genes in different developmental stages of fenugreek

The simplest analysis tool for gene expression data visualization is the scatter plot. In a scatter plot each point represents the expression value of a gene in two experiments, one plotted on the x-axis and the other one on the y. In such a plot genes with equal expression values would line up on the identity line (diagonal), with higher expression values further away from the origin. Points below the diagonal represent genes with higher expression in the experiment plotted on the x-axis. Similarly, points above the diagonal represent genes with higher expression values in the experiment plotted on the y-axis. The further away the point is from the identity line, the larger is the difference between its expression in one experiment compared with the other.

In present study, differential gene expression analysis with group-1 (vegetative stage) and group- 2 (reproductive stage) was carried out. In this graphs, most of the genes were present in the both vegetative stage and reproductive stage were highly expressed in both the stages, some of the genes were found far away from the origin and above the diagonal

showing high expression in reproductive stage and vice versa, with $r = 0.98$ (ratio intensity) (Figure 3.2).

Expression analysis with group- 1 (vegetative stage) and group- 3 (maturity stage) showing that, in this graphs, some of the genes (points) were found below the diagonal highly expressed in vegetative stage than in maturity stage, some genes were found above the diagonal were highly expressed in maturity stage, the genes were found away from the origin and above the diagonal were highly expressed in maturity stage and *vice versa*, with $r = 0.36$. The genes which were found on the diagonal were highly expressed in both the stages *i.e.*, vegetative stage and maturity stage (Figure 3.1).

Expression analysis with group- 2 (reproductive stage) and group- 3 (maturity stage) showing that, in this graphs, some of the genes (points) were found below the diagonal highly expressed in reproductive stage than in maturity stage, some genes were found above the diagonal were highly expressed in maturity stage, the genes were found away from the origin and above the diagonal were highly expressed in maturity stage and *vice versa*, with $r = 0.43$. The genes which were found on the diagonal were highly expressed in both the stages *i.e.*, reproductive stage and maturity stage (Figure 3.3).

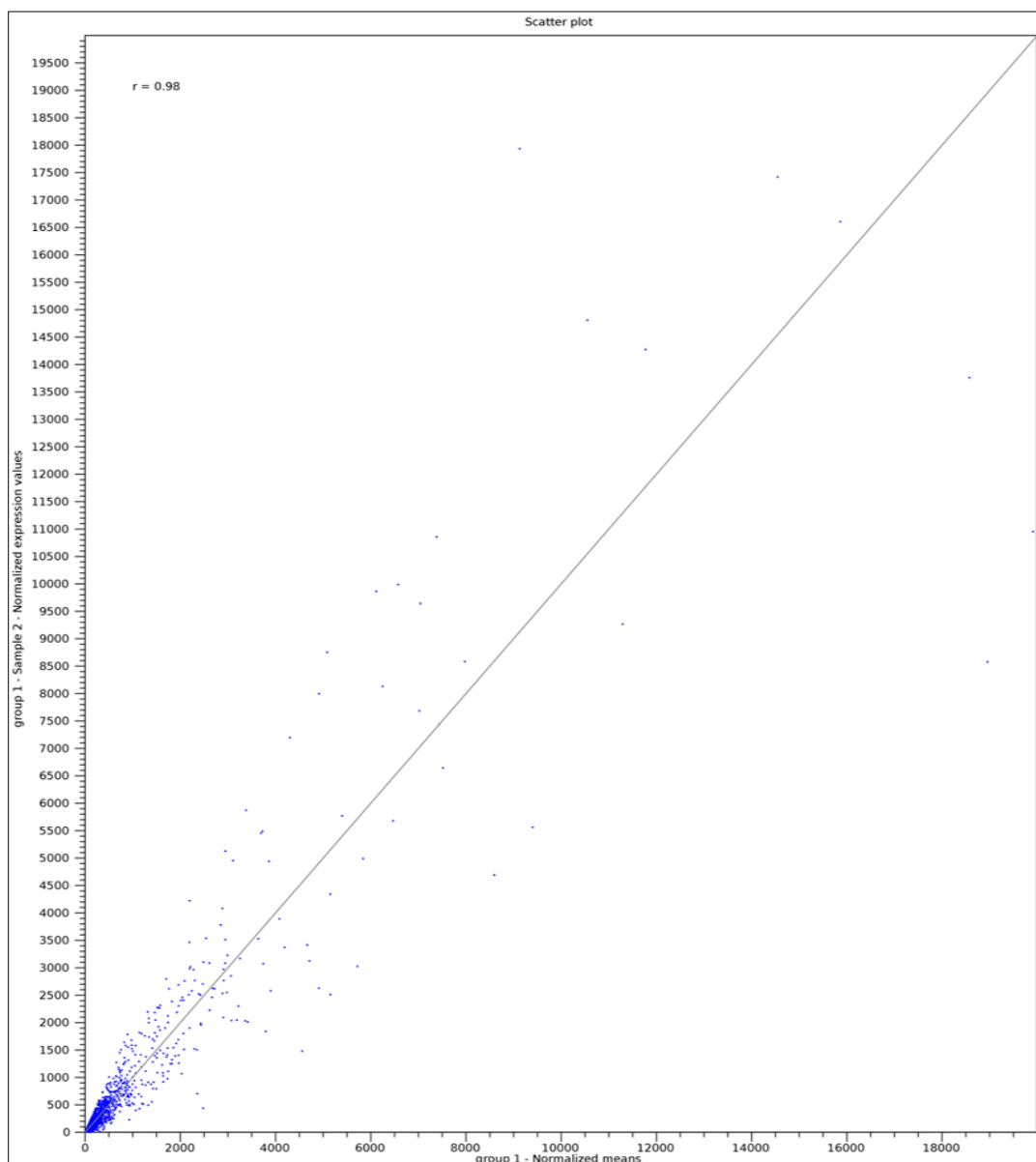


Fig 3.1: Scatter plot graph differential gene expression analysis between vegetative stage and reproductive stage

- a) Group- 1: Vegetative stage
- b) Group- 2: Reproductive stage

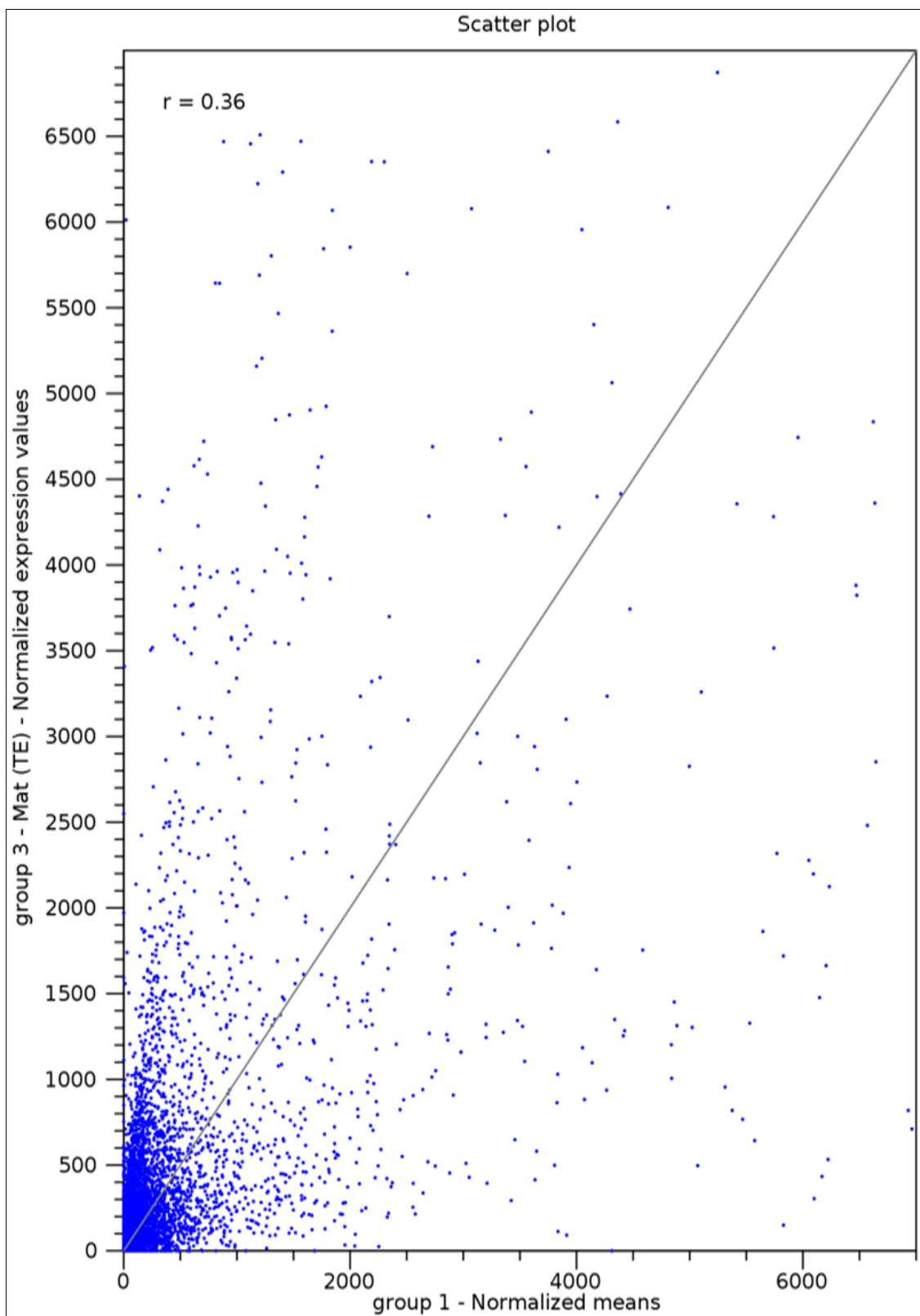


Fig 3.2: Scatter plot graph differential gene expression analysis between vegetative stage and maturity stage
a) Group- 1 : Vegetative stage
b) Group- 3 : Maturity stage

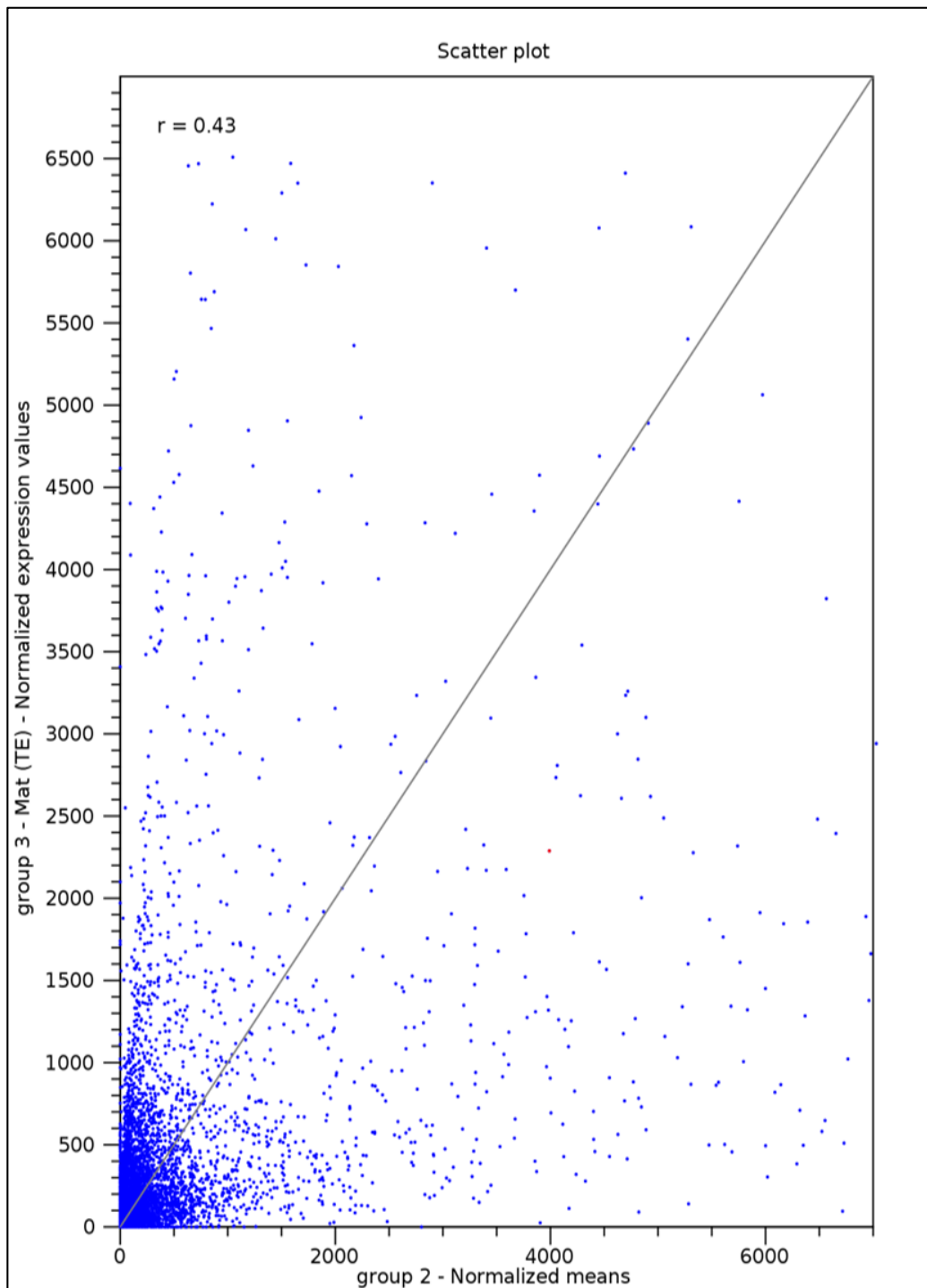


Fig 3.3: Scatter plot graph differential gene expression analysis between reproductive stage and maturity stage

- a) Group- 2: Reproductive stage
- b) Group- 3: Maturity stage

Conclusion

Fenugreek (*Trigonella foenum-graecum* L.) is extensively used as a spice crop in India and the Mediterranean region and is known to possess a number of medicinal properties. 445 putative genes were found from all the developmental stages of fenugreek. 48 putative genes in vegetative stage from 86 contigs, no genes were found in reproductive stage, instead of that GO IDs were found and 397 genes in maturity stage from 57 contigs. Through differential gene expression analysis the data generated from transcriptome were mapped with reference genome *Arabidopsis thaliana*, from that top 10 highly expressed and top 10 lower expressed genes were identified and their expression level in all the three developmental stages were identified. Results provide a

valuable resource for fenugreek research. The developed molecular markers are foundation for further genetic linkage analysis and gene localization and they will be essential to accelerate the process of breeding. It is a viable alternative source for different metabolites like, isoleucine, sapogenins and galactomannans production.

5. References

1. BBC. Food - Fenugreek recipes, 2017.
2. Raju Sajad S, Pradyuman K. Fenugreek: A review on its nutraceutical properties and utilization in various food product. *Journal of the Saudi Society of Agricultural Sciences*. 2004; 10:23-26.